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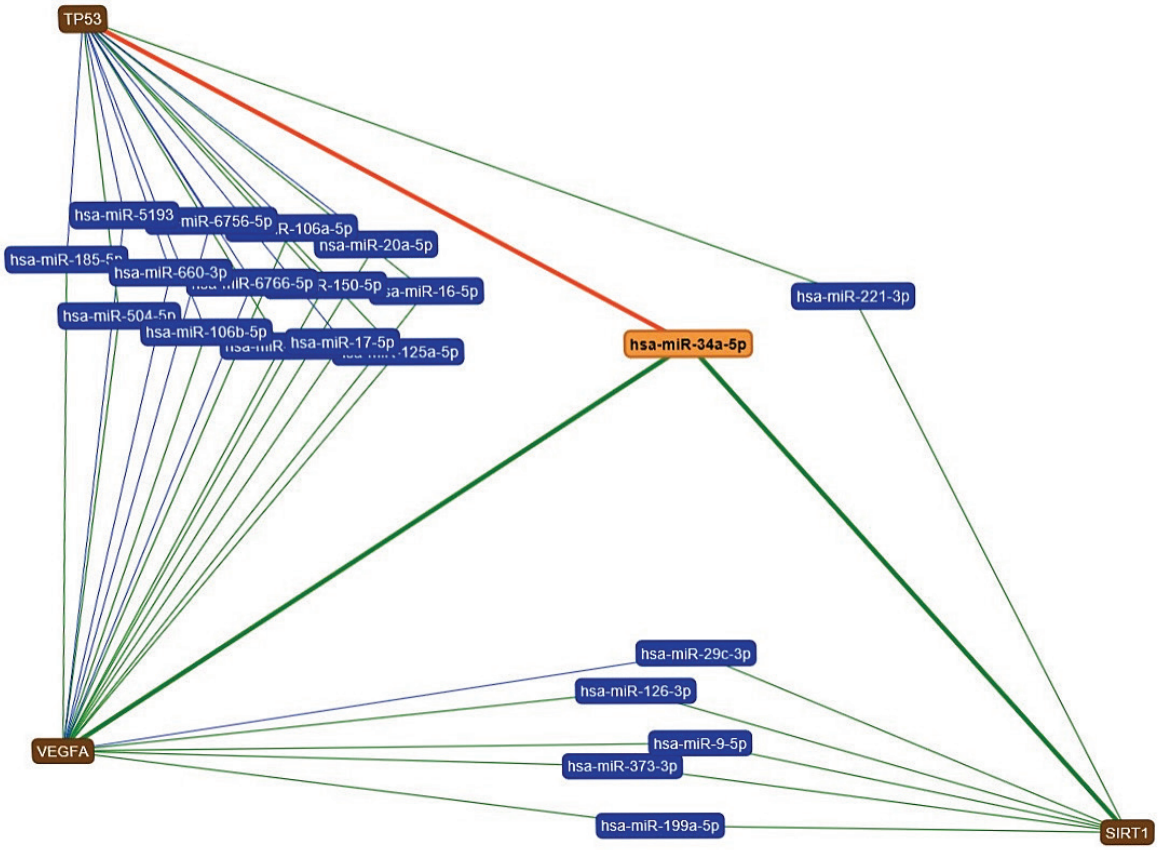



Interaction network of genes targeted by micro-RNA 34a (miR-34a) and playing a significant role in endothelial function. This analysis was done using (miRTargetLinkdatabase) (https://ccb-web.cs.uni-saarland.de/mirtargetlink/index.php) and retrieved that miR-34a is the only miRNA that targets the three major genes, vascular endothelial growth factor, *sirtuin 1 (SIRT1)* and *p53*, involved in endothelial function (green line indicates a strong interaction and red line indicates a weak interaction)

Association of Exosomal miR-34a with Markers of Dyslipidemia and Endothelial Dysfunction in Children and Adolescents with T1DM

Ibrahim AA et al.

Page: 401-409

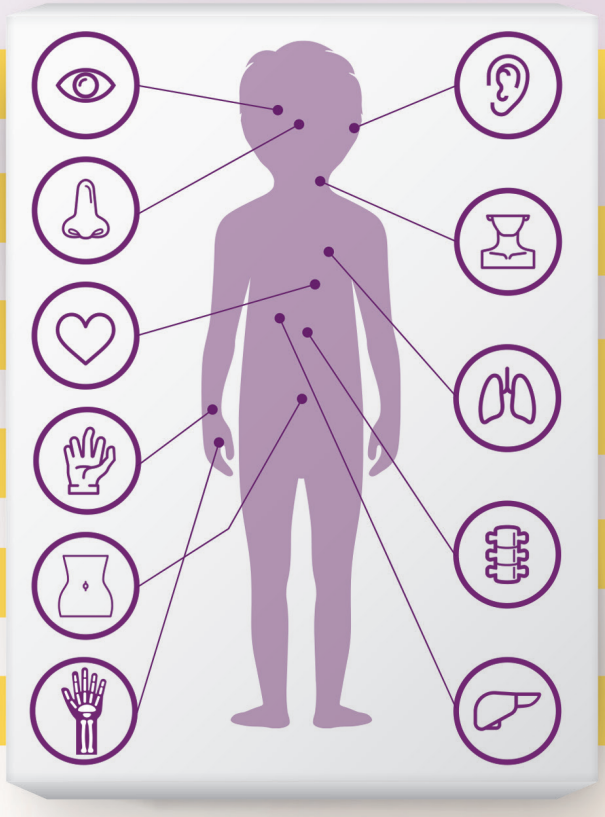

Official Journal of
Turkish Pediatric Endocrinology
and Diabetes Society

galenos yayınevi

# Kısa Boy
## Hafif MPS1'e İşaret Eden **Bir Şifre** Olabilir.[1-3]

**Kısa boyun** yanı sıra, hafif MPS1'li hastalarda aşağıdaki semptomlardan bir veya daha fazlası görülebilir[4-7]

- Korneal bulutlanma
- Kronik sinüzit
- Kardiyovasküler hastalık
- Eklemlerde kontraktürler
- Umbilikal/inguinal herni
- Karpal tünel sendromu
- Kronik otit
- Kısa boyun
- Respiratuar enfeksiyonlar ve hastalık
- Kifoz ve omurilikte baskı
- Hepatosplenomegali



**ALDURAZYME®, Mukopolisakkaridoz I (MPS I; a-L-iduronidaz eksikliği) tanısı konmuş hastalarda, hastalığın norolojik olmayan bulgularını tedavi etmek amacıyla uzun süreli enzim replasman tedavisinde endikedir.[8]**

**Referans: 1.** Morishita K and Petty RE. Rheumatology 2011;50v19-v25. **2.** Malkoç İ., Van Tıp Dergisi: 13 (2):67-70, 2006. **3.** Wilma Oostdijk Diagnostic Approach in Children with Short Stature Horm Res 2009;72:206-217. **4.** Wraith EJ. Espert Opin. Pharmacother. 2005;6(3):489-506. **5.** Pastores GM, Arn P, Beck M, et al. Molecular Genetics and Metabolism 2007;91:37-47. **6.** Muenzer J, Wraith JE and Clarke LA. Pediatrics 2009;123:19-29 **7.** Beck M, Arn P, Giugliani R, et al. Genet MEd 2014;16(10):759-65. **8.** Aldurazyme Kısa Ürün Bilgisi

**Aldurazyme® 100U/ml IV infüzyon için konsantre çözelti:** ▼ Bu ilaç ek izlemeye tabidir. Bu üçgen yeni güvenlilik bilgisinin hızlı olarak belirlenmesini sağlayacaktır. Ruhsatlandırma sonrası şüpheli ilaç advers reaksiyonlarının raporlanması büyük önem taşımaktadır. Raporlama yapılması, ilacın yarar/risk dengesinin sürekli olarak izlenmesine olanak sağlar. Sağlık mesleği mensuplarının herhangi bir şüpheli advers reaksiyonu Türkiye Farmakovijilans Merkezi (TÜFAM)'ne bildirilmesi gerekmektedir (www.titck.gov.tr; e-posta: tufam@titck.gov.tr; tel: 0 800 314 00 08; faks: 0 312 218 35 99). Her bir Aldurazyme flakonu 500U laronidaz içermektedir. 1 ml 100U (yaklaşık 0.58mg) laronidaz içermektedir. İnfüzyon için konsantre çözelti. Berrak/hafif opelasans ve renksiz /açık sarı renkli çözelti. Ambalaj miktarı: 1 flakonluk ambalajlarda. **Endikasyonları:** Aldurazyme® mukopolisakkaridoz I (MPS I; α-L-iduronidaz eksikliği) tanısı konmuş hastalarda, hastalığın nörolojik olmayan bulgularını tedavi etmek amacıyla uzun süreli enzim replasman tedavisinde endikedir. **Kullanım şekli ve dozu:** Aldurazyme® tedavisi, MPS I veya diğer kalıtımsal metabolik hastalıkların tedavisinde deneyimli olan hekimler tarafından takip edilmelidir. Aldurazyme® uygulaması, acil durumlarda kullanılmak üzere hayata döndürücü cihazların olduğu uygun klinik koşullarda yapılmalıdır. Aldurazyme®'ın tavsiye edilen dozu vücut ağırlığına göre her hafta bir kez intravenöz infüzyon yoluyla verilen 100U/kg'dır. Başlangıçtaki infüzyon hızı olan 2U/kg/saat, hasta tarafından tolere ediliyorsa, her 15 dakikada artırılarak maksimum 43 U/kg/saat değerine kadar çıkabilir. Uygulanacak toplam hacim yaklaşık 3-4 saat içerisinde verilmelidir. İnfüzyon için konsantre çözelti, aseptik teknik kullanılarak % 0.9 NaCl (i.v.) çözeltisi ile seyreltilmelidir. Seyreltilen Aldurazyme® çözeltisinin 0.2 mikrometre'lik iç filtresi olan bir infüzyon seti ile uygulanması tavsiye edilmektedir. Belirlenen flakon, uygulamadan 20 dakika önce oda sıcaklığına gelmesi için buzdolabından çıkartılırak; seyreltme öncesi yabancı madde ve renklenme açısından göz ile kontrol edilir. Çözelti herhangi bir gözle görülebilir partikül içermemelidir. Yabancı madde içeren veya renklenme görülen flakonlar kullanılmamalıdır. Vücut ağırlığı 20 kg'dan az veya eşit ise 100 ml'ye, vucüt ağırlığı 20 kg'dan fazla ise 250 ml'ye % 0.9 NaCl (i.v.) ile seyreltilir. **Uyarılar/Önlemler:** Aldurazyme® ile tedavi edilen hastalarda infüzyon sırasında veya infüzyon yapılan günün sonuna kadar olan sürede infüzyona bağlı reaksiyonlar oluşabilir. Tedavi edilen hastalar yakından takip edilmelidir. Altta yatan akut bir hastalığı bulunanlar, advers reaksiyon açısından daha büyük risk taşırlar. Özellikle, ciddi üst solunum yolu tutulumu olan hastalarda, infüzyon ile ilgili şiddetli reaksiyonlar bildirilmiştir, bu sebeple özellikle bu hastalar yakından takip edilmelidir. Antikor oluşum durumu düzenli olarak takip edilmeli ve rapor edilmelidir. Bu tıbbi ürün sodyum içerir ve intravenöz %0.9 Sodyum klorür ile uygulanır; bu sebeple sodyum diyetindeki hastalarda göz önünde bulundurulmalıdır. Araç ve makina kullanma üzerine etkisi incelenmemiştir. Böbrek/karaciğer yetmezliği bulunan hastalarda ve geriyatrik popülasyonda Aldurazyme®'ın güvenlilik ve etkililiği değerlendirilmemiştir. Dolayısıyla bu hastalarda herhangi bir doz rejimi tedavisi yapılamamaktadır. Pediyatrik popülasyonda doz ayarlaması gerekli değildir. **Gebelik/Laktasyon Döneminde Kullanım:** Gebelik kategorisi B'dir. Çocuk doğurma potansiyeli olan kadınlar ve kontrasepsiyon ile ilgili veri yoktur. Aldurazyme® açıkça gerekli olmadığı sürece gebelik süresinde kullanılmamalıdır. Laronidaz süte geçebilir. Yeni doğanların anne sütü yoluyla laronidaza maruz kalmasının neden olacağı etkiler ile ilgili yeterli veri olmadığından, Aldurazyme® kullanırken emzirmenin durdurulması tavsiye edilmektedir. Aldurazyme®'in insanlarda üreme yeteneğine etkisi ile ilgili bilgi bulunmamaktadır. **Yan Etkiler/Kontrendikasyonlar:** Etkin maddeye veya formülasyonda yer alan yardımcı maddelerden herhangi birine karşı şiddetli aşırı duyarlılık (anaflaktik reaksiyon). Klinik çalışmalardaki istenmeyen etkilerin büyük bir kısmı (Faz 3'te %53 ve Faz 4'te %35) infüzyon ile ilişkili olay olarak sınıflandırılmıştır. İnfüzyona bağlı advers etkilerin bazıları şiddetlidir. Zamanla birlikte bu reaksiyonların sayıları azalır. En sık ilaç advers etkiler: Baş ağrısı, bulantı, karın ağrısı, kaşıntı, artralji, sırt ağrısı, ekstremitelerde ağrı, flushing, yüksek ateş, infüzyon bölgesinde reaksiyonlar, kan basıncı artışı, oksijen satürasyon düşüşü, taşikardi ve tiremedir. **Doz Aşımı:** Doz aşımı vakası bildirilmemiştir. **İlaç Etkileşimleri:** Tıbbi ürünler ile ilgili herhangi bir etkileşim çalışması yapılmamıştır. Metabolizması nedeniyle laronidazın sitokrom p450'den kaynaklanan etkileşimler için uygun bir aday olduğu söylenemez. Aldurazyme®, laronidazın hücreler tarafından alımında potansiyel etkileşim riski nedeni ile klorokin veya prokainle birlikte kullanılmamalıdır. **Raf ömrü/Saklama Koşulları:** Raf ömrü 36 aydır. Mikrobiyolojik güvenlilik açısından ürün hemen kullanılmalıdır. Eğer hemen kullanılmazsa, kullanmadan önce saklanma ve koşulları kullanıcının sorumluluğundadır ve 24 saatten fazla olmayacak şekilde, 2-8C'de, ışıktan korunarak saklanmalıdır. **Ruhsat tarihi ve numarası:** 20.10.2007; 123/17 **KÜB revizyon tarihi:** 05.11.2014 **Ruhsat Sahibinin İsim ve Adresi:** Genzyme Europe B.V. Hollanda lisansı ile Sanofi Sağlık Ürünleri Ltd. Şti. Büyükdere Cad. No: 193 Levent-Şişli İstanbul Tel:0212 339 10 00 www.sanofi.com. Daha geniş bilgi için firmamıza başvurunuz. **Reçete ile satılır.** 19/02/2020 tarihi itibariyle KDV dahil parekende satış fiyatı Aldurazyme® 100U/ml IV infüzyon için konsantre çözelti: 3.584,61TL'dir. **KÜB ÖZETİ Onay Kodu:** GZTR.ALDU.20.02.0104b

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## AIMS AND SCOPE

The Journal of Clinical Research in Pediatric Endocrinology (JCRPE) publishes original research articles, reviews, short communications, letters, case reports and other special features related to the field of pediatric endocrinology. JCRPE is published in English by the Turkish Pediatric Endocrinology and Diabetes Society quarterly (March, June, September, December). The target audience is physicians, researchers and other healthcare professionals in all areas of pediatric endocrinology.

JCRPE is indexed in **EBSCO, SCOPUS, EMBASE, Engineering Village, Reaxys, Index Copernicus, CINAHL, ProQuest, GALE, Turk Medline, Tübitak Ulakbim TR Index, Index Medicus/PubMed, Turkiye Citation Index, PubMed Central (PMC), Science Citation Index-SCI-E, Hinari, GOALI, ARDI, ROOT INDEXING, OARE, PubMed/MEDLINE, J-GATE, Idealonline and DOAJ.**

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****The 5-year impact factor 1.9 in 2019.**
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**What is already known on this topic?**

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These two items must be completed before submission. Each item should include at most 2-3 sentences and at most 50 words focusing on what is known and what this study adds.

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All clinical investigations described in submitted manuscripts must have been conducted in accordance with the guidelines in the Declaration of Helsinki and has been formally approved by the appropriate institutional review committees. All manuscripts must indicate that such approval was obtained and that informed consent was obtained from subjects in all experiments involving humans. The study populations should be described in detail. Subjects must be identified only by number or letter, not by initials or names. Photographs of patients' faces should be included only if scientifically relevant. Authors must obtain written consent from the patient for use of such photographs.

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Limitations of the study should be detailed. In addition, an evaluation of the implications of the obtained findings/results for future research should be outlined.

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The conclusion of the study should be highlighted.

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Number of References: Case Report max 30 / Original Articles max 50
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### Sample References
*Papers Published in Periodical Journals:* Gungor N, Saad R, Janosky J, Arslanian S. Validation of surrogate estimates of insulin sensitivity and insulin secretion in children and adolescents. J Pediatr 2004;144:47-55.

*Papers Only Published with DOI Numbers:* Knops NB, Sneeuw KC, Brand R, Hile ET, de Ouden AL, Wit JM, Verloove-Vanhorick SP. Catch-up growth up to ten years of age in children born very preterm or with very low birth weight. BMC Pediatrics 2005 doi: 10.1186/1471-2431-5-26.
*Book Chapters:* Darendeliler F. Growth Hormone Treatment in Rare Disorders: The KIGS Experience. In: Ranke MB, Price DA, Reiter EO (eds). Growth Hormone Therapy in Pediatrics: 20 Years of KIGS. Basel, Karger, 2007;213-239.

*Books:* Practical Endocrinology and Diabetes in Children. Raine JE, Donaldson MDC, Gregory JW, Savage MO. London, Blackwell Science, 2001;37-60.

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**Referanslar: 1.** Omnitrope® KÜB. **2.** Genotropin KÜB. **3.** Norditropin KÜB. **4.** Humatrope KÜB. **5.** Saizen KÜB. **6.** Romer T et al. Seven years of safety and efficacy of the recombinant human growth hormone Omnitrope® in the treatment of growth hormone deficient children: results of a phase III study. Horm Res 2009; 72: 359-369. **7.** Rapaport R, et al. Med Devices (Auckl) 2013;6:141-146. **8.** Partsch CJ, et al. Med Devices (Auckl) 2015;8:389-393. **9.** Omnitrope® Resmi Websitesi. https://www.sandoz.com/our-work/biopharmaceuticals/sandoz-biosimilars Erişim tarihi: Mart 2019

▼ **Bu ilaç ek izlemeye tabidir.** Bu üçgen yeni güvenlilik bilgisinin hızlı olarak belirlenmesini sağlayacaktır. Sağlık mesleği mensuplarının şüpheli advers reaksiyonları TÜFAM'a bildirmeleri beklenmektedir. Raporlama yapılması, ilaçın yarar/risk dengesinin sürekli olarak izlenmesine olanak sağlamaktadır. Herhangi bir şüpheli advers reaksiyonu Türkiye Farmakovijilans Merkezi (TUFAM)'ne (www.titck.gov.tr; e-posta: tufam@titck.gov.tr; tel: 0312 218 30 00, 0800 314 00 08; faks: 0 312 218 35 99) ve/veya ilgili firma yetkililerine bildirmeniz gerekmektedir.

OMNITROPE® 5 mg (15 IU)/1.5 mL ve 10 mg (30 IU)/1.5 mL ve OMNITROPE® 15 mg (45 IU)/1,5 mL SC enjeksiyon için çözelti içeren kartuş **Etkin madde:** OMNITROPE® 5 mg kartuş 5 mg Somatropin'e eşdeğer (15 IU) 1.5 mL, OMNITROPE® 10 mg kartuş, 10 mg Somatropin'e eşdeğer (30 IU) 1.5 mL, OMNITROPE® 15 mg Somatropin'e eşdeğer (45 IU) 1,5 mL çözelti içerir. Somatropin rekombinant DNA teknolojisi ile Escherichia coli'den üretilmiştir. **Terapötik endikasyonlar:** İnfantlar, çocuklar ve adolesanlar: Büyüme hormonunun (GH) yetersiz salınımından kaynaklanan büyüme bozuklukları, Turner sendromu ile ilişkili büyüme bozuklukları, kronik böbrek yetmezliği ile ilişkili büyüme bozuklukları, -2 standart sapma (SD) altındaki doğum ağırlığı ve/veya doğum boyu olan 4 yaşında veya daha sonraki yaşlarda büyümede geri kaldığı gösterilen (son yıl süresince uzama hızı (HV) SDS<0), gestasyonel yaşa göre (SGA) küçük doğmuş kısa boylu çocuklardaki (mevcut uzunluk standart sapma oranı (SDS) <-2.5 ve parental ayarlanmış SDS<-1) büyüme bozuklukları, Prader-Willi sendromunda (PWS), büyüme ve vücut kompozisyonunun düzeltilmesi için. Yetişkinler: Belirgin bir büyüme hormonu yetmezliği olan yetişkinlerde replasman tedavisi için. **Posoloji:** Çocuklarda büyüme hormonunu salgılama yetersizliğine bağlı büyüme bozukluğu: Genel olarak günlük 0.025-0.035 mg/kg (günlük 0.7-1.0 mg/m²) tavsiye edilmektedir. Daha yüksek dozlar da kullanılmıştır. Prader-Willi Sendromu olan çocuklarda büyüme ve vücut kompozisyonunun düzeltilmesi için: Genel olarak günlük 0.035 mg/kg (günlük 1.0 mg/m²) tavsiye edilmektedir. Günlük doz 2.7 mg'ı aşmamalıdır. Turner sendromuna bağlı büyüme bozukluğu: Günlük 0.045-0.050 mg/kg (günlük 1.4 mg/m²) tavsiye edilmektedir. Kronik böbrek yetmezliğine bağlı büyüme bozukluğu: Günlük 0.045-0.050 mg/kg (1.4 mg/m²) doz tavsiye edilmektedir. Gestasyonel yaşa göre küçük doğmuş (SGA) çocuklarda / adolesanlarda büyüme bozukluğu: Genellikle en son uzunluğa ulaşılıncaya kadar günlük 0.035 mg/kg (günlük 1 mg/m²) tavsiye edilmektedir. Yetişkinlerde büyüme hormonu yetersizliği: Günlük 0.15-0.3 mg gibi düşük bir dozda tedaviye başlanmalıdır. Doz, IGF-1 konsantrasyonuna göre belirlenen bireysel hasta gereksinimlerine göre aşamalı olarak artırılmalıdır. **Uygulama sıklığı ve süresi:** OMNITROPE® günde bir defa, akşamları uygulanır. Büyüme hormonu eksikliği tedavisi normal olarak, uzun süreli tedavi gerektirir. Dozaj ve tedavi süresi hastanın tedaviye vereceği yanıta göre bireyselleştirilmelidir. **Uygulama şekli:** Subkütan enjeksiyon şeklinde uygulanır. Yalnızca OMNITROPE® 5 mg (15 IU)/1.5 mL'lik kullanım için özel olarak geliştirilmiş bir enjeksiyon aracı (enjeksiyon kalemi) olan SurePal™ 5; OMNITROPE® 10 mg (30 IU)/1.5 mL'lik kullanım için özel olarak geliştirilmiş bir enjeksiyon aracı (enjeksiyon kalemi) olan SurePal™ 10; OMNITROPE® 15 mg (45 IU)/1.5 mL'lik kullanım için özel olarak geliştirilmiş bir enjeksiyon aracı (enjeksiyon kalemi) olan SurePal™ 15 ile uygulanmalıdır. **Özel popülasyonlara ilişkin ek bilgiler: Böbrek yetmezliği:** Kronik böbrek yetmezliği durumunda, tedaviye başlamadan önce böbrek fonksiyonları normalin %50'sinin altında olmalıdır. Büyüme bozukluğunu doğrulamak için tedavinin başlatılmasından önce bir yıl süreyle büyüme takip edilmelidir. Bu dönem süresince böbrek yetmezliği için konservatif tedavi (asidoz, hiperparatiroidizm ve beslenme durumu kontrolünü içeren) uygulanmalı ve tedavi süresince sürdürülmelidir. **Karaciğer yetmezliği:** Karaciğer fonksiyon bozukluğu olan hastalarda somatropin klerensinde azalma görülebilmektedir, ancak bu durumun klinik önemi bilinmemektedir. **Pediyatrik popülasyon:** Somatropin dozu ve uygulama takvimi her hastaya göre bireysel olarak ayarlanmalıdır. Epifiz füzyonu oluşmuşsa tedaviye devam edilmemelidir. Büyüme hormonu tedavisine yanıt zamanla azalma eğilimi gösterir. **Kontrendikasyonlar:** Somatropin veya herhangi bir yardımcı maddeye karşı aşırı duyarlılık. Somatropin; tümör aktivitesine dair herhangi bir bulgu olduğu zaman kullanılmamalıdır ve tedaviye başlamadan önce anti-tümör tedavisi tamamlanmış olmalıdır. Somatropin epifizleri kapanmış çocuklarda büyümenin uyarılması için kullanılmamalıdır. Açık kalp ameliyatı, abdominal cerrahi, multiple kaza travması, akut solunum yetmezliği veya benzer durumlar gibi akut kritik hastalığı olanlarda somatropin ile tedavi uygulanmamalıdır. **Özel kullanım önerileri:** Eğer somatropin almakta olan bir kadın oral östrojen tedavisine başlarsa, serum IGF-1 seviyelerinin yaşa göre uygun aralıkta muhafaza edilebilmesi için somatropin dozunun artırılması gerekli olabilir. Büyüme hormonu, T4'ün T3'e tiroid bezi dışındaki dönüşümünü artırabilir, bu durum serum T4 seviyesinde azalma ve serum T3 konsantrasyonlarında bir artışla sonuçlanabilir. Malign hastalıkların tedavisine sekonder olarak görülen bir büyüme hormonu yetmezliği varsa malignitenin nüks belirtilerine dikkat edilmesi tavsiye edilmektedir. Şiddetli ve yinelenen baş ağrısı, görme problemleri, mide bulantısı ve/veya kusma durumlarında papilödemi için fundoskopinin yapılması tavsiye edilmektedir. Eğer papilödemi varlığı doğrulanırsa, iyi huylu intrakraniyal hipertansiyon tanısı düşünülmelidir ve eğer uygunsa büyüme hormonu tedavisi kesilmelidir. OMNITROPE® 5 mg (15 IU)/1.5 mL içeren form her mL'de 9 mg benzil alkol içerir. Benzil alkol varlığından dolayı prematüre bebekler ve yeni doğanlara uygulanmamalıdır. Bebeklerde ve 3 yaşına kadar olan çocuklarda toksik reaksiyonlara ve anafilaktoid reaksiyonlara sebebiyet verebilir. **Gebelik ve laktasyon Genel tavsiye** Gebelik kategorisi: C **Gebelik dönemi:** OMNITROPE® gebelik döneminde kullanılmamalıdır. Emziren kadınlarda somatropin içeren ürünlerle yapılan klinik çalışma yoktur. Emzirmenin durdurulup durdurulmayacağına ya da OMNITROPE® tedavisinin durdurulup durdurulmayacağına/ tedaviden kaçınılıp kaçınılmayacağına ilişkin karar verilirken, emzirenin çocuk açısından faydası ve OMNITROPE® tedavisinin emziren anne açısından faydası dikkate alınmalıdır. **İstenmeyen etkiler:** Büyüme hormonu yetmezliği olan hastalar, ekstrasellüler hacim eksikliği ile karakterizedir. Somatropin ile tedaviye başlandığı zaman bu hacim hızla düzelmektedir. Yetişkin hastalarda periferal ödem, ekstremitelerde tutukluk, artralji, miyalji ve parestezi gibi sıvı retansiyonu ile ilgili advers etkiler yaygın olarak meydana gelmektedir ve kendiliğinden veya doz azaltılması ile birlikte hafifler. Çocuklarda bu gibi advers etkiler yaygın değildir. Somatropin, hastaların yaklaşık %1'inde antikor oluşumuna neden olmaktadır. Bu antikor oluşumu klinik değişikliklere neden olduğu saptanmamıştır ve bağlanma kapasitesi düşüktür. Somatropinin serum kortizol seviyelerini büyük olasılıkla taşıyıcı proteinleri etkilemek suretiyle ya da artan hepatik klirens yoluyla azalttığı rapor edilmiştir. Klinik önemi sınırlı olabilir ancak kortikosteroid replasman tedavisi, tedaviye başlamadan önce optimize edilmelidir. Somatropinle tedavi gören büyüme hormonu yetmezliği olan çocuklarda seyrek ya da çok seyrek olarak fesmi vakaları meydana geldiği rapor edilmiştir ve bu durum pazarlama sonrası deneyimde yer almıştır. Beyin ve kafatya radyasyon uygulaması gibi hazırlayıcı faktörler olmaksızın lösemi riski artışına dair bir kanıt bulunmamaktadır. GH ile tedavi edilen çocuklarda femur başı epifiz kayması ve Legg-Calvé-Perthes hastalığı raporlanmıştır. Femur başı epifiz kayması, endokrin bozukluklar halinde daha sık meydana gelmektedir ve boy kısalığı halinde Legg-Calvé-Perthes daha sıktır. Pazara erişim sonrası deneyimde, her ne kadar nedensel bir ilişki gösterilemese de somatropin ile tedavi edilen Prader-Willi sendromlu hastalarda seyrek olarak ani ölüm raporlanmıştır. Somatropin ile tedavi edilirken bu patolojilerin daha sık olup olmadığı bilinmemektedir. Azalan insülin duyarlılığı nedeniyle hiperglisemi somatropine ait sınıf etkisi olduğu düşünülebilir. Ayrıntılı bilgi için TITCK onaylı KÜB bakınız. **Doz aşımı ve tedavisi:** Akut doz aşımı başlangıçta hipoglisemiye ve daha sonra hiperglisemiye neden olabilir. Uzun süreli doz aşımı, yüksek doz insan büyüme hormonunun bilinen etkileri ile uyumlu belirti ve bulgularla sonuçlanabilir. **Farmakokinetik özellikler:** Emilim: Sağlıklı gönüllülerde ve büyüme hormonu yetersizliği olan çocuklarda subkütan olarak uygulanan somatropinin biyoyararlanımı yaklaşık %80'dir. Sağlıklı yetişkenlerde 5 mg/1,5 mL OMNITROPE®'un subkütan enjeksiyonundan sonra Cmaks ve tmaks değerleri sırasıyla 72 ± 28 mikrogram/L ve 4 ± 2 saattir. Sağlıklı yetişkenlerde 10 mg/1,5 mL OMNITROPE®'un 5 mg subkütan enjeksiyonundan sonra Cmaks ve tmaks değerleri sırasıyla 74 ± 22 mikrogram/L ve 3,9 ± 1,2 saattir. Sağlıklı yetişkenlerde 15 mg/1,5 mL OMNITROPE®'un 5 mg subkütan enjeksiyonundan sonra Cmaks ve tmaks değerleri sırasıyla 52 ± 19 mikrogram/L ve 3,7 ± 1,2 saattir. Eliminasyon: Büyüme hormonu yetersizliği olan yetişkinlerde intravenöz uygulamadan sonra somatropinin ortalama terminal yarı ömrü yaklaşık 0,4 saattir. Ancak, OMNITROPE® 5 mg/1,5 mL ve 10 mg/1,5 mL'nin subkütan uygulamasından sonra 3 saatlik, OMNITROPE® 15 mg/1,5 mL'nin subkütan uygulamasından sonra ise 2,76 saatlik bir yarı ömre ulaşılmıştır. Gözlenen farklılığa subkütan uygulamayı takiben enjeksiyon bölgesinden yavaş emilimin neden olması muhtemeldir. **Raf ömrü:** OMNITROPE® 5 mg (15 IU)/1.5 mL için 24 ay, OMNITROPE® 10 mg (30 IU)/1.5 mL için 18 ay, OMNITROPE® 15 mg (45 IU)/1.5 mL için 18 aydır. İlk kullanımdan sonra raf ömrü: İlk kullanımdan sonra kartuş enjeksiyon kaleminin içinde kalmalıdır. Açıldıktan sonra buzdolabında (2°C-8°C) saklanması koşulu ile 28 gün içerisinde kullanılmalıdır. Dondurulmamalıdır. Orijinal enjeksiyon kaleminin içerisinde ışıktan korunarak saklanmalıdır. **Saklamaya yönelik özel tedbirler:** Açılmamış kartuş: Buzdolabında (2°C-8°C) saklanmalı ve taşınmalıdır. Dondurulmamalıdır. Orijinal ambalajında ışıktan korunarak saklanmalıdır. **Ambalajın niteliği ve içeriği:** Bromobütil tıpası ve alüminyum çek-çıkar kapağı olan renksiz, 1.5 mL'lik Tip I kartuş ile ambalajlanır. **KDV DAHİL PERAKENDE SATIŞ FİYATI:** OMNITROPE® 5 mg (15 IU)/1.5 mL SC enjeksiyon için çözelti içeren kartuş 330,07 TL KDV Dahil (19.02.2020), OMNITROPE® 10 mg (30 IU)/1.5 mL SC enjeksiyon için çözelti içeren kartuş 635,71 TL KDV dahil (19.02.2020),OMNITROPE® 15 mg (45 IU)/1,5 mL SC enjeksiyon için çözelti içeren kartuş 997,18 TL KDV dahil (19.02.2020). **RUHSAT SAHİBİ:** Sandoz İlaç San. ve Tic. A.Ş. Suryapı & Aksel İş Merkezi Rüzgarlıbahçe Mah. Şehit Sinan Eroğlu Cad. 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Sağlık mesleği mensuplarının herhangi bir şüpheli advers reaksiyonu Türkiye Farmakovijilans Merkezi (TÜFAM)'ne bildirmeleri gerekmektedir (www.titck.gov.tr; e- posta: tufam@titck.gov.tr; tel: 0 800 314 00 08; faks: 0 312 218 35 99). Sandoz ürünleri ile ilgili advers olayları drug_safety.turkey@novartis.com adresine e-posta göndererek ya da 0216 681 22 11 numarasına faks çekerek Hasta Güvenliği Departmanı'na bildirebilirsiniz.
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CONTENTS

# BH tedavisi
# işte bu kadar kolay[1,2*]


Saizen® Likit*, 25 derecenin altında art arda 7 gün boyunca saklanabilir.[2**]

* Enjeksiyonluk çözelti içeren kartuş

** Saizen® kartuş orijinal ambalajında buzdolabında 2°C-8°C arasında 18 ay boyunca saklanabilir. Kartuş açıldıktan sonra oda ısısında (25°C veya altında) birbirini takip eden 7 gün, buzdolabında (2°C-8°C) ise en fazla 28 gün boyunca saklanabilir.

**Referanslar: 1.** Dahlgren J et al. Curr Med Res Opin. 2007; 23 (7): 1649-1655. **2.** Saizen® enjeksiyonluk çözelti içeren kartuş kısa ürün bilgisi

**SAİZEN 12 mg/1,5 mL enjeksiyonluk çözelti içeren kartuş.** Her bir kartuş 1,5 mL çözeltide 12 mg somatropin* içerir. **SAİZEN 20 mg/2,5 mL enjeksiyonluk çözelti içeren kartuş.** Her bir kartuş 2,5 mL çözeltide 20 mg somatropin* içerir.

**Farmakolojik Özellikler:** Enjeksiyonluk çözelti mL başına 8 mg somatropin içerir. SAİZEN, memeli hücrelerinden genetik mühendisliğiyle üretilmiş rekombinant insan büyüme hormonu içerir. **Endikasyonlar:** Çocuklarda ve ergenlerde: Çocuklarda endojen büyüme hormonu salgısının olmaması veya azlığı ile meydana gelen büyüme geriliği / Kromozom analizleri ile teyid edilmiş, gonadal disgenezili kızlarda büyüme geriliği (Turner sendromu) / Kronik böbrek yetmezliğinden ötürü prepubertal çocuklarda büyüme geriliği (CRF) / Doğum ağırlığı ve/veya boyu –2 SD altında olan, gestasyonel yaşa göre küçük doğmuş (SGA) ve 4 yaş ya da üzeri itibariyle büyümeyi yakalayamamış (son yıl içinde HV SDS < 0) kısa çocuklarda büyüme bozukluğu (mevcut boy SDS < -2,5 ve ayarlanmış parental boy SDS < -1) Yetişkinlerde: Yetişkinlerde, büyüme hormonu eksikliğini ölçen tek dinamik test ile teşhis edilen belirgin büyüme hormonu eksikliğinin replasman tedavisi. Hastalar aşağıdaki kriterlere uymalıdır: Çocuklukta başlayan: Çocukluk esnasında büyüme hormonu eksikliği teşhis edilmiş hastalar, tekrar test edilmelidir ve SAİZEN ile replasman tedavisine başlamadan önce büyüme hormonu eksiklikleri teyid edilmelidir./ Yetişkinlerde başlayan: İzole GH yetmezliğinde, 2 dinamik test ile, ve hipofiz veya hipotalamus hastalığına bağlı durumlarda diğer hipofiz ön lob hormonlarından en az birinin (prolaktin hariç) eksikliği durumunda ise 1 dinamik test ile yetmezliğin kanıtlanması halinde eksikliği yerine koymak amacıyla kullanılır. **Kontrendikasyonlar:** SAİZEN içeriğindeki etkin maddeye ya da yardımcı maddelerden birine karşı aşırı hassasiyeti olan hastalarda kontrendikedir. Somatropin,epifizleri kapanmış çocuklarda büyümeyi artırmak için kullanılmamalıdır. Somatropin, herhangi bir aktif malign tümor bulgusu varlığında kontrendikedir. Tedaviye başlamadan önce, aktif intrakranial tümörler inaktif olmalı ve antitümör tedavi tamamlanmalıdır. Tümör büyümesine dair bir bulgu varlığında tedavi kesilmelidir. Proliferatif veya preproliferatif diyabetik retinopati durumunda Somatropin kullanılmamalıdır. Açık kalp ameliyatı, karın ameliyatı, çoklu kaza travması, akut solunum yetmezliği, ya da be nzer durumlarla ilişkili akut hastalığı olan bireylerde somatropin kullanılmamalıdır. Kronik böbrek yetmezliği olan çocuklarda, böbrek nakli durumunda somatropin tedavisi kesilmelidir. **Uyarılar / Önlemler:** Neoplazi: Büyüme hormonu ile tedavi alan remisyondaki intra ya da ekstrakranial neoplazili hastalar hekim tarafından dikkatle ve düzenli aralıklarda kontrol edilmelidir. Prader-Willi sendromu: SAİZEN, beraberinde büyüme hormonu eksikliği teşhisi konulmadığı sürece, büyüme geriliği olan, genetik olarak Prader-Willi sendromu olduğu doğrulanmış pediatrik hastaların uzun dönem tedavisi için endike değildir. Lösemi: Büyüme hormonu ile tedavi edilmiş veya edilmemiş büyüme hormonu eksikliği olan çocuklarda kısıtlı sayıda lösemi vakaları rapor edilmiştir. Bununla beraber, predispoze faktörlerin yokluğunda büyüme hormonu kullananlarda lösemi insidansının arttığına dair bir bulgu yoktur. İnsülin duyarlılığı: Somatropin, insülin duyarlılığını azaltabileceğinden dolayı, hastalar glukoz intoleransı yönünden gözlenmelidir. Diabetes mellitusu ve glukoz intoleransı olan hastaların somatropin tedavisi süresince yakın takip edilmeleri gerekir. Retinopati: İlerlemeyen retinopati olgularında somatropin replasman tedavisi kesilmemelidir. Tiroid fonksiyonu: Somatropin ile tedaviye başladıktan sonra ve doz ayarı sonrası tüm hastalara tiroid fonksiyon testi yapılması tavsiye edilir. Benign intrakranial hipertansiyon: Şiddetli ya da tekrarlayan baş ağrısı, görsel problemler, bulantı ve/veya kusma durumlarında, papilödem için funduskopi önerilir. Papilödem varsa, iyi huylu intrakranial basınç artışı (psödotümor serebri) tanısı düşünülmeli ve uygunsa SAİZEN tedavisi kesilmelidir. Pankreatit: Nadir görülmesine rağmen, özellikle karın ağrısı gelişen çocuklarda olmak üzere somatropin ile tedavi edilen hastalarda pankreatit düşünülmelidir. Skolyoz: Somatropinin skolyozun insidansını veya şiddetini arttırdığı gösterilmemiştir. Antikorlar: Tüm somatropin içeren preparatlarda olduğu üzere, bazı hastalarda somatropin'e karşı antikorlar oluşabilir. Femur başı epifizlerinde kayma: SAİZEN ile tedavi edilen hastalarda diz ağrısı ya da kalça şikayetleri ya da topallama gelişmesine karşı hekimler ve hasta yakınları dikkatli olmalıdır. Kronik böbrek yetmezliğine bağlı büyüme geriliği: Femur başı epifiz kayması ya da femur başının avasküler nekrozları, ilerlemiş renal osteodistrofisi olan çocuklarda görülebilir ve bu problemlerin büyüme hormonu tedavisinden etkilendiği kesin değildir. Tedavi başlamadan önce kalçanın röntgeni alınmalıdır. Kronik böbrek yetmezliği olan çocuklarda, tedaviye başlamadan önce böbrek fonksiyonu normalin %50 altına kadar düşmüş olmalıdır. Büyüme bozukluğunu doğrulamak için, tedaviye başlamadan önce 1 yıl büyüme takip edilmelidir. Tedavi böbrek nakli esnasında kesilmelidir. Gestasyonel yaşa göre küçük doğan çocuklar: Diyabet varlığı kesin ise, büyüme hormonu uygulanmamalıdır. SGA hastalarında, ergenlik başlangıcında tedaviye başlanması konusundaki deneyim sınırlıdır. Silver-Russell sendromuna sahip SGA hastalarındaki deneyimler sınırlıdır. Sıvı retansiyonu: Yetişkinlerde doz azaltmak gerekebilir. Akut kritik hastalık: Büyüme hormonu ile tedavinin olası yararları; olabilecek potansiyel riskler düşünülerek değerlendirilmelidir. Glukokortikoidlerle etkileşim: Glukokortikoid dozunun ayarlanması gerekebilir. Oral östrojen tedavisi ile kullanım: Somatropin alan bir kadın oral östrojen tedavisine başlarsa, somatropinin dozunun artırılması gerekebilir. Tersine, somatropin kullanmakta olan bir kadın oral östrojen tedavisini bırakırsa, somatropin dozunun azaltılması gerekebilir. Lipoatrofiyi önlemek için enjeksiyon farklı yerlere yapılmalıdır. Yetişkinlerde büyüme hormonu eksikliği ömür boyu süren ve tedavi edilmesi gereken bir durumdur. Ancak 60 yaş üstü hastalarda ve uzun süreli kullanımlarla ilgili sınırlı deneyim bulunmaktadır. Biyoteknolojik ürünlerin takip edilebilirliğinin sağlanması için uygulanan ürünün ticari ismi ve seri numarası mutlaka hasta dosyasına kaydedilmelidir. **İstenmeyen Etkiler:** Hastaların %10'a kadarı enjeksiyon yerinde kızarıklık ve kaşıntı yaşayabilir. Çocukluk döneminde büyüme hormonu eksikliği tanısı konmuş yetişkin hastalarda ortaya çıkan yan etkiler, hastalığın yetişkin dönemde ortaya çıktığı hastalara göre daha azdır. Hastaların küçük bir yüzdesinde somatropin'e karşı antikorlar oluşabilir. Büyüme hormonu ile tedavi edilmiş veya edilmemiş büyüme hormonu eksik çocuklarda bazı lösemi vakaları rapor edilmiştir. Bununla beraber, predispoze faktörlerin yokluğunda büyüme hormonu kullananlarda lösemi insidansı arttığına dair bir bulgu yoktur. İstenmeyen etkiler, ciddiyetleri açısından azalan sıra içinde yer almaktadır. Bilinmiyor: Lokal veya yaygın aşırı duyarlılık reaksiyonları; Çok seyrek: Hipotiroidizm; Yaygın: Yetişkinlerde: Sıvı tutulması: periferal ödem, sertlik, artralji, myalji, paraestezi; Yaygın olmayan: Çocuklarda: Sıvı tutulması: periferal ödem, sertlik, artralji, myalji, paraestezi; Bilinmiyor: İnsülin rezistansı, hiperinsülinizm ve nadiren hiperglisemi ile sonuçlanabilir; Yaygın: Baş ağrısı (izole), karpal tünel sendromu (yetişkinlerde); Yaygın olmayan: İdiopatik intrakranial hipertansiyon (benign intrakranial hipertansiyon), karpal tünel sendromu (çocuklarda); Bilinmiyor: Pankreatit; Çok seyrek: Femur başı epifiz kayması, ya da femur başının avasküler nekrozları; Yaygın olmayan: Jinekomasti; Yaygın: Enjeksiyon yeri reaksiyonları, Lokalize lipoatrofi (enjeksiyon yeri değiştirilerek önlenebilir). **Gebelik Kategorisi:** C'dir. Gebelerde yapılmış klinik veri mevcut değildir. Somatropin içeren ilaçlar gebelikte ve kontrasepsiyon kullanmayan çocuk doğurma potansiyeli olan kadınlarda önerilmez. Somatropinin insan sütüyle atılıp atılmadığı bilinmemektedir. **İlaç Etkileşimleri ve Diğer Etkileşimler:** Eşzamanlı olarak glukokortikoid kullanılması, somatropinin büyümeyi artırıcı etkisini engeller. ACTH eksikliği olan hastalarda büyüme hormonu tedavilerinin engellenmemesi için glukokortikoid tedavileri çok dikkatli ayarlanmalıdır. Büyüme hormonu kortizonun kortizole dönüşmesini azaltır ve daha önceden keşfedilmemiş santral hipoadrenalizmi ortaya çıkartabilir veya düşük glukokortikoid replasman dozlarını etkisiz hale getirebilir. Büyüme hormonu eksikliği olan erişkin hastalarda yapılan bir çalışma, büyüme hormonu uygulamasının özellikle, CYP P450 3A4 hepatik enzimler ile metabolize olduğu bilinen ilaçlarla (ör. seks stereoidleri, kortikosteroidler, antikonvülsanlar ve siklosporin) somatropin ile eşzamanlı kullanıldığı zaman, bu ilaçların klirensi artarak plazma seviyelerinde düşmeye sebep olabilir. **Kullanım Şekli ve Dozu:** Çocuklarda ve ergenlerde: Endojen büyüme hormonu salgısının yetersiz olduğu büyüme geriliği gösteren hastalarda: Subkutan uygulama ile günde 0,7-1,0 mg/m² vücut yüzey alanı ya da günde 0,025-0,035 mg/kg vücut ağırlığı. / Gonadal disgeneziden ötürü büyüme geriliği gösteren kızlarda (Turner sendromu): Subkutan uygulama ile günde 1,4 mg/m² vücut yüzey alanı ya da günde 0,045- 0,050 mg/kg vücut ağırlığı. / Kronik böbrek yetmezliğinden ötürü büyüme geriliği gösteren prepubertal çocuklarda (CRF): Subkutan uygulama ile günde 1,4 mg/m² vücut yüzey alanı ya da günde 0,045-0,050 mg/kg vücut ağırlığı. / Gestasyonel yaşa göre küçük doğmuş kısa çocuklarda büyüme geriliği (SGA): Tavsiye edilen günlük doz, subkutan uygulama yoluyla 0,035 mg/kg vücut ağırlığı (ya da 1 mg/m²/gün)'dır. Uygun yetişkin boya ulaşınca ya da epifizler kapanınca tedavi kesilmelidir. Eğer boy uzama hızı SDS'si +1'in altında ise,bir yıl sonunda tedavi sonlandırılmalıdır. Yetişkinlerde: Büyüme hormonu eksikliğinde; Somatropin tedavisi başlangıcında, günlük subkutan enjeksiyon olarak 0,15-0,3 mg gibi düşük dozlar önerilir. Doz, İnsulin-benzeri Büyüme Faktörü 1 (IGF-1) değerleri ile kontrollü şekilde ayarlanmalıdır. Önerilen en fazla büyüme hormonu dozu nadiren 1,0 mg/gün değerini aşar. Genellikle en düşük etkili doz uygulanmalıdır. Zaman içerisinde IGF-1 duyarlığında artış gösteren erkeklere kıyasla kadın hastalarda daha yüksek dozlar gerekebilir. Bu,erkekler fazla tedavi almış olurken, özellikle oral östrojen tedavisindeki kadınların eksik tedavi alma riskinin bulunduğu anlamına gelir Yaşlılarda veya kilolu hastalarda, daha düşük dozlar gerekli olabilir. **Uygulama Şekli:** SAİZEN enjeksiyonluk çözeltinin uygulanması için kullanma talimatında ve seçilen oto enjektör (iğnesiz cool.click oto enjektörler, easypod oto enjektör veya aluetta kalem enjektör) ile birlikte verilen kullanma kılavuzundaki talimatlar takip edilmelidir. Hedeflenen easypod kullanıcıları ağırlıklı olarak 7 yaşından büyük çocuklar ve erişkinlerdir. Bu cihazların çocuklar tarafından kullanımı daima yetişkin gözetiminde yapılmalıdır. **Doz Aşımı ve Tedavisi:** Önerilen dozları aşmak yan etkilere sebep olabilir. Doz aşımı hipoglisemi ve sonrasında hiperglisemiye yol açabilir. Bundan başka, somatropin doz aşımı sıvı retansiyonuna sebep olabilir. **Saklama Koşulları:** Kullanılmayan SAİZEN kartuşu 2°C-8°C arasında buzdolabında saklanmalıdır. Dondurmayınız. Işıktan korumak için orijinal ambalajında saklayınız. İlk enjeksiyondan sonra, SAİZEN kartuşu, içinde SAİZEN kartuşu bulunan easypod oto enjektör veya içinde SAİZEN kartuşu bulunan aluetta kalem enjektör maksimum 28 gün süresince 2°C-8°C arasında buzdolabında saklanmalıdır. Bu süre boyunca, en fazla 7 gün süresince, 25°C altında veya buzdolabı dışında tutulabilir. SAİZEN kartuşu 7 gün boyunca buzdolabının dışında saklandığında, yeniden buzdolabına konulmalı ve ilk enjeksiyon tarihinden itibaren hesaplanacak şekilde 28 gün içinde kullanılmalıdır. Easypod oto enjektör veya aluetta kalem enjektör kullanılırken, kartuş cihazın içinde tutulmalıdır. Cool.click iğnesiz oto enjektör, daima SAİZEN kartuşundan ayrı bir şekilde ve buzdolabının dışında saklanmalıdır. Kullanılmış kartuşu ışıktan koruyunuz. **Raf ömrü:** 18 aydır. **Ruhsat Sahibi:** Merck İlaç Ecza ve Kimya Tic.A.Ş. Atatürk Mh. Ertuğrul Gazi Sk. 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# Metabolic Bone Disease in Premature Neonates: An Unmet Challenge

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## Abstract

Metabolic bone disease (MBD) is an important cause of morbidity in premature, very low birth weight (VLBW) and sick infants and, if left undiagnosed, may lead to structural deformities and spontaneous fractures. MBD is defined as impaired bone mineralization in a neonate with lower than expected bone mineral levels in either a fetus or a neonate of comparable gestational age and/or weight, coupled with biochemical abnormalities with or without accompanying radiological manifestations. MBD has been reported to occur in 16% to 40% of extremely low birth weight neonates and presents by 6-16 weeks after birth. Insufficient calcium and phosphorous stores during the phase of accelerated growth predispose to MBD in neonates along with the use of some medications such as caffeine or steroids, prolonged parenteral nutrition and chronic immobilization. Enhanced physical activity in preterm infants facilitates bone mineralization and weight gain. Biochemical abnormalities tend to worsen significantly, as the severity of disease progresses. These abnormalities may include hypocalcemia, hypophosphatemia, hyperphosphatasia and secondary hyperparathyroidism. In addition, urinary phosphate wasting and hypovitaminosis D can be additional complications. Conversely, biochemical abnormalities may not be accompanied by rachitic changes. Newer diagnostic modalities include non-invasive bone densitometry by quantitative ultrasound over the mid-tibial shaft. The management of MBD includes adequate calcium, phosphorous and vitamin D supplementation, along with optimum nutrition and physical activity. Similarly, preventive strategies for MBD should target nutritional enhancement in combination with enhanced physical activity. MBD usually results in preventable morbidity in preterm and VLBW neonates. Treatment consists of optimum nutritional supplementation and enhanced physical activity.

**Keywords:** Extremely premature, hypocalcemia, hypophosphatemia, neonate, osteopenia, premature, rickets, very low birth weight

## Introduction

Metabolic bone disease (MBD) in neonates is associated with reduced bone mineral content (BMC) leading to impaired skeletal mineralization. It is also known as osteopenia of prematurity and is a common consequence of numerous nutritional and biomechanical factors in premature neonates. BMC is inversely proportional to gestational age and birth weight and is influenced by the adequacy of calcium and phosphorus intake in postnatal life (1,2). MBD may or may not be accompanied by rachitic changes (3,4). Although advanced neonatal intensive care has led to improved survival of extremely preterm infants, this has not resulted in the abolition of morbidity and achieval of optimum growth (5,6,7).

MBD is defined as decreased bone mineralization in neonates when compared to *in utero* or *ex utero* bone mineral density of neonates with equivalent gestational age or birth weight along with biochemical evidence and or radiological findings (8,9).

## Magnitude of the Problem

It has been reported that 55% of infants with extremely low birth weight (ELBW) (ELBW ≤1000 grams birth weight) and 23% of infants with very LBW (VLBW) (VLBW < 1500 and > 1000 grams) have MBD. Similarly, it is more frequent in neonates under 28 weeks of gestation (10,11). The incidence of MBD in breastfed, premature infants is 40% and in formula-fed, preterm infants (with oral calcium and phosphorus supplements) is 16%. As the proportion of extremely preterm and ELBW neonates is increasing, the incidence of MBD is on the rise. Osteopenia occurs in about 50% of VLBW neonates and the majority of ELBW infants at

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40 weeks of post conceptional age, without adequate calcium and phosphorous supplementation (10,11,12,13,14,15). It could be more prevalent in both developing and developed countries as more and more sick preterm neonates are being catered with enhanced survival (5,14,15).

## Etiology

MBD is often multifactorial (see Table 1). The leading causes are inadequate mineralization and include intrauterine growth restriction, prolonged parenteral nutrition (PN) (without phosphate) and delayed enteral nutrition. Neonates with insufficient calcium, phosphorous and vitamin D intake are at further risk of MBD when subjected to extended periods of immobilization (3,4,5,6,7,8,9,10,11,12). The onset of MBD ensues between the 6th and 16th week of life or by 40 weeks of corrected age, although it may go unnoticed until marked demineralization takes place (loss of 20-40% of BMC). The physiological basis of MBD is inadequate calcium and phosphorus stores in the face of accelerated fetal growth during the third trimester. *In utero* calcium and phosphorus accretion occurs at the rate of 120 mg/kg/day and 60 mg/kg/day, respectively (15,16). However, impaired supplementation and absorption of these minerals in post natal life leads to a sub-optimally mineralized new and remodelled skeletal system. Preterm, human milk has insufficient calcium, phosphorous and vitamin D *per se*, necessitating supplementation. Vitamin D concentration in human milk is 25 to 50 IU/L which is grossly insufficient to maintain serum 25-hydroxyvitamin D [25(OH)D] levels greater than 20 ng/mL in premature infants. This vitamin D deficiency leads to hypocalcemia, secondary hyperparathyroidism which in turn leads to phosphaturia. In addition, undue fluid restriction, use of term formula, and soy-based and lactose-free formulas in preterm neonates can contribute to MBD (17,18,19,20,21). However, VLBW neonates can produce adequate 1,25-dihydroxyvitamin D levels after the initial few weeks of life if they have optimum dietary vitamin D supplementation. Some medications used in preterm infants including frusemide, steroids and methyl xanthines, which enhance osteoclastic activity, decrease osteoblastic proliferation, reduce calcium absorption and promote renal calcium wasting may lead to osteopenia (19,20,21). Similarly, extended administration of phenobarbital or phenytoin in neonates with seizures can lead to enhanced 25(OH)D metabolism and osteopenia (21).

**Table 1. Etiological factors of metabolic bone disease**

| | |
|---|---|
| *In utero* | - Deficient maternal calcium and phosphorus stores |
| | - Maternal vitamin D deficiency |
| | - Accelerated physiological fetal growth in 3rd trimester |
| *Ex utero* | **Maternal** |
| | - Inadequate nutritional supplementation to lactating mother (calcium, phosphorus, vitamin D) |
| | **Neonatal** |
| | - Insufficient supplementation of calcium, phosphorus, vitamin D |
| | - Excessive fluid restriction in VLBW neonates |
| | - Urinary calcium wasting (phosphorus deficiency) |
| | - Use of term formula in preterm infants |
| | - Use of soy based or lactose free formula |
| | - Neonatal cholestasis |
| | - Hereditary pseudovitamin D deficiency: type 1 (abnormal or absent 1-α-hydroxylase activity) or type 2 (1,25-dihydroxyvitamin D resistance in tissues) |
| | - Medications: furosemide, steroids, methylxanthines, phenobarbitone, phenytoin |

VLBW: very low birth weight

Maternal vitamin D deficiency often occurs when lactating mothers have inadequate vitamin D (less than 600 IU/day) supplementation. This often manifests as neonatal hypocalcemia and can result in congenital rickets. Neonates with cholestatic liver disease may have exaggerated malabsorption and impaired 25(OH)D, which further aggravates osteopenia. Rare causes of hypovitaminosis D, such as hereditary pseudovitamin D deficiency type 1, due to abnormal or absent 1-α-hydroxylase activity or type 2 due to 1,25-dihydroxyvitamin D resistance in tissues, can also lead to MBD (21). Chronic renal failure can also result in renal osteodystrophy and osteopenia.

Maintaining calcium and phosphorus levels in PN is difficult due to restricted solubility and temperature lability. Aminoacid, glucose, lipid concentration, pH and methods of preparation of calcium salts determine the bioavailability of calcium and phosphorus. Lowering the pH with cysteine enhances solubility.

## Pathophysiology

### Calcium and Phosphorus Homeostasis

The structural matrix of the skeletal system largely made up of calcium, phosphorus and magnesium and their homeostasis play a key role in bone integrity. The majority of total body calcium (99%) and phosphorus (80%) are present in bone as microcrystalline hydroxyapatite. The remainder of total body calcium (1%) lies within the extracellular fluids and soft tissues. However, only 50% of total serum calcium is biologically active as being in the

ionized form. The remaining calcium is bound to proteins (albumin and globulin: 40%) and the rest (10%) to organic and inorganic acids. Similarly, a major proportion of magnesium (60%) is present in the bone matrix. Numerous factors such as vitamin D, parathyroid hormone (PTH), and calcitonin, followed by dietary calcium and phosphorous content, intestinal absorption, bone accretion, resorption and, finally, rate of urinary excretion determine calcium and phosphorus homeostasis (15).

### Role of PTH

Soon after the birth, irrespective of gestational age and persisting mineral requirement, there is a fall in calcium, with a nadir attained at 24-30 hours after birth in preterm infants. As a result, there is a PTH surge. PTH augments calcium reabsorption in the kidney and also results in urinary phosphate wasting. PTH aids in the production of calcitriol [$1,25(OH)_2D$] by activating renal $25(OH)D_3$-1-alpha-hydroxylase, which increases intestinal calcium and phosphate absorption. PTH also promotes bone resorption and subsequent release of calcium and phosphate. On the whole, PTH has the greatest action in the kidney for regulating calcium metabolism. When there is insufficient calcium intake for prolonged periods, as with MBD, these metabolic changes persist (5).

### Fetal Bone Homeostasis

The amount of minerals required for proper accretion of the skeleton vary according to the age of the baby. The fetus has a higher rate of skeletal growth, especially during the last trimester. There is an enormous increase in bone volume with advanced gestational age due to bone remodelling and augmented bone synthesis as seen by increased trabecular thickness. It has been shown that the rate of trabecular thickening is 240 times more rapid in the fetus compared to children (15). Fundamentally, osteoblasts produce osteoid/organic bone matrix into which calcium and phosphate hydroxyapatite are incorporated. This osteoblastic activity increases exponentially, involving 80% of mineral accretion, during the period of 24 to 37 weeks of gestation (22,23,24,25,26).

Normally the fetal nutrient supply of protein, energy and minerals is ample for fetal growth and skeletal development (1.2 cm/week). The physical density of bone (expressed as bone mass divided by bone volume) is highest in term neonates. The calcium and phosphate deposition during the last trimester of fetal life is around 20 grams and 10 grams respectively, which corresponds to a calcium and phosphate accretion rate of 100-120 mg/kg/day and 50-65 mg/kg/day respectively (15,16).

The placenta has a pivotal role in fetal skeletal development as calcium is actively transported transplacentally with the aid of a calcium pump in the basement membrane (22,23,24,25,26) with a maternal to fetal calcium gradient of 1:4. In addition, activation of vitamin D to 1,25-dihydroxy cholecalciferol also occurs in the placenta, which is an essential element of transplacental phosphate transfer (26). Thus, there is a hypercalcemic status in fetal life due to increased estrogen levels, resulting in enhanced bone modelling and endocortical bone formation (27). All these processes are interupted in preterm neonates, predisposing them to under mineralization of the bone. In addition chronic placental inflammation (chorioamnionitis), or placental insufficiency, as indicated by intrauterine growth retardation, impairs transplacental transfer of calcium and phosphorous creating an osteopenic *milieu* in the fetus. As placental calcium levels and fetal bone accretion depend on maternal dietary calcium intake, calcium supplementation of 2 grams on or after 22 weeks of gestation to pregnant women enhances neonatal BMC (15,26).

### Neonatal Bone Homeostasis

It has been noted that from birth to six months of age, bone physical density is reduced by one third in term neonates (15,27). This results is because of the preferential rapid widening of the bone marrow cavity compared to the cortical surface area. However, term neonates generally maintain bone integrity, unlike preterm infants. There is a fall in transplacentally transferred estrogens and serum calcium levels after birth leading to a rise in PTH (28,29). However, within the first 48 hours of life, falling serum calcium levels do not result in a corresponding rise in serum PTH levels which in turn leads to a nadir in serum calcium levels. It is notable that serum PTH concentrations in term neonates remain within the optimum range for term neonates or adults; they show a falling trend from foetal levels based on measurement in large cohorts of foetuses and neonates not necessarily measured in the same neonates at different points of time (5,15,17).

Calcium absorption in post natal life is a function of type and amount of calcium intake, gastrointestinal function, including both active and passive transport of calcium, and vitamin D levels in the mother. Preterm neonates with reduced intake and inefficient absorption of calcium and phosphorous from the gut are at a twofold disadvantage and are prone to MBD. Oral calcium bioavailability is compromised in cases of large gastric aspirates, vomiting, abdominal distension and constipation, which are often seen in preterm neonates. The interplay of calcium and phosphorous absorption is such that when the dietary levels

are disproportionate, one reduces the other's absorption. Apart from nutritional supplementation, another important factor regulating osteoblastic activity is physical activity during fetal life such as quickening against the uterine wall, which may be lost in sick, preterm neonates who are less active in post natal life. Reduced physical activity enhances osteoclastic activity and inhibits osteoblastic activity, leading to bone resorption and urinary calcium wasting (30,31,32,33).

Interestingly, the *in utero* rise in bone mineral apparent density (BMAD) is faster than that found in *ex utero* babies. BMAD is measured by dividing BMC with the surface area of bone (BMC/BA = g/cm$^3$) and is a measure of volumetric BMD. It initially falls after birth but is maintained later on (33). Preterm neonates will have a fall in mineral accretion when compared to fetal life, although skeletal growth remains comparable, and thus leads to osteopenia of prematurity. However, after adequate nutritional supplementation, catch up bone growth begins in preterm VLBW infants.

## Clinical Features and Signs

The clinical manifestations are diverse depending up on the degree of demineralization. MBD can either remain unnoticeable or can present with florid rickets. It can also present as arrested growth velocity and with features of hypocalcemia such as jitteriness or tetany. Affected neonates may have a large head, craniotabes, frontal bossing, sutural separation in the skull, wide fontanelle, costochondral thickening, hypotonia, and protruding abdomen, although this is not consistently present. MBD may manifest with multiple pathological or spontaneous fractures of the ribs and long bones, which is seen in 10% of premature neonates and these present as pain while handling (see Table 2) (3). Rib softening and/or fractures may lead to deranged pulmonary function and respiratory distress around 5 to 11 weeks of age (34,35). These infants can have prolonged ventilator requirement or difficulty in weaning from the ventilator.

**Table 2. Clinical features of metabolic bone disease**

- Arrested growth velocity (reduced linear growth with normal head growth)
- Features of hypocalcemia (jitteriness, tetany)
- Features of rickets
- Spontaneous fractures of ribs and long bones
- Pain while handling
- Respiratory distress
- Deranged pulmonary function
- Difficulty in weaning from ventilator

## Diagnosis

### Biochemistry

The mainstay of diagnosis is by estimation of biochemical markers which should include serum calcium, phosphorous, PTH and alkaline phosphatase (ALP) and urinary calcium concentrations (see Table 3). The predominant biochemical change includes decreased serum phosphorus levels. Hypophosphatemia is an early indicator of disrupted calcium metabolism and manifests by 7-14 days of life. This can occur either due to isolated phosphate deficiency or to elevated PTH levels. Phosphate depletion increases calcitriol synthesis and may lead to hypercalcemia which supresses PTH levels. In addition phosphate reabsorption is increased by the kidney and thus tubular reabsorption of phosphate is also a useful measure of phosphate homeostasis.

Serum ALP levels ≥900 IU/L show 100% sensitivity and 70% specificity for MBD and ALP concentrations may increase fivefold in MBD (35). Caution should be exercised in interpretation of elevated ALP levels as these may be a symptom of hepatic and/or gastrointestinal diseases because this enzyme is also produced by the liver and gastrointestinal tract. Hence, estimation of the bone iso-enzymeof ALP is more specific for a skeletal cause and thus to diagnose MBD (36,37,38). PTH levels have better specificity than ALP in diagnosing MBD. PTH levels > 180 pg/mL or phosphate concentration < 4.6 mg/dL at three weeks after delivery have 100% sensitivity and 94% specificity for the diagnosis of severe MBD (6,15,17,36,37,38). Ryan et al

**Table 3. Diagnosis of metabolic bone disease**

**Biochemical**

- Decreased serum phosphorus levels [< 3.5 to 4 mg/dL (1.1 to 1.3 mmol/L)],
- Increased serum alkaline phosphatase levels
- Elevated bone isoenzymes of alkaline phosphatase
- Low or normal serum calcium levels
- Low or normal serum 25(OH)D levels
- Elevated serum PTH levels (often variable)
- Low urinary calcium and phosphorus levels

**Radiological**

- Radiograph of long bone: widening of epiphyseal growth plates; metaphysis rarefaction, cupping, fraying, subperiosteal new bone formation and osteopenia
- Radiograph of the skull, spine, scapula. Demonstration of **osteopenia**
- Radiograph of the chest: osteopenia and rachitic changes, pathologic fractures in ribs
- DEXA: reduced bone mineral content
- Quantitative ultrasound: reduced bone SOS

25(OH)D: 25-hydroxyvitamin D, PTH: parathyroid hormone, DEXA: dual energy X-ray absorptiometry, SOS: speed of sound

(37) in a cohort of 108 preterm neonates, failed to find any association between serum ALP levels and BMC when they reached term.

These biomarkers should be estimated at initial diagnosis and later, during follow-up at four-weekly intervals, to monitor the response to treatment (Figure 1). The fundamental principle in treating these neonates is to establish normocalcemia, normophosphatemia and to prevent urinary calcium wasting. With the normalization of calcium, phosphorus and ALP, evaluation of these parameters should be performed every month up to six months of age and can be done once every three months, thereafter.

### Imaging

Various imaging modalities have been used to diagnose MBD (see Table 3). Plain X-rays will show osteopenia, reduced cortical thickness, rib fractures, widening of the epiphysis, and uneven margins (39). Dual energy X-ray absorptiometry (DEXA) is an imaging tool to detect even small changes in BMC and BMD and to predict the probability of impending fractures. DEXA has been standardized in both term and preterm neonates. Although DEXA has diagnostic precision for bone mineralization, it involves exposure to ionizing radiation and cannot be performed at the bedside (40,41).

Another newer, non-invasive diagnostic modality for MBD is measuring bone speed of sound (SOS) by quantitative ultrasound. This method does not expose to radiation, can be done bedside and has reference standards for both term and preterm infants, both at birth and during follow up. SOS by quantitative ultrasound measures bone density, delineates the structure and enables prediction of bone turnover in preterm infants. This is usually performed using the mid-tibial shaft. Bone SOS is increased in term infants (median 3079 m/s) compared with preterm infants (median 2911 m/s). Similarly, there is a good correlation between gestational age and bone SOS. Also, bone SOS was noted to be low in preterm infants even at a corrected age of 40 weeks when compared with term infants (42,43,44,45).

### Management

The principles of management of MBD in preterm neonates are multidimensional (see Figure 2, Table 4) (29).

### Mineral Requirements of Infants

The requirements of calcium and phosphorus are based on intrauterine bone mineral accretion rates. The ideal calcium to phosphorus ratio for optimum skeletal mineralization is 1.7:1 (46). While on PN, use of soluble forms of calcium and phosphorus such as sodium and potassium phosphate, glycerol phosphate or sodium-glucose phosphate, will improve bioavailability (15,21).

Vitamin D requirement is a function of gestational age and maternal vitamin D levels. The fetus is capable of metabolizing vitamin D to 1,25-dihydroxyvitamin D from

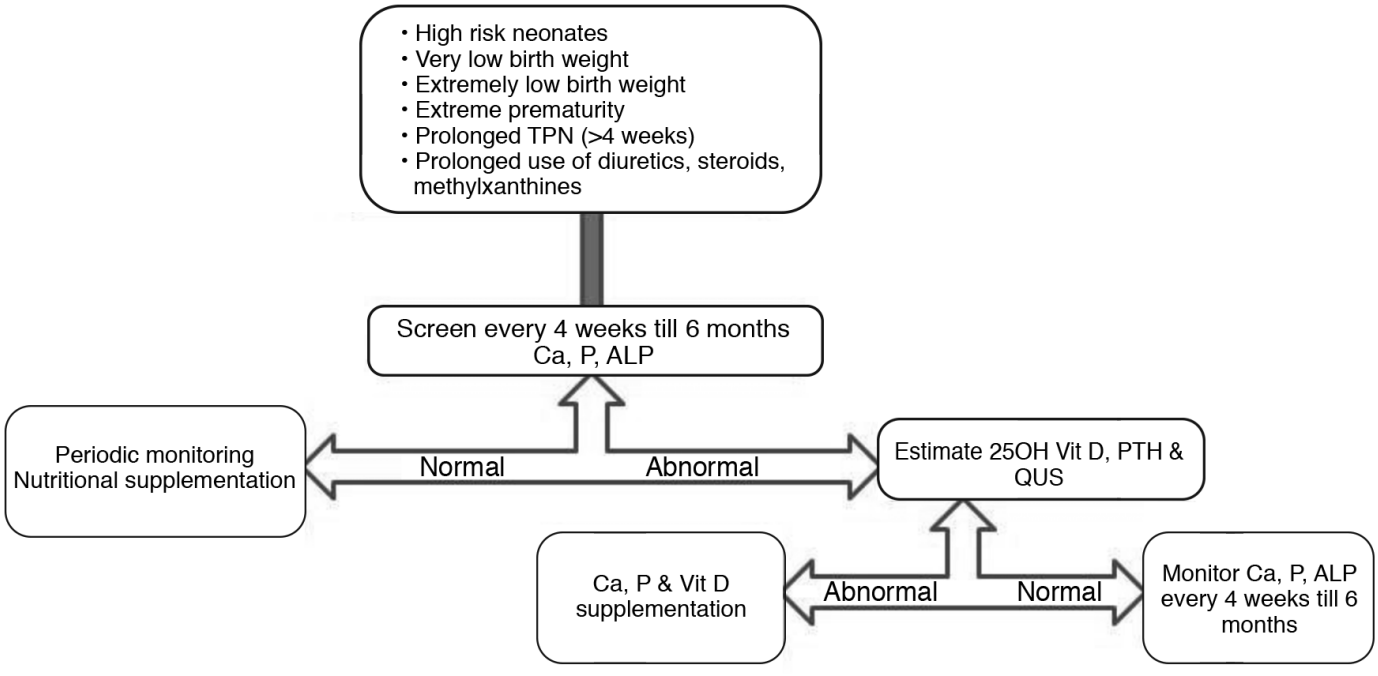


**Figure 1.** Algorithm for screening of metabolic bone disease

*ALP: alkaline phosphatase, PTH: parathyroid hormone, TPN: total parenteral nutrition*

the 24th week of gestation. It is recommended to provide 400 IU of vitamin D daily for all premature neonates after establishment of full feeds (21).

Calcium and phosphorus requirements in preterm neonates are 123 to 185 mg Ca/100 kcal and 80 to 110 mg P/100 kcal, respectively. This can be achieved with fortification of human milk and with formula milk. Calcium is in the form of soluble calcium glycerol phosphate in formula milk achieving 90 mg/kg/day of calcium absorption (88% of the total). Thus, fortification and supplementation is often mandatory in preterm neonates.

The effects of human milk fortifiers on skeletal mineralization are inconclusive as reported in the Cochrane systematic review and meta analysis of Kuschel and Harding (46). Use of milk fortifiers may predispose to necrotizing enterocolitis with higher doses of calcium, due to increased gastrointestinal transit time, fecal calcium and reduced absorption of fat.

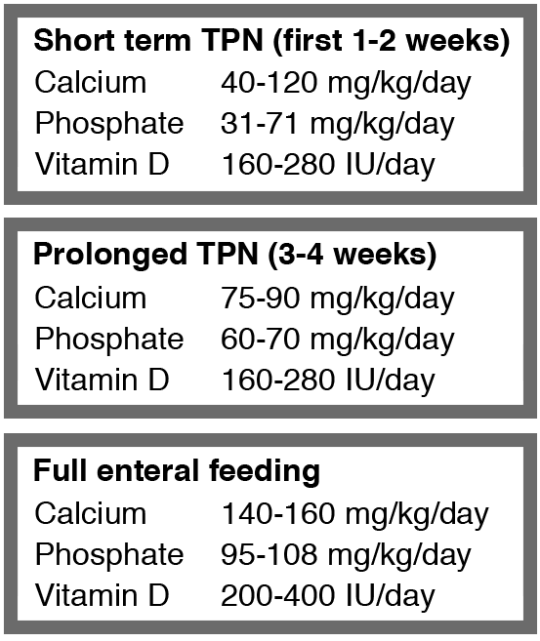


**Figure 2.** Mineral requrements during total parenteral nutrition and enteral feeds The American Academy of Pediatrics

Ref. 29,65,66

*TPN: total parenteral nutrition*

**Table 4. Treatment of metabolic bone disease**

| |
|---|
| - Early enteral feeding |
| - Fortified human milk |
| - Premature formulas |
| - Adequate calcium and phosphorus intake |
| - Vitamin D supplementation of 400 IU/day (ensures adequate vitamin D stores) |
| - Preferable use of thiazide diuretics over furosemide |
| - Assisted physical exercises |
| - Avoid forceful chest physiotherapy |
| - Calcitriol (special circumstances) |

### Prognosis and Outcome

As MBD resolves spontaneously with adequate calcium, phosphorous and vitamin D supplementation, it carries a good prognosis. Although there are differences of opinions about duration, amount and route of mineral supplementation, it has been reported that infants receiving formula feeds until nine months of age have higher BMC (6,15). Also, it has been stated that preterm infants have an adequate catch up by one year of life with optimum supplementation as demonstrated by quantitative ultrasound and DEXA measurements (21,33,39). Skeletal mineralization of term and preterm infants is comparable in later childhood. Similarly, studies have shown reduced spinal BMC in later childhood in LBW neonates who are stunted (3,5,6,15).

Assisted physical exercise is a newer preventive modality which adds to nutritional management in stable premature neonates. Chen et al (43) found that early assisted exercise in VLBW neonates enhanced bone strength. The assisted physical exercise gives either tactile stimulation with moderate pressure strokes or kinaesthetic stimulation with passive flexion and extension of both upper limbs and lower limbs. It was shown to enhance body weight, bone mineralization and osteogenesis. Some studies have shown that exercise may attenuate postnatal reduction in bone SOS (47,48,49,50,51).

It is interesting that nutrition plays a dual role in MBD, both therapeutic and preventive (52,53,54,55,56,57,58). Supplementing mothers with 600 IU/day of vitamin D univerasally has also been shown to help in preventing MBD (59). Focusing on the optimum supply of minerals and of vitamin D, by using human milk fortifier, calcium and phosphorous supplementation or preterm formula is vital to prevent MBD (60,61,62,63,64,65,66).

## Conclusion

Optimum nutritional supplementation of neonates with calcium, phosphorus, and vitamin D, along with assisted physical exercise has been shown to be effective in preventing much MBD. These measures inhibit pathological bone resorption in the initial few weeks of life and enhance the growth of premature infants. It is also vital to identify the biochemical abnormalities characteristic of MBD in a timely manner to

initiate therapeutic interventions at the earliest opportunity and thus prevent spontaneous/pathological fractures. Periodic estimation of phosphate and alkaline phosphatase concentrations is important to estimate the risk of osteopenia, along with assessment of treatment efficacy. Similarly, DEXA and quantitative ultrasound enable quantification of bone mineralization and assist in nutritional rehabilitation. Additionally, maternal vitamin D supplementation is another essential preventive strategy for MBD.

### Ethics

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Swathi Chacham, Rachna Pasi, Najeeb Ahmad, Concept: Swathi Chacham, Rachna Pasi, Madhuradhar Chegondi, Najeeb Ahmad, Design: Swathi Chacham, Rachna Pasi, Madhuradhar Chegondi, Najeeb Ahmad, Data Collection or Processing: Najeeb Ahmad, Shanti Bhusan Mohanty, Analysis or Interpretation: Swathi Chacham, Rachna Pasi, Madhuradhar Chegondi, Najeeb Ahmad, Shanti Bhusan Mohanty, Literature Search: Swathi Chacham, Rachna Pasi, Madhuradhar Chegondi, Najeeb Ahmad, Shanti Bhusan Mohanty, Writing: Swathi Chacham, Rachna Pasi, Madhuradhar Chegondi, Najeeb Ahmad, Shanti Bhusan Mohanty.

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REVIEW

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# Bronchial Carcinoid Tumour as a Rare Cause of Cushing's Syndrome in Children: A Case Report and Review of Literature

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## Abstract

Cushing's syndrome (CS) is rare in childhood and adolescence. The most common paediatric cause of CS is exogenous administration of glucocorticoids; either topical, inhaled or oral corticosteroids. Endogenous causes can be classified into adrenocorticotropic hormone (ACTH) independent and ACTH dependent causes. Herein, we report our experience of managing a 12 year old girl who presented with features of CS and was found to have an ectopic, ACTH-secreting bronchial carcinoid tumour, which was resected surgically. Our patient was managed successfully by multidisciplinary approach and has recovered from hypertension and Cushing's habitus. The English language literature was searched from 2019 back, using PubMed, Google and Google Scholar. Keywords used for the search were; "Ectopic ACTH syndrome (EAS) in children", "bronchial carcinoid in children" and "Cushing's Syndrome in children". Children with bronchial carcinoid tumours causing EAS were identified. Case variables such as age, sex, type of carcinoid, investigations, surgery, recurrences and outcome were reviewed. Fourteen cases of paediatric bronchial carcinoid producing ACTH were found with a mean age of 15.8 years and female preponderance. Most of the patients had a right lung lesion and histological appearance was typical of carcinoid tumour. Bronchial carcinoid is extremely rare in children and only 4% are associated with CS. The postoperative treatment of CS is challenging with a high prevalence of hypertension, increased body mass index and visceral fat mass, impaired cognitive function and decreased quality of life. A careful follow up is indispensable for monitoring recurrence of carcinoid and complete remission of CS.
**Keywords:** Paediatric Cushing's syndrome, Ectopic ACTH syndrome, paediatric bronchial carcinoid

## Introduction

Paediatric Cushing's syndrome (CS), is a condition which occurs due to excessive amount of glucocorticoids in body, either produced endogenously or administered exogenously. The most common cause for the condition is iatrogenic, like in adults due to excessive administration of glucocorticoids. The endogenous paediatric CS is a rare condition, which is broadly classified into adrenocorticotropic hormone (ACTH) dependent and ACTH independent CS. When the excessive ACTH is secreted by pituitary adenoma, it is called as CS and if the source of ACTH production is outside pituitary, it is called as, Ectopic ACTH syndrome (EAS). Athough rare, the bronchial carcinoids are the most common causes for EAS in children. The overall incidence for bronchial carcinoids is 3-5 tumors per million people per year and 4% of pulmonary carcinoids are associated with CS. The median age of presentation is 9.5 years, with a female predominance. We describe a case of ectopic ACTH secreting bronchial carcinoid presented to us with symptoms and signs of CS and review the present literature for paediatric cases of EAS due to bronchial carcinoid.

Literature review was performed from the year 2019 back to the oldest available report in English, to analyse all cases of bronchial carcinoid tumours causing EAS in children. The online databases searched were PubMed/MEDLINE, Google Scholar and Google using the following keywords: "EAS in children"; "Bronchial carcinoid in children"; and "Cushing's Syndrome in children". All articles that described paediatric patients with bronchial carcinoid tumours causing EAS were identified. Case

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variables such as age, sex, type of carcinoid, diagnosis, surgery and recurrences were reviewed. We also describe a teenage girl with an ectopic ACTH-secreting bronchial carcinoid tumour who presented with symptoms and signs of CS.

## Literature Search

Fourteen paediatric and adolescent patients with bronchial carcinoid tumours causing EAS were identified from nine case series and reports (1-9) excluding our patient. The mean age of these patients was 15.8 ± 3.36 years. There were nine females (70 %) and four male (30 %) children and in one case gender of the patient was not specified. These cases included one atypical carcinoid tumour and four typical carcinoid tumours, mostly involving right lung. There was lymph node metastasis in six patients. All of them were managed by surgical excision of the tumour, although one patient underwent bilateral adrenalectomy due to relapse, two patients had bilateral adrenalectomy and one patient underwent hypophysectomy prior to surgery. There was one death reported and three patients had recurrence.

In a major series of ninety patients with EAS reported by Ilias et al (10), 35 patients with bronchial carcinoid tumours causing EAS were included but the ages ranged from 8-72 years (Table 1) with almost half of the patients having lymph node involvement requiring lymph node dissection. There were three deaths and two relapses reported.

A 12-year old girl presented with complaints of excessive weight gain, dry skin and weakness of limbs. There was no history of steroid intake or previous illness. On examination, she showed typical Cushing's habitus, including "moon face", abdominal striae, growth retardation, muscle weakness, dry and thick skin and excessive hair growth all over her body (Figure 1a).

She was hypertensive (130/90 mmHg) with a body mass index (BMI) of 22.7 kg/cm$^2$ (height 134.5 cm and weight of 41 kg) with BMI standard deviation score (SDS) of 1.22. Her SDS for height for age and weight for age were -2.2, and -0.10 respectively. On evaluation, her midnight and evening serum cortisols concentrations were high (Table 2). A low dose dexamethasone suppression test was done to confirm CS which again showed non-suppressed serum cortisol (26.94 µg/dL). A high dose dexamethasone suppression test (HDDST) showed < 50 % suppression (baseline serum cortisol = 59.55 µg/dL; suppressed cortisol = 35.67 µg/dL). Bilateral inferior petrosal sinus sampling (BIPSS) was done to localise ACTH secretion with stimulation by desmopressin 10 I.U. intravenously. BIPSS ruled out pituitary secretion and suggested peripheral ACTH secretion (Figure 1b). To identify the peripheral ACTH secreting tumour, contrast enhanced computed tomography (CT) of thorax and abdomen were done, which identified a 1.5 cm nodule in the apical segment of the upper lobe of the right lung with no mediastinal lymph node enlargement (Figure 1c, 1d). The abdominal viscera and adrenals were normal. A core needle biopsy of the lung nodule was inconclusive on two separate attempts.

After management of hypokalemia and hypertension, she underwent right upper lobectomy as the location of tumour did not allow segmentectomy (Figure 2a). The histopathological examination showed a well circumcscribed tumour with a nest of small cells around bronchial cartilage (Figure 2b) and no nuclear atypia or mitotic activity. On immunohistochemistry, the cells were immune-reactive for synaptophysin and chromogranin-A (CgA) (Figure 2c) suggesting typical bronchial carcinoid.

The patient had vomiting in the immediate postoperative period for which hydrocortisone was started and tapered over a period of one week. There was persistent hypertension postoperatively for which calcium channel blocker and beta-blocker was continued. The patient became eucortisolemic three days after surgery with a cortisol concentration of 7.68 µg/dL (Table 2). Over six months of follow up, she lost 10 kg of body weight, facial puffiness and body hair have decreased and she currently does not need antihypertensives and steroids (Figure 2d). She also had normal serum calcium (9.84 mg/dL), phosphorus (6.35 mg/dL), parathormone (17.8 pg/mL) and insulin like growth factor-1 (355.7 ng/mL).

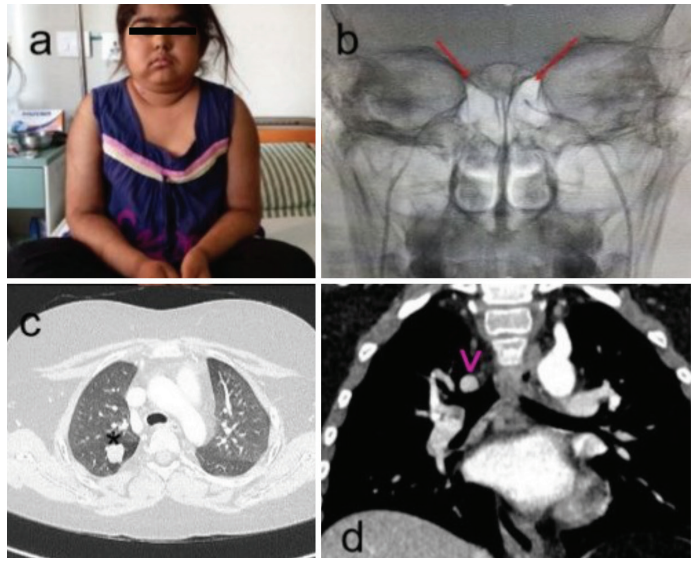


**Figure 1.** (a) Pre-operative photograph of patient showing cushingoid facies, hirsutism and obesity, (b) bilateral inferior petrosal sinus sampling with red arrows indicating the micro-catheters in petrosal sinuses, (c) axial and, (d) coronal view of computed tomography chest showing a smoothly margined nodule of 15 mm diameter in the apical segment of the right upper lobe

**Table 1. Review of literature of bronchial carcinoid tumours causing ectopic adrenocorticotropic hormone syndrome**

| Reference | S. no | Year | Age | Sex | EAS tumours | Location | LN metastasis | Imaging | Treatment | Outcome | Recurrence |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ward et al (1) | 1 | 1983 | 16 | F | BC | | | CT + | Metyrapone- Surgery | | |
| Wang et al (2) | 2 | 1993 | 19 | F | BC | Right mddle lobe | Yes | X-ray | Transspenoidal hypopysectomy f/b wedge resection | | |
| Magiakou et al (3) | 3 | 1994 | 15 | F | BC | | | | Bilateral (B/L) adrenalectomy f/b surgical resection | | |
| Weber et al (4) | 4 | 1995 | 17 | F | BC | | | | | | |
| Dias et al (5) | 5 | 2006 | 18 | ND | BC | | | | | | |
| Salgado et al (6) | 6 | 2006 | 11 | F | BC | | | | Surgery | Surgical | |
| Bhansali et al (7) | 7 | 2009 | ND ND | M | BC | Right upper lobe | | | Surgery f/b Recurrence-B/L adrenalectomy, CT, RT | Alive | 1 |
| | 8 | | 10 | M | BC | Right middle | | | Surgery-ARDS | Death | |
| More et al (8) | 9 | 2005 | 20 | M | TC 2 in no | | Yes | CT-, SRS-, FDG + , F-, DOPA + | KC f/b surgery | Alive after 3 yr of f/u | 0 |
| | 10 | 1996 | 19 | F | TC | | Yes | CT + , SRS + | Metyrapone f/b surgery | Alive after 14 yr of f/u | 2 |
| | 11 | 1995 | 13 | M | TC | | Yes | CT-, SRS + , MRI + , | Surgery | Alive after 4 yr of f/u | 0 |
| | 12 | 1995 | 15 | F | TC | | Yes | CT + , SRS- | KC + mitotane f/b Surgery | Alive after 16 yr of f/u | 4 |
| | 13 | 1988 | 13 | F | AC | | Yes | CT-, MRI + after 4 years | KC + , mitotane + , B/L adrenalectomy, surgery when identified | Alive after 16 yr of f/u | 0 |
| Potter et al (9) | 14 | 2018 | 20 | F | BC | Right lower lobe-superior segment | Not assessed | CT | Wedge resection | Alive | 0 |
| Ilias et al (10) | 35 patients | | | 19 M | 15 TC; 13 AC | 4-right upper lobe | 17 localised | CT, MRI | Lobetomy and LN dissection | 3 deaths | 2 relapse |
| | | | | 16 F | | 3-right middle lobe | 17 LN involvement | | | | |
| | | | 8-72 years | | | 2-right lower lobe | 1 metastatic | | | | |
| | | | | | | 2-left lower lobe | | | | | |
| | | | | | | 1-left upper lobe | | | | | |
| Present case | | 2019 | 12 | F | TC | Right upper lobe | No | CT | Right upper lobectomy | Alive and well after 1 year f/u | NIL |

TC: typical carcinoid, AC: atypical carcinoid, SRS: somatostatin receptor scintigraphy, FDG-DOPA: flurodeoxyglucose-18-L-dihyroxyphenylalanine, KC: ketoconazole, f/b-followed by, f/u-follow-up, MRI: magnetic resonance imaging, LN: lymph node, CT: computed tomography, yr: year, ARDS: adrenocorticotropic hormone, EAS: ectopic ARDS syndrome, RT: radiation therapy

Chest X-ray demonstrated expansion of the right middle and lower lobes to occupy the chest cavity.

## Discussion

### Endogenous CS

Endogenous CS is a rare disorder in children and adolescents due to increased glucocorticoids, which in early childhood is more common in males but with a female preponderance in older children (11,12). The most common cause CS is iatrogenic, due to administration of exogenous glucocorticoids (13,14). The causes of endogenous CS are classified into ACTH dependent and ACTH independent CS (Table 3).

**Table 2. Patient cortisol and adrenocorticotropic hormone concentrations at various points during diagnosis and treatment**

| Investigations | Patient values | Reference values |
|---|---|---|
| Midnight serum cortisol (preoperative value) | 17.52 µg/dL | < 7.5 µg/dL (11) |
| Evening serum cortisol (preoperative value) | 59.47 µg/dL | 1.8-6.5 µg/dL |
| LDDST (post 1 mg dexamethasone at 8:00 am) | 26.94 µg/dL | < 1.8 µg/dL (11) |
| HDDST (preoperative value) | 35.67 µg/dL | 1.8-6.5 µg/dL |
| ACTH (adrenocoticotrophic hormone, plasma) preoperative value | 76.3 pg/mL | < 29 pg/mL (11) |
| Serum cortisol (morning sample postoperative day 3) | 7.68 µg/dL | 4.5-24.0 µg/dL |
| Serum cortisol (morning sample six months postoperative) | 3.27 µg/dL | 4.5-24.0 µg/dL |

LDDST: low dose dexamethasone suppression test, HDDST: high dose dexamethasone suppression test, ACTH: adrenocoticotrophic hormone

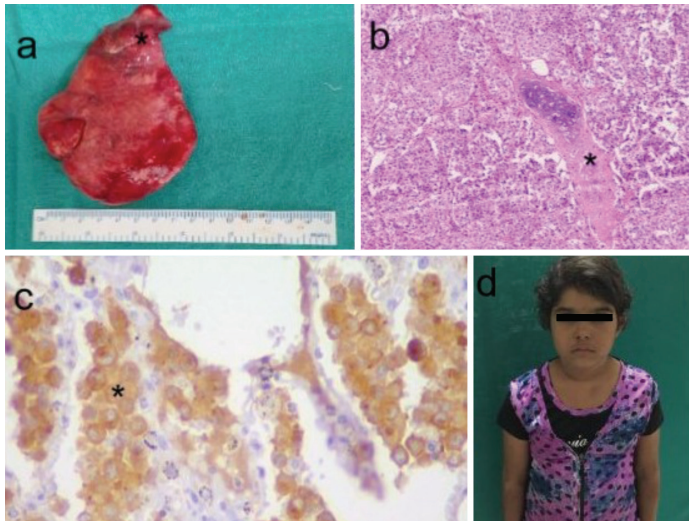


**Figure 2.** (a) Resected upper lobe of right lung, (*) tumour in apical segment, (b) haematoxylin and eosin image of nests of tumour around bronchial cartilage (*), 10x image (c) immunohistochemistry for synaptophysin, (*) showing strong cytoplasmic positivity at 40x magnification (d) postoperative photograph showing resolution of Cushing's habitus

In ACTH-dependent causes of CS, when the ACTH is produced by a pituitary adenoma, it is known as Cushing's disease (CD). CDis the most common cause of CS in children older than six years of age and is responsible in 75-90% of paediatric CS (11,14). Adrenal causes of CS are more common in young children and may present with features of virilisation (11,15,16). A less common cause is when ACTH is secreted by a non-pituitary tumor, known as EAS (14). EAS in young children is much rarer than in adults where it accounts for 15% of cases (8).

CS patients can present with various signs and symptoms which differ depending on patients age and causes of CS (13). Growth failure and associated weight gain are the most common presenting features of paediatric CS (13,17). Other common features are hypertension (50-60%), hirsutism (80%) and striae (61%) (17).

The differentiation between ACTH dependent and independent causes of CS is made by measuring 8 am plasma ACTH concentrations. A value of ACTH >29 pg/mL has a sensitivity of 70% to diagnose an ACTH-dependent cause of CS (11). To identify cases of CS, corticotropin-releasing hormone (CRH) test and the HDDST may be helpful but they are not reliable for differentiating between CS and other causes of ectopic ACTH secretion. This is because patients with ectopic CS may also show a decrease in cortisol level, which happened in our patient (8,13,18). BIPSS is considered to be the gold standard for lateralisation of lesions and in distinguishing CS from EAS (8,13,18). In our patient, BIPSS gradients confirmed that there was no lesion in the pituitary and thus the secretion of ACTH was ectopic.

**Table 3. Causes of endogenous Cushing's syndrome in children**

| ACTH-dependent CS | ACTH-independent CS |
|---|---|
| 1. Cushing's disease (ACTH secreting pituitary adenoma) | 1. Adrenocortical tumours (adenoma or carcinoma) |
| 2. Ectopic ACTH syndrome | 2. Primary adrenocortical hyperplasia |
| | - PPNAD, Carney complex/MEN |
| | - Macronodular adrenal hyperplasia |
| | - McCune-Albright syndrome |

CS: Cushing's syndrome, ACTH: adrenocoticotrophic hormone, PPNAD: primary pigmented adrenocortical disease, MEN: multiple endocrine neoplasia

Ref. 13.

If ACTH-independent CS is diagnosed, adrenal CT or magnetic resonance imaging (MRI) is required to differentiate between adrenocortical tumour and primary nodular hyperplasia. When an ectopic ACTH secreting lesion is suspected, CT of the neck, thorax, abdomen and pelvis should be performed to localise the lesion. Octreotide scan, positron emission tomography (PET), DOTATE scan (Ga-68 DOTA-1,4,7,10-tetraazacyclododecane-tetraacetic acid, TATE-Tyr3-octreotate) and octreotide PET scan can also help in identification of lesion (11,18). In our patient CT of the thorax using 0.5 cm sections identified a lesion of 1.5 cm in the apical segment of the upper lobe of her right lung, which was the source of the ectopic ACTH secretion.

## Ectopic ACTH Syndrome

EAS is very rare in children in comparison to adults and is more common in female children from 10 year of age (11,13). The majority of EAS cases result from carcinoid tumours of the bronchus or thymus (9) but have been reported from appendiceal, kidney and duodenal tumours (10,19). EAS may also occur due to ACTH secretion from adrenal neuroblastoma, clear cell sarcoma, pancreatic tumour, gastrinoma, pheochromocytoma, Wilm's tumour and sacrococcygeal tumour (10,20,21,22,23,24,25). Muscle weakness, hypertension and hypokalemia is significantly more common in patients with EAS compared to those with CS (8,10). In addition, when compared to CS, patients with EAS have statistically significant higher levels of urinary free cortisol, ACTH (sensitivity 80% and specificity 74% for ACTH levels of 1.6 times the upper limit of normal) and mean ACTH increase is lower on CRH testing (sensitivity 83% and specificity 81% for differential increase of 31% in plasma ACTH) (8). Inferior petrosal sinus sampling is considered to be the gold standard for the diagnosis of EAS (10). The localization of the ACTH secreting tumor is difficult, and CT, MRI and octreotide scan, should all be used to find the tumour in EAS (10). Although biochemical tumor markers are less helpful, serum calcitonin can be used as it is known to be elevated in carcinoid tumours, medullary thyroid cancer and neuroendocrine tumors and is normal in CS (10). The surgical resection of an ACTH producing tumor is the optimal treatment, but bilateral adrenalectomy is required in refractory cases to control hypercortisolemia (10).

## Bronchial Carcinoid Tumours in Children

Although bronchial carcinoid tumours are the most common intrabronchial primary tumour in children (9,26) only 4% of them are associated with CS (27). Bronchial carcinoid tumours can arise from main, lobar or segmental bronchi and they can present with obstructive symptoms including atelectasis, dyspnea, pleuritic pain or obstructive pneumonitis (28) although our patient did not have any respiratory symptoms. Carcinoid tumors arise from Kolschitzky cells found in the basal layer of the bronchial epithelium. The overall incidence is 3-5 tumours per million people per year. However, the exact incidence of bronchial carcinoid tumours in children is not known but they constitute 70-80 percent of all primary malignant lung tumors in children (2,27,29).

Contrast-enhanced CT of the chest, with 5 mm thick sections or MRI of neck, chest and abdomen is considered first line for diagnosing ectopic ACTH secreting lesions (18,27,30). In our patient, the suspicious lesion was detected on chest CT with 5 mm sections. Octreotide scan may be useful to diagnose the primary lesion and to detect metastasis and recurrence of carcinoid tumours (9,29) but some studies suggested it to be less helpful in bronchial carcinoids, as one third of them do not express somatostatin receptors (31).

Travis et al (32,33) reported the tumour appearance as follows. Grossly, the cut surface was a homogenous tan colour with foci of haemorrhage. Microscopically they were composed of small uniform cells arranged in a mosaic pattern with interlacing fibrovascular stroma (32). The average size of these tumours is 2-4 cm and they may infiltrate the bronchial wall and surrounding lung tissue (33). The prognosis depends upon histology, lymph node status and size of tumour (30). They are classified as atypical (10%) and typical (90%) carcinoids depending upon the presence or absence of necrosis and elevated mitotic index (>2 mitoses/HPF) (9,33). Both of them can be positive for biomarkers including Chromogranin A (CgA) and synaptophysin. Typical carcinoid tumours tend to be central in location while atypical tumours tend to be peripheral (9). Although typical carcinoid tumours are considered to be benign, both variants are capable of metastasizing to regional lymph nodes, liver, bones and brain (30,33). Our patient had a typical carcinoid tumour with no evidence of necrosis and a low mitotic index and thus a good prognosis.

The treatment of choice for a bronchial carcinoid tumour is complete surgical resection with removal of involved lymph nodes (9,27). Lymph nodes are involved in up to 20 percent of paediatric cases of both types (9). Lymph node resection is more important for atypical carcinoid tumours owing to the greater malignant potential. Radiation and chemotherapy can be used where complete surgical resection is not possible (31). Somatostatin analogues, interferon $\alpha$ and temozolomide analogues have been used in adults with advanced disease (9). The surgery should be parenchymal-preserving whenever possible and sleeve

resections and bronchoplastic procedures should be considered for central lesions (34). In our patient, it was not possible to remove the tumour while preserving the upper lobe.

A typical carcinoid tumour has a good 5-year survival rate of 88-92% and that of atypical carcinoid ranges from 60-75% (9,28,30,35). The ACTH secreting bronchial carcinoid tumours are considered aggressive variants, as lymph node positivity and recurrences are observed, even in typical carcinoid tumours (11,36). Annual serum ACTH and tumour markers should be measured as part of follow-up in order to achieve early detection of recurrence (27). CT scan of neck and chest every six to 12 months is required in node positive cases (9).

Although the surgical removal of the source of the hypercortisolaemia is the treatment of choice, medical agents such as antihypertensives and inhibitors of steroidogenesis, such as metyrapone and ketoconazole, can be used in the preoperative period to reduce the surgical risk, or when surgery is contraindicated and in postoperative period when the patient is not cured by surgical resection (18). The resolution of hypertension is more common in children compared to adults, due to vascular protective mechanism and shorter lasting hypercortisolemia (37) as is seen in our patient who became normotensive within three months after surgery.

There was requirement for postoperative hydrocortisone in our patient, as she developed postoperative vomiting, headache and weakness, which was tapered and discontinued over a period of one month. This is similar to other reports (18,38) where discontinuation of hydrocortisone within 1-2 years was noted.

The postoperative treatment of CS is challenging with a high prevalence of hypertension, increased BMI and visceral fat mass, impaired cognitive functions and decreased quality of life (18,39,40,41). However, our patient lost 10 kg weight and performing well in school. However, her growth chart needs close monitoring since patient did not have CS. Following medical or surgical treatment for CS, monitoring of growth and pubertal development is important, as growth hormone deficiency is the most common pituitary deficiency in children with CS, followed by ACTH deficiency (18).

The complete remission of CS is a rare phenomenon, which was achieved successfully in our patient by multidisciplinary approach and she has recovered completely from hypertension and Cushing's habitus. The timely diagnosis of cause of hypercortisolism and its appropriate management is the cornerstone of successful management.

## Conclusion

Paediatric pulmonary carcinoid tumours causing EAS and leading to CS is a very rare entity and the algorithm of investigations should be followed to reach to diagnosis. BIPSS is the investigation of choice to differentiate EAS CS from CS. CT of neck and chest will help to locate the site of EAS tumours. The treatment of choice is surgical resection of tumor and involved lymph nodes with the intention of achieving negative margins and to preserve as much lung parenchyma as possible The complete remission is possible in children with EAS but bronchial carcinoid tumours causing EAS are aggressive in nature making good follow up mandatory in these cases to monitor for recurrence.

### Ethics

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: Rahul Saxena, Manish Pathak, Ravindra Shukla, Arvind Sinha, Poonam Elhence, Jyotsna N. Bharti, Pushpinder Khera, Design: Rahul Saxena, Manish Pathak, Ravindra Shukla, Arvind Sinha, Poonam Elhence, Jyotsna N. Bharti, Pushpinder Khera, Data Collection or Processing: Rahul Saxena, Manish Pathak, Ravindra Shukla, Arvind Sinha, Poonam Elhence, Jyotsna N. Bharti, Pushpinder Khera, Analysis or Interpretation: Rahul Saxena, Manish Pathak, Ravindra Shukla, Arvind Sinha, Poonam Elhence, Jyotsna N. Bharti, Pushpinder Khera, Literature Search: Rahul Saxena, Manish Pathak, Ravindra Shukla, Arvind Sinha, Poonam Elhence, Jyotsna N. Bharti, Pushpinder Khera, Writing: Rahul Saxena, Manish Pathak, Ravindra Shukla, Arvind Sinha, Poonam Elhence, Jyotsna N. Bharti, Pushpinder Khera.

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# Gender Identity and Assignment Recommendations in Disorders of Sex Development Patients: 20 Years' Experience and Challenges

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### What is already known on this topic?

Gender assignment in disorders of sex development (DSD) patients is always very difficult, complex and demanding experience in the management for both families and clinicians, particularly in cases where the gender appropriate for the clinical diagnosis is incompatible with the psychological gender of the patient. Gender assignment councils must have an experienced and multidisciplinary approach.

### What this study adds?

Here, we present 20 years of experience and challenges in gender assignment, the causes and clinical characteristics of patients with DSD. This study is the longest timeframe, is the most comprehensive and has the largest number of cases in terms of gender assignment recommendation and assessing the factors affecting gender assignment from Turkey.

## Abstract

**Objective:** Gender assignment in infants and children with disorders of sex development (DSD) is a stressful situation for both patient/families and medical professionals.

**Methods:** The purpose of this study was to investigate the results of gender assignment recommendations in children with DSD in our clinic from 1999 through 2019.

**Results:** The mean age of the 226 patients with DSD at the time of first admission were 3.05±4.70 years. 50.9% of patients were 46,XY DSD, 42.9% were 46,XX DSD and 6.2% were sex chromosome DSD. Congenital adrenal hyperplasia (majority of patients had 21-hydroxylase deficiency) was the most common etiological cause of 46,XX DSD. In 46,XX patients, 87 of 99 (89.7%) were recommended to be supported as a female, 6 as a male, and 4 were followed up. In 46,XY patients, 40 of 115 (34.8%) were recommended to be supported as a female, and 70 as male (60.9%), and 5 were followed up. In sex chromosome DSD patients, 3 of 14 were recommended to be supported as a female, 9 as a male. The greatest difficulty in making gender assignment recommendations were in the 46,XY DSD group.

**Conclusion:** In DSD gender assignment recommendations, the etiologic diagnosis, psychiatric gender orientation, expectation of the family, phallus length and Prader stage were effective in the gender assignment in DSD cases, especially the first two criteria. It is important to share these experiences among the medical professionals who are routinely charged with this difficult task in multidisciplinary councils.

**Keywords:** Gender assignment, disorders of sex development, ambiguous genitalia, congenital adrenal hyperplasia

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## Introduction

According to Jost's paradigm, the first sexual development stage begins with the identification of the chromosomal sex at the time of fertilization and is completed as a result of many biological process (1). Money et al. (2) added the theory of psychosexual development to this paradigm. This theory is influenced by hormonal and genetic status, environmental and psychosocial experiences, and social and parental behavior (3,4,5). Any defect occurring during this complicated process of sexual differentiation may lead to a discordant development of chromosomal, gonadal, and anatomical sex/phenotype and is defined as disorders of sex development (DSD) (6,7,8). DSD are a heterogeneous group of rare conditions which include various etiologies and presentations (9,10,11). The incidence of DSD is almost 1 in 4,500-5,500 (10,11,12).

The long-term physical, social and psychological outcomes of patients with DSD are still unclear. There are increasing concerns regarding early decisions about gender assignment in recent reports (13,14,15,16,17,18). Studies have been generally conducted regarding psychosexual and surgical outcomes in this group of patients (19,20,21,22). Gender assignment of a child with DSD is the most difficult and stressful condition for both the family and the clinician, especially in cases of ambiguous genitalia (6,23,24). Families will always want to know the actual gender of their DSD baby as soon as possible and give their baby a gender appropriate name. The primary goal in DSD is for gender identity to be consistent with the gender assigned (6). In this respect, a multidisciplinary approach is required for the diagnosis and treatment of DSD (25). Influencing factors to consider when debating gender assignment include medical diagnosis, external genital appearance, potential of fertility and sexuality, therapeutic and/or surgical intervention options, views and desires of the patients and their families, sociocultural factors, and the psychological gender development status of the child (26,27,28).

There is a multidisciplinary council to make gender assignment recommendations in DSD patients which, in our clinic, consists of pediatric endocrinology, pediatric surgery, pediatric psychiatry, medical genetics and forensic science specialists. Here, we present 20 years of experience at a single regional referral center in assistance with gender assignment in DSD patients.

## Methods

The purpose of this study was to investigate the results of gender assignment recommendations in children with DSD and the factors affecting these results in our clinic. In the present study, the file records of the 226 children with DSD admitted to the Department of Pediatric Endocrinology of Çukurova University between the years of 1999 and 2019 were reviewed. The clinical diagnosis of a DSD was supported by anatomical examination findings, gonadal and pelvic ultrasound, cytogenetic studies, determination of serum electrolytes, 17-hydroxyprogesterone levels, the ratio of testosterone-dihydrotestosterone (basal and hCG stimulated) and molecular genetic testing. 21-hydroxylase deficiency (21-OHD) (72 of 88), 11-beta-hydroxylase deficiency (6 of 6), 17-beta-hydroxysteroid dehydrogenase type 3 deficiency (4 of 4), Steroidogenic Acute Regulatory Protein *(STAR)* gene mutations (5 of 5), complete androgen resistance (8 of 9), incomplete androgen resistance (6 of 6), 5-alpha-reductase deficiency, (19 of 19), Leydig cell aplasia/hypoplasia (2 of 2), 17-alpha-hydroxylase deficiency, (1 of 1), DSS-AHC Region on Human X Chromosome (*DAX1*; also known as *NR0B1*) (2 of 2), *NR5A1 (SF1)* (2 of 2), Persistent Mullerian Duct syndrome (1 of 1), and Klinefelter syndrome (2 of 2) were diagnosed by cytogenetic studies and molecular genetic analyses. However, mixed gonadal dysgenesis, gonadal dysgenesis, ovotestis and Sertoli cell only syndrome were diagnosed by laparoscopy with gonadal biopsy, and molecular genetic testing. All the genetic testing was performed for diagnostic purposes after consent from the patients or child's legal representative.

Laparoscopy and gonadal biopsy were performed in selected DSD patients for determination of gonadal histology. Cystoscopy was performed in order to examine urethra, uterus and uterine remnants.

Our center is the first, and the oldest and largest 'Gender Evaluation Council' in the region. This council consists of pediatric endocrinologists, pediatric surgeons, child psychiatrists, specialists in forensic medicine and a medical geneticist. Gender assignment recommendations were made by this council. The role of the council is to evaluate medical data, to conduct expert discussion, and to provide information and medical advice to the patient and/or family. The council ensures that ample time and opportunities are provided to patient and families for their questions, concerns, and counseling needs.

Exclusion criteria for this study were: DSD patients who did not need gender assignment (therefore not discussed in the council) such as Turner syndrome and isolated hypospadias. Written inform consent was obtained after the council from the parents or legal guardians of all the patients before participation. The study protocol was approved by the Ethics Committee of Çukurova University and performed in

accordance with the ethical standards of the Declaration of Helsinki (ethical decision no: 452018.77/10).

Background clinical data obtained from medical file records included age at the time of first admission and meeting, reason for admission, genital examination findings, Prader stage, karyotype, diagnosis, psychiatric gender orientations, gender patient was being raised as, parents' views and requests for the gender, number of council meetings held for each patient, and gender assigned. Although genital phenotype evaluation according to the Sinnecker classification is more appropriate for 46,XY DSD cases (29), all patients were evaluated via Prader classification in order to avoid confusion (30).

The patients were classified into three main groups on the basis of the karyotype of the affected individual, according to The Lawson Wilkins Pediatric Endocrine Society and the European Society for Paediatric Endocrinology consensus (8,9,31). These groups are: 46,XX DSD; 46,XY DSD; and sex chromosome DSD.

The psychological evaluation for gender orientation was based on psychiatric interview with children and according to Diagnostic and Statistical Manual of Mental Disorders-5 diagnostic criteria (32).

### Statistical Analysis

All analyses were performed using SPSS, version 20.0 statistical software package (IBM Inc., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation (SD). Chi-square test was used to compare categorical variables between the groups. The normality of distribution for continuous variables was confirmed with the Shapiro-Wilk test. For comparison of continuous variables between two groups, the Student's t-test or Mann-Whitney U test was used depending on the distribution being normal or non-parametric, respectively. For comparison of continuous variables between more than two groups, Kruskal Wallis test was used. Bonferroni adjusted Mann-Whitney U test was used for pairwise comparisons of groups. The statistical level of significance for all tests was considered to be 0.05.

## Results

A total of 226 patients were classified as 46,XY DSD (n = 115, 50.9%), 46,XX DSD (n = 97, 42.9%) or sex chromosome DSD (n = 14, 6.2%) (Table 1). The mean ± SD age at first admission of the patients was 3.05 ± 4.70 (range 0-17.58) years.

Of the 226 patients, ambiguous genitalia (n = 141, 62.4%) was the most frequent cause of admission for all three groups (Table 1).

When the diagnostic distribution of the patients was examined, congenital adrenal hyperplasia (CAH) was the most common cause of DSD. Among the 46,XX DSD (n = 97) patients, 21-OHD was the most common (n = 88, 90.7%) (Table 1). The most common cause amongst 46,XY DSD cases (n = 115) was 5-alpha reductase deficiency (n = 19, 16.5%). This was followed by complete androgen insensitivity syndrome (CAIS) and incomplete androgen resistance (PAIS) (total n = 15, 13%). Forty-two (18.6%) of all cases had undetermined causes for DSD. The vast majority of these were 46,XY DSD cases (40/42, 95.2%) (Table 1, 2).

The psychiatric evaluation of cases showed that only about half of the 46,XX DSD patients had female and one in three of the 46,XY DSD patients had male gender orientation. In the sex chromosome DSD cases, female gender was 4/15 and male gender was 5/14 patients and 5/15 patients had no sexual orientation (Figure 1).

The median age of all cases was 1.90 (mean: 4.46 ± 4.98, range 0.12-18.63) years at the time of the council meeting. For each of the categories 46,XX DSD, 46,XY DSD and sex chromosome DSD patients these median ages were 1.60 (mean: 3.20 ± 3.92, range 0.12-18.56), 2.98 (mean: 5.49 ± 5.41, range 0.13-18.38) and 1.67 (mean: 4.77 ± 6.15, range 0.21-18.63) years, respectively (p = 0.004). While 200 (88.5%) of 226 patients had gender assignment at the first council meeting, 26 patients (11.5%) had more than one council meeting of whom 18/26 were 46,XY DSD, six were 46,XX DSD and two were sex chromosomal DSD patients. It is notable that patients requiring more than one meeting were mostly 46,XY DSD cases.

The mean age intervals of presentation and being considered at the meeting for 46,XX DSD, 46,XY DSD, and sex chromosome DSD were 1.19 ± 2.03 (range 0.06-10.96) years, 1.45 ± 2.12 (range 0.03-11.97) years and 2.73 ± 4.58 (range 0.02-15.08) years, respectively. It was found that, these intervals were not different according to the DSD diagnosis (p = 0.113), Prader stage (p = 0.949) and decision (p = 0.062).

In 46,XY DSD patients, 40 of 115 (34.8%) were recommended to be assigned as a female gender (Figure 1). The female gender assignment recommendation in these cases was made for all of the CAIS, Leydig cell aplasia/hypoplasia, *STAR* gene mutations, 17-alpha hydroxylase and *DAX1 (NR0B1)* mutation cases according to the genetic diagnosis (Table 2).

**Table 1. Distribution of admission reasons and etiological causes of patients**

| | | Reason for admission | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ambiguous genitalia | Swelling in the groin | Adrenal crisis | Primary amenorrhea | No testes | Ambiguous genitalia history in family | Micropenis | Absence of vaginal meatus | Short stature | Total |
| | | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% |
| **46,XX DSD** | 21-OHD | 62 | | 14 | 2 | 10 | | | | | 88 |
| | | 70.5 | | 15.9 | 2.3 | 11.4 | | | | | 100 |
| | 11-OHD | 4 | | | | 2 | | | | | 6 |
| | | 66.7 | | | | 33.3 | | | | | 100 |
| | Sertoli cell only syndrome | 1 | | | | | | | | | 1 |
| | | 100 | | | | | | | | | 100 |
| | Undetermined causes | 2 | | | | | | | | | 2 |
| | | 100 | | | | | | | | | 100 |
| **46,XY DSD** | SRD5A2 | 10 | 4 | | 3 | | 2 | | | | 19 |
| | | 52.6 | 21.1 | | 15.8 | | 10.5 | | | | 100 |
| | CAIS | | 5 | | 3 | | 1 | | | | 9 |
| | | | 55.6% | | 33.3 | | 11.1 | | | | 100 |
| | PAIS | 5 | | | 1 | | | | | | 6 |
| | | 83.3 | | | 16.7 | | | | | | 100 |
| | *STAR* | | 1 | 4 | | | | | | | 5 |
| | | | 20.0 | 80.0 | | | | | | | 100 |
| | HSD17B3 | 3 | 1 | | | | | | | | 4 |
| | | 75.0 | 25.0 | | | | | | | | 100 |
| | CYP17A1 | | 1 | | | | | | | | 1 |
| | | | 100 | | | | | | | | 100 |
| | Leydig cell aplasia/hypoplasia | | 2 | | | | | | | | 2 |
| | | | 100 | | | | | | | | 100 |
| | DAX-1 | | 2 | | | | | | | | 2 |
| | | | 100 | | | | | | | | 100 |
| | *NR5A1 (SF1)* | | 2 | | | | | | | | 2 |
| | | | 100 | | | | | | | | 100 |
| | Progesterone treatment in pregnancy | 6 | 1 | | | | | | | | 7 |
| | | 85.7 | 14.3 | | | | | | | | 100 |
| | Gonadal dysgenesis | 4 | | | 1 | 1 | 1 | 1 | | | 8 |
| | | 50.0 | | | 12.5 | 12.5 | 12.5 | 12.5 | | | 100 |
| | Mixed gonadal dysgenesis | 4 | | | | | | | | | 4 |
| | | 100 | | | | | | | | | 100 |
| | Ovotestis | | | | 1 | 1 | | | 1* | | 3 |
| | | | | | 33.3 | 33.3 | | | 33.3 | | 100 |
| | Persistent Mullerian duct syndrome | | | | | 1 | | | | | 1 |
| | | | | | | 100 | | | | | 100 |
| | Vanishing testis | | | | | 1 | | | | | 1 |
| | | | | | | 100 | | | | | 100 |
| | Burn | 1** | | | | | | | | | 1 |
| | | 100 | | | | | | | | | 100 |
| | Undetermined causes | 27 | 4 | | 5 | 3 | | 1 | | | 40 |
| | | 67.5 | 10.0 | | 12.5 | 7.5 | | 2.5 | | | 100 |

**Table 1. Continued**

| | | Reason for admission | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ambiguous genitalia | Swelling in the groin | Adrenal crisis | Primary amenorrhea | No testes | Ambiguous genitalia history in family | Micropenis | Absence of vaginal meatus | Short stature | Total |
| | | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% | n<br>% |
| **Sex chromosome DSD** | Mixed gonadal dysgenesis | 10<br>83.3 | | | 1<br>8.3 | | | | | 1<br>8.3 | 12<br>100 |
| | Klinefelter | 2<br>100 | | | | | | | | | 2<br>100 |

DSD: disorders of sex development, 21-OHD: 21-hydroxylase deficiency, 11-OHD: 11-beta-hydroxylase deficiency, SRD5A2: 5-alpha-reductase deficiency, CAIS: complete androgen insensitivity syndrome, PAIS: incomplete androgen resistance, *STAR*: Steroidogenic Acute Regulatory Protein, HSD17B3: 17-beta-hydroxysteroid dehydrogenase type 3 deficiency, CYP17A1: 17-alpha-hydroxylase deficiency, F: female, M: male.

*Female patient with Prader stage 1 was referred for short stature; palpable gonads were identified in the inguinal region and she had 46,XY chromosome structure by cytogenetic analysis.

**Patient was admitted to the council due to burn-induced ambiguous genitalia.

Eleven of 226 cases (4.8%) were followed without a gender assignment (Figure 1). The common characteristic of all these cases who were not assigned a gender was that the family's gender expectation was not compatible with chromosomal analysis, specific diagnosis, Prader stage and/or psychiatric evaluation.

When the effect of phallus length on the assignment recommendation was examined, it was found that in all three groups, phallus length was significantly higher in male assignments than in female assignments (Table 3).

According to the Prader classification with gender assignments recommendation, lower Prader stages (especially stage 1) were effective in making a female gender assignment in 46,XY DSD and sex chromosomal DSD cases. In addition, as the Prader stage increased, the decision-making ratio was gradually increased in favor of the male gender. However, the higher Prader stages were not associated with making a male gender assignment in 46,XX DSD cases. Moreover, the gender assignment of patients with Prader stage 1-4 was the female gender in a very large number of the 46,XX DSD cases. In general, it was found that a lower Prader stage was more effective in making a female gender assignment recommendation, than making a male gender assignment recommendation with a higher Prader stage (Table 4).

## Discussion

In this study, 20 years of experience in helping gender assignment, the causes and clinical characteristics of patients with DSD in a single referral clinic are presented. Gender assignment is always very difficult, complex and demands experience in the management of patients with DSD for both families and clinicians, particularly in cases where the gender appropriate for the clinical diagnosis is incompatible with the psychological gender of the patient. It should be recognized that every DSD is unique and has to be treated with individualized care. To our knowledge, this study has the longest timeframe, is the most comprehensive and has the largest number of cases in terms of gender assignment recommendation and assessing the factors affecting gender assignment from Turkey.

DSD are a heterogeneous group of conditions, which has an estimated incidence of 1:4500-5500 (10,11,12,33,34). In a recent study from Turkey by Aydin et al (35), it was found that the DSD newborn with ambiguous genitalia rate was 1.3/1000 newborns. However, this rate may be higher in our region where there is an increase in autosomal recessive forms of DSD due to higher rates of consanguinity, around 20% to 25% (35). This is in contrast to the consanguineous marriage rate reported by Aydin et al (35) (3 families of total 18 DSD patients). Nordenvall et al (36) remarked that the developmental anomalies of the external genitalia may be seen in 1:300 infants. However, not all of these conditions require gender assignment, including relatively common conditions such as isolated undescended testis and/or hypospadias.

Previous studies have reported a higher incidence of 46,XY DSD compared to 46,XX or sex chromosome DSD

(35,37,38,39,40,41,42). In accordance with this the most common DSD group in our cohort was 46,XY DSD (50.9%). In a study with 117 patients from Thailand, it was reported that most of the cases were sex chromosome DSD (53%) (43). However, the majority of these patients were Turner syndrome. Girls with Turner syndrome were excluded from the present study because there is no necessity for gender assignment. Two Klinefelter syndrome patients were included because of ambiguous genitalia but other patients with Klinefelter syndrome without ambiguous genitalia, and thus without requirement for a gender assignment process were excluded.

Most patients with DSD are referred with ambiguous genitalia (35,37,38,39,43). In this study, ambiguous genitalia was the most common cause of admissions for all three DSD classifications (Table 1).

Despite the current advanced genetic analyses, a definitive genetic diagnosis can only be made in about 20% of cases of DSD (11,12,31,37). Compatible with this information, the rate of patients with undetermined causes of DSD was 18.6% (42/226) in our study. There were only two patients (2%) with undetermined causes in the 46,XX DSD group whereas this was 40/115 (34.7%) amongst the 46,XY DSD

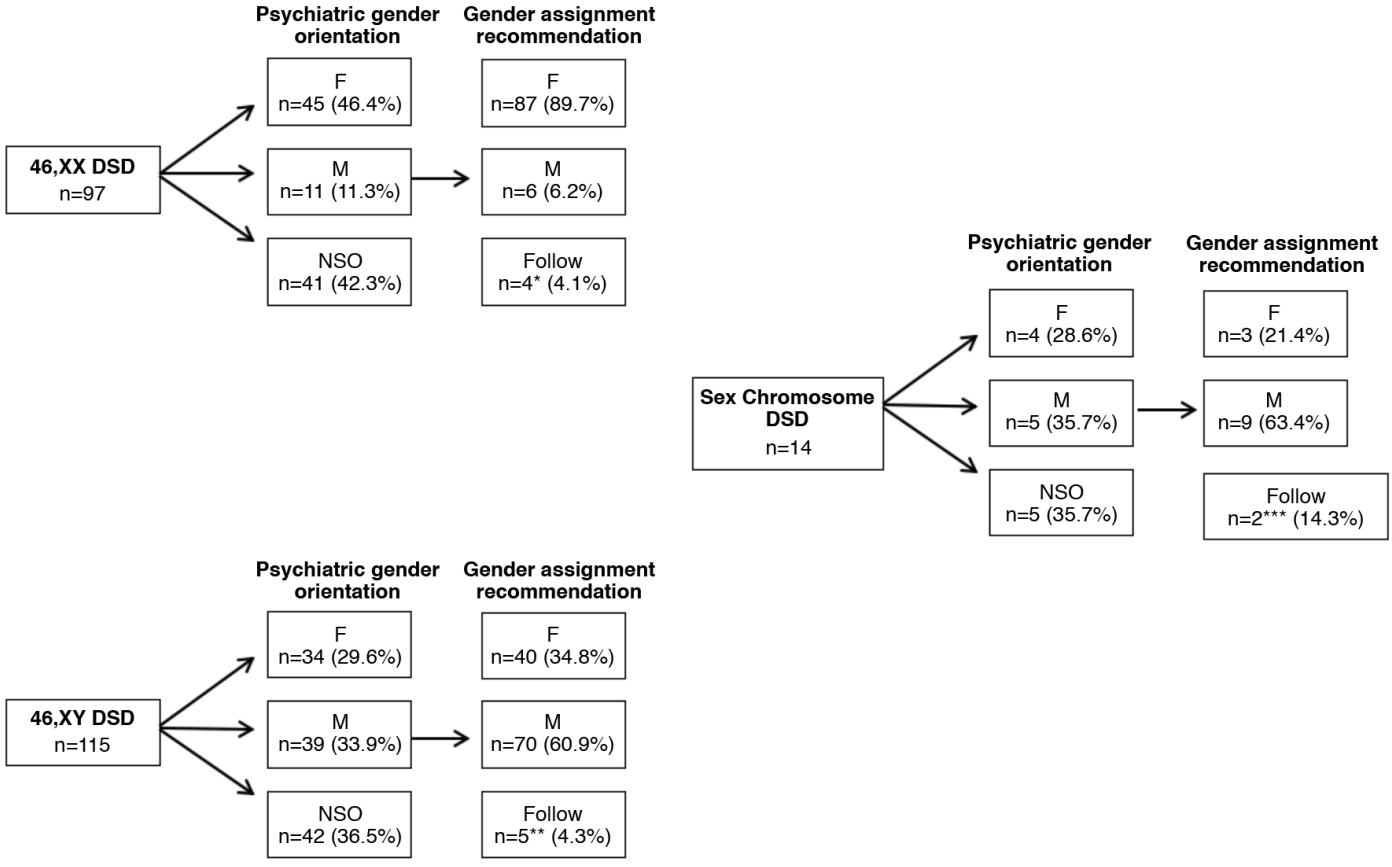


**Figure 1.** Gender orientations and gender assignment recommendations

*DSD: disorders of sex development, F: female, M: male, NSO: no sexual orientation*

**Two of the 3 cases with 46,XX related to 21-hydroxylase deficiency were raised as a male and their families insisted on an assignment recommendation as the male gender. The other one case had female gender orientation, but the family wanted to raise as the male gender. The remaining one 46,XX DSD patient had 11-OH deficiency, and raised as the male gender. Moreover, patient's family wanted to raise as the male gender although the patient had menstrual bleeding.*

***For 2 cases with 46,XY DSD diagnosed with 5-alpha reductase deficiency a follow-up recommendation was made, who were raised as female gender instead of male gender by their parents. Families were persistently wanting for a female assignment to be made. The other two 46,XY DSD cases had a diagnosis of gonadal dysgenesis and had not yet developed a gender orientation. The one 46,XY DSD patient had 17-beta-hydroxysteroid dehydrogenase type 3 deficiency, was raised as a female and the family asked for a male gender assignment.*

****The one Klinefelter syndrome case was raised as a female and her family wanted to raise as the female gender. The other one patient was mixed gonadal dysgenesis and had no gender orientation yet.*

**Table 2. Etiological causes of disorders of sex development with gender assignment recommendations**

| | | Gender assignment | | | |
|---|---|---|---|---|---|
| | | F | M | Follow up | Total |
| | | n | n | n | n |
| | | % | % | % | % |
| **46,XX DSD** | 21-OHD | 80 | 5 | 3 | 88 |
| | | 90.9 | 5.7 | 3.4 | |
| | 11-OHD | 5 | | 1 | 6 |
| | | 83.3 | | 16.7 | |
| | Sertoli cell only syndrome | | 1 | | 1 |
| | | | 100 | | |
| | Undetermined causes | 2 | | | 2 |
| | | 100 | | | |
| **46,XY DSD** | SRD5A2 | 1 | 16 | 2 | 19 |
| | | 5.3 | 84.2 | 10.5 | |
| | CAIS | 9 | | | 9 |
| | | 100 | | | |
| | PAIS | 2 | 4 | | 6 |
| | | 33.3 | 66.7 | | |
| | *STAR* | 5 | | | 5 |
| | | 100 | | | |
| | HSD17B3 | | 3 | 1 | 4 |
| | | | 75 | 25 | |
| | CYP17A1 | 1 | | | 1 |
| | | 100 | | | |
| | Leydig cell aplasia/hypoplasia | 2 | | | 2 |
| | | 100 | | | |
| | DAX-1 | 2 | | | 2 |
| | | 100 | | | |
| | NR5A1 | 1 | 1 | | 2 |
| | | 50 | 50 | | |
| | Progesterone treatment in pregnancy | 1 | 6 | | 7 |
| | | 14.3 | 85.7 | | |
| | Gonadal dysgenesis | 2 | 4 | 2 | 8 |
| | | 25 | 50 | 25 | |
| | Mixed gonadal dysgenesis | 3 | 1 | | 4 |
| | | 75 | 25 | | |
| | Ovotestis | 2 | 1 | | 3 |
| | | 66.7 | 33.3 | | |
| | Persistent Mullerian duct syndrome | | 1 | | 1 |
| | | | 100 | | |
| | Vanishing testis | | 1 | | 1 |
| | | | 100 | | |
| | Burn | | 1 | | 1 |
| | | | 100 | | |
| | Undetermined causes | 9 | 31 | | 40 |
| | | 22.5 | 77.5 | | |
| **Sex chromosome DSD** | Mixed gonadal dysgenesis | 3 | 8 | 1 | 12 |
| | | 25 | 66.7 | 6.3 | |
| | Klinefelter | | 1 | 1 | 2 |
| | | | 50 | 50 | |

DSD: disorders of sex development, 21-OHD: 21-hydroxylase deficiency, 11-OHD: 11-beta-hydroxylase deficiency, SRD5A2: 5-alpha-reductase deficiency, CAIS: complete androgen resistance, PAIS: incomplete androgen resistance, *STAR*: Steroidogenic Acute Regulatory Protein, HSD17B3: 17-beta-hydroxysteroid dehydrogenase type 3 deficiency, CYP17A1: 17-alpha-hydroxylase deficiency, F: female, M: male

cases, thus constituting 40/42 (95%) of the patients without a definitive genetic diagnosis.

The etiologic cause of most of the patients with 46,XX DSD is CAH due to 21-OHD (37,38,39,44). In this study, CAH was the most common underlying etiological condition of 46,XX DSD (Table 1). CAH due to 21-OHD and 11-OHD accounted for 97.9% of 46,XX DSD in our series. Similarly, Ocal et al (39) found that 21-OHD and 11-OHD were the most frequent etiology (88.8%) of their 46,XX DSD group. De Paula et al (38) from Brazil with a 408 case series of genital ambiguity, Al-Mutair et al (45) from Saudi Arabia with a total of 120 DSD patients, and Al-Agha et al (46) from Australia report that the main etiology of 46,XX DSD was 21-OHD. However, Ganie et al (37) reported that the main referring cause of 46,XX DSD was ovotesticular in patients from sub-Saharan Africa.

**Table 3. Evaluation of patients' phallus length with gender assignment recommendations**

| | Mean phallus length (cm) | | | |
|---|---|---|---|---|
| **Assignment** | **46,XX DSD** | **46,XY DSD** | **Sex chromosome DSD** | **Total** |
| F | 2.70 ± 1.24 | 0.82 ± 0.71 | 1.0 ± 0.86 | 2.08 ± 1.40 |
| M | 6.0 ± 2.34 | 2.71 ± 0.98 | 3.31 ± 1.39 | 3.01 ± 1.42 |
| Follow up | 4.7 ± 1.7 | 1.80 ± 0.75 | 2.5 ± 0.70 | 3.0 ± 1.77 |

DSD: disorders of sex development, F: female, M: male

**Table 4. Prader classification with gender assignment recommendations**

| | | Gender assignment | | |
|---|---|---|---|---|
| | | **F** | **M** | **Follow up** |
| | **Prader stage** | **n**<br>**%** | **n**<br>**%** | **n**<br>**%** |
| **46,XX DSD** | 1 | 1<br>100 | | |
| | 2 | 7<br>100 | | |
| | 3 | 49<br>98 | 1<br>2 | |
| | 4 | 20<br>90.9 | 1<br>4.5 | 1<br>4.5 |
| | 5 | 10<br>58.8 | 4<br>23.5 | 3<br>17.6 |
| **46,XY DSD** | 1 | 28<br>90.3 | 2<br>6.5 | 1<br>3.2 |
| | 2 | 8<br>44.4 | 9<br>50 | 1<br>5.6 |
| | 3 | 2<br>6.1 | 28<br>84.8 | 3<br>9.1 |
| | 4 | 2<br>7.4 | 25<br>92.6 | |
| | 5 | | 6<br>100 | |
| **Sex chromosome DSD** | 1 | 2<br>100 | | |
| | 3 | 1<br>14.3 | 4<br>57.1 | 2<br>28.6 |
| | 4 | | 5<br>100 | |

DSD: disorders of sex development, F: female, M: male

It has been reported that only 50% of patients with 46,XY DSD can be given a definite diagnosis (44). In our study, the rate of 46,XY DSD patients with diagnosed causes was higher (n=75, 65.2%). The reason for this difference may be due to the further development of genetic understanding over the years. 5-alpha reductase deficiency was the most common etiology followed by CAIS and PAIS in 46,XY DSD (Table 1,2). The etiological distributions of both 46,XX DSD and 46,XY DSD patients were similar to previous studies (38,39,41,45, 46,47). Contrary to this, Ganie et al (37) report that the main etiological cause of 46,XY DSD was disorder of androgen synthesis or action.

Mixed gonadal dysgenesis was the most common etiology in the sex chromosome DSD group in our study (85.7%) which excluded Turner syndrome. Jaruratanasirikul and Engchaun (43) from Thailand reported that the most common sex chromosome DSD was Turner syndrome followed by Klinefelter syndrome and 45,X/46,XY DSD. Similar to this report, Ganie et al (37) from South Africa, with a total 346 cases diagnosed with DSD, noted that Turner syndrome constituted the largest proportion of the sex chromosome DSD group (61%), followed by mixed gonadal dysgenesis.

Gender identity is a characteristic which is influenced by various prenatal and postnatal variables. Psychosexual development plays an important role in the formation of sexual identity and is the main component of sexual identity, which is influenced by genetic status, pre/postnatal exposure to androgens, sociocultural factors, and family dynamics (6,39,48,49). Gender assignment is an important problem in DSD patients who have a virilized brain with undervirilized external genitalia (13,14,15,39).

Eleven of 97 46,XX patients (11.3%) had male gender orientation in the psychological evaluation, and were raised as the male gender by parents (nine were 21-OHD, one was 11-OHD, and one had Sertoli cell only syndrome; mean age of cases was 9.92±4.96 years). At the council meeting, six of these 11 cases were gender assignment recommendation male, two as female and three were not assigned and were recommended to be followed up.

Five of the patients who received a male assignment recommendation were 46,XX 21-OHD CAH and the other one was Sertoli cell only syndrome (Table 2). The mean age at presentation and at the time of the meeting of these five 21-OHD CAH patients was 7.56±5.26 years and 10.66±3.88 years, respectively. It was found that all of these patients were Prader stage 4-5, raised as male and their psychologic gender orientation was male, and all of the parents demanded a male gender assignment. The factors most strongly influencing recommended gender assignment in 46,XX cases included etiological diagnosis, age, psychologic gender and Prader staging (Table 2, 4).

Similar to our study, Khattab et al (13) report three 46,XX with 21-OHD CAH patients who were reared as male gender. In another study, of 50 DSD patients, 4/11 cases diagnosed with 46,XX DSD due to CAH had assumed a male social gender (15). This condition occurs due to prenatal and/or postnatal exposure to high levels of androgens that promote the masculinization of gender behavior (16,50). With the recent implementation of national neonatal CAH screening, it is hoped that late diagnosis of CAH, and therefore ambiguous genitalia, will be prevented.

For our council, the greatest difficulty in making gender assignment recommendations was in the 46,XY DSD group. The mean length of the phallus of patients who received a female assignment was 0.82±0.71 cm and 90% were Prader stage 1-2; etiological causes of these cases is shown in Table 2. Most of the 46,XY DSD patients who had no etiological diagnosis and had female gender assignment recommendations were Prader stage 1-2. Interestingly, psychological evaluation of these cases showed 8/9 had female gender and 1/9 had no gender orientation.

The majority, 93.7%, of the 46,XY cases with a male gender assignment recommendation and no etiological diagnosis were Prader stage 3-5. Moreover, 62.5% of these patients had no gender orientation yet. These findings suggest that, besides the etiologic diagnosis, the expectation of the family, phallus length and Prader stage were effective in the female assignment recommendations in 46,XY DSD cases. Furthermore, if there is no definite etiologic diagnosis, the most important factors in determining the gender assignment recommendation in 46,XY DSD patients were Prader stage and psychological gender orientation.

### Study Limitations

The major limitation of this study was the patients were only considered from presentation until the final decision for each individual by the gender assignment recommendation council. Due to ethical concerns, follow-up of patients after gender assignment recommendation was not included and thus there is no measure of agreement or discordance with the decision of the council reported.

## Conclusion

The most difficult aspect of managing a patient with DSD diagnosis who has ambiguous genitalia is the assignment of an appropriate gender. Specific diagnosis and psychological gender are more effective in gender assignment of DSD patients with an etiologic cause. Phallus length and Prader

stage are important criteria in the gender assignment of patients with undiagnosed DSD. In this cohort none of the clinical, etiological or genetic features of the patients dominated the gender assignment decision. Gender assignment should be determined by evaluating the patient's chromosome structure, specific diagnosis, fertility, Prader stage, phallus length, psychological orientation, family wish and the consensus opinion of experienced specialist physicians. Gender assignment becomes more difficult, especially if there is a mismatch of the gender the child is raised as, with the etiologic diagnosis. Gender assignment councils must have an experienced and multidisciplinary approach to the diagnosis, medical and/or surgical treatment, psychosocial support, and genetic counseling of patients with DSD. We hope that by publishing our extensive experience in this challenging clinical area we will help other clinicians and patients facing these difficult choices.

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### Ethics

**Ethics Committee Approval:** The study protocol was approved by the Ethics Committee of Çukurova University and performed in accordance with the ethical standards of the Declaration of Helsinki (ethical decision no: 452018.77/10).

**Informed Consent:** Written inform consent was obtained after the council from the parents or legal guardians of all the patients before participation.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Murat Alkan, Ünal Zorludemir, Fatih Gürbüz, Gonca Çelik, Atıl Bişgin, Necmi Çekin, İlker Ünal, Ali Kemal Topaloğlu, Ayşe Avcı, Bilgin Yüksel, Concept: Atıl Bişgin, Ali Kemal Topaloğlu, Bilgin Yüksel, Murat Alkan, Gonca Çelik, Design: Fatih Gürbüz, Murat Alkan, Gonca Çelik, Atıl Bişgin, Ali Kemal Topaloğlu, Bilgin Yüksel, Data Collection or Processing: Fatih Gürbüz, Murat Alkan, Gonca Çelik, Atıl Bişgin, Bilgin Yüksel, Analysis or Interpretation: Fatih Gürbüz, Murat Alkan, Gonca Çelik, Atıl Bişgin, Ali Kemal Topaloğlu, Bilgin Yüksel, Literature Search: Fatih Gürbüz, Murat Alkan, Gonca Çelik, Atıl Bişgin, Ali Kemal Topaloğlu, Bilgin Yüksel, Writing: Fatih Gürbüz, Murat Alkan, Gonca Çelik, Atıl Bişgin, Necmi Çekin, İlker Ünal, Ali Kemal Topaloğlu, Ayşe Avcı, Bilgin Yüksel,

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# Detection of *SHOX* Gene Variations in Patients with Skeletal Abnormalities with or without Short Stature

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### What is already known on this topic?

The phenotypic spectrum of *SHOX* deficiency ranges from Langer mesomelic dysplasia at the severe end of the spectrum to idiopathic short stature at the mild end of the spectrum. Partial or whole *SHOX* gene deletions are usually detected in most of the patients.

### What this study adds?

*SHOX* deficiency should be especially considered in patients who have disproportionate short stature or forearm anomalies with or without short stature. *SHOX* gene sequencing should also be performed in suspected patients who do not have any deletion/duplication in *SHOX* gene. Additionally, hearing loss might be found in addition to the skeletal and the other clinical features.

## Abstract

**Objective:** *SHOX* gene mutations constitute one of the genetic causes of short stature. The clinical phenotype includes variable degrees of growth impairment, such as Langer mesomelic dysplasia (LMD), Léri-Weill dyschondrosteosis (LWD) or idiopathic short stature (ISS). The aim of this study was to describe the clinical features and molecular results of *SHOX* deficiency in a group of Turkish patients who had skeletal findings with and without short stature.

**Methods:** Forty-six patients with ISS, disproportionate short stature or skeletal findings without short stature from 35 different families were included. *SHOX* gene analysis was performed using Sanger sequencing and multiplex ligation-dependent probe amplification analysis.

**Results:** Three different point mutations (two nonsense, one frameshift) and one whole *SHOX* gene deletion were detected in 15 patients from four different families. While 4/15 patients had LMD, the remaining patients had clinical features compatible with LWD. Madelung's deformity, cubitus valgus, muscular hypertrophy and short forearm were the most common phenotypic features, as well as short stature. Additionally, hearing loss was detected in two patients with LMD.

**Conclusion:** This study has presented the clinical spectrum and molecular findings of 15 patients with *SHOX* gene mutations or deletions. *SHOX* deficiency should be especially considered in patients who have disproportionate short stature or forearm anomalies with or without short stature. Although most of the patients had partial or whole gene deletions, *SHOX* gene sequencing should be performed in suspected cases. Furthermore, conductive hearing loss may rarely accompany these clinical manifestations.

**Keywords:** *SHOX* gene, short stature, multiplex ligation-dependent probe amplification, sequence analysis, Madelung's deformity, hearing loss

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## Introduction

Short stature is defined as a height more than two standard deviations (SDs) below the mean for age and sex, compared with national height standards, and affects 2-3% of individuals in the general population. It is a multifactorial disorder as strong genetic and environmental factors are involved (1,2). Several monogenic genetic causes have been identified in short stature and one of these is the short stature homeobox-containing gene *(SHOX)* (3). The *SHOX* gene, which is located in pseudoautosomal region 1 (PAR1) on the short arm of the sex chromosomes Xp22.33 and Yp11.32, escapes X-inactivation. It encodes a nuclear protein which acts as an important transcription factor during limb development (4,5).

The loss of both *SHOX* alleles causes an extreme phenotype of skeletal dysplasia called Langer mesomelic dysplasia (LMD) while *SHOX* haploinsufficiency is associated with a wide spectrum of short stature phenotypes including Turner syndrome, Léri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). LWD is characterized by short stature, mesomelic shortening of the limbs, and characteristic abnormality of the wrists known as Madelung's deformity. The phenotype can be also highly variable, even within the same family (6,7).

The sensitivity of clinical characteristics in identifying patients with ISS are usually insufficient, especially in younger children in whom skeletal disproportions are not so prominent (1,8). In many cases short stature is also the only clinical manifestation. Mutations or deletions of *SHOX* or *SHOX* regulatory regions have been detected in 75% of the cases with LMD and 60% of the cases with LWD. Additionally mutations of this gene are detected in 2-22% of ISS (9,10). However, partial or complete *SHOX* duplications have been described in a few patients with LWD and ISS (11). Moreover, more than 380 mutations in the coding regions of the gene and mutations in the downstream or upstream enhancer elements have been identified but a clear genotype-phenotype correlation has not been reported (1,12).

The aim of this study was to determine the clinical findings and molecular results of *SHOX* deficiency in a group of Turkish patients with LWD, LMD or ISS.

## Methods

### Patient Selection

Forty-six patients with ISS, disproportionate short stature or skeletal findings without short stature from 35 different families, who were examined at Clinic of Pediatric Genetics, Medical Genetics and Pediatric Endocrinology of Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital and Department of Medical Genetics of Ege University from Turkey, between June 2014 and July 2019, were included in this study. Data collected included the age, sex, weight, height, body mass index (BMI), and upper segment/lower segment ratios of 15 patients from four different families with *SHOX* gene variation. The clinical and dysmorphic features, anthropometric measurements, skeletal findings including appearance of muscular hypertrophy, cubitus valgus, forearm bowing, Madelung's deformity, and molecular findings were recorded. A Rappold scoring system was used, which was designed to identify the most appropriate patients for gene testing, and the results were calculated from the medical records of the patients. The score combines three anthropometric variables [arm span/height ratio $<96.5\%$ (2 points), sitting height/height ratio $>55.5\%$ (2 points) and BMI $>50^{th}$ percentile (4 points)], with five clinical variables [cubitus valgus (2 points), short forearm (3 points), bowing of forearm (3 points), muscular hypertrophy (3 points) and dislocation of the ulna at the elbow (5 points)], each of which represents at least two points in the score system. A score greater than 4 out of a total possible score of 24 is more valuable as a clinical indicator to detect patients with *SHOX* deficiency (1).

ISS is defined as a condition characterized by a height more than two SDs below the mean of the age and sex-matched population in a subject with normal birth size, normal body proportions, normal nutrition, no evidence of chronic disease, no psychiatric or emotional disturbance and no endocrine deficiency (13).

### Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp., Armonk, NY, USA). Data was presented with descriptive statistics (median with $25^{th}$-$75^{th}$ percentiles for continuous variables; frequency and percentage for categorical variables). Student's t-test or Mann-Whitney U test was used to compare continuous variables, as appropriate. The significance level was accepted as $p<0.05$ in all statistical analyses. The Local Ethics Committee approved the study (Dr. Behçet Uz Children's Hospital, Clinical Research Ethics Committee, İzmir; approval number: 2020/01-07), and written informed consent was obtained from all individuals involved.

### Molecular Analysis

### DNA Isolation and Sanger Sequencing

Genomic DNA from peripheral blood lymphocytes of all individuals were extracted with Zinexts MagPurix Blood

DNA Extraction Kit (Zinexts Life Science Corp., New Taipei City, Taiwan) using standard procedures. All coding exons and exon-intron boundaries of the *SHOX* gene were amplified by polymerase chain reaction. The sequences were evaluated using SeqScape Software 3 sequencing program (Applied Biosystems SeqScape Software 3, Life Technologies Corporation, 5791 Van Allan Way, Carlsbad, California 92008). "Ensembl.org" database (GRCh38.p12) with ENST00000381578.6 transcript ID of the *SHOX* gene was used to compare individual and reference sequences. All variations were checked using mutation and SNP databases (Human Genome Mutation Database, National Center for Biotechnology Information, ensembl.org). Each variation was confirmed by bidirectional sequencing. Variation descriptions were done according to the nomenclature recommended by the Human Genomic Variation Society. Furthermore, in silico programmes, such as SIFT, PolyPhen 2, and Mutation Taster were used to describe the pathogenity of novel variations in coding exons and exon-intron boundaries.

Multiplex ligation-dependent probe amplification (MLPA) analysis was performed to detect large deletions and duplications using P018 SALSA MLPA Kit (MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands). The PCR products were analysed by ABI 3500 capillary electrophoresis system (Applied Biosystems 3500/3500xL Genetic Analyzer Life Technologies Corporation, 5791 Van Allan Way, Carlsbad, California 92008) and Coffalyser Software (MRC-Holland, Amsterdam, The Netherlands; http://www.mrc-holland.com). The area under the peak for each amplified fragment was measured and normalized to the peak areas of normal control individuals.

## Results

Forty-six patients from 35 families with idiopathic or disproportional short stature or skeletal findings without short stature were screened for deletions and intragenic mutations of the *SHOX* gene (Figure 1). Mutations in *SHOX* were identified in 15 patients from four different families; three different point mutations and one heterozygous whole *SHOX* gene deletion were detected (Table 1). The skeletal findings (cubitus valgus, Madelung's deformity, mesomelic shortening, radial bowing) of the mutation positive patients were variable, even within the same family (Figure 2). The median age of the patients with *SHOX* deletion/mutation at referral was 12 years (range, 8-36 years) and five (33%) of them were male. Anthropometric parameters of the cases showed great variation in terms of clinical diagnosis. While the median height SD score (SDS) of the patients with LMD with severe Madelung deformity was -5.5 [range, (-7.1)-(-4.8)], the median height SDS of the patients with LWD was -1.5 [range, (-1.9)-(-1.3)]. The median BMI of the fifteen patients with *SHOX* mutation/deletion was 22.8 (range, 18.3-28.7). The Rappold score was higher than 4 points in all of the patients with *SHOX* deficiency. The other clinical characteristics and molecular findings of the cases are detailed in Table 2. Patients with *SHOX* deficiency also showed significantly higher BMI SDS levels than patients without *SHOX* deficiency [BMI SDS 1.4 (range, 0.3-2.3), vs. -0.68 (range, -1.56-0.92), $p < 0.05$]. Furthermore, there

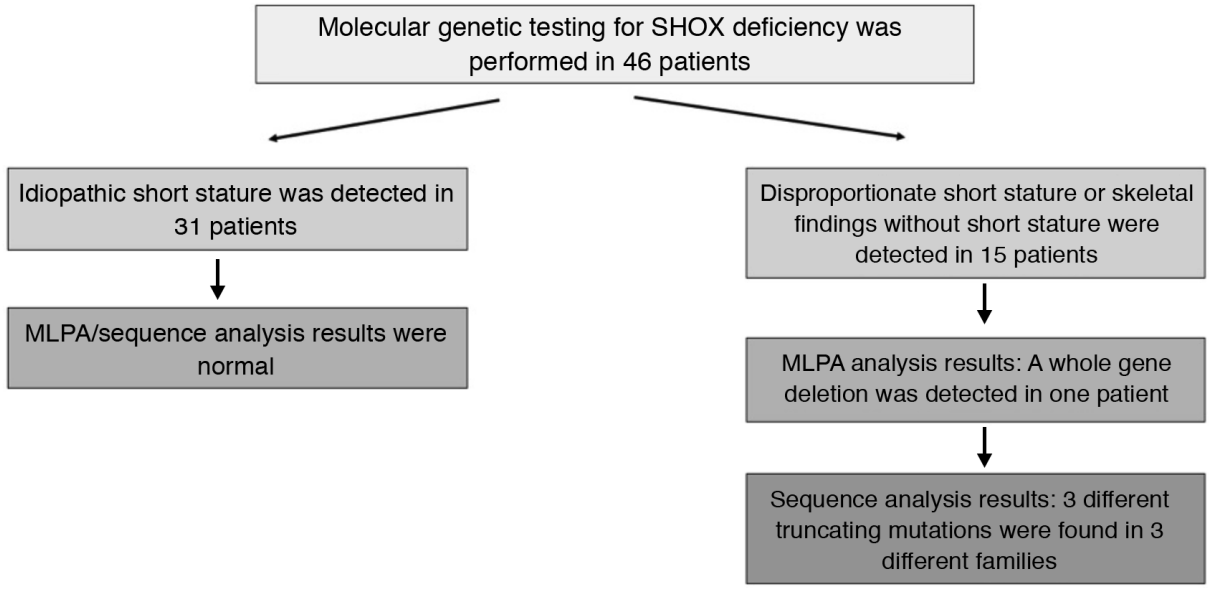


**Figure 1.** This scheme provides an approach to the study design and molecular results of the patients with/without *SHOX* deficiency

was no significant difference between these two groups, regarding height and height SDS. The comparison of the demographic features of these two groups were shown in Table 3.

In the first family, a frameshift mutation, c.42delG (p.Ser16AlafsTer60), was detected in all family members (five siblings and the parents). The parents had a consanguineous marriage. A 9 year-old boy (patient 2) and his three affected sisters were found to be homozygous for that mutation and were diagnosed with LMD. In contrast, their other brother and the parents were heterozygous for the same mutation, which favored the diagnosis of LWD. Karyotype analysis was also normal in patient 2 (the index patient). On physical examination, disproportionate short stature, short and webbed neck, low hairline, pectus excavatus, bilateral severe Madelung's deformity with ulnar deviation, camptodactyly of the 3rd-4th digits in right hand and fourth digit in left hand were noted in the patients with LMD. Antero-posterior and lateral radiographs demonstrated the bowing and shortening of the distal radius, widening of the distal radial-ulnar joint, and triangulation of the distal radial epiphysis, producing an ulnar slant to the articular surface (Figure 2). Additionally, audiometric test showed that two of them had bilateral conductive hearing loss (patient 1: right 45 dB, left 45 dB; patient 4: right 45 dB, left 65 dB). Patient 5 had only Madelung's deformity, which was detected by radiological examination. Besides, the mother had short stature (142 cm) and Madelung's deformity and was diagnosed as LWD.

**Table 1. The molecular details of the *SHOX* gene alterations**

| Family no | Nucleotide change | Aminoacid change | Exon | Transmission | Phenotype in SHOX mutation database | Phenotype in the present study |
|---|---|---|---|---|---|---|
| 1 | c.42delG | p.S16AfsTer60 | 2 | Both of the parents were heterozygous | LWD | LMD, LWD |
| 2 | c.631C > T | p.Q211X | 5 | Both of the parents were heterozygous | ISS | LWD |
| 3 | c.492G > A | p.W164X | 4 | From the father | ISS | LWD |
| 4 | Whole gene deletion | | | - | | LWD |

LWD: Léri-Weill dyschondrosteosis, ISS: idiopathic short stature, PVS1: pathogenic very strong, PM2: pathogenic moderate, PP3: pathogenic supporting, PM1: pathogenic moderate, SHOX mutation database: LOVD X-chromosome gene database short stature homeobox (https://databases.lovd.nl/shared/genes/SHOX)

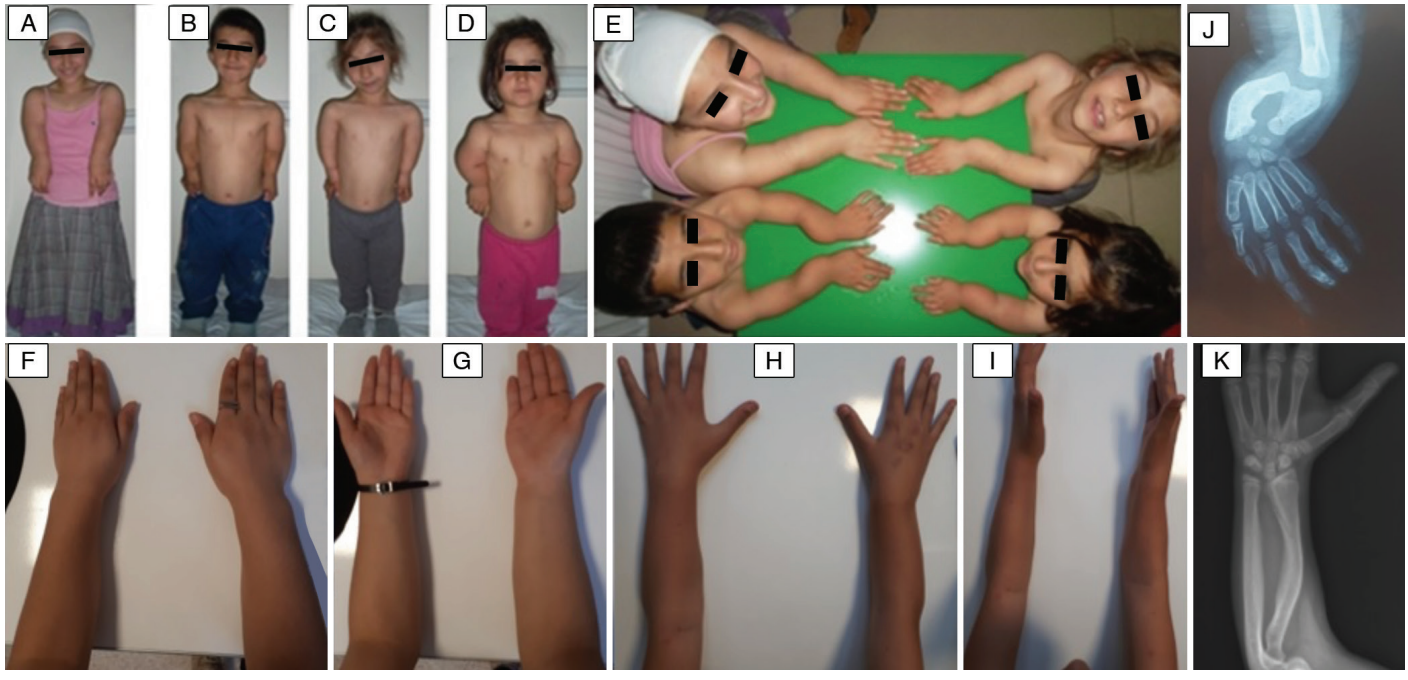


**Figure 2.** The clinical and radiological findings of the mutation positive patients. A, B, C, D, E) The clinical features of the patients with langer mesomelic dysplasia. F, G) Madelung deformity of patient 15. H, I) Madelung deformity and short forearm of patient 13. J) The direct radiography of patient 2 revealed bowing and shortening of the distal radius, widening of the distal radial-ulnar joint, and triangulation of the distal radial epiphysis, producing an ulnar slant to the articular surface. K) Bowing of forearm, radial bowing and ulnar shaft thickening of patient 13

**Table 2. Clinical and molecular findings of the mutation/deletion positive patients**

| Family no | Patient no | Age/ Sex | Clinical diagnosis | Height cm | Height SDS | BMI | BMI (SDS) | Upper segment/ lower segment ratio | Madelung deformity | Cubitus valgus | Scoliosis | Muscular hypertrophy | Rappold scoring results | Genetic defects | Zygosity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 14/F | LMD | 115 | -7.65 | 33.2 | 3.1 | 2.57 (> 2 SDS) | + (severe) | + | - | + | 19 | c.42delG (p.Ser16AlafsTer60) | ***Homozygous*** |
| | 2 | 9/M | LMD | 102 | -5.43 | 24.03 | 2.3 | 2.15 (> 2 SDS) | + (severe) | + | - | + | 19 | c.42delG (p.Ser16AlafsTer60) | ***Homozygous*** |
| | 3 | 8/F | LMD | 102,2 | -4.65 | 22.98 | 2.19 | 2.11 (> 2 SDS) | + (severe) | + | - | + | 19 | c.42delG (p.Ser16AlafsTer60) | ***Homozygous*** |
| | 4 | 6/F | LMD | 88,6 | -5.7 | 22.8 | 2.59 | 2.34 (> 2 SDS) | + (severe) | + | - | + | 19 | c.42delG (p.Ser16AlafsTer60) | ***Homozygous*** |
| | 5 | 2/M | LWD | 86 | -0.76 | 16.2 | -0.15 | NA | + (mild) | - | - | + | 14 | c.42delG (p.Ser16AlafsTer60) | *Heterozygous* |
| | 6 (Mother) | 35/F | LWD | 142 | NA | 26.7 | NA | NA | + (mild) | - | - | + | NA | c.42delG (p.Ser16AlafsTer60) | *Heterozygous* |
| | 7 (Father) | 36/M | LWD | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | c.42delG (p.Ser16AlafsTer60) | *Heterozygous* |
| 2 | 8 | 7/F | LWD | 113 | -1,63 | 18.01 | 1.11 | 1.48 (> 2 SDS) | - | - | - | + | 14 | c.631C > T (p.Q211X) | *Heterozygous* |
| | 9 | 9/F | LWD | 123 | -1.57 | 18.5 | 0.93 | 1.41 (> 2 SDS) | - | - | - | + | 14 | c.631C > T (p.Q211X) | *Heterozygous* |
| | 10 | 9/F | LWD | 123 | -1.57 | 22.4 | 1.88 | 1.38 (> 2 SDS) | - | - | - | + | 14 | c.631C > T (p.Q211X) | *Heterozygous* |
| | 11 (Father) | 36/M | LWD | 158 | NA | 28.4 | NA | NA | + | + | - | + | NA | c.631C > T (p.Q211X) | *Heterozygous* |
| | 12 (Mother) | 36/F | LWD | 154 | NA | 29.9 | NA | 1.26 (> 2 SDS) | + | + | - | + | 16 | c.631C > T (p.Q211X) | *Heterozygous* |
| 3 | 13 | 12/F | LWD | 143 | -1.57 | 16.1 | -1.21 | 1.11 (> 2 SDS) | + (severe with pain) | + | - | + | 13 | c.492G > A (p.W164X) | *Heterozygous* |
| | 14 (Father) | 45/M | LWD | 157 | NA | 31.2 | NA | 1.3 (> 2 SDS) | - | - | - | - | 15 | c.492G > A (p.W164X) | *Heterozygous* |
| 4 | 15 | 14/F | LWD | 142 | -3.1 | 21.8 | 0.46 | 1.29 (> 2 SDS) | + | + | - | + | 17 | Whole gene deletion | *Heterozygous* |

F: female, M: male, LWD: Léri-Weill dyschondrosteosis, LMD: Langer mesomelic dysplasia, SDS: standard deviation score, BMI: body mass index, NA: not applicable

We could not have contact with the father; only his blood samples were analyzed and we obtained related data from his photographs and wife.

In the second family, a heterozygous nonsense mutation, c.631C>T (p.Q211X), was detected in a 7 year-old girl (patient 8) who had the diagnosis of LWD. Her height was 113 cm (-1.63 SDS) and weight was 23 kg (0.03 SDS). Mild muscular hypertrophy, short forearm, and bowing of the tibia were observed. Madelung deformity and cubitus valgus were not obvious. Her parents and two sisters were found to have similar clinical features and the same mutation. Additionally, the parents had cubitus valgus and Madelung deformity.

In the third family, patient 13 and her father had another heterozygous nonsense mutation, c.492G>A (p.W164X). This 12 year-old girl was referred for multiple skeletal findings. Her height was 143 cm (-1.57 SDS) and mesomelic shortening was detected in upper and lower extremities. Madelung's deformity with pain and restriction of the flexion/extension were observed in the right forearm. On her radiologic examination, bowing of forearm, especially radial bowing, Madelung's deformity and ulnar shaft thickening were detected (Figure 2). Abdominal ultrasonography revealed right renal ptosis. Her father, who was 157 cm, had only short stature with mild mesomelic shortening.

Whole *SHOX* gene deletion was detected with MLPA analysis in patient 15, the only member of the fourth family to be affected, who was referred for disproportional short stature. The patient was 14 years old and her height was 142 cm (-3.1 SDS). On her physical examination, cubitus valgus, bowing of the forearm, Madelung's deformity and short forearm were noticeable. Abdominal ultrasonography was normal. Karyotype analysis was 46,XX. The clinical features and molecular tests were also normal in other members of the family.

## Discussion

In the present study *SHOX* gene molecular defects in patients with LMD, LWD and ISS and the phenotype-genotype spectrum of *SHOX* deficiency were evaluated. In the current literature, point mutations and deletions of the *SHOX* gene have been reported in patients with ISS at an estimated prevalence ranging from 2-22% (9,10). Nevertheless, forearm anomalies and short stature with an increased sitting height/height ratio are most likely to be associated with *SHOX* haploinsufficiency (14,15).

The *SHOX* gene belongs to a family of transcriptional regulators and is essential for the development of the skeleton; especially in the growth and maturation of bones in the arms and legs (16). The clinical expression of *SHOX* deficiency is highly variable and the phenotype usually becomes more pronounced with age, and typical manifestations appear over time (17). While LMD, which is a much more severe skeletal dysplasia than LWD, results from biallelic (homozygous or compound heterozygous) *SHOX* pathogenic variants, *SHOX* haploinsufficiency is associated with ISS and LWD (6). In the present study, four patients from the first family had a homozygous *SHOX* gene mutation and severe skeletal findings, whereas the clinical features of other family members, who had heterozygous mutation, were compatible with LWD. In the second family,

**Table 3. Demographic and anthropometric findings of the patients with/without *SHOX* deficiency**

| Variable | *SHOX* | Non-*SHOX* | p value |
|---|---|---|---|
| Diagnosis (n) | 15 | 31 | |
| LMD | 4 | - | |
| LWD | 11 | - | |
| ISS | - | 31 | |
| Age (years) | 12 (range, 8-36) | 11 (range, 6-14) | 0.24 |
| n <18 | 10 | 27 | |
| n >18 | 5 | 4 | |
| Males/females n (%) | 5 (33%)/10 (67%) | 12 (39%)/19 (61%) | 0.7 |
| Height (cm) | 123 (range, 102-145) | 120 (range, 112-141) | 0.77 |
| Height (SDS) | -2.3 [range, (-5.4)-(-1.5)] | -3.1 [range, (-3.4)-(-1.9)] | 0.93 |
| BMI | 22.8 (range, 18.3-28.7) | 16.7 (range, 14.8-22.3) | 0.005 |
| BMI (SDS) | 1.4 (range, 0.3-2.3) | -0.68 [range, (-1.56)-(0.92)] | 0.004 |

Median and range values are given.

SDS: standard deviation score, BMI: body mass index, NA: not applicable, LWD: Léri-Weill dyschondrosteosis, LMD: Langer mesomelic dysplasia, ISS: idiopathic short stature

the parents had more obvious skeletal manifestations than their daughters. Additionally, while the father of patient 13 had only mild short forearm with short stature, the daughter had Madelung's deformity, radial bowing and ulnar shaft thickening on limb radiographs. Consistent with the literature, the clinical findings of these patients highlight an intrafamilial phenotypic variability.

In the patients presented, short stature, increased upper segment/lower segment ratio, short forearm, bowing of tibia and appearance of muscular hypertrophy were the most common phenotypic features. Three out of four index patients had at least one affected family member. Additionally, in the first and second family, the parents had a consanguineous marriage and both of them had a heterozygous mutation. As reported in the literature, it is not uncommon for patients with *SHOX* haploinsufficiency to have an affected parent (3).

The combination of dyschondrosteosis and hearing loss has been reported in several cases. In 1970 Nassif et al (18,19) described five siblings with dyschondrosteosis and two of the affected patients had a conductive hearing loss with middle ear deformities. The audiogram revealed bilateral conductive hearing loss of approximately 40-50 dB in both of the patients. In 2003, De Leenheer et al (19,20) reported a patient with a diagnosis of LWD who had a deletion in *SHOX* gene. The patient had short stature, mesomelic shortening and Madelung's deformity with shortening and bowing of the radius and dorsal dislocation of the ulnar head. The audiogram showed that the patient had unilateral 35 dB conductive hearing loss in the left ear. In our study, bilateral conductive hearing loss was detected in two patients with LMD from the first family. Hearing tests were normal in the other affected siblings. On the basis of these findings and earlier evidence, we suggest that conductive hearing loss may be a rare manifestation of *SHOX* deficiency and a hearing evaluation should be performed in these patients.

The most common mutation is a deletion of part or the entire *SHOX* locus (i.e., 80-90% of cases), whereas point mutations appear to be less frequent (10-20%). The *SHOX* protein contains three characteristic domains: a homeodomain, an SH3 binding domain and an OAR domain. Most of the mutations have been described in the homeobox domain which spans exons 3 and 4. The OAR domain is localized at the C terminal end of the gene and is essential for transactivation (11,16). The homeodomain of the *SHOX* gene mediates several key functions that include nuclear localization, DNA binding and protein-protein interactions. Therefore, mutations located in this region may impair these processes and lead to bone defects (20). Furthermore, the cis-regulatory region of *SHOX* extends to ~ 1Mb of the PAR1 and alterations of these regions may be the cause of the phenotype (21). In the present study, deletion of the whole *SHOX* gene was detected in only one patient. Additionally, three different point mutations (two nonsense, one frameshift) were observed in 14 patients from three different families. Nonsense and frameshift mutations that lead to truncation of the SHOX protein can cause absence of the OAR domain at the C-terminal end, resulting in lack of transactivating function. In our study group, the first family had a heterozygous or homozygous frameshift mutation in exon 2, c.42delG (p.Ser16AlafsTer60), which caused the lack of the HD, SH3 and OAR domains. While the second family had a nonsense mutation, c.631C>T (p.Q211X), which was located in exon 5, another nonsense mutation, c.492G>A (p.W164X), which was located in exon 4 and the homeodomain region was detected in the third family. Although the rate of gene deletions is high in *SHOX* deficiency, gene sequencing should be performed in suspected cases. There is also a wide range of phenotypic variations associated with mutations or deletions in the *SHOX* gene. In the current study, point mutations were detected in different exons, but no correlation was found between the severity of phenotype and the underlying *SHOX* pathogenic variant.

### Study Limitation

The major limitation of our study is the relatively small size of the sample.

## Conclusion

In conclusion, the clinical findings and molecular manifestations of four different *SHOX* alterations in four different families are presented. Screening for *SHOX* deficiency should be considered in children with disproportionate short stature or forearm abnormalities with and without short stature. Furthermore, the fact that conductive hearing loss may accompany clinical manifestations should be kept in mind. Genetic diagnosis is essential for the management of the disease and prediction of prognosis. Future studies and identification of further *SHOX* modifier genes will allow better understanding of the phenotype-genotype correlation.

### Ethics

**Ethics Committee Approval:** The Local Ethics Committee approved the study (Dr. Behçet Uz Children's Hospital, Clinical Research Ethics Committee, İzmir; approval number: 2020/01-07).

**Informed Consent:** Written informed consent was obtained from all individuals involved.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Medical Practices: Semra Gürsoy, Filiz Hazan, Özlem Nalbantoğlu, Concept: Ayça Aykut, Korcan Demir, Özgür Çoğulu, Design: Hüseyin Anıl Korkmaz, Behzat Özkan, Özgür Çoğulu, Data Collection or Processing: Hüseyin Anıl Korkmaz, Korcan Demir, Semra Gürsoy, Filiz Hazan, Analysis or Interpretation: Ayça Aykut, Özgür Çoğulu, Semra Gürsoy, Filiz Hazan, Literature Search: Ayça Aykut, Hüseyin Anıl Korkmaz, Behzat Özkan, Korcan Demir Writing: Behzat Özkan, Semra Gürsoy, Filiz Hazan, Özgür Çoğulu.

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# Diagnostic Performance of Neck Circumference and Cut-off Values for Identifying Overweight and Obese Pakistani Children: A Receiver Operating Characteristic Analysis

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### What is already known on this topic?

Childhood obesity is a growing problem in Pakistan. Therefore there is a need to identify a quick and simple tool for screening obesity. Neck circumference (NC) may be a valuable tool for screening individuals with overweight/obesity.

### What this study adds?

This is the first study evaluating the correlation between NC and body mass index among Pakistani children. The optimal cut-off values of NC for identification of overweight and obesity were identified in pre-pubertal and pubertal boys and girls using receiver operating characteristic analysis.

## Abstract

**Objective:** Neck circumference (NC) is considered to be an alternative screening method for obesity. The aims were: (1) to examine the correlation between body mass index (BMI) and NC; and (2) to determine diagnostic performance including the best cut-off values of NC for identification of overweight and obese Pakistani children.

**Methods:** The study sample was 7,921 children, aged 5-14 years, by cross-sectional survey carried-out in four major cities of Pakistan. Receiver operating characteristic analysis was used to investigate the diagnostics performance of NC and to determine the optimal cut-off points for identifying children with overweight and obesity.

**Results:** The mean of each anthropometric variable (i.e., height, weight, BMI and NC) increased with age in both sexes. In the whole sample, NC had a strong positive correlation ($r = 0.61$, $p < 0.01$) with BMI. NC optimal cut-off points for identifying overweight and obesity in Pakistani boys ranged between 25.00 to 30.35 cm and the corresponding values for the girls were 24.00 to 31.62 cm. In the prepubertal period, NC cut-off points indicative overweight, in both boys and girls were 26.36 cm and 25.27 cm, respectively; the corresponding values for obesity were 26.78 cm and 25.02 cm. During puberty, the cut-off values for overweight and obesity respectively were 28.32 cm and 28.57 cm in boys and 28.70 cm and 28.82 cm in girls.

**Conclusion:** NC may be used as a simple and widely applicable measure for identification of overweight and obesity with reasonable accuracy in Pakistani children.

**Keywords:** Body mass index, LMS method, neck circumference, obesity, receiver operating characteristic curve

## Introduction

In recent decades, obesity has become an increasing global public health issue (1,2,3,4). Children and adolescents are the worst affected group with an estimated 10% of the world's school children being overweight and one quarter of these being obese (4,5). In developing countries including Pakistan, childhood obesity is also growing at a fast pace. Different studies (6,7,8,9) in various settings show that the prevalence of overweight and obesity in Pakistani children ranges from 8% to 19.3% and 6% to 7.5%, respectively.

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To measure obesity prevalence in children and adults, there are various anthropometric measures. However, epidemiological researchers usually use the internationally recognized and established measure body mass index (BMI), which is calculated by taking an individual's weight in kilograms (kg) and dividing by height in meters squared (2). Despite the popularity of BMI and ease of use, it is becoming increasingly clear that it is not a good measure for regional adiposity, especially upper body fat distribution of an individual (10).

Currently, neck circumference (NC) is an alternative screening method, proposed as a potential proxy for BMI (11,12). Measurement of NC is an easy, quick and inexpensive method and various investigators have attempted to use it for screening of overweight and obese children (13,14). Studies with different pediatric samples showed that NC performed well as an index of high BMI in young children and adolescents (13,15,16).

However in Pakistan, there is a scarcity of data about the use of NC as an indicator of overweight and obesity in children. Only one investigator (17) has attempted to use NC to screen for high BMI among young adults aged 18-20 years. Given this gap in the evidence base the present study was undertaken with the following objectives: i) to evaluate the correlation between NC and BMI in children and ii) to determine diagnostic performance and the best NC cut-off values for identification of overweight and obese Pakistani children.

## Methods

This was a school-based, cross-sectional study and was conducted between March and June, 2016. The details of the sampled population and sampling methodology of this study have been described previously (18,19,20). Some of the aspects of the sampling procedure should be reiterated. Sampling was conducted in four major cities of Pakistan. These were: Lahore which is the second most populous city of Pakistan, with a high human development index (HDI = 0.877); the city of Multan in the center of Pakistan with an HDI = 0.718; and two adjacent cities, Rawalpindi and Islamabad, the latter being the capital city of Pakistan, with HDI of 0.871 and 0.875, respectively (21). A grade-wise complete list of schools (i.e., primary and secondary schools) of the selected cities was obtained from Punjab and the Federal Department of Education (Schools). Schools were chosen using simple random sampling from the lists. In each selected school, classes were also selected randomly and all the children who were present on the day of data collection were invited to participate in the study. For this investigation, a sample of 7,921 children, aged 5-14, were recruited from a total of 68 schools of which 28 were Public schools and 40 were Private schools.

After obtaining written consent from the school's head master and verbal consent from each child's parents or guardians, data collection activities were performed. All information related to age (years), sex, residential city, and anthropometric measurements including height (cm), weight (kg) and NC (cm) of each child were chronicled in a self-designed questionnaire. Age of each child was confirmed from the school register and physical measurements were taken in a standing position using a standard protocol (20,22). For anthropometric measurements, a stadiometer (Seca model SCA 217, Hamburg, Germany) was used for height and a weighing machine (Westpoint model WF 7009, Karachi, Pakistan) for weight. NC of the children was measured in centimeters using a non-stretchable plastic tape measure. Measurement was made in a horizontal plane, with the participants' shoulders down and looking straight ahead, at a point just below the thyroid cartilage and perpendicular to the long axis of the neck. This location was chosen, as it is the most easily palpable landmark of the pediatric airway. During the measurement process, attention was paid not to engage the trapezoid muscles of the shoulder and neck. The average of two readings was used for the analysis. All NC measurements were performed by three well-trained data collection teams, supervised by the principal investigator. The BMI of each child was calculated using the standard formula: weight (kg)/height ($m^2$). Age-and sex-specific BMI z-scores were obtained by using the LMS method (23). For defining overweight and obesity of a child, World Health Organization 2007 z-scores cut-offs [$> +1$ standard deviation (SD) i.e. BMI z-score $>1$ for overweight; and $> +2$ SD i.e. BMI z-score $>2$ for obesity] were used. If BMI z-score is $<-2$, the child will be considered as underweight (24,25).

### Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), version 21.0 was used for all the statistical analyses (IBM Inc., Armonk, NY, USA). For descriptive analysis, means $\pm$ SD and 95% confidence intervals (CI) were estimated for each sex, based on age in years for each year and age groupings (5-9 and 10-14 years old). Mean differences of NC between two groups were determined using an unpaired t-test. For both sexes, the correlation between NC and other quantitative variables were estimated using Pearson's correlation. Odds ratios (ORs) were also computed to determine the strength of association. Age-and sex-specific diagnostic ability and cut-off values of NC were calculated with receiver operating

characteristic (ROC) curve analysis according to two dependent variables; overweight defined by BMI z-score > 1 and obesity defined by BMI z-score > 2 (24,25). An NC value with the highest Youden's index was chosen for best cut-off point. The diagnostic ability of NC to discriminate children with or without overweight and obesity was assessed using area under the curve (AUC). The diagnostic test was considered to be "highly accurate if, $0.65 \leq AUC \leq 1.00$" and "moderately accurate if, $0.50 \leq AUC \leq 0.65$" (26,27). The likelihood ratios (positive [$LR^P$] and negative [$LR^N$]) for NC were also computed for each age and sex as described by Nafiu et al (28). Sex-specific NC cut-off points according to puberty periods were also determined. Boys between 5-11 years and girls between 5-10 years were considered to be in the prepubertal period; boys and girls over 11 and 10 years, respectively were in pubertal period. These age-groupings were chosen as previously described (13).

For this study, exclusion criteria were: (a) children who refused to perform anthropometry (b) children who had goiter or any physical disability and (c) children who were absent at the time of data collection. The study was approved by the Departmental Ethics Committee of Bahauddin Zakariya University, Multan, Pakistan (IRB# SOC/D/2715/19).

## Results

A total of 7921 children, aged 5-14, years were included in the study. The mean BMI and NC were 16.16 $Kg/m^2$ and 26.00 cm, respectively. Age-and sex-specific mean ($\pm$ SD) and 95% CI of each anthropometric measurement are listed in Table 1. For each anthropometric variable, as expected, mean increased with age in both boys and girls. Generally, boys had higher mean values than girls with few exceptions.

Table 2 presents age-and sex-specific mean comparison of NC according to overweight and obesity status. Overweight and obesity prevalence in overall subjects were 16.0% and 3.3%, respectively. Moreover, 1.9% children were underweight (i.e., BMI < -2 SD) in the study. For both genders in different age groups, it was observed that the mean value of NC was higher in subjects that were overweight or obese than in the other subjects. The results were statistically significant at different ages with the exception of 7-year old obese boys.

The correlation coefficients of NC with other anthropometric measurements are displayed in Table 3a. NC had a strong positive correlation with age and all the other anthropometric measures in both genders, as well as in all the subjects studied. Logistic regression analysis confirmed that NC had a statistically significant positive association with overweight and obesity. The crude ORs for overweight and obesity were 1.43 (95% CI: 1.39, 1.46) and 1.42 (95% CI: 1.36, 1.49) and adjusted ORs for overweight and obesity were 1.74 (95% CI: 1.67, 1.80) and 1.76 (95% CI: 1.67, 1.86), respectively (Table 3b).

Table 4 displays the results of AUC for boys and girls of all ages (5-14 years). In all age-groups of both genders, diagnostic performance of NC was 'highly accurate' in classifying the individuals to overweight (AUC = 0.67 to 0.83) and obesity (AUC = 0.66 to 0.97). Diagnostic performance comparison between participants in the prepubertal and pubertal periods showed that the AUC was statistically lower in the prepubertal period. For example, for prepubertal boys the AUC of overweight (0.75) and obesity (0.78) was lower than the AUC values for pubertal overweight (0.78) and obese boys (0.85). The ROC curves accurately define overweight and obesity of the whole cohort regardless of age for both sexes of Pakistani children (see Figure 1).

Based on ROC analysis, sensitivities, specificities, and cut-off values for NC for each age-group, by gender, are presented in Table 5. NC cut-off values for overweight and obesity increased from 25.00 to 30.35 cm for boys and 24.00 to 31.62 cm for girls between 5 and 14 years. In the prepubertal period, NC cut-off values for overweight and obesity were 26.36 and 26.78 cm in boys and 25.27 and 25.02 cm, in girls, respectively. For the pubertal period, these cut-off values were 28.32 and 28.57 cm in boys and 28.70 and 28.82 cm in girls. Considering all the children included in the study, the cut-off points for NC that identified overweight and obesity in boys and girls were 27.05 cm and 27.56 cm for boys and 26.55 cm and 27.81 cm for girls, respectively. The LRs for each cut-off point were also calculated. For example, $LR^P$ for a 14-years old boy with NC > 30.35 cm indicates that he is 2.64 times more likely to be overweight than a 14-year old boy with an NC value below this cut-off point.

## Discussion

Obesity in children is now considered to be a serious chronic health issue in most populations (29) and its worldwide prevalence is growing (30). Various studies (3,31) have reported increased adverse health outcomes of childhood obesity with both short-term and long-term consequences. Early prevention and treatment of childhood obesity are important priorities for health practitioners and these require accurate diagnostic measures (32). Different practical methods such as BMI, waist circumference (WC), and waist-to-hip ratio are applicable for assessing obesity. However, in circumstances where these methods are not feasible, measurement of NC may be an alternative. NC is a

**Table 1. Descriptive statistics (95% confidence interval) for height, weight, body mass index and neck circumference by age**

| Age (years) | Height (cm) | | Weight (kg) | | BMI (kg/m²) | | NC (cm) | |
|---|---|---|---|---|---|---|---|---|
| | Boys | Girls | Boys | Girls | Boys | Girls | Boys | Girls |
| 05 (n = 621) | 112.78 ± 7.13 (111.87, 113.69) | 111.30 ± 7.38 (110.55, 112.03) | 19.05 ± 3.77 (18.57, 19.53) | 18.16 ± 3.26 (17.83, 18.49) | 14.92 ± 2.28 (14.63, 15.21) | 14.64 ± 2.14 (14.43, 14.85) | 23.93 ± 1.88 (23.75, 24.23) | 23.42 ± 1.62 (23.27, 23.59) |
| 06 (n = 697) | 119.36 ± 7.81 (118.46, 120.26) | 117.15 ± 6.99 (116.46, 117.83) | 21.39 ± 4.23 (20.90, 21.87) | 20.07 ± 3.51 (19.73, 20.42) | 14.94 ± 2.11 (14.70, 15.18) | 14.58 ± 1.85 (14.40, 7.76) | 24.33 ± 1.77 (24.13, 24.54) | 23.71 ± 1.76 (23.54, 23.88) |
| 07 (n = 660) | 123.70 ± 7.18 (122.85, 124.54) | 122.21 ± 7.64 (121.44, 122.99) | 23.19 ± 4.59 (22.65, 23.73) | 22.51 ± 4.65 (22.04, 22.98) | 15.07 ± 2.21 (14.82, 15.33) | 14.98 ± 2.11 (14.76, 5.19) | 24.48 ± 1.82 (24.26, 24.69) | 24.10 ± 1.62 (23.94, 24.27) |
| 08 (n = 649) | 128.21 ± 7.63 (127.30, 129.12) | 127.55 ± 8.07 (126.73, 128.37) | 25.14 ± 5.23 (24.52, 25.76) | 25.38 ± 5.69 (24.81, 25.96) | 15.20 ± 2.19 (14.94, 15.46) | 15.48 ± 2.34 (15.24, 15.72) | 24.88 ± 1.81 (24.68, 25.10) | 24.91 ± 1.84 (24.72, 25.09) |
| 09 (n = 583) | 132.65 ± 7.18 (131.75, 133.55) | 130.73 ± 6.65 (130.01, 131.44) | 27.33 ± 5.44 (26.65, 28.01) | 27.14 ± 5.60 (26.54, 27.74) | 15.45 ± 2.31 (15.16, 15.74) | 15.79 ± 2.48 (15.52, 16.05) | 25.16 ± 2.02 (24.90, 25.41) | 25.36 ± 2.07 (25.14, 25.58) |
| 10 (n = 879) | 139.19 ± 7.76 (135.44, 136.93) | 132.59 ± 7.93 (132.26, 133.71) | 29.44 ± 6.32 (28.83, 30.04) | 28.64 ± 5.55 (28.13, 29.15) | 15.75 ± 2.37 (15.53, 15.98) | 16.14 ± 2.47 (15.51, 16.37) | 25.64 ± 1.78 (25.47, 25.81) | 25.78 ± 2.05 (25.59, 25.96) |
| 11 (n = 764) | 140.09 ± 7.89 (139.36, 140.84) | 138.37 ± 8.05 (137.49, 139.25) | 31.75 ± 6.08 (31.18, 32.32) | 31.67 ± 7.03 (31.90, 33.44) | 16.11 ± 2.42 (15.89, 16.35) | 16.96 ± 2.75 (16.68, 17.26) | 26.30 ± 1.78 (26.13, 26.46) | 26.56 ± 2.16 (26.33, 26.80) |
| 12 (n = 1111) | 144.45 ± 8.28 (143.82, 145.07) | 142.97 ± 8.60 (142.15, 143.78) | 34.31 ± 7.03 (33.78, 34.84) | 35.50 ± 7.31 (34.81, 36.19) | 16.35 ± 2.57 (16.16, 16.55) | 17.27 ± 2.63 (17.02, 17.09) | 26.69 ± 2.03 (26.53, 26.84) | 27.17 ± 2.04 (26.98, 27.37) |
| 13 (n = 1053) | 149.07 ± 9.28 (148.33, 149.82) | 148.96 ± 8.77 (148.16, 149.76) | 37.81 ± 7.93 (33.17, 38.45) | 39.73 ± 7.70 (39.03, 40.44) | 16.90 ± 2.59 (16.69, 17.11) | 17.83 ± 2.66 (17.58, 18.07) | 27.43 ± 2.02 (27.27, 27.60) | 27.83 ± 2.19 (27.63, 28.03) |
| 14 (n = 904) | 156.52 ± 9.28 (156.14, 157.68) | 150.50 ± 8.87 (149.56, 151.44) | 43.53 ± 8.81 (42.80, 44.26) | 41.89 ± 7.89 (41.04, 42.74) | 17.57 ± 2.63 (17.35, 17.78) | 18.42 ± 2.72 (18.13, 18.71) | 28.54 ± 2.13 (28.57, 28.92) | 28.76 ± 2.15 (28.33, 28.99) |
| 5-9 (n = 3210) | 123.36 ± 9.96 (122.82, 123.90) | 121.49 ± 10.11 (121.04, 121.96) | 23.22 ± 5.45 (22.92, 23.51) | 22.51 ± 5.64 (22.25, 22.76) | 15.11 ± 2.22 (14.99, 15.23) | 15.07 ± 2.23 (14.97, 15.17) | 24.57 ± 1.90 (24.46, 24.67) | 24.27 ± 1.91 (24.18, 24.36) |
| 10-14 (n = 4711) | 146.08 ± 11.05 (145.66, 146.50) | 142.60 ± 10.72 (142.13, 143.06) | 35.83 ± 8.81 (35.50, 36.17) | 35.53 ± 8.58 (35.15, 35.90) | 16.60 ± 2.61 (16.50, 16.69) | 17.29 ± 2.75 (17.17, 17.40) | 27.05 ± 2.23 (26.97, 27.14) | 27.14 ± 2.32 (27.04, 27.24) |

Values expressed as mean ± standard deviation.

BMI: body mass index, NC: neck circumference, CI: confidence interval, Kg: kilograms

reliable and easy to use index that is generally acceptable to patients and health practitioners (12,13,15). Some studies (12,13) in the pediatric age group have confirmed that NC value measurements could be used as an index of overweight and obesity. In response to these reports, this study was planned to assess the use of NC in Pakistani children using BMI SDS scores to define overweight and obesity.

Validation of NC versus WC and BMI, reported by Hatipoglu et al (13), showed that NC could serve as an easy way to determine overweight and obesity in children with good correlation to cardiovascular risk factors. A study in Greek children, aged 9-13 years, also indicated that NC is associated with cardiovascular risk factors (33). Moreover, the NC measurement was confirmed as a reliable anthropometric index to predict children with cardio-metabolic disease (34).

In the present study it was shown that NC has a good correlation with BMI and other anthropometric characteristics. These findings are consistent with earlier studies (14,35) that reported that NC had a significant positive correlation with age and anthropometric variables in both genders. The NC increased with age in both genders and mean values of NC were higher in overweight and obese children as compared to normal weight subjects. These findings are in accordance with a previous population-based study of Iranian children and adolescents, aged 6-18 years (36). Also consistent with more recent studies (37,38), the

**Table 2. Mean comparison of neck circumference according to overweight and obesity status in children by age and sex**

| Age (years)-sex group | Overweight status | | Obesity status | |
|---|---|---|---|---|
| | Yes | No | Yes | No |
| Age group-boys | | | | |
| 05 | 25.33 ± 2.29 | 23.79 ± 1.73* | 25.74 ± 1.88 | 23.93 ± 1.85* |
| 06 | 26.34 ± 2.27 | 24.09 ± 1.54* | 27.50 ± 2.60 | 24.21 ± 1.61* |
| 07 | 26.05 ± 2.27 | 24.18 ± 1.56* | 25.25 ± 2.61 | 24.46 ± 1.80 |
| 08 | 26.49 ± 1.98 | 24.63 ± 1.65* | 27.50 ± 2.17 | 24.77 ± 1.71* |
| 09 | 26.96 ± 2.39 | 24.88 ± 1.82* | 27.46 ± 2.44 | 25.07 ± 1.90* |
| 10 | 27.25 ± 1.91 | 25.34 ± 1.59* | 28.29 ± 1.83 | 25.54 ± 1.70* |
| 11 | 27.55 ± 1.88 | 26.04 ± 1.65* | 28.55 ± 1.60 | 26.21 ± 1.74* |
| 12 | 28.70 ± 2.06 | 26.31 ± 1.80* | 30.24 ± 1.79 | 26.56 ± 1.92* |
| 13 | 29.33 ± 1.97 | 26.97 ± 1.75* | 29.57 ± 2.03 | 27.37 ± 1.99* |
| 14 | 30.51 ± 1.67 | 28.39 ± 2.04* | 31.22 ± 1.37 | 28.63 ± 2.09* |
| 5-9 | 26.23 ± 2.27 | 24.31 ± 1.69* | 26.81 ± 2.44 | 24.49 ± 1.83* |
| 10-14 | 28.84 ± 2.22 | 26.69 ± 2.05* | 29.79 ± 2.00 | 26.96 ± 2.17* |
| Overall (5-14 years) | 28.11 ± 2.52 | 25.88 ± 2.24* | 28.82 ± 2.56 | 26.14 ± 2.37* |
| Age group-girls | | | | |
| 05 | 24.42 ± 1.98 | 23.29 ± 1.51* | 24.57 ± 2.14 | 23.37 ± 1.57* |
| 06 | 25.26 ± 2.17 | 23.44 ± 1.53* | 25.40 ± 2.05 | 23.66 ± 1.73* |
| 07 | 25.77 ± 2.10 | 23.86 ± 1.38* | 25.65 ± 2.22 | 24.05 ± 1.57* |
| 08 | 26.85 ± 2.11 | 24.50 ± 1.49* | 27.80 ± 2.59 | 24.80 ± 1.70* |
| 09 | 27.20 ± 2.74 | 24.99 ± 1.69* | 28.89 ± 3.83 | 25.27 ± 1.94* |
| 10 | 27.21 ± 2.24 | 25.51 ± 1.91* | 27.39 ± 2.90 | 25.73 ± 2.01* |
| 11 | 29.23 ± 2.48 | 26.15 ± 1.78* | 31.59 ± 1.06 | 26.44 ± 2.02* |
| 12 | 29.16 ± 2.11 | 26.75 ± 1.76* | 30.24 ± 2.24 | 27.07 ± 1.96* |
| 13 | 29.51 ± 1.90 | 27.47 ± 2.08* | 30.56 ± 1.79 | 27.77 ± 2.16* |
| 14 | 30.02 ± 2.00 | 28.24 ± 2.05* | 31.11 ± 1.06 | 28.51 ± 2.14* |
| 5-9 | 25.97 ± 2.44 | 23.97 ± 1.64* | 26.20 ± 2.88 | 24.20 ± 1.84* |
| 10-14 | 29.00 ± 2.32 | 26.77 ± 2.14* | 29.93 ± 2.54 | 27.07 ± 2.28* |
| Overall (5-14 years) | 27.63 ± 2.81 | 25.41 ± 2.37* | 27.86 ± 3.30 | 25.70 ± 2.53* |

Values expressed as mean ± standard deviation.

*Significant values $p < 0.01$

present study yields NC in overweight/obese adolescents that are significantly higher than adolescents with normal BMI ($p < 0.001$).

In our study, results for AUC values between 70% and 90% in various age-groups were similar to those found in the Iranian cross-sectional study (36), suggesting that NC could

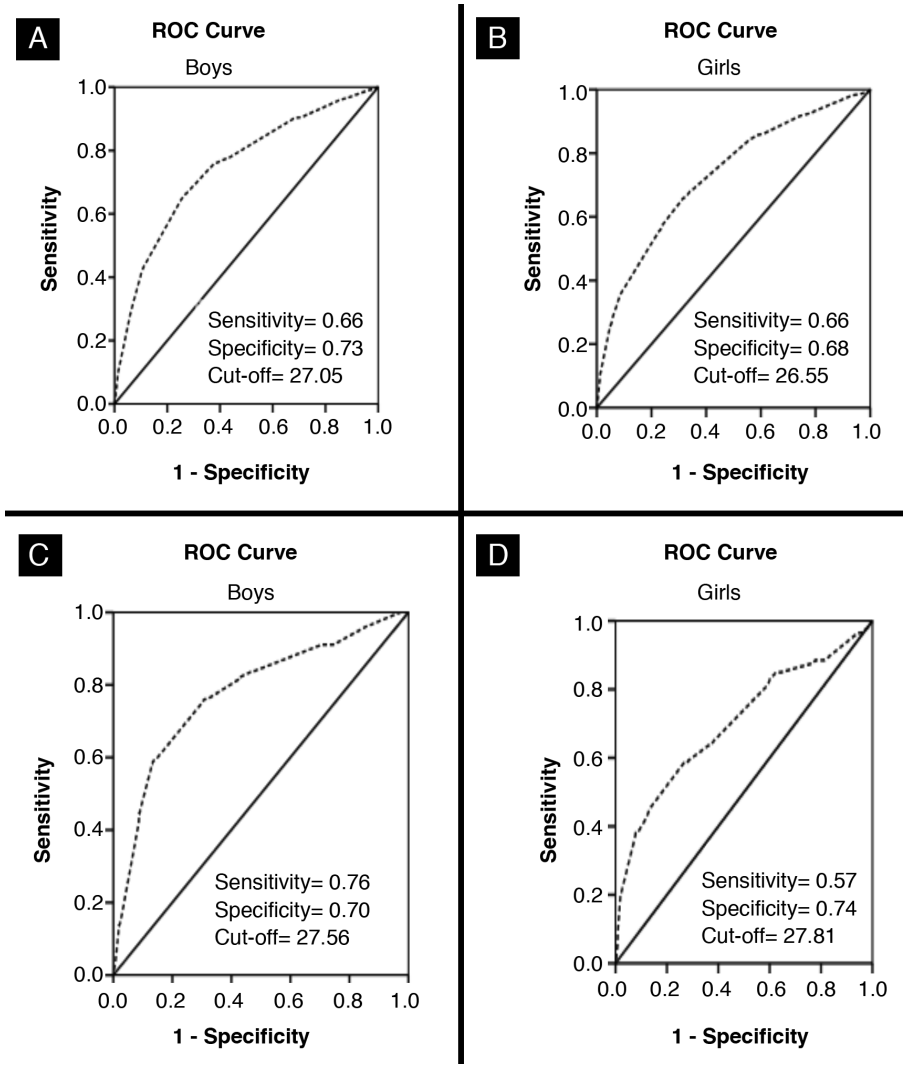


**Figure 1.** Receiver operating characteristic curve of neck circumference as an indicator of overweight (A + B) and obese (C + D) Pakistani children aged, 5-14 years in both genders

*ROC: receiver operating characteristic*

**Table 3a. Correlation co-efficient between neck circumference and other anthropometric characteristics in children**

| | Neck circumference (cm) | | |
|---|---|---|---|
| | Sex | | |
| **Anthropometric characteristics** | **Boy** | **Girls** | **Total** |
| Age (years) | 0.58* | 0.65* | 0.62* |
| Height (cm) | 0.68* | 0.70* | 0.69* |
| Weight (kg) | 0.79* | 0.80* | 0.79* |
| BMI (kg/m$^2$) | 0.59* | 0.64* | 0.61* |

BMI: body mass index, *Significant values $p < 0.01$.

**Table 3b. Association of neck circumference with overweight (i.e. body mass index z-score > 1) and obesity (i.e. body mass index z-score > 2) in a logistic regression model**

| | Model | Overweight OR (95% CI) | Obesity OR (95% CI) |
|---|---|---|---|
| **Neck circumference (cm)** | Model I | 1.43 (1.39-1.46)* | 1.42 (1.36-1.49)* |
| | Model II | 1.74 (1.67-1.80)* | 1.76 (1.67-1.86)* |

Model I: without adjustment.

Model II: Adjusted for age, sex and city living area.

*Significant values $p < 0.01$.

BMI: body mass index, CI: confidence interval, OR: Odds ratio

**Table 4. Area under the curve for detection of overweight and obesity based on the neck circumference in children by age and sex**

| Age (years)-sex group | Overweight status | | | Obesity status | | |
|---|---|---|---|---|---|---|
| | AUC (95% CI) | SE | p value | AUC (95% CI) | SE | p value |
| Age group-boys | | | | | | |
| 05 | 0.699 (0.592-0.806) | 0.054 | < 0.001 | 0.765 (0.625-0.905) | 0.071 | 0.007 |
| 06 | 0.789 (0.695-0.884) | 0.048 | < 0.001 | 0.855 (0.726-0.983) | 0.065 | < 0.001 |
| 07 | 0.749 (0.660-0.839) | 0.046 | < 0.001 | 0.555 (0.313-0.797) | 0.123 | 0.620[NS] |
| 08 | 0.763 (0.680-0.945) | 0.042 | < 0.001 | 0.830 (0.685-0.975) | 0.074 | < 0.001 |
| 09 | 0.790 (0.692-0.867) | 0.050 | < 0.001 | 0.792 (0.589-0.996) | 0.104 | 0.005 |
| 10 | 0.783 (0.718-0.848) | 0.033 | < 0.001 | 0.855 (0.744-0.965) | 0.056 | < 0.001 |
| 11 | 0.729 (0.666-0.792) | 0.032 | < 0.001 | 0.836 (0.758-0.914) | 0.040 | < 0.001 |
| 12 | 0.805 (0.755-0.855) | 0.026 | < 0.001 | 0.908 (0.833-0.984) | 0.038 | < 0.001 |
| 13 | 0.814 (0.769-0.859) | 0.023 | < 0.001 | 0.785 (0.666-0.904) | 0.061 | < 0.001 |
| 14 | 0.787 (0.740-0.833) | 0.024 | < 0.001 | 0.844 (0.784-0.903) | 0.030 | < 0.001 |
| 5-9 | 0.753 (0.710-0.796) | 0.022 | < 0.001 | 0.768 (0.688-0.847) | 0.040 | < 0.001 |
| 10-14 | 0.761 (0.737-0.786) | 0.012 | < 0.001 | 0.826 (0.784-0.867) | 0.021 | < 0.001 |
| **Prepubertal** | **0.752 (0.721-0.783)** | **0.016** | **< 0.001** | **0.784 (0.725-0.843)** | **0.030** | **< 0.001** |
| **Pubertal** | **0.786 (0.758-0.814)** | **0.014** | **< 0.001** | **0.850 (0.805-0.895)** | **0.023** | **< 0.001** |
| Overall | 0.747 (0.726-0.769) | 0.011 | < 0.001 | 0.776 (0.733-0.819) | 0.022 | < 0.001 |
| Age group-girls | | | | | | |
| 05 | 0.672 (0.580-0.764) | 0.047 | < 0.001 | 0.664 (0.496-0.831) | 0.085 | 0.027 |
| 06 | 0.774 (0.704-0.843) | 0.036 | < 0.001 | 0.749 (0.620-0.877) | 0.066 | 0.003 |
| 07 | 0.792 (0.714-0.870) | 0.040 | < 0.001 | 0.768 (0.596-0.941) | 0.088 | 0.002 |
| 08 | 0.824 (0.765-0.883) | 0.030 | < 0.001 | 0.841 (0.710-0.972) | 0.067 | < 0.001 |
| 09 | 0.758 (0.686-0.830) | 0.037 | < 0.001 | 0.771 (0.531-1.000) | 0.123 | 0.009 |
| 10 | 0.724 (0.657-0.792) | 0.034 | < 0.001 | 0.668 (0.486-0.849) | 0.093 | 0.040 |

**Table 4. Continued**

| Age (years)-sex group | Overweight status | | | Obesity status | | |
|---|---|---|---|---|---|---|
| | AUC (95% CI) | SE | p value | AUC (95% CI) | SE | p value |
| Age group-girls | | | | | | |
| 11 | 0.836 (0.768-0.904) | 0.035 | < 0.001 | 0.976 (0.957-0.995) | 0.010 | < 0.001 |
| 12 | 0.810 (0.752-0.868) | 0.030 | < 0.001 | 0.860 (0.739-0.981) | 0.062 | < 0.001 |
| 13 | 0.770 (0.714-0.827) | 0.029 | < 0.001 | 0.840 (0.736-0.944) | 0.053 | < 0.001 |
| 14 | 0.758 (0.688-0.828) | 0.036 | < 0.001 | 0.867 (0.765-0.970) | 0.052 | 0.002 |
| 5-9 | 0.761 (0.728-0.795) | 0.017 | < 0.001 | 0.720 (0.643-0.797) | 0.039 | < 0.001 |
| 10-14 | 0.761 (0.731-0.790) | 0.015 | < 0.001 | 0.802 (0.726-0.877) | 0.038 | < 0.001 |
| **Prepubertal** | **0.748 (0.719-0.778)** | **0.015** | **< 0.001** | **0.703 (0.634-0.773)** | **0.036** | **< 0.001** |
| **Pubertal** | **0.788 (0.758-0.819)** | **0.016** | **< 0.001** | **0.877 (0.825-0.929)** | **0.026** | **< 0.001** |
| Overall | 0.728 (0.705-0.750) | 0.012 | < 0.001 | 0.694 (0.637-0.751) | 0.029 | < 0.001 |

SE: standard error, NS: not significant, CI: confidence interval, AUC: area under the curve, NS: not significant

serve to accurately identify children who are overweight or obese. Another Brazilian study, Souza et al (39), has also established NC as an adequate indicator to identify adolescents with high BMI. Similar to two recent studies (40,41), our results also suggest that NC has good diagnostic ability, as indicated by an AUC > 0.65, for identifying overweight and obesity in children and adolescents and could be used to screen for excess body weight in routine medical practice. Furthermore, the cut-off point of NC to identify children who are overweight in different age-groups was between 25.00-30.35 cm and 24.00-29.33 cm for boys and girls, respectively. The cut-off points for NC to identify children who are obese in different age-groups was between 25.27-30.35 cm and 25.00-31.62 cm, for boys and girls; respectively. Larger NC cut-offs, between 28.0 to 38.0 cm in boys and 27.0 to 34.5 cm in girls were reported by Hatipoglu et al (13) for a Turkish study for the prediction of overweight and obesity, defined as BMI above the 85$^{th}$ percentile of the BMI reference curve. Similarly, larger cut-off values of NC for the prediction of overweight (defined as BMI between the 85$^{th}$ and 94$^{th}$ centiles for age and sex) or general obesity (defined as obesity as BMI equal to or greater than the sex-specific 95$^{th}$ centile), were also noted in an Iranian population-based study (36). Taheri et al (16) compared the reported NC cut-offs, and associated sensitivity and specificity from different countries and this revealed a notable variation in these values from country to country. Differences in the methods used to define overweight and obesity might partially explain the heterogeneity in the optimal cut-offs among different populations. The variation in sensitivity and specificity of the NC method between studies may be explained due to sample size and age range differences. Furthermore, in our study, BMI-for-age z-scores were calculated by using the LMS method. No other study in the literature calculated BMI-for-age z-scores using this method. Such methodological diversity can also influence these values. The optimal cut-off may vary according to age and additional studies using the same methodology and assessing a wide age range are needed.

Our study has several strengths. Firstly, we have taken a large sample. Secondly, our results using ROC curve analysis are likely to be representative of today's children and these results are applicable at the national level. Thirdly, there is no similar study to determine the best cut-off points of NC for identification of overweight and obese Pakistani children using a multi-ethnic data set.

Moreover, NC measures were collected by the same researcher, which reduces possible inter-observer biases.

**Table 5. Cut-off point, sensitivity and specificity of neck circumference for detecting overweight and obesity in children by sex and age groups**

| Age (year) Sex group | Overweight | | | | | Obesity | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cut-off point | Sensitivity | Specificity | $LR^P$ | $LR^N$ | Cut-off point | Sensitivity | Specificity | $LR^P$ | $LR^N$ |
| Age group-boys | | | | | | | | | | |
| 05 | 25.28 | 0.63 | 0.75 | 2.52 | 0.50 | 25.27 | 0.78 | 0.72 | 2.78 | 0.30 |
| 06 | 25.28 | 0.74 | 0.70 | 2.47 | 0.37 | 26.17 | 0.73 | 0.90 | 7.30 | 0.30 |
| 07 | 25.00 | 0.71 | 0.70 | 2.37 | 0.41 | 27.31 | 0.29 | 0.94 | 4.83 | 0.76 |
| 08 | 25.02 | 0.78 | 0.54 | 1.70 | 0.41 | 26.42 | 0.73 | 0.86 | 5.21 | 0.31 |
| 09 | 27.00 | 0.70 | 0.85 | 4.67 | 0.35 | 27.00 | 0.75 | 0.80 | 3.75 | 0.31 |
| 10 | 27.00 | 0.73 | 0.78 | 3.32 | 0.34 | 28.00 | 0.80 | 0.84 | 5.00 | 0.24 |
| 11 | 26.54 | 0.75 | 0.61 | 1.92 | 0.41 | 28.00 | 0.80 | 0.73 | 2.96 | 0.27 |
| 12 | 27.30 | 0.78 | 0.71 | 2.69 | 0.31 | 29.08 | 0.88 | 0.88 | 7.33 | 0.14 |
| 13 | 28.00 | 0.66 | 0.84 | 4.12 | 0.41 | 28.32 | 0.77 | 0.76 | 3.21 | 0.30 |
| 14 | 30.35 | 0.74 | 0.72 | 2.64 | 0.36 | 30.35 | 0.88 | 0.66 | 2.59 | 0.18 |
| 5-9 | 25.78 | 0.51 | 0.88 | 4.25 | 0.56 | 25.78 | 0.59 | 0.85 | 3.93 | 0.48 |
| 10-14 | 27.56 | 0.78 | 0.63 | 2.11 | 0.35 | 29.08 | 0.73 | 0.81 | 3.84 | 0.33 |
| Prepubertal | **26.36** | **0.67** | **0.82** | **3.30** | **0.49** | **26.78** | **0.70** | **0.77** | **3.13** | **0.38** |
| Pubertal | **28.32** | **0.68** | **0.78** | **3.06** | **0.41** | **28.57** | **0.88** | **0.74** | **3.37** | **0.16** |
| Overall | 27.05 | 0.66 | 0.73 | 2.45 | 0.47 | 27.56 | 0.76 | 0.70 | 2.53 | 0.34 |
| Age group-girls | | | | | | | | | | |
| 05 | 24.00 | 0.62 | 0.63 | 1.68 | 0.60 | 25.02 | 0.63 | 0.83 | 3.71 | 0.45 |
| 06 | 24.76 | 0.67 | 0.81 | 3.53 | 0.41 | 25.00 | 0.67 | 0.76 | 2.79 | 0.43 |
| 07 | 25.02 | 0.74 | 0.77 | 3.22 | 0.34 | 25.27 | 0.83 | 0.74 | 3.19 | 0.23 |
| 08 | 26.00 | 0.63 | 0.90 | 6.30 | 0.41 | 25.78 | 0.77 | 0.83 | 4.53 | 0.28 |
| 09 | 26.54 | 0.61 | 0.81 | 3.21 | 0.48 | 27.68 | 0.75 | 0.89 | 6.82 | 0.28 |
| 10 | 26.79 | 0.54 | 0.84 | 3.37 | 0.55 | 27.68 | 0.58 | 0.81 | 3.05 | 0.52 |
| 11 | 27.17 | 0.77 | 0.74 | 2.96 | 0.31 | 30.22 | 1.00 | 0.93 | 14.28 | 0.00 |
| 12 | 28.32 | 0.60 | 0.88 | 5.00 | 0.45 | 28.32 | 0.86 | 0.82 | 4.78 | 0.17 |
| 13 | 28.19 | 0.75 | 0.73 | 2.78 | 0.34 | 28.19 | 0.89 | 0.66 | 2.62 | 0.17 |
| 14 | 29.33 | 0.71 | 0.74 | 2.73 | 0.39 | 31.62 | 0.67 | 0.92 | 8.37 | 0.36 |
| 5-9 | 25.27 | 0.71 | 0.72 | 2.54 | 0.40 | 25.02 | 0.74 | 0.66 | 2.18 | 0.39 |
| 10-14 | 28.32 | 0.59 | 0.82 | 3.28 | 0.50 | 28.32 | 0.76 | 0.76 | 3.17 | 0.32 |
| Prepubertal | **25.27** | **0.73** | **0.67** | **2.16** | **0.40** | **25.02** | **0.75** | **0.66** | **1.88** | **0.41** |
| Pubertal | **28.70** | **0.67** | **0.80** | **3.33** | **0.42** | **28.82** | **0.92** | **0.72** | **3.31** | **0.10** |
| Overall | 26.55 | 0.66 | 0.68 | 2.06 | 0.50 | 27.81 | 0.57 | 0.74 | 2.19 | 0.58 |

$LR^P$: Likelihood ratio for positive, $LR^N$: Likelihood ratio for negative

## Study Limitations

The first limitation of this study is that the causality underlying the observed relationships could not be investigated, due to the cross-sectional design. A second limitation is that our study does not cover all age ranges of children and adolescents from birth to 18 years of age. A third limitation is the completely urban and relatively wealthy study population. Findings of the study do not truly cover the rural and relatively poor population of children and adolescents in Pakistan. It should be noted that NC measurements for obesity/overweight screening may be unreliable for individuals with different health problems affecting the neck, such as malignancy or thyroid diseases, cervical spinal disorders, short neck, craniofacial anomalies or neurological conditions or underlying cardiac or pulmonary disease. In spite of the limitations, we believe that the results of this study will contribute new information for knowledge of Public Health.

## Conclusion

NC had good correlation with BMI and also had good diagnostic performance for identifying overweight and obese children. Therefore, NC may be a simple and valuable tool for screening children for weight problems. The results suggested that the Pakistani boys and girls, aged 5-14 years with NC range ≥25.00 to 30.35 cm and ≥24.00 to 31.62 cm, respectively, could be considered to be overweight and obese. As previous epidemiological studies have reported an association between NC and cardiovascular and metabolic risk in obese children and adults, further studies in Pakistani children and young adults should be undertaken to investigate the usefulness of NC as an index of adiposity.

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### Ethics

**Ethics Committee Approval:** The study was approved by the Departmental Ethics Committee of Bahauddin Zakariya University, Multan, Pakistan (IRB# SOC/D/2715/19).

**Informed Consent:** After obtaining written consent from the school's head master and verbal consent from each child's parents or guardians, data collection activities were performed.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: Muhammad Asif, Muhammad Aslam, Design: Muhammad Asif, Muhammad Aslam, Data Collection or Processing: Muhammad Asif, Shakeel Ahmed, Analysis or Interpretation: Muhammad Asif, Muhammad Aslam, Saima Altaf, Literature Search: Muhammad Asif, Shakeel Ahmed, Saima Altaf, Writing: Muhammad Asif, Saima Altaf, Justyna Wyszynska.

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# Girls with Premature Thelarche Younger than 3 Years of Age May Have Stimulated Luteinizing Hormone Greater than 10 IU/L

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### What is already known on this topic?

A gonadotropin-releasing hormone (GnRH) stimulation test is the gold standard for the diagnosis of central precocious puberty and a peak stimulated luteinizing hormone (LH) level of 5 mIU/L is considered pubertal by many endocrinologists.

### What this study adds?

GnRH stimulation test reference values differ in young girls with premature thelarche (PT) compared to older girls. In all girls with PT under three years of age, peak LH/follicle stimulating hormone ratio was ≤0.43, regardless of peak LH levels which were usually >5 IU/L in two thirds and were even >10 IU/L in some girls.

## Abstract

**Objective:** Premature thelarche (PT) is defined as isolated breast development in girls before eight years of age. Gonadotropin-releasing hormone (GnRH) stimulation test is sometimes used to distinguish between PT and central precocious puberty (CPP), although the interpretation of the test at early ages is challenging. The objective of this study was to determine the follicle stimulating hormone (FSH) and luteinizing hormone (LH) responses to GnRH stimulation in girls with PT below 3 years of age.

**Methods:** A standardized GnRH stimulation test, bone age and pelvic ultrasound were evaluated and those without pubertal progression after a minimum of one-year follow up were included in the study.

**Results:** On GnRH stimulation test, the median (range) baseline LH was 0.29 (0.10-0.74) IU/L, baseline FSH was 4.96 (3.18-7.05) mIU/mL, and the peak median LH was 5.75 (3.31-8.58) IU/L with the peak mean ± standard deviation FSH was 40.38 ± 20.37 mIU/mL. Among the patients, 33.3% (n=10) had baseline LH >0.3 IU/L, 67% (n=20) had peak LH >5 IU/l and 16.6% (n=5) >10 IU/L. The mean peak LH/FSH ratio was 0.17 ± 0.09 and was ≤0.43 in all participants.

**Conclusion:** Although consensus statements usually define baseline LH >0.3-0.5 IU/L, peak LH >5 IU/L, and LH/FSH ratios >0.66-1.0 as diagnostic cut-offs for CPP, in children below 3 years of age, the baseline and peak LH values may be similar to pubertal values, possibly due to mini-puberty. A dominant FSH response on GnRH stimulation test is more valuable than the peak LH response in the diagnosis of PT.

**Keywords:** GnRH stimulation test, central precocious puberty, young girls

## Introduction

Precocious puberty (PP) has been an increasing concern in recent years due to both an increase in the number of related outpatient visits and the existing challenge of determining the patients who require treatment. Most girls with signs of puberty in the first three years of life are diagnosed with premature thelarche (PT), which is a benign and non-progressive condition. While early skeletal maturation, increase in height growth rate and decrease in adult height are seen in central PP (CPP), these findings are not seen in PT. How often girls initially diagnosed with PT progress to

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CPP and how much monitoring is required is contentious. Certain clinicians perform gonadotropin-releasing hormone (GnRH) stimulation tests based on the clinical findings and/or randomly requested baseline hormonal tests in these patients (1,2). The consensus statements on PP recommend to use the same threshold values in the interpretation of the GnRH stimulation tests in all children below eight years of age. These thresholds values are a stimulated luteinizing hormone (LH) >5 IU/L or a LH/follicle stimulating hormone (FSH) ratio above 0.66 or 1.0; these results support a diagnosis of CPP (3,4,5). However, patients in the younger age group present challenges in the interpretation of diagnostic tests, due to the impact of the activation of the hypothalamic-pituitary-gonadal axis in the first months of life, termed 'mini-puberty' (6). Although a limited number of studies report that higher stimulated LH responses may be observed following GnRH stimulation tests conducted in children below three years old (7), this information is not highlighted in consensus statements (1,8).

In the present study, GnRH stimulation test results of children diagnosed with PT before the age of three years were evaluated and the validity of the criteria used in pediatric age groups to distinguish between pubertal and prepubertal responses was investigated.

## Methods

Thirty girls with PT under the age of three years, admitted to the Pediatric Endocrinology Department outpatient clinic of Kocaeli University, between January 2010 and June 2016, were prospectively evaluated. All parents received oral and written information before being asked for consent. The study was approved by the Local Ethics Committee of Kocaeli University Institutional Review Board (KÜ GÖKAEK 2016/70).

PT was diagnosed based upon the following criteria: isolated breast development without other signs of puberty; and bone age within ±2 standard deviation (SD) of mean for chronological age. The diagnosis of PT was confirmed by a lack of pubertal progression over at least one-year follow-up. Signs suggestive of pubertal progression were growth acceleration with a height velocity >1 SD score (SDS) and/or progression to Tanner breast stage >3 and/or bone age acceleration.

During the initial assessment of the girls presented with isolated breast development, a detailed patient history was taken and a physical examination was performed. The length of the children aged <2 years of age was measured in the supine position on the head-foot board (Seca Limited, Hammer Steindamm, Hamburg, Germany), while the height of children >2 years of age was measured in a standing position using a Harpenden Stadiometer (Holtain Limited, Crymych, Dyfed, UK). The SDS for height was calculated based on Turkish Child growth standards (9). Height velocity SDS was calculated using Tanner's growth charts (10). Puberty stage was assessed by physical examination according to Tanner's criteria for breast development in females (11). The left hand and wrist X-rays for bone aging, pelvic ultrasonography (USG), and GnRH stimulation tests were performed at the time of the diagnosis. Bone age was assessed using the Greulich & Pyle method by the same pediatric endocrinologist and repeated every 6 to 12 months (12). Bone age acceleration was defined as Δ bone age/Δ calendar age >1. Longitudinal diameter of the uterus >34 mm at pelvic USG was defined resulting from estrogen exposure (13).

### GnRH Stimulation Test Procedure

An intravenous (IV) cannula was inserted into the antecubital region for blood sampling and GnRH analogue injection. After the baseline blood was drawn for LH and FSH measurement, gonadorelin acetate (LH-RH ferring ampul, 0.1 mg/mL, Ferring İlaç San. ve Tic. Ltd., İstanbul, Turkey) 0.1 mg/m$^2$ body surface area (max 0.1 mg) was injected as an IV bolus and a second blood sample was obtained at the 40$^{th}$ minute (14). LH and FSH were measured using an immuno-chemiluminescense assay using an Immulite 1000 apparatus and commercial kits (Diagnostic Products Corp.-Medlab, Los Angeles, CA) (15). For LH, the intra- and inter-assay coefficients of variation (CVs) were 4.8% and 10.7%, respectively. For FSH, the intra- and inter-assay CVs were 3.4% and 5.4%, respectively. The minimum detectable concentration was 0.1 IU/L for both FSH and LH.

### Statistical Analysis

Statistical evaluation was performed using the SPSS, version 20.0 (IBM Inc., Chicago, IL, USA). The normality of the distribution was assessed with the Kolmogorov-Smirnov Test. Numeric variables were expressed as mean +/- SD and median plus 25$^{th}$ to 75$^{th}$ percentile (interquartile range) and frequency (percentage). Independent t-tests were used when comparing continuous variables that were normally distributed. Continuous variables that were not normally distributed were assessed using the Mann-Whitney U test. Categorical variables were presented as numbers and percentages. Differences between categorical variables, such as Tanner stage, were assessed using a chi-square test. The relationship between the variables within the normal distribution was evaluated using Pearson's correlation analysis, while the relationship between the variables outside the normal distribution was analysed through

Spearman's correlation analysis. Statistical significance was assumed with a value of $p < 0.05$.

## Results

Thirty girls aged under three years with PT, in whom the diagnosis was confirmed by lack of pubertal progression in at least one-year of follow-up, were enrolled in the study. Clinical characteristics of patients are shown in Table 1. The bone age was within mean ± 2 SD of chronologic age range in all the patients. The pelvic USG results showed that the uterine sizes and ovarian volumes were consistent with the ages of the patients and no pathologies were observed. The results of the GnRH stimulation tests are presented in Table 2. The distribution of the baseline LH and FSH, peak LH and FSH, and the peak LH/FSH ratios according to age are shown in Figure 1. The baseline LH value was > 0.3 IU/L in

**Table 1. Clinical characteristics of girls with premature thelarche**

| | |
|---|---|
| No. of girls | 30 |
| Age (months) | 20 ± 7[a] |
| Bone age (months) | 17.0 [12.0-24.0][b] |
| Bone age advancement (months) | 4.0 [1.0-16.0][b] |
| BMI-SDS | 0.78 (-0.94 ± 0.98)[a] |
| Breast stage 2 | 8 (26.6)[c] |
| Breast stage 3 | 22 (73.3)[c] |
| Height SDS | 1.5 [0.5-3.0][b] |
| Height velocity SDS | -0.19 [-2.1-3.7][b] |

[a]Mean ± standard deviation, [b]median [interquartile range], [c]number (%).
BMI-SDS: body mass index-standard deviation score

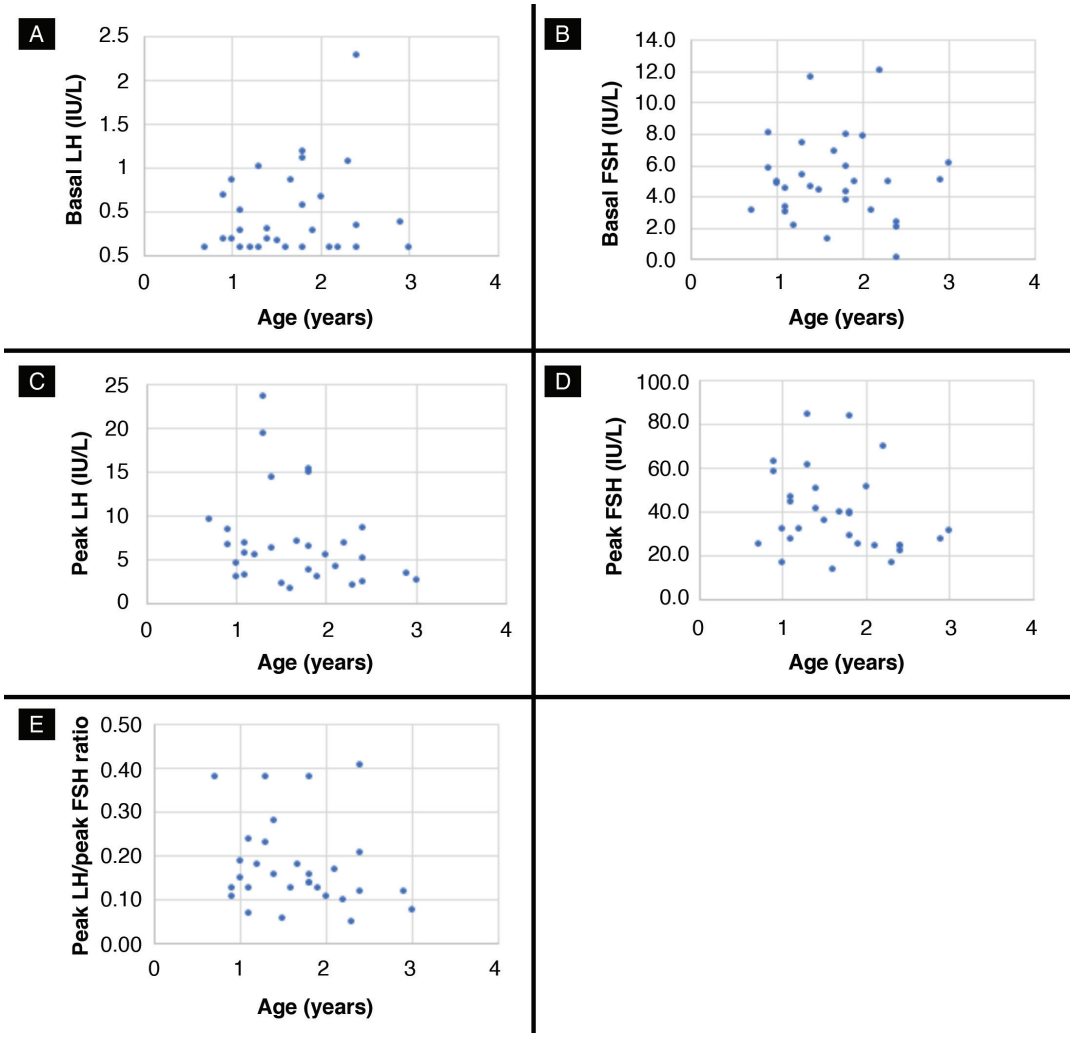


**Figure 1.** A) The distribution of the baseline luteinizing hormone (LH) levels according to age. B) The distribution of the peak LH responses according to age. C) The distribution of the baseline follicle-stimulating hormone (FSH) levels according to age. D) The distribution of the peak FSH responses according to age. E) The distribution of the peak LH/ peak FSH ratio according to age

10/30 patients (33.3%). In 67% of the patients (n = 20), the peak LH value was > 5 IU/L, while it was > 10 IU/L in 16.6% (n = 5). In all patients, the peak LH/FSH ratio was ≤0.43.

No significant relationship was observed between the baseline LH and the peak LH (r = 0.054, p = 0.776). However, there was a positive correlation between stimulated FSH and stimulated LH (r = 0.647, p < 0.001).

There were no significant differences between subjects with basal LH values > 0.3 IU/L and < 0.3 IU/L in terms of Tanner breast stage, bone age, basal FSH, estradiol concentration, peak LH and peak FSH values (Table 3). Similarly, there were no significant differences in Tanner stage, bone age, basal LH, basal FSH, estradiol concentration and peak FSH values between the groups with peak LH value > 5 IU/L and peak LH value < 5 IU/L (Table 4).

**Table 2. Hormonal results following gonadotropin-releasing hormone stimulation test in girls with premature thelarche**

| | Statistics |
|---|---|
| Basal LH (IU/L) | 0.29 [0.10-0.74] |
| Basal FSH (mIU/mL) | 4.96 [3.18-7.05] |
| Peak LH (IU/L) | 5.75 [3.31-8.58] |
| Peak FSH (mIU/mL) | 40.38 ± 20.37 |
| Peak LH/peak FSH ratio | 0.17 ± 0.09 |

Median [interquartile range] or mean ± standard deviation.
LH: luteinizing hormone, FSH: follicle-stimulating hormone

## Discussion

Signs of puberty, especially breast development, in girls up to 3 years of age are a source of concern both for the parents and physicians. At such an early age, isolated PT is the most likely diagnosis and often all that is needed is observation. On the other hand, CPP, although rare, is also observed in this age group or cases diagnosed initially as isolated PT may progress to CPP (1,16). Clinically, breast development in patients with isolated PT is unaccompanied by areola and nipple maturation, breast development shows fluctuations, and no growth spurt is observed (16). However, in a study from Israel comprising the follow up of 139 patients with PT for a decade, CPP was reported in 13%, regardless of the age of the diagnosis and the clinical progression (17). In this study, cases progressing to CPP were significantly less frequent in patients under the age of two compared with girls over 2 years of age (3.8% vs. 52.6%). Other studies from Italy and Denmark also showed 14% of girls with an initial diagnosis of PT progressed to CPP on follow-up (18,19). It is therefore warranted to perform further studies, including GnRH stimulation tests, in certain cases.

**Table 3. The comparison of girls with basal luteinizing hormone value < 0.3 IU/L and > 0.3 IU/L**

| | Basal LH < 0.3 IU/L | Basal LH > 0.3 IU/L | p |
|---|---|---|---|
| Number of girls (n) | 15 | 15 | |
| Tanner stage (n, 2/3) | 4-11 | 3-12 | 0.367 |
| Bone age (years) | 12 [9-24][b] | 24 [12-24][b] | 0.539 |
| Basal FSH (mIU/mL) | 4.57 [3.1-5.1][b] | 6.91 [4.3-8]b | 0.102 |
| Estradiol (pg/ml) | 13.3 [5-20][b] | 17.4 [12.5-20][b] | 0.886 |
| Peak LH (IU/L) | 5.77 [3.2-7.04][b] | 5.6 [3.9-14.5][b] | 0.325 |
| Peak FSH (mIU/mL) | 35.9 ± 16.1[a] | 46.2 ± 24.3[a] | 0.089 |

[a]Mean ± standard deviation, [b]median [interquartile range].
LH: luteinizing hormone, FSH: follicle-stimulating hormone

**Table 4. The comparison of girls with peak luteinizing hormone value < 5 IU/L and > 5 IU/L**

| | Peak LH < 5 IU/L | Basal LH > 5 IU/L | p |
|---|---|---|---|
| Number of girls | 12 | 18 | |
| Tanner stage (n, 2-3) | 4-8 | 4-14 | 0.14 |
| Bone age (years) | 19 [12-27][b] | 12 [9.75-24][b] | 0.689 |
| Basal LH (IU/L) | 0.32 [0.16-0.54][b] | 0.24 [0.1-0.82][b] | 1.000 |
| Basal FSH (mIU/mL) | 4.7 [3.31-5.03][b] | 5.6 [3.3-7.96][b] | 0.156 |
| Estradiol (pg/mL) | 11.5 [2.0-19.5][b] | 17.4 [7.72-20.0][b] | 0.472 |
| Peak FSH (mIU/mL) | 27.2 ± 9.32[a] | 43.1 ± 21.2[a] | 0.342 |

[a]Mean ± standard deviation, [b]median [interquartile range].
LH: luteinizing hormone, FSH: follicle-stimulating hormone

However, the lack of established reference values for GnRH stimulation test responses in girls below three years taking into account the effect of mini-puberty has led to some clinical confusion. In a study from Italy including 450 patients, progression to CPP was observed in only 2% of the patients diagnosed with PT below the age of two and the baseline hormone levels, including the GnRH test, were found to be unhelpful in predicting progression (2). In this study, 97 patients were evaluated through endocrine tests and imaging methods in addition to a 3-month clinical follow up and 85 patients were diagnosed with PT, nine patients were diagnosed with CPP and three were attributed the diagnosis of peripheral PP. Among the patients with a final diagnosis of PT, 36.4% had peak LH levels >5 IU/L (100% among the patients with CPP). On the other hand, baseline LH values >0.2 IU/L were observed in only 1.17% of the patients with PT. All girls with isolated thelarche had a FSH predominant response with peak LH/FSH ratio <1, while girls with complete sexual development showed a ratio >1. A study from Taiwan also reported similar results (7). In this study, 36 patients with the final diagnosis of isolated PT were classified into two groups; patients under the age of 4 (group A) and over the age of 4 (group B). Their GnRH stimulation test results were compared. In group A, the peak mean LH was 13.0±6.06 IU/L, while it was 8.5±4.10 in group B and the peak LH response was significantly higher in group A compared to group B ($p<0.05$). In addition, the peak mean FSH in group A was 120.5±45.87, while it was 48.7±24.05 IU/L in group B and the peak FSH response was significantly higher in group A compared to group B ($p<0.001$). The peak LH/FSH ratio was <1 in all the patients.

Vestergaard et al (20) investigated the physiological LH, FSH and LH/FSH response to GnRH stimulation test in healthy girls below six years of age with no signs of PP. The study showed an age-dependent response to the GnRH test with larger LH and FSH responses in girls aged from 10 months to 3 years compared to girls aged from 3-6 years (the 30-min LH response 5.2±4.0 and 2.9±2.5 IU/L, the 30-min FSH response 23.3±16.2 and 14.5±10.3 IU/L). The peak LH/FSH ratio was 0.23±0.19 (range 0.06-0.43).

In the present study, the ratio of both the baseline LH values >0.3 IU/L (33%) and the peak LH values >5 IU/L (67%) were high. Also, 16.6% of the patients (n=5) had values of peak LH >10 IU/L. These latter patients can easily be misdiagnosed with CPP with a traditional approach. Indeed, GnRH analogue therapy had been suggested for some girls referred to our clinic based on these results, However, all patients had peak LH/FSH ratio values ≤0.43.

The main focus of our study was to analyse GnRH stimulation test results among patients with a confirmed diagnosis of isolated PT. The results showed that high gonadotropin response, even when peak LH value was >10 IU/L, was not associated with progression to true PP in our cohort as all girls had confirmed isolated PT. Thus the treatment decision should not be based solely upon these criteria. The peak LH/FSH ratio appears to be a useful parameter for defining pubertal activation. Indeed, both our study results and other studies have demonstrated that after mini-puberty, although the baseline LH and FSH values are undetectable, a GnRH test may induce a "pubertal gonadotropin response" in girls under the age of three years (2,6). Although the association of this response with the etiology of PT is yet to be elucidated, it may be a crucial diagnostic factor.

### Study Limitations

As the study was conducted as an observation of young girls with PT, the number of participants was restricted. Further studies with larger sample sizes may yield more definitive results. Also, it was not possible to compare PT patients with CPP patients or healthy girls with no signs of pubertal progression in the age group of interest because of either insufficient numbers or ethical issues.

## Conclusion

In conclusion, a peak LH value, even if >10 IU/L, is inadequate to make an accurate therapy decision in patients with PT below three years of age. Elevated LH responses to GnRH stimulation test are common, but not related to PP. The diagnosis of CPP solely based on the response to a GnRH test is often misleading in the first three years of life, leading to overestimation of CPP. In our opinion the peak LH/FSH ratio is more valuable for distinguishing between a pubertal and a prepubertal response. As highlighted in recent years, there is still uncertainty regarding the diagnosis and treatment of early puberty, and the evaluation of the biochemical results with the clinical findings and the age of the patient is one of the most important points to avoid unnecessary treatment (21,22).

### Ethics

**Ethics Committee Approval:** The study was approved by the Local Ethics Committee of Kocaeli University Institutional Review Board (KÜ GÖKAEK 2016/70).

**Informed Consent:** All parents received oral and written information before being asked for consent.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Gülcan Seymen Karabulut, Müge Atar, Concept: Şükrü Hatun, Gülcan Seymen Karabulut, Design: Şükrü Hatun, Gülcan Seymen Karabulut, Data Collection or Processing: Gülcan Seymen Karabulut, Müge Atar, Filiz Mine Çizmecioğlu Jones, Analysis or Interpretation: Gülcan Seymen Karabulut, Literature Search: Şükrü Hatun, Gülcan Seymen Karabulut, Müge Atar, Writing: Şükrü Hatun, Gülcan Seymen Karabulut.

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# Different Potent Glucocorticoids, Different Routes of Exposure but the Same Result: Iatrogenic Cushing's Syndrome and Adrenal Insufficiency

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### What is already known on this topic?

The most common cause of Cushing's syndrome (CS) in childhood is the administration of high doses of synthetic glucocorticoids (GC) for treatment purposes or misuse of these steroids.

### What this study adds?

This is the largest series presenting iatrogenic CS (ICS) and adrenal insufficiency (AI) caused by potent steroids in childhood. AI is a rare cause of hypercalcemia in infancy and childhood but hypercalcemia was detected in two infants of fourteen patients in this study. In addition, an infant with ICS had exposure to a cream and the patient's urine and blood steroid analyses revealed exposure to high-dose steroids.

## Abstract

**Objective:** Potent glucocorticoids (GC) cause iatrogenic Cushing's syndrome (ICS) due to suppression of hypothalamo-pituitary-adrenal (HPA) axis and may progress to adrenal insufficiency (AI). The aim was to review the clinical and laboratory findings of patients with ICS and to investigate other serious side effects.

**Methods:** The possibility of AI was investigated by low-dose adrenocorticotrophic hormone test. Hydrocortisone was started in patients with adrenal failure.

**Results:** Fourteen patients (five boys) with ages ranging from 0.19 to 11.89 years were included. The duration of GC exposure ranged from 1 to 72 months. Ten patients were prescribed topical GC and the rest had oral exposure. Moon face and abdominal obesity were detected in all patients. At presentation, 12 of 14 had AI and two infants had hypercalcemia and nephrocalcinosis. Of 11 patients, ultrasonography revealed hepatosteatosis in five. A cream for diaper dermatitis was used in one infant and the active ingredient was listed as panthenol. However, blood and urine steroid analyses revealed that all endogenous steroids were suppressed. Median (range) time to normalization of HPA axis function was 60 (30-780) days.

**Conclusion:** The majority (85%) of patients had life-threatening AI and two patients had hypercalcemia. These results highlight the serious side-effects of inappropriate use of potent GCs, especially in infants. The recovery of the HPA axis in children might take as long as three years. Parents should be informed regarding the possibility of some products containing unlisted synthetic GC and to be aware of their side effects.

**Keywords:** Cushing's syndrome, adrenal insufficiency, glucocorticoids, adverse effect, hypercalcemia, non-alcoholic fatty liver disease

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## Introduction

Cushing's syndrome (CS) is very rare in childhood and the most common cause is the administration of high doses of synthetic glucocorticoids (GC) for treatment purposes or misuse of these steroids (1). GC are one of the most widely used drugs in the treatment of numerous diseases, including hematological diseases, oncological malignancies, respiratory system diseases, rheumatologic diseases, neurological diseases, kidney diseases, organ transplantations and adrenal insufficiency (AI). As the potency and duration of administration of GC increase, the risk of serious side effects also increases. These side effects include hypercortisolism, hypothalamo-pituitary-adrenal axis (HPA) suppression, non-alcoholic fatty liver disease (NAFLD), osteoporosis, or even adrenal atrophy. Hypercortisolism due to exogenous steroids is known as iatrogenic CS (ICS) (2).

Synthetic GC exogenously given via oral, iv, intramuscular, intra-articular, topical, inhaled, intra-ocular or intra-nasal routes can cause ICS. It is known that GC, which are frequently administered orally and topically in childhood, cause ICS (3,4). In particular, application of topical steroids to the diaper region of an infant may lead to CS and subsequent AI (5). Such use of exogenous GC may even facilitate the spread of infectious disease through suppression of immune function that might be fatal (6). In addition to potent steroids, long-term and high-dose administration of low-potency GC such as hydrocortisone (HC), prednisolone (PZ) and methylprednisolone (MPZ), which are frequently used in the treatment of several diseases, may cause similar side effects (7,8).

In this article, the clinical and laboratory findings of 14 patients with ICS due to oral GC treatment prescribed by physicians for treatment purposes and topical steroids applied by their parents are presented. The aim of this study was to review the clinical and laboratory findings of patients with ICS and to investigate and demonstrate other rare but important side effects.

## Methods

### Patients

In this retrospective study, all data was obtained from patients' medical records. Only those exposed to high-dose potent GC were included in this study. Anthropometric measurements were recorded. Body mass index (BMI) of the patients was calculated. Height, weight and BMI-standard deviation (SD) score (SDS) of the patients were calculated using "Child metrics" (9). Fourteen patients including nine girls and five boys, aged between 0.19 and 11.89 years, were identified. All patients had been given a high dose of moderate to high potency GC either orally or topically. Those who were exposed to topical potent GC for more than a week or had used oral potent steroids for more than 15 days were included in the study.

Ten patients had been given topical GC, such as clobetasol-propionate, diflucortolone-valerate, MPZ-aceponate and betamethasone exposure. The remaining four had been given MPZ and PZ orally.

The mother of one patient (case 12) had been using an ointment to prevent diaper dermatitis since birth, believing that it contains panthenol. The manufacturer stated that there was no GC in the cream.

In addition to the side effects of steroids in these patients, AI due to suppression of the HPA axis, frequently seen in ICS patients, was investigated. Low-dose adrenocorticotrophic hormone (LD-ACTH) test was performed to investigate AI in all patients except two. These two were not tested because the family of one did not consent and the other patient, with severe thrombocytopenia, could not be tested since intravenous (iv) Synacthen was not available at the time.

The equivalent daily dose (EDD) of GC exposure was calculated in five patients, according to HC equivalence. However, the EDD could not be predicted for those who were exposed to topical steroids. The potency of GCs according to HC was determined (2).

Ophthalmologic examination was performed in seven patients and abdominal ultrasonography was performed in 12 patients. Detailed clinical information about all patients is given in supplemental file.

### Laboratory Investigations

After an overnight fast (in children for at least eight hours and in infants for at least six hours) blood samples were taken. Serum concentrations of glucose, calcium, phosphorus, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, total cholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C) and triglyceride (TG) were measured. Fasting concentrations of serum TC, TG and LDL-C were considered high when these were above 200 mg/dL, 100 mg/dL and 130 mg/dL, respectively. The desirable level of HDL-C was above 40 mg/dL, so the cut-off point was accepted as 40 mg/dL. Serum parathyroid hormone, magnesium, 25-hydroxyvitamin D [25-$(OH)D_3$] and 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$] levels were measured in the two patients with hypercalcemia. Spot urine calcium and creatinine ratio also was calculated.

A 24-hour urine sample and a morning fasting blood sample were obtained from case 12 before treatment was initiated. Blood steroid analysis was performed at Marmara University Medical Faculty Hospital, Biochemistry Laboratory and high-performance liquid chromatography was used in the analysis. In addition, urine steroid metabolites of this infant were measured using quantitative gas chromatography-mass spectrometry in selected ion monitoring at the University of Birmingham, College of Medical and Dental Sciences, Steroid Metabolome Analysis Core, Institute of Metabolism and System Research.

A LD (1 μg) ACTH test was performed in 12 patients (Synacthen 250 μg, iv, Novartis, Basel, Switzerland) for adrenal investigation. A stimulated peak cortisol level on LD-ACTH test of more than 500 nmol/L (equivalent 18 μgr/dL) was considered adequate (10). Lower levels of cortisol (<500 nmol/L) demonstrate AI. In patients who received HC, MPZ or PR with a drug reduction scheme, the LD-ACTH test was performed 4-7 days after the discontinuation of the drug.

### Therapy and Follow-up

Nine of the patients had been treated with HC (15-20 mg/m$^2$/day), and one patient with PR (4-5 mg/m$^2$/day) for 2-3 weeks for prevention of GC withdrawal syndrome. Case 1 had been treated with MPZ (15 mg/m$^2$/d), since a total of 5000 mg MPZ po was given over five days for severe immune thrombocytopenic purpura before admission. HC, MPZ or PR doses were gradually decreased over the 2-3 weeks. According to the age of the patients, LD-ACTH test was re-performed with an interval of one to two months. The HPA axis was considered to be recovered when stimulated cortisol increased above 18 μgr/dL.

Written informed consent was obtained from the parents. The hospital ethics committee approved this study (Zeynep Kamil Women and Children Diseases Training and Research Hospital Clinical Research Ethics Committee, 116/18.12.2019).

### Statistical Analysis

All analysis was done using the Statistical Package for the Social Sciences, version 21 (IBM Inc., Armonk, NY, USA). In order to determine whether the data was normally distributed the Shapiro-Wilk method was used. Descriptive statistics of the data which were normally distributed are summarized as mean ± SD. For non-parametric data summary is given as median (interquartile range: IQR). For all tests, a p-value of less than 0.05 was accepted as statistically significant.

## Results

A clinical summary of the patients is given in Table 1. The median age was 1.76 (11.05) years (0.19-11.89). Mean BMI of the patients was 24.3 ± 7.8 kg/m$^2$, median height was 79.7 (80.23) cm and median weight was 12 (58.5) kg. Mean height SDS of the patients was -0.46 ± 1.32, weight SDS was 1.32 ± 2.1, and BMI SDS was 1.82 ± 2.0. Only one patient had short stature, a girl who received high dose MPZ orally for two years; all other patients had height or length within the expected range. At presentation, six patients (four girls) were obese and three were overweight. However, BMI SDS of the two infants were -1.44 and -1.63. Systolic and diastolic blood pressure was normal in all patients in whom blood pressure could be measured (mean systolic: 108 ± 15 mmHg and mean diastolic: 69 ± 4.9 mmHg).

Five patients were exposed to potent GCs orally, and nine patients via the transdermal route. Median duration of high dose GC exposure was 4 (22.1) (range 1-72) months. In five patients who were exposed orally the equivalent HC daily dose was 93 ± 78 mg/m$^2$/d. However, the EDD could not be estimated in nine patients, since the amount applied to skin and its absorption could not be calculated.

Median basal ACTH and cortisol levels of the patients were 16.9 (22.91) pg/mL and 3.73 (6.37) mcg/dL, respectively. Mean stimulated cortisol was 8.55 ± 6.5 mcg/dL. At presentation, 100% of the infants were found to have AI, while overall 85% of patients had AI.

Persistent AI was detected in case 2 due to adrenal atrophy. On presentation the HPA axis was normal in two patients. However, HPA axis normalization time was not predicted in three patients because they were lost to follow up after initial discharge. In the remainder the normalization of the suppressed HPA axis occurred at a median of 60 (IQR 160; range 30-780) days in nine patients. Patients were followed for a median of 240 (825) days.

Although the majority of the patients' transaminases were within normal range (AST 31.1 ± 14 IU and ALT 35.3 ± 23 IU), these were elevated in two patients. Mean fasting serum TG level was 131 ± 63 mg/dL. Hypertriglyceridemia was detected in six of nine patients whose fasting TG levels were measured. The youngest patient with hypertriglyceridemia was two months old. Hypercholesterolemia and increased LDL-C was found in the same two patients. Low HDL-C was detected in other two patients (Table 2). AI was detected in 12 patients with serum basal and/or stimulated cortisol (Table 2). Bilateral posterior segment cataract was detected in case 1 on ophthalmologic examination. Abdominal

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**Table 1. Clinical findings of the patients with iatrogenic Cushing's syndrome at presentation**

| Patient's number | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Age (years) | 12 | 0.21 | 9 | 0.56 | 0.41 | 2.45 | 1.08 |
| Gender | F | M | F | F | F | M | M |
| Complaint | CS suspicion during treatment of ITP | Puffiness on the face and abdomen | Maculopapular rash | Pubic hair | Rapid weight gain | Hair on the face | Widespread hypertrichosis |
| Causes of exposure and corticosteroid (CS) type | 91250 mgr pulse MPZ was given 2-3 times a month by the pediatrician in one year for ITP treatment | Prednisolone was given by the pediatrician before congenital cataract surgery | MPZ was given by the pediatrician for the treatment skin rashes | Diflucortolone valerate 0.3% and isoconazole nitrate containing cream was used by her mother for the treatment of diaper dermatitis | Diflucortolone valerate 0.3% and isoconazole nitrate containing cream was used by her mother for the treatment of diaper dermatitis | Clobetasol propionate 0.05% containing cream was used by his mother for the treatment of diaper dermatitis | Clobetasol propionate 0.05% containing cream was used by his mother for the treatment of diaper dermatitis |
| Duration of exposure | 24 months | 1.6 months | 1 months | Since birth | Since birth | 7 months | 2 months |
| CS potency/ HC equivalent dose, mg/m$^2$/d | 5 times/ 180 | 4 times/148 | 5 times/165 | 100-150 times/ unpredictable | 100-150 times/ unpredictable | Up to 600 times/ unpredictable | Up to 600 times/ unpredictable |
| CS application route | Oral | Oral | Oral | Topical | Topical | Topical | Topical |
| Weight SDS | 0.04 | 0.08 | 2.75 | 0.74 | 5.23 | -1.25 | -1.93 |
| Height SDS | -3.35 | -1.33 | 0.6 | 0.03 | -0.22 | -1.47 | -1.21 |
| BMI SDS | 1.98 | 1.57 | 2.9 | 0.91 | 6.21 | -0.3 | -1.63 |
| Physical examination findings | Buffalo hump, moon face, central obesity, purple striae | Moon face, central obesity, facial acne, oral and diaper candidiasis | Moon face, central obesity, widespread maculopapular rash in the whole body | Diffuse fine hair in the genital and sacral region | Moon face, central obesity, prominent skin folds | Moon face, central obesity, widespread hypertrichosis | Moon face |
| Ophthalmologic examination | Bilateral posterior segment cataract | Right congenital cataract-left operated | Normal | Not performed | Not performed | Not performed | Not performed |
| Blood pressure (mmHg) | 120/75 | | 110/60 | | | | |

F: female, M: male, CS: Cushing's syndrome, ITP: immune thrombocytopenic purpura, MPZ: methylprednisolone, BMI SDS: body mass index standard deviation score

ultrasonographic examination was performed in 11 patients. Five patients had hepatosteatosis. Two patients with hypercalcemia had nephrocalcinosis. In case 12, all adrenal steroids including androgen precursors and GC and their precursors were found to be decreased in the blood steroid profile. The urine results also showed low androgen precursors, GC and their precursors. TH-aldosterone was slightly increased above normal, but others were normal or low. This profile was not consistent with endogenous CS, but compatible with exogenous administration of GC and subsequent suppression of endogenous GC production.

## Discussion

In this study, clinical and laboratory results of children who were prescribed high-dose and potent GC by a doctor or

| 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|
| 11.56 | 11.89 | 10.88 | 0.46 | 0.19 | 11.4 | 6 |
| F | M | F | F | F | M | F |
| Weight gain | Weight gain, psoriasis | Weight gain | Pubic hair, ichthyosis | Weight gain | Weight gain | Weight gain |
| Clobetasol propionate 0.05% containing cream was used intermittently by physician for the treatment of atopic dermatitis | MPZ contained tablets and clobetasol propionate 0.05% containing cream was used by physician for the treatment of psoriasis | MPZ was given by pediatric neurologist for the treatment of Rasmussen encephalitis | MPZ, dexamethasone, betamethasone valerate were used by the dermatologist for five-day periods, respectively, to treat psoriasis. She was also given dexamethasone nasal drops | A cream for diaper dermatitis, which is thought to contain only panthenol was used by his mother | MPZ was given by the pediatrician for the treatment of allergic asthma | Clobetasol propionate 0.05% containing cream was used by his mother for treatment diaper dermatitis until two months before admission |
| 3 years | 6 years | 2 years | 1 month | 2 months | 1 month | 3 months |
| Up to 600 times/ unpredictable | Up to 600 times/ unpredictable | 5 times | 5 times/30 times/25 times | Unknown | 5 times | Up to 600 times/ unpredictable |
| Topical | Topical and oral | Oral | Topical | Topical | Oral | Topical |
| 2.43 | 2.27 | 4.06 | -2.1 | 2.26 | 2.12 | 1.79 |
| -1.24 | 1.37 | 0.58 | -1.7 | 1.45 | -0.03 | -0.01 |
| 3.12 | 2.08 | 3.74 | -1.44 | 1.58 | 2.47 | 2.22 |
| Moon face, buffalo hump, central obesity, purple striae | Moon face, central obesity, widespread white-yellow plaques in the whole body | Moon face, central obesity, purple striae | Moon face, hepatomegaly fine hair on mons pubis | Moon face, central obesity, prominent skin folds | Moon face, Buffalo hump, central obesity, purple striae | Moon face, central obesity |
| Normal | Normal | Normal | Not performed | Not performed | Normal | Not performed |
| 90/70 | 130/70 | 92/70 | | | 110/70 | |

given them by their parents were investigated. In the present study, six of the 14 patients were younger than two years of age and five of them were exposed to high-dose GC via the transdermal route. Topical steroids were administered to these patients by their parents to prevent or treat diaper dermatitis. An LD-ACTH test was used in all of them except for case 5, and all revealed AI. In infants diagnosed with AI, low potency GC, such as PZ and MPZ, as well as high potency GCs, such as diflucortolone valerate 0.3% and clobetasol propionate 0.05% were used.

It is accepted that GCs, when used traditionally in doses less than 20 mg/day for less than three weeks, will not cause AI. However, it was reported that AI might be seen in adult patients who are exposed to prednisone or an equivalent dose of GCs at doses greater than 20-30 mg/

**Table 2. Laboratory findings in the patients**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Fasting glucose md/dL | 348 | 91 | 168 | 99 | NA | | 99 |
| Insulin, µIU/mL | 17.07 | - | - | 3.5 | NA | | |
| Ca, mg/dL | 9.1 | 10.2 | 8.7 | 10.3 | NA | 9.9 | 10.1 |
| P, mg/dL | 4.8 | 5.9 | 3.5 | 5.5 | NA | 5.4 | 5.5 |
| [25-(OH)$D_3$], ng/mL | 18,93 | 37.10 | | | NA | | |
| [1,25-(OH) $D_3$], pg/mL, 15-90 | | 2.30 | | | NA | | |
| PTH | | < 3 | | | NA | | |
| ALT, U/L | 13 | 67 | 331 | 28 | NA | 41 | 11 |
| AST, U/L | 11 | 44 | 254 | 52 | NA | 17 | 28 |
| ALP, U/L | 99 | | 180 | | NA | 325 | 308 |
| Total cholesterol, mg/dL | 186 | 361 | 142 | 149 | NA | | |
| Triglyceride, mg/dL | 186 | 143 | 270 | 75 | NA | NA | 69 |
| LDL, mg/dL | 87 | 263 | 66 | 98 | NA | | |
| HDL, mg/dL | 62 | 69 | 22 | 36 | NA | | |
| Basal cortisol, µgr/dL | 3.73 | 2.1 | 0.6 | 4.52 | < 1 | < 1 | 7.85 |
| Basal ACTH, pg/mL | < 5 | 7.48 | 15.3 | 19 | < 5 | 19.5 | 37 |
| Stimulated cortisol, µgr/dL | ND | 5.4 | 6.21 | 16.3 | ND | 3 | 9.44 |
| Abdominal USG | Splenomegaly | Bilateral nephrocalcinosis | Hepatosteatosis grade 2 | Normal | NA | Normal | NA |
| Other | BMD-SD L1-L4: -0.2 | | | | | | |
| Normalization time of the HPA axis, day | 40 | None | 150 | 51 | Unknown | 60 | Unknown |
| Therapy | MPZ started as 15 mg/$m^2$/d. Splenectomy for ITP; metformin for hyperglycemia | HC started as 20 mg/$m^2$/d and FC 0.1 mg/d for adrenal atrophy; furosemide and pamidronate for hypercalcemia | HC started as 20 mg/$m^2$/d | None | Parents refused to all investigations and therapy | HC started as 20 mg/$m^2$/d | HC started as 20 mg/$m^2$/d |

HC: hydrocortisone, FC: fludrocortisone, PR: prednisolone, MPZ: methylprednisolone, 25-(OH)$D_3$: 25-hydroxyvitamin D, 1,25-$(OH)_2D_3$: 1,25-dihydroxyvitamin D, PTH: parathormon, AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, TC: total cholesterol, LDL: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, MPZ: methylprednisolone, TG: triglyceride, HC: hydrocortisone, USG: ultrasonography, HPA: hypothalamo-pituitary-adrenal, BMD: bone mineral density, SD: standard deviation, OGTT: oral glucose tolerance test, NA: not applicable, ND: not defined

day for more than five days (11). In addition, studies in adults have shown that high-dose GC therapy can cause HPA axis suppression even with a five day-treatment (12). Different results have been published regarding the recovery times from HPA axis suppression in children. In children with asthma who received PZ for five days, a full improvement in HPA axis was observed in 10 days, whereas in infants who received 12-25 weeks of high dose PZ, this period was 6-12 weeks (13,14). Moreover, it has been demonstrated that the HPA axis returns to normal within 1-2 weeks following discontinuation of PZ treatment for more than six months (15). In this study, it was found that the HPA axis returned to normal between 30 and 780 days.

AI might also occur in patients exposed to topical, inhaled, or intra-nasal GCs, depending on dose and potency (3,4). Patients in this series had been exposed to potent GCs for at least seven months. AI was inevitable due to the patients being children, and half of them were infants, and the vast

| 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|
| | 85 | 87 | 80 | | 73 | |
| 24 | 29.9 | 40.5 | | | | |
| 9.8 | | | | 11.2 | 10.1 | 10.9 |
| | | | | 4.9 | 5.1 | |
| | | | | 24 | | |
| | | | | 21 | | |
| 148 | | | | 3 | | |
| 23 | 46 | 14 | 50 | 22 | 80 | 15 |
| 22 | 31 | 17 | 29 | 28 | 55 | 42 |
| | | | 294 | | 228 | 372 |
| 140 | 230 | 138 | | | 137 | |
| 140 | 102 | 124 | NA | NA | 75 | |
| 70 | 153 | 52 | | | 74 | |
| 42 | 62 | 61 | | | 48 | |
| 3.76 | 6.87 | < 1.0 | < 1.0 | < 1.0 | 11.7 | 12.4 |
| 19 | 16.9 | 9.1 | < 5 | < 5 | 35.2 | 56 |
| 4.57 | 12.6 | 6 | < 1.0 | 1.1 | 18.1 | 21.2 |
| Hepatosteatosis, hepatosplenomegaly | Hepatosteatosis | Hepatosteatosis | Normal | Hepatosteatosis grade 1, nephrocalcinosis at right kidney | Hepatosteatosis grade 2, hepatomegaly | NA |
| | Hyperinsulinism at the OGTT | | | Spot urine Ca/ creatinin ratio: 1.12 | BMD-SD L1-L4:-0.6 | |
| Unknown | 780 | 240 | 30 | 66 | HPA axis was normal | HPA axis was normal |
| HC started as 20 mg/ $m^2$/d | PR equivalent to 20 mg/$m^2$/d hydrocortisone | HC started as 20 mg/$m^2$/d | HC started as 20 mg/ $m^2$/d | HC started as 20 mg/ $m^2$/d | None | None |

majority of them were exposed to topical potent-GCs with unpredictable doseage, due to the route of application.

Topical GCs with mild and moderate potency, used in children to treat atopic dermatitis, have rarely been demonstrated to suppress the HPA axis, even if used for a long time (16,17). However, potent or high-potency topical GCs or combinations of other GCs are well recognized to suppress the HPA axis and cause AI (3,4). In addition, it has been shown that the severity of HPA suppression was negatively correlated with cortisol response in the LD-ACTH test (17). Suppression of the HPA axis is dose dependent after systemic GC treatment (18). In older children, potent GCs usually cause mild to moderate AI, to a greater degree than HC would, but they rarely cause severe AI (16).

Although obesity is an expected finding in patients with CS, seven patients had a BMI SDS < +2 in this study, the three infants and one child . Two of these infants were underweight for their age. The reasons for not gaining weight, such as

malabsorption, food intolerance, or gastroesophageal reflux in these patients could not be investigated. The reason for weight loss may be central AI caused by suppression of the HPA axis by potent GCs. Anorexia, nausea, and weight loss are well-known signs of AI (19,20) and may be the reason for poor weight gain in these infants. In addition, the girl infant was born prematurely, was followed up in the neonatal intensive care unit and had a history of cardiac arrest on the 4th day of her life. She was being followed up with a diagnosis of ichthyosis since birth. On admission, she was still being treated with a number of potent GCs simultaneously by the dermatologist. At presentation there was no sign of catch-up growth. These findings do not permit comment on the underlying reasons for failure to gain weight in infants in this study.

In this study, AI was absent on admission in two patients who were exposed to potent steroids. Clobetazol-containing pomade was not applied to case 13 for two months prior to presentation. Although she was obese, the cause of the absence of AI on LD-ACTH test was the improvement in the HPA axis within these two months. Oral MPZ was given to case 14 for treatment of asthma for a month. A month before his admission, MPZ treatment was abruptly stopped due to weight gain. In this patient, AI was not detected at admission since the duration of MPZ usage was short and it is possible that HPA axis function improved within this one month respite from MPZ therapy. Depending on the dosage of GCs, the route of administration, and the duration of drug administration, complete recovery of the HPA axis after discontinuation may vary from one week to several weeks (12,13,14,15). We hypothesize that the reason that AI was not detected following ICS in these patients was the complete recovery of HPA in the period between discontinuation of GC and their admission to the clinic.

The metyrapone test is not recommended in the diagnosis of AI in children, and is even more strongly discouraged in infants, since it could trigger adrenal crisis. Furthermore, the insulin tolerance test (ITT) is inconvenient in younger children, and in patients with a history of seizure or cardiac insufficiency (21). Since both the ITT and the metyrapone test carry major risks, such as precipating acute AI, corticotrophin analog stimulation test has been introduced. The LD (1 µg of corticotrophin) ACTH stimulation test is easier to perform than the ITT and carries a very low risk of side effects (10,22). In a recent study in adults, the stimulated cortisol values of the 1 mcg ACTH test were compared and it was suggested that the number of false positive patients would be significantly reduced by accepting the stimulated cortisol cut-off value of 401.5 nmol/L (14.55 mcg/dL) instead of 500 nmol/L (18.12 mcg/dL) (23). However, in studies conducted in children, it was stated that values above 500 nmol/L (8) or 550 nmol/L (24) of stimulated cortisol in the 1 mcg ACTH test may exclude AI. In this study, in 10 of the 12 patients tested, the stimulated cortisol response was inadequate, whereas in only two patients, stimulated cortisol was greater than 18 mcg/dL.

It has been shown that GCs increase lipid production in rats and also increase circulating TG, as well as resulting in hepatosteatosis (25). GCs increase the conversion of carbohydrates to fatty acids in hepatocytes, and decrease fatty acid oxidation and also cause an increase in the synthesis of TG in hepatocytes using increased fatty acids (26). In the liver of a baby with AI caused by topical clobetasol propionate, we demonstrated for the first time that macrovesicular fat was present in the liver (6). After our original report, a subsequent study regarding non-alcoholic hepatosteatosis (NAFLD) detected by ultrasonography in infants with ICS was published (5). In the present series, NAFLD was detected in six of 11 patients who had ultrasonographic examination. The youngest patient with NAFLD was 2.2 months old, while the others were older than nine years. The mother of the baby with NAFLD was applying a cream to prevent diaper dermatitis. Blood and urine samples taken before treatment revealed that the synthesis of this patient's endogenous steroids was suppressed. Since this patient had not been prescribed any GC treatment, it was suggested that the cream used for diaper dermatitis contained potent GC. Misuse of a potent corticosteroid was responsible for hepatosteatosis in our patients. Hypertriglyceridemia, as well as an increase in transaminases, are expected findings in patients with NAFLD (26,27). In the present study, increased transaminase associated with hypertriglyceridemia level was detected in an infant without NAFLD. Interestingly, serum lipids and transaminases were normal in another infant with NAFLD. Among the patients presented here, hypertriglyceridemia was detected in two thirds of the patients in whom this was measured. These abnormal lipid profiles might be due to exposure to potent GCs.

Hypercalcemia has been reported in patients with primary or secondary AI (28,29,30). In addition, the reduction of glomerular filtration due to fluid loss in adrenal crisis, acute kidney injury and hypercalcemia were also found in adult patients (31,32,33). Decreased glomerular filtration results in a reduced filtered load of calcium, and increased calcium renal reabsorption occurs, due to volume depletion in AI. Enhanced calcium mobilization from bone in AI also contributes to the development of hypercalcemia. The

postulated mechanism for AI to cause hypercalcemia is through a combination of increased calcium flux into the extracellular space and reduced calcium renal excretion (34). Stanniocalcin, secreted from the adrenal gland, reduces circulating calcium (35). The level of calcium is also reduced due to a decreased production in stanniocalcin in the state of AI. Endogenous GCs decrease the absorption of calcium from the intestines and increase the excretion of calcium in the urine (36). It is postulated that increased calcium absorption is caused by GC insufficiency (37) and GC replacement therapy has been shown to improve hypercalcemia.

In the presented series, hypercalcemia was detected in two infants. Both patients were less than three months old and had been exposed to GCs since the first days of their lives. Vitamin D hypervitaminosis and subcutaneous fat necrosis were not detected in these patients. Nephrocalcinosis was found in both babies. Nephrocalcinosis persisted in one patient until the age of 2.5 years when he was lost to follow-up. CYP24A1 mutation was investigated in this patient and no mutation was detected. The other baby's father also had nephrolithiasis. However, nephrocalcinosis resolved by 30 months of age in this second case. AI due to exposure to exogenous potent GCs may be the cause of nephrolithiasis in these infants.

### Study Limitations

The first limitation was that not all patients were examined for possible side effects of GC, such as intracranial benign hypertension, osteoporosis, myopathy, glaucoma and neuropsychiatric symptoms (depression, mood changes). The second limitation was that not all cases could be followed up for a long time.

## Conclusion

This study highlights, yet again, the high frequency of AI in children exposed to high-dose oral and topical potent GCs. Recovery of HPA axis function from this AI may be as long as 780 days in children. It is notable that NAFLD was detected even in very young infants exposed to potent GC. It should be also kept in mind that hypercalcemia and nephrocalcinosis may be detected following exposure to potent GC. Clinicians should alert parents to the potentially serious side effects, when prescribing GC to their children.

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### Ethics

**Ethics Committee Approval:** The hospital ethics committee approved this study (Zeynep Kamil Women and Children Diseases Training and Research Hospital Clinical Research Ethics Committee, 116/18.12.2019).

**Informed Consent:** Written informed consent was obtained from the parents.

**Peer-review:** Externally peer-reviewed.

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# Endocrine Disruptors and Polycystic Ovary Syndrome: Phthalates

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### What is already known on this topic?

In animal and *in vitro* studies, there are some significant findings suggesting that di-2-ethylhexyl phthalate (DEHP) and/or mono (2-ethylhexyl) phthalate (MEHP) might have a role in the development of polycystic ovary syndrome (PCOS). There are only a few human studies exploring the relationship between PCOS and phthalates in the literature.

### What this study adds?

We found significant correlations between DEHP/MEHP and insulin resistance in adolescents with PCOS, suggesting that phthalates might have a possible effect on energy metabolism in this population.

## Abstract

**Objective:** We aimed to investigate a possible role of the endocrine disruptors phthalates, di-2-ethylhexyl phthalate (DEHP) and mono (2-ethylhexyl) phthalate (MEHP), in polycystic ovary syndrome (PCOS) aetiopathogenesis. We also wished to evaluate the relationship between phthalates and metabolic disturbances in adolescents with PCOS.

**Methods:** A total of 124 adolescents were included. Serum MEHP and DEHP levels were determined by high-performance liquid chromatography. Insulin resistance was evaluated using homeostasis model assessment-insulin resistance, quantitative Insulin Sensitivity Check Index, fasting glucose/insulin ratio, Matsuda index, and total insulin levels during oral glucose tolerance test. Participants were further subdivided into lean and obese subgroups according to body mass index (BMI).

**Results:** Sixty-three PCOS and 61 controls, (mean age 15.2 ± 1.5; range: 13-19 years) were enrolled. Serum DEHP and MEHP concentrations were not significantly different between PCOS and control groups. The mean (95% confidence interval) values of DEHP and MEHP were 2.62 (2.50-2.75) µg/mL vs 2.71 (2.52-2.90) µg/mL and 0.23 (0.19-0.29) µg/mL vs 0.36 (0.18-0.54) µg/mL in PCOS and the control groups respectively, $p > 0.05$. Correlation analysis, adjusted for BMI, showed that both phthalates significantly correlated with insulin resistance indices and serum triglycerides in adolescents with PCOS.

**Conclusion:** Serum DEHP and MEHP concentrations were not different between adolescents with or without PCOS. However, these phthalates are associated with metabolic disturbances such as dyslipidemia and insulin resistance, independently of obesity, in girls with PCOS.

**Keywords:** Phthalate, di-(2-ethylhexyl)-phthalate, mono-(2-ethylhexyl)-phthalate, endocrine disrupter, polycystic ovary syndrome

## Introduction

Phthalates are a group of industrial chemicals that are commonly used as plasticisers in the production of soft toys, flooring, food packaging, paints, plastic bags, medical devices, cosmetics, and air fresheners (1). Di-2-ethylhexyl phthalate (DEHP), the most commonly used phthalate, and its metabolites including mono (2-ethylhexyl) phthalate (MEHP), are known to be endocrine disruptors and have been related to some health problems such as obesity (2), abnormal genital development (3), low semen quality (4), precocious puberty (5), and gynaecomastia (6) in humans.

Polycystic ovary syndrome (PCOS) is a common endocrine disorder characterised by menstrual irregularity, hyperandrogenism (HA) and polycystic ovaries. The

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pathogenesis of the disorder has not yet been fully clarified. In several animal studies, DEHP exposure has been shown to result in prolonged oestrous cycles and decreased ovulation rate (7,8). Davis et al (8) demonstrated that DEHP exposure caused hypo-oestrogenic, hypo-progestinic anovulatory cycles in previously regularly menstruating rats. Morphologically, polycystic ovaries developed in these rats.

There are only a few human studies investigating the relationship between PCOS and phthalates in the literature. However, the reported findings are not in agreement (9,10,11,12).

The aim of the present study was to evaluate serum MEHP and DEHP concentrations in adolescents with PCOS and compare with healthy controls. In addition, we wished to investigate the possible relationship between these endocrine disruptors and metabolic abnormalities in this population.

## Methods

The study was approved by the Ethical Committee of Erciyes University (approval no: 211-159). An informed consent was taken from each adolescent in addition to an informed consent obtained from the parents of the participants.

The study included adolescent girls who presented to our Paediatric Endocrinology Outpatient Clinic because of irregular menstrual bleeding and/or hirsutism between January 2011 and August 2012. An age-matched cohort who had regular menstruation and did not have hirsutism served as the control group. Exclusion criteria included the presence of any major disease, a history of taking insulin sensitising or antiandrogenic medication within the past one year and the current use of any drugs.

The participants partly served as the study population of another study, which has been published previously in which the methods of anthropometric measurements, assays, and the definitions of obesity, PCOS and insulin resistance are given in detail (13). In brief, modified Rotterdam criteria (14) was used for the diagnosis of PCOS and the adolescents who had a body mass index (BMI) ≥95$^{th}$ percentile according to age and sex were defined as obese (15). Subjects required any two of the following three criteria to be present to be diagnosed with PCOS: oligo/anovulation (OA), clinical and/or biochemical evidence of HA and polycystic ovarian morphology (PCOM) on ultrasound, with other endocrinopathies excluded. At the beginning of the study 112 adolescents were diagnosed with PCOS. Twenty-six of the patients had classic phenotype (phenotype 1) with HA, OA and PCOM. Thirty-seven patients had only OA and HA (phenotype 2), 46 patients had only HA and PCOM (phenotype 3) and three patients had only OA and PCOM (phenotype 4). However, as the current Endocrine Society guidelines (16,17) recommend the presence of both HA and chronic anovulation for PCOS diagnosis in adolescence, we excluded the patients with phenotypes 3 and 4. Therefore, in total 63 girls with PCOS were included in the study. All the participants were at least 1-year postmenarcheal at their inclusion into the study. The presence of symptoms of oligomenorrhoea for at least two years was required to be used as a criterion for PCOS diagnosis. Biochemical HA was defined as serum total testosterone concentration ≥55 ng/dL. Hirsutism was diagnosed when Ferriman-Gallwey score was eight or more. When 17-hydroxyprogesterone (17-OH-P) concentrations were above 2 ng/mL, an adrenocorticotropic hormone (ACTH) stimulation test was carried out for differential diagnosis of congenital adrenal hyperplasia. Homeostasis model assessment-insulin resistance (HOMA-IR), Quantitative Insulin Sensitivity Check Index (QUICK-I), fasting glucose/fasting insulin ratio (FGIR), Matsuda index and total insulin levels during oral glucose tolerance test were used for the estimation of insulin resistance and sensitivity indexes (18).

Venous blood samples, for DEHP and MEHP measurement, were taken into glass test tubes to avoid plastic contamination. A clean aluminium foil was used to cover the mouths and surrounds of the tubes to protect the sample from contact with the screw caps and sunlight. The serum was separated by centrifugation at 800 g, and the samples were immediately taken into glass vials to be stored in a freezer at -80 °C until analysis.

The blood samples for the hormonal assays, including serum 17-OH-P, androstenedione, dehydroepiandrosterone sulphate (DHEA-S), total and free testosterone, follicle stimulating hormone (FSH), luteinising hormone (LH), oestradiol, and sex hormone binding globulin (SHBG) were taken in the morning during the early follicular phase (second to fifth day) of a spontaneous menstrual cycle or at any time in patients with amenorrhoea. Progesterone was measured in the second phase of the menstrual period. A chemiluminescence immunoassay method (Siemens Healthcare Diagnostics Products, Llanberis, UK) was used to measure serum prolactin, thyrotropin (TSH), free thyroxine free triiodothyronine, LH, FSH, and oestradiol concentrations. A further chemiluminescence immunoassay method (Siemens Healthcare Diagnostics Inc., Flanders, USA) was used to test plasma cortisol, and serum ACTH and insulin concentrations. Serum concentrations of 17-OH-P, DHEA-S, androstenedione, total testosterone and free testosterone were measured by

a radioimmunoassay method. SHBG concentrations were analysed by immunoradiometric assay.

Routine enzymatic methods on an Abbott Architect c16000 analyser (Abbott Diagnostics, USA) were used to test serum fasting glucose, triglycerides, high-density lipoprotein-cholesterol (HDL-C), total cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations. An oral glucose tolerance test (OGTT) was performed using a dose of 1.75 g glucose/kg body weight (maximum 75 g) in all PCOS patients and obese controls. Venous blood samples were obtained at 0, 30, 60, 90, and 120 minutes to measure plasma glucose and plasma insulin concentrations in the morning, after an overnight fast.

### DEHP and MEHP Measurements

Determination of MEHP and DEHP concentrations was performed on a high-performance liquid chromatography (HPLC) analyzer equipped with an auto-sampler (Hewlett Packard Agilent 1100 Series, Vienna, Austria) and using a UV detector (230 nm). A Spherisorb C18 ODS2 column was used (250 mm x 4.6 mm I.D., 5 µm, Waters, Milford, MA, USA). Separations were performed at room temperature. The mobile phase was orthophosphoric acid 0.1% (acetonitrile [90:10, vol/vol]), and the flow rate was 1 mL/min. For the extractions, to a sample of 200 µL serum were added 400 µL of Na OH 1N, 100 µL of 50% $H_3PO_4$ and 600 µL of acetonitrile. After each addition, the sample was agitated by vortex for 30 seconds. After centrifugation for 10 minutes at 3500 rpm, the supernatant was separated, and the residue was again extracted with 600 µL of acetonitrile. After centrifugation under the same conditions, the collected supernatants were evaporated, reconstituted with 400 µL of mobile phase and injected into the chromatograph. The injection volume was 100 µL. Stock solutions containing DEHP or MEHP (2000 ppm) were prepared by dissolving a weighed amount of substance in acetonitrile. Standard solutions were prepared by dilution of the above stock solutions with the mobile phase and by varying the concentration in the range 0.05-5.0 ppm (6,19). The concentrations of DEHP and MEHP in the samples were calculated by using the calibration curve of the peak area prepared for DEHP and MEHP standards. The detection limits were determined as 0.05 ppm for DEHP and as 1 ppm for MEHP.

The retention times for DEHP and MEHP were 23 minutes and 3.7 minutes, respectively. Recovery studies were performed on blank samples of serum, and the mean ± SD percentage recoveries were found to be 92 ± 1.12% for DEHP and 99 ± 1.10% for MEHP on 20 occasions. Between-run coefficient of variation (CV) were 6.44 ± 0.12% for DEHP and 8.03 ± 1.05% for MEHP. Within-day precisions were 8.75 ± 0.43% CV for DEHP and 4.83 ± 0.21% for MEHP. DEHP and MEHP were purchased from Merck (Merck GmbH, Hohenbrunn, Germany) and Cambridge Isotope Laboratories (Cambridge Isotope Laboratories Inc., Andover, MA, USA), respectively. Acetonitrile (HPLC grade) and all other analytical-grade reagents were obtained from Sigma-Aldrich Co. (Sigma-Aldrich Co., St Louis, MO, USA).

### Statistical Analysis

The IBM SPSS Statistics, version 21.0, statistics program was used to perform all statistical analyses (IBM Inc., Armonk, NY, USA). Data are given as frequencies or means with 95% confidence intervals. The distributions of continuous variables were analysed in terms of skewness and kurtosis and were transformed logarithmically, when appropriate. We used t-test to test differences between the groups. Mann-Whitney U was used for variables without normal distributions. The comparison of prevalences was performed by using chi-square test. We used correlation tests (Pearson or Spearman as appropriate) to analyse the relationship between the parameters. The value of $p < 0.05$ was taken for statistical significance.

## Results

Subjects with PCOS consisted of 63 adolescent girls; 36 obese and 27 lean with a mean age of 15.3 ± 1.3 (range: 13-19 years). The control group consisted of 61 age-matched healthy female adolescents (35 obese and 26 lean). The clinical and laboratory characteristics of the study population are shown in Tables 1 and 2. Serum DEHP and MEHP concentrations were not different between adolescent girls with or without PCOS (Table 2). In bivariate correlation analysis, correlations were investigated between the phthalates, DEHP and MEHP, and the following parameters: age, FSH, LH, oestradiol, progesterone, total and free testosterone, androstenedione, 17-OH-P, DHEA-S, BMI, waist circumference, glucose, triglyceride, HDL, low-density lipoprotein, total cholesterol, ALT, AST, insulin, HOMA-IR, QUICKI, Matsuda index, FGIR, and total insulin levels during OGTT. Serum MEHP and DEHP levels were correlated ($r = 0.32$, $p = 0.02$). We did not find any correlations between DEHP or MEHP and androgens, sex steroids and gonadotropins in either the entire group or in the PCOS subgroup. In contrast, significant correlations were present between MEHP or DEHP and insulin resistance indices, as well as serum lipids, in patients with PCOS. The parameters with statistically significant correlations with MEHP or DEHP in the PCOS group are shown in Table 3. These correlations were even more pronounced in the obese PCOS subgroup (Table 4). To eliminate the possible effect of obesity, we performed correlation analysis after adjustment for BMI in adolescents with PCOS, which revealed that both

**Table 1. Clinical features of adolescents with and without polycystic ovary syndrome**

| | Entire group (n = 124) | | p |
|---|---|---|---|
| | PCOS | Controls | |
| Age, (years) | 15.4 (15.1-15.7) | 14.9 (14.5-15.4) | NS |
| Ferriman-Gallwey score* | 14.2 (12.4-16.4) | 2.69 (2.20-3.31) | < 0.001 |
| Acne (%) | 38.1 | 19.7 | 0.030 |
| Acanthosis nigricans (%) | 31.1 | 18.0 | NS |
| BMI (kg/m$^2$) | 27.3 (25.5-28.9) | 25.6 (24.1-27.1) | NS |
| Waist circumference (cm) | 87.1 (82.9-86.6) | 83.5 (79.9-87.2) | NS |
| Body fat percentage (%) | 36.2 (33.4-38.6) | 34.6 (30.3-38.9) | NS |
| Systolic BP (mmHg) | 115.2 (111.5-119.1) | 109.7 (105.1-114.5) | NS |
| Diastolic BP (mmHg) | 72.6 (69.9-75.2) | 73.8 (69.7-78.2) | NS |

Data are given as mean [95% confidence interval (CI)], (%), or *geometric mean (95% CI) for log-transformed variables, PCOS: polycystic ovary syndrome, NS: not significant, BMI: body mass index, BP: blood pressure

**Table 2. Laboratory measurements and serum di-2-ethylhexyl phthalate and mono (2-ethylhexyl) phthalate concentrations in adolescents with and without polycystic ovary syndrome**

| | PCOS (n = 63) | Controls (n = 61) | p |
|---|---|---|---|
| Phthalates and hormone profiles | | | |
| DEHP (µg/mL) | 2.62 (2.50-2.75) | 2.71 (2.52-2.90) | NS |
| MEHP (µg/mL)* | 0.23 (0.19-0.29) | 0.36 (0.18-0.54) | NS |
| FSH (mIU/mL)* | 5.51 (4.96-6.12) | 5.4 (4.3-6.5) | NS |
| LH (mIU/mL) | 10.73 (9.32-12.15) | 4.4 (2.5-7.8) | **0.009** |
| Estradiol (pg/mL)* | 54.89 (49.21-61.23) | 65.7 (45.9-94.1) | NS |
| Progesterone (ng/mL)* | 0.66 (0.52-0.84) | 0.6 (0.2-1.8) | NS |
| 17-OH-Progesterone (ng/mL) | 2.01 (1.71-2.31) | 1.0 (0.8-1.4) | NS |
| Androstenedione (ng/mL) | 2.53 (2.18-2.88) | 1.3 (1.1-1.6) | **< 0.001** |
| DHEA-S (ng/mL) | 2517.9 (2246.2-2789.5) | 1909.1 (1612.3-2206.0) | **0.006** |
| Total testosterone (ng/dL) | 101.6 (89.5-113.6) | 40.5 (33.7-47.2) | **< 0.001** |
| Free testosterone (pg/mL) | 2.78 (2.32-3.23) | 1.9 (1.7-2.1) | **0.009** |
| SHBG (nmol/L)* | 24.11 (18.73-31.02) | 26.4 (19.5-33.4) | NS |
| Liver enzymes | | | |
| Aspartate aminotransferase (U/L)* | 22.80 (20.74-25.07) | 22.3 (20.6-23.9) | NS |
| Alanine aminotransferase (U/L)* | 20.66 (18.09-23.61) | 19.2 (17.2-21.5) | NS |
| Serum lipids | | | |
| Triglycerides (mg/dL)* | 96.54 (85.65-108.81) | 108.7 (95.3-122.2) | NS |
| HDL-cholesterol (mg/dL) | 43.83 (41.25-46.42) | 46.1 (42.4-49.8) | NS |
| LDL-cholesterol (mg/dL) | 86.47 (80.33-92.61) | 86.2 (77.9-94.4) | NS |
| Total cholesterol (mg/dL) | 150.8 (143.5-158.2) | 155.5 (146.7-164.4) | NS |
| Insulin resistance | | | |
| Fasting glucose (mg/dL) | 82.72 (80.45-84.98) | 86.1 (82.9-89.3) | NS |
| HOMA-IR * | 2.95 (2.33-3.73) | 3.3 (2.5-4.3) | NS |
| Matsuda index* | 3.57 (2.87-4.43) | 3.3 (2.7-4.1) | NS |
| QUICK-I | 0.33 (0.326-0.35) | 0.32 (0.31-0.33) | NS |
| FGIR* | 5.36 (4.32-6.65) | 5.16 (4.05-6.56) | NS |

Data are given as mean [95% confidence interval (CI)], or *geometric mean (95% CI) for log-transformed variables.

DEHP: di-2-ethylhexyl phthalate, MEHP: mono (2-ethylhexyl) phthalate, FSH: follicle-stimulating hormone, LH: luteinising hormone, NS: not significant, DHEA-S: dehydroepiandrosterone sulphate, SHBG: sex hormone binding globulin, HDL: high-density lipoprotein, LDL: low-density lipoprotein, HOMA-IR: homeostasis model assessment-insulin resistance, QUICK-I: Quantitative Insulin Sensitivity Check Index, FGIR: fasting glucose/fasting insulin ratio

phthalates remained significantly correlated with insulin resistance indices and serum triglycerides (Table 5). There was no correlation between DEHP or MEHP and any other parameters, neither in the control group nor in the obese non-PCOS subgroup, when taken separately.

## Discussion

Although the evidence is limited, accumulating data are indicating the potential role of endocrine disruptors in the pathogenesis of adipogenesis and diabetes (20). It has been reported that DEHP exposure and insulin resistance

**Table 3. Correlations between mono (2-ethylhexyl) phthalate or di-2-ethylhexyl phthalate and metabolic parameters in adolescents with polycystic ovary syndrome**

| | DEHP | | MEHP | |
|---|---|---|---|---|
| | r | p | r | p |
| Triglyceride | **0.417** | **0.020** | -0.050 | 0797 |
| Glucose | 0.168 | 0.359 | **0.443** | **0.014** |
| Matsuda index | **-0.405** | **0.018** | 0.024 | 0.896 |
| HOMA-IR | **0.515** | **0.003** | 0.216 | 0.251 |
| QUICK-I | **-0.496** | **0.003** | 0.056 | 0.759 |
| Total insulin during OGTT | **0.386** | **0.024** | 0.078 | 0.672 |
| Cholesterol | **0.405** | **0.024** | -0.052 | 0.788 |

HOMA-IR: homeostasis model assessment-insulin resistance, QUICK-I: Quantitative Insulin Sensitivity Check Index, DEHP: di-2-ethylhexyl phthalate, MEHP: mono (2-ethylhexyl) phthalate, OGTT: oral glucose tolerance test

**Table 4. Correlations between mono (2-ethylhexyl) phthalate or di-2-ethylhexyl phthalate and metabolic parameters in obese adolescents with polycystic ovary syndrome**

| | DEHP | | MEHP | |
|---|---|---|---|---|
| | r | p | r | p |
| Glucose | 0.155 | 0.492 | **0.454** | **0.034** |
| Triglyceride | **0.493** | **0.020** | -0.221 | 0.323 |
| Insulin | **0.683** | **< 0.001** | 0.063 | 0.782 |
| Matsuda index | **-0.508** | **0.016** | 0.058 | 0.797 |
| HOMA-IR | **0.683** | **< 0.001** | 0.124 | 0.584 |
| QUICK-I | **-0.635** | **0.001** | 0.058 | 0.797 |
| Total insulin during OGTT | **0.482** | **0.023** | 0.067 | 0.766 |
| Cholesterol | **0.524** | **0.012** | -0.101 | 0.654 |
| FGIR | **-0.653** | **0.001** | -0.257 | 0.248 |

DEHP: di-2-ethylhexyl phthalate, MEHP: mono (2-ethylhexyl) phthalate, HOMA-IR: homeostasis model assessment-insulin resistance, QUICK-I: Quantitative Insulin Sensitivity Check Index, OGTT: oral glucose tolerance test, FGIR: fasting glucose/fasting insulin ratio

**Table 5. Correlations of mono (2-ethylhexyl) phthalate and di-2-ethylhexyl phthalate with metabolic parameters adjusted for body mass index in adolescents with polycystic ovary syndrome**

| | DEHP | | MEHP | |
|---|---|---|---|---|
| | r | p | r | p |
| Waist circumference | 0.365 | 0.056 | 0.337 | 0.079 |
| Glucose | **0.460** | **0.014** | **0.465** | **0.013** |
| Triglyceride | **0.521** | **0.004** | 0.169 | 0.391 |
| Insulin | **0.495** | **0.007** | 0.298 | 0.123 |
| Matsuda index | **-0.439** | **0.020** | -0.133 | 0.501 |
| HOMA-IR | **0.558** | **0.002** | **0.631** | **< 0.001** |
| Total insulin during OGTT | **0.548** | **0.003** | 0.275 | 0.157 |

HOMA-IR: homeostasis model assessment-insulin resistance, OGTT: oral glucose tolerance test, DEHP: di-2-ethylhexyl phthalate, MEHP: mono (2-ethylhexyl) phthalate

are associated in adolescents (21). Insulin resistance, which is a well-known aetiological factor in PCOS development, is reported in 50-80% of women with PCOS (22,23). To our knowledge, this is the first report of an association of phthalates with insulin resistance in a PCOS cohort in the English literature.

In the current study, we found significant correlations between DEHP and insulin resistance and dyslipidaemia, suggesting that DEHP might have a direct or indirect effect on energy metabolism. This association was even stronger in the PCOS group and absent in the control group. Interestingly, in the PCOS group, after adjustment for BMI, the correlations of both DEHP and MEHP with insulin resistance indices and serum triglycerides remained strikingly significant. These findings lead us to think that there might be another mechanism, other than obesity, by which phthalates affect insulin resistance-one of the key pathologies in PCOS development - and dyslipidaemia in patients with PCOS.

In animal and *in vitro* studies, there are some significant findings suggesting that phthalates might have a role in the aetiopathogenesis of PCOS (7,8,24,25,26,27,28,29,30). Previous studies showed that DEHP exposure in rats results in prolonged oestrous cycles and decreased ovulation rates, altered circulating FSH, LH, testosterone, and progesterone levels (7,8,24,25). DEHP exposure resulted in suppressed oestradiol levels in granulosa cells which could not stimulate the LH surge necessary for ovulation. This consequently caused hypo-estrogenic anovulatory cycles and polycystic ovaries in adult female rats (8). Moreover, MEHP stimulates basal steroidogenesis (25), inhibits progesterone production in rat granulosa cells and decreases aromatase concentrations causing a hyperandrogenaemic, hypo-progestinic milieu which is similar to that seen in PCOS (29). In humans, in a study on granulosa-lutein cells from women planning *in vitro* fertilisation, MEHP was reported to inhibit oestradiol production and affected steroidogenesis similarly to the findings in rats (31).

Based on the results of these studies and given the fact that hyperfunctioning of theca and relative hypofunctioning of granulosa cells accompany the acyclicity of PCOS in humans, we thought that it was worthwhile investigating the PCOS-phthalate relationship in humans. In another paper inspiring us to conduct this study, Svechnikova et al (32) reported that DEHP exposure in female rats caused increased LH response to GnRH by pituitary and inhibition of progesterone production in granulosa cells.

There are only four studies in the literature, investigating PCOS-phthalate relationship in humans, which report conflicting findings (9,10,11,12). In the present study, we did not find any relation between DEHP or MEHP and gonadotropins or sex hormones. It is known that the effects of phthalates on hormones are both complex and multifactorial. Hence, considering the results of experimental studies, one might suggest that random measurements of serum concentrations of these compounds are not enough to elucidate a causal relationship between DEHP/MEHP and the disorder. Phthalates have been reported to be associated with gynecomastia and the risk of shortened anogenital distance (6,33). It may be argued that higher levels of DEHP/MEHP might mask PCOS findings, due to its antiandrogenic effects, which could be investigated in a non-hyperandrogenic PCOS group, such as in patients with Rotterdam criteria phenotype 4 (14).

In a very recent study, Jin et al (10) reported significantly increased DEHP levels in follicular fluid of women with PCOS compared to controls. They also showed that DEHP is associated with lower pregnancy rate and DEHP exposure resulted in significant increase in androgen production in human granulosa cells. We speculate that, in our cohort, DEHP might have a role in PCOS development through insulin resistance at the follicular level, which is not reflected in the serum concentrations of the participants.

### Study Limitations

One of the limitations of this study is its cross-sectional design, which does not allow for the identification of any causal relationship. The second limitation was that we measured serum FSH, LH, progesterone, and phthalate levels at different times of the menstrual cycle in patients, which might have also masked some associations.

## Conclusion

In this study, serum DEHP and MEHP concentrations in adolescents with PCOS were not different from those in their non-PCOS peers. However, both DEHP and MEHP significantly correlated with insulin resistance and metabolic disturbances in patients with PCOS. We believe that further well-designed studies are needed to evaluate the possible role of phthalates in PCOS development in humans.

### Ethics

**Ethics Committee Approval:** The study was approved by the Ethical Committee of Erciyes University (approval no: 211-159).

**Informed Consent:** An informed consent was taken from each adolescent in addition to an informed consent obtained from the parents of the participants.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Leyla Akın, Concept: Leyla Akın, Mustafa Kendirci, Design: Leyla Akın, Mustafa Kendirci, Data Collection or Processing: Leyla Akın, Nihal Hatipoğlu, Analysis or Interpretation: Figen Narin, Ferhan Elmalı, Leyla Akın, Literature Search: Leyla Akın, Figen Narin, Selim Kurtoğlu, Writing: Leyla Akın, Mustafa Kendirci.

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# Association of Exosomal miR-34a with Markers of Dyslipidemia and Endothelial Dysfunction in Children and Adolescents with T1DM

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### What is already known on this topic?

Dyslipidemia and endothelial dysfunction are common disorders and major predisposing factors for atherosclerosis and cardiovascular diseases in patients with type 1 diabetes mellitus (T1DM). However, their pathophysiology in children and adolescents with T1DM is still under evaluated.

### What this study adds?

Association of exosomal micro-RNA 34a serum expression with markers of dyslipidemia and endothelial dysfunction was identified in children and adolescents with T1DM, suggesting its role in regulation of lipid metabolism and endothelial function in T1DM.

## Abstract

**Objective:** Dyslipidemia and endothelial dysfunction are common disorders and major causative factors for atherosclerosis in patients with type 1 diabetes mellitus (T1DM). However, their pathophysiology in young patients with T1DM is still under evaluated. We aimed, for the first time, to assess the expression of exosomal micro-RNA 34a (miR-34a) in serum of children and adolescents with T1DM and correlate this expression with markers of dyslipidemia and endothelial dysfunction.

**Methods:** The study included 120 T1DM patients and 100 control subjects. Assessment of miR-34a was performed using quantitative real-time polymerase chain reaction. Lipid profile was assessed on an automated analyzer and serum endoglin and intracellular adhesion molecule (ICAM) concentrations were measured using immunometric methods.

**Results:** Relative expression of miR-34a and serum endoglin and ICAM concentrations were higher in patients than controls ($p = 0.001$) and in patients with dyslipidemia (42 patients) compared to patients without dyslipidemia (78 patients) ($p = 0.01$). Linear regression analysis revealed a strong independent association between exosomal miR-34a expression and total cholesterol, low-density lipoprotein, serum endoglin and serum ICAM after adjustment for other cofactors. The utility of miR-34a as an indicator for associated dyslipidemia was tested using receiver operator characteristic curve analysis which revealed area under the curve: 0.73 with confidence interval: 0.63-0.83 and $p = 0.001$.

**Conclusion:** This was the first study to show the altered expression of exosomal miR-34a among children and adolescents with T1DM. Moreover, association of miR-34a with markers of dyslipidemia and endothelial dysfunction was identified, suggesting that it could play a role in regulation of lipid metabolism and endothelial function in T1DM.

**Keywords:** miR-34a, dyslipidemia, endothelial dysfunction, type 1 diabetes mellitus, endoglin, intracellular adhesion molecule

## Introduction

Type 1 diabetes mellitus (T1DM) is a complex, multifactorial, autoimmune illness and continues to increase in prevalence among children and adolescents (1). T1DM affects about 35 million persons all over the world with annual increase ranging between 3-5% (2).

Dyslipidemia and endothelial dysfunction are very common metabolic abnormalities in these patients (3,4). Both are major causative factors for atherosclerosis which is a major

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precursor of cardiovascular disease (CVD), the leading cause for morbidity and mortality in T1DM (5). However, the pathophysiology underlying the occurrence of dyslipidemia and endothelial dysfunction in young patients with T1DM remains unclear. It is suggested that the pathogenesis involves an interaction between genetic, environmental and eventually epigenetic factors. Epigenetic factors, including microRNAs, not only represent a key for understanding the complexity of vascular diseases in these patients but also represent a new field of investigation to discover new diagnostic and prognostic markers (6).

MicroRNA (miRNA, miR) is a class of small, noncoding RNA, which play a significant role in regulating gene expression. Therefore, miRNAs could contribute to the pathogenesis of a number of different physiological and pathological processes (7). Exosomes are nanovesicles originating from all cells, whether healthy or diseased, and can be found in all body fluids. The lipid bilayer surrounding exosomes enable exosomal-enclosed miRNAs to be stably expressed in body fluids much more so than free circulating miRNAs. Consequently, recent studies have focused on exosomal-enclosed miRNAs as contributing factors in various human diseases (8).

Accumulating data have demonstrated that miR-34a contributes to β-cell apoptotic pathways, suggesting a role in T1DM (9). Among different miRNA candidates, miR-34a is of special interest regarding lipid metabolism and endothelial function because of its interaction with many genes involved in both pathways (10). Genomic data for exosomal miR-34a were retrieved from the extracellular vesicles miRNA database (11,12), while the predicted miRNA target genes were analyzed by using DIANA-miRPath v1.1 webserver (13). Accordingly, its role in adipogenesis, atherosclerosis progression, inflammation and CVD development and progression, has been suggested.

The current study aimed, for the first time, to assess the expression of exosomal miR-34a in the serum of children and adolescents with T1DM and to evaluate the association between exosomal miR-34a expression and markers of dyslipidemia, such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG), as well as the serum levels of markers of endothelial dysfunction, including serum endoglin and intracellular adhesion molecule (ICAM).

## Methods

This study is a pilot cross-sectional study. A total of 120 T1DM patients, with disease duration more than five years, were randomly selected from Diabetes, Endocrine and Metabolism Pediatric Unit, Pediatric Department, Faculty of Medicine, Cairo University, with respect to inclusion and exclusion criteria (see below). Confirmation of T1DM diagnosis was based on criteria of American Diabetes Association (ADA) (14).

- Exclusion criteria in the current study included: any type of diabetes other than T1DM; T1DM with any microvascular complication; hypertension; heart, liver, or renal insufficiency; acute diabetic complications; systemic inflammatory diseases; other endocrine disorders; neoplastic disorders; and family history of dyslipidemic diseases.

- One hundred age and sex matched healthy subjects served as control group and were recruited from the National Research Centre (NRC) during regular check-up.

- The included patients were subsequently categorized into patients with dyslipidemia (42 patients) and patients without dyslipidemia (78 patients). According to the ADA, dyslipidemia was defined by the presence of one or more of the following criteria: TC ≥200 mg/dL, LDL-C ≥130 mg/dL, HDL-C ≤35 mg/dL, and TG ≥150 mg/dL (15).

- Informed consent was obtained from each participant. This study was approved by the Ethics Committee of the NRC, under approval number 16/285, in accordance with the Declaration of Helsinki 2015.

All participants were subjected to full history taking and full clinical examination.

### Biochemical Analysis

- Venous blood samples were collected from all subjects after 12 hours of overnight fast. Serum was separated and parameters of dyslipidemia including, TC, TG, HDL and LDL levels were quantified using Erba Mannheim XL300 Chemistry Analyzer (ERBA Diagnostics Mannheim, Medical EXPO, India).

- Markers of endothelial dysfunction including serum endoglin and ICAM concentrations were assessed using enzyme linked immunosorbent assay sandwich technique (Quantikine, R&D Systems, Minneapolis, USA).

Assessment of exosomal miR-34a was done by quantitative reverse transcriptase real-time polymerase chain reaction (qRT PCR) technique:

Exosomes were isolated from eight hundred microliters of serum according to exoRNeasy Serum/Plasma midi kit (Qiagen, Hilden, Germany). The characteristics of isolated exosomes were confirmed by transmission electron microscopy (16). Isolation, purification and elution of

exosomal RNA was done according to exoRNeasy Serum/Plasma midi kit's protocol and 3.5 microns of synthetic spike in control Cel-miR-39 was added to each sample as the internal control. Concentration of isolated RNA was assessed using a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA). Complementary DNA (cDNA) was synthesized using a miScript reverse transcription kit (Qiagen, Hilden, Germany) and then all specimens were stored at -80 °C. Quantitative PCR was run on Applied technologies, Stratagene Mx3000P using miScript SYBR green PCR kit (Qiagen, Hilden, Germany) for detection of miR-34a (ID: MS00003318). The relative expression of miRNA was described as fold change using the calculated formula; $2^{-\Delta\Delta CT}$. Methods have been described in detail elsewhere (8).

### Statistical Analysis

Patients' clinical and laboratory quantitative and qualitative data were presented as mean ± standard deviation and frequencies respectively, while the levels of relative miR-34 expression were presented as median [interquartile range (IQR)]. Non-parametric tests were used to evaluate expression difference of miR-34a between patients and healthy controls and between patients' groups. To assess the relationships between exosomal miR-34a and different patients' parameters, Pearson's correlation and linear regression were performed. The utility of miR-34a as an indicator for associated dyslipidemia among patients was tested using receiver operating curve (ROC) technique and area under the curve (AUC) was calculated. All tests were two-sided and a $p < 0.05$ was considered statistically significant.

## Results

The clinical, demographic and routine laboratory test results for patients and healthy controls are presented in Table 1. Both patients and controls were matched for age, gender and body mass index (BMI). Glycated hemoglobin (HbA1c), TC, TGs and LDL were significantly increased in patients compared to controls, while HDL showed no significant difference between the two groups.

Regarding miRNA expression, miR-34a showed significant higher expression among T1DM patients (median: 22.6, IQR: 4.2-111.7 fold change) than healthy controls (median: 6, IQR: 0.1-12 fold change) ($p = 0.001$) (Figure 1). Frequency of patients with miR-34a overexpression, defined as expression more than two-fold change, among T1DM patients was 90%. Association of miR-34a expression and T1DM was confirmed using logistic regression analysis after adjustment for age, gender and BMI ($p = 0.01$).

Comparison between patients regarding associated dyslipidemia showed that the relative expression of exosomal miR-34a was higher in patients with dyslipidemia (median: 78; IQR: 18.1-2388 fold change) compared to patients without dyslipidemia (median: 4.8; IQR: 3.7-34.2 fold change) ($p = 0.001$) (Figure 1). There was no significant difference between patients with dyslipidemia and those without dyslipidemia regarding age, gender, BMI and HbA1c. Disease duration, serum TC, TGs and LDL-C were significantly higher in patients with dyslipidemia compared to patients without dyslipidemia, while HDL showed no significant difference between the two groups (Table 2). The most common disorder of dyslipidemia was hypercholesterolemia (95%). Prevalence of high TG, low HDL-C and high LDL-C was 9.5%, 12% and 45% respectively.

**Table 1. Clinical, demographic and biochemical laboratory data of patients and controls**

| Characteristic | Controls (number = 100) | T1DM (number = 120) | p value |
|---|---|---|---|
| **Age (years)** | 12.1 ± 2.8 | 13.5 ± 3.2 | 0.6 |
| **Gender (male/female)** | 41/59 | 54/66 | 0.5 |
| **BMI (kg/m²)** | 18.1 ± 3.1 | 18.9 ± 2.3 | 0.7 |
| **Diabetes duration (years)** | - | 7.6 ± 1.9 | - |
| **HbA1c (%)** | 4 ± 0.6 | 8 ± 1.2 | 0.001 |
| **Total cholesterol (mmol/L)** | 127 ± 25 | 181 ± 42 | 0.001 |
| **Triglycerides (mg/dL)** | 75 ± 35 | 88 ± 30.7 | 0.005 |
| **HDL-C (mg/dL)** | 52 ± 4.4 | 53 ± 8.8 | 0.2 |
| **LDL-C (mg/dL)** | 80 ± 19.1 | 106 ± 33 | 0.001 |
| **Serum endoglin (ng/mL)** | 28.5 (16.8-43) | 31 (19-75) | 0.01 |
| **ICAM (ng/mL)** | 231 (129-321) | 293 (240-693) | 0.001 |

T1DM: type 1 diabetes mellitus, BMI: body mass index, HbA1c: glycated hemoglobin, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, ICAM: intracellular adhesion molecule

Levels of serum endoglin and serum ICAM were significantly higher in patients with T1DM than healthy subjects (p = 0.01 and p = 0.001, respectively) (Table 1) and higher in T1DM patients with dyslipidemia compared to patients without dyslipidemia (p = 0.01) (Table 2). Serum endoglin and serum ICAM showed no significant correlation with age, BMI, disease duration, glycated hemoglobin and parameters of the lipid profile (Table 3).

Pearson's correlation revealed positive correlation between miR-34a and both serum endoglin and serum

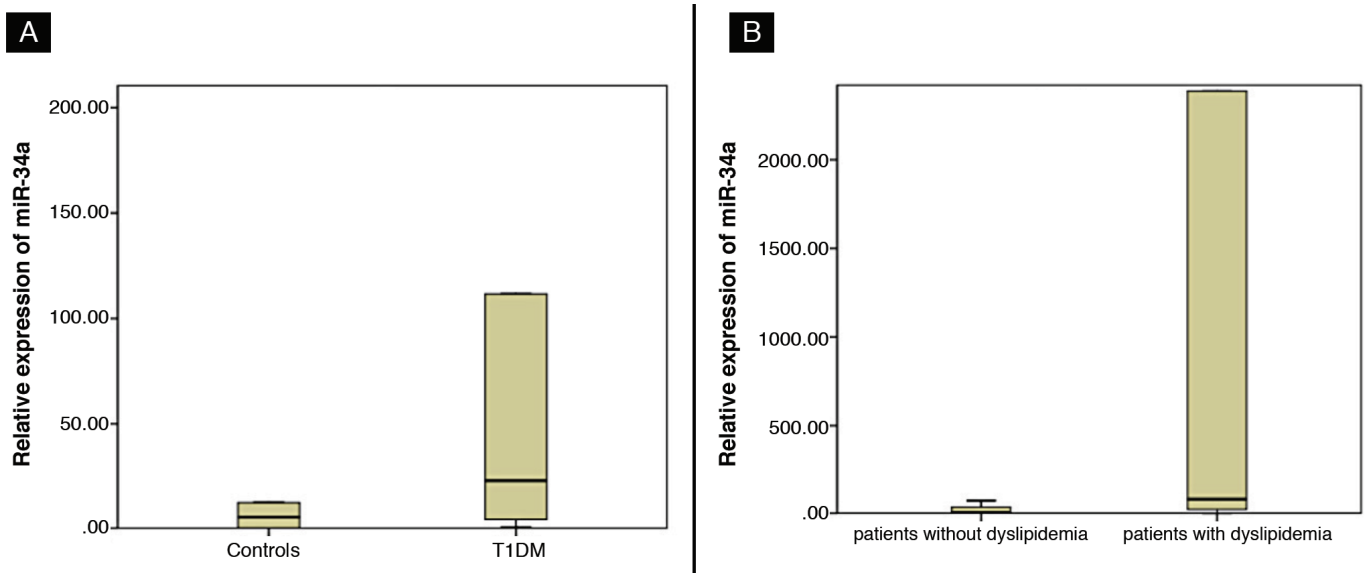


**Figure 1.** Expression of exosomal micro-RNA 34a (miR-34a) among studied groups. A) Shows significant higher expression of miR-34a in patients with type 1 diabetes mellitus [median: 22.6, interquartile range (IQR): 4.2-111.7] compared to healthy controls (median: 6, IQR: 0.1-12). B) Shows increased expression of exosomal miR-34a in patients with dyslipidemia (median: 78, IQR: 18.1-2388) compared to patients without dyslipidemia (median: 4.8, IQR: 3.7-34.2)

*miR-34a: micro-RNA 34a, T1DM: type 1 diabetes mellitus*

**Table 2. Comparison between patients with dyslipidemia and patients without dyslipidemia**

| Parameter | Patients without dyslipidemia (78 patients) | Patients with dyslipidemia (42 patients) | p value |
|---|---|---|---|
| **Age** (years) | 13.6 ± 3.2 | 13.2 ± 3.4 | **0.5** |
| **Gender** (male/female) | 32/46 | 22/20 | 0.2 |
| **BMI** (kg/m$^2$) | 18.8 ± 3.1 | 17.9 ± 3.6 | 0.9 |
| **Disease duration** (years) | 7.2 ± 1.6 | 8.3 ± 2.2 | 0.03 |
| **HbA1c** (%) | 7.9 ± 1.1 | 8.3 ± 1.2 | 0.1 |
| **Total cholesterol** (mmol/L) | 157 ± 23.2 | 227 ± 20.3 | 0.001 |
| **Triglycerides** (mg/dL) | 80.3 ± 43 | 103 ± 25.7 | 0.001 |
| **HDL-C** (mg/dL) | 52.6 ± 8.3 | 54 ± 9.7 | 0.2 |
| **LDL-C** (mg/dL) | 87.8 ± 21.4 | 139.5 ± 25.3 | 0.001 |
| **Frequency of disorders** | | | |
| Hypercholesterolemia (> 200 mmol/L) | - | 40/42 | |
| Hypertriglyceridemia (> 150 mg/dL) | - | 4/42 | - |
| Decreased HDL-C (< 35 mg/dL) | - | 5/42 | |
| Increased LDL-C (> 130 mg/dL) | - | 19/42 | |
| **Serum endoglin** (ng/mL) | 29 (18-57) | 48 (21-90) | 0.02 |
| **ICAM** (ng/mL) | 342 (234-509) | 435 (346-657) | 0.005 |

BMI: body mass index, HbA1c: glycated hemoglobin, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, ICAM: intracellular adhesion molecule

ICAM concentration, but failed to demonstrate significant association between miR-34a with parameters of lipid profile (Table 4). Linear regression analysis was used to confirm the association of exosomal expression of miR-34a with parameters of lipid metabolism and endothelial dysfunction in patients with T1DM after adjustment for age, gender, BMI and disease duration. This analysis revealed a strong independent association between exosomal miR-34a with TC, serum endoglin and serum ICAM (Table 4). The utility of miR-34a as indicator for associated dyslipidemia was tested using ROC curve which revealed AUC: 0.73 with confidence intervals: 0.63-0.83 (p = 0.001) (Figure 2).

To validate and strengthen our results, the analysis of miRNAs that target different genetic pathways involved in lipid metabolism and endothelial function was done using https://ccb-web.cs.uni-saarland.de/mirtargetlink/index.php. This analysis retrieved miR-34a as one of the three miRNAs that can target both the *hepatocyte nuclear factor 4 (HNF4)* and *sirtuin 1 (SIRT1)* genes that have been identified as playing major roles in lipid metabolism (Figure 3); In addition miR-34a was the only miRNA that targeted the three major genes, vascular endothelial growth factor (VEGF), *SIRT1* and *p53*, involved in endothelial function (Figure 4) (17).

## Discussion

Pathogenesis of associated endothelial dysfunction and dyslipidemia in children and adolescents with T1DM is still under-evaluated. This is the first study to evaluate exosomal miR-34a expression in children and adolescents with T1DM and to correlate this expression with markers of dyslipidemia and endothelial dysfunction in the studied patients.

The role of miR-34a in the development of diabetes is under the spotlight. Accumulating data indicate that miR-34a plays significant roles in glucose sensing, insulin secretion, and increasing sensitivity of β cells to cytokine-induced apoptosis (18,19,20). In the current study, expression of miR-34a was increased in patients with T1DM compared to healthy subjects, suggesting its role in the pathogenesis of T1DM. This is in agreement with other studies that demonstrated over-expression of miR-34a in T1DM (21,22), especially in recent-onset T1DM, compared to high-risk and healthy children (23).

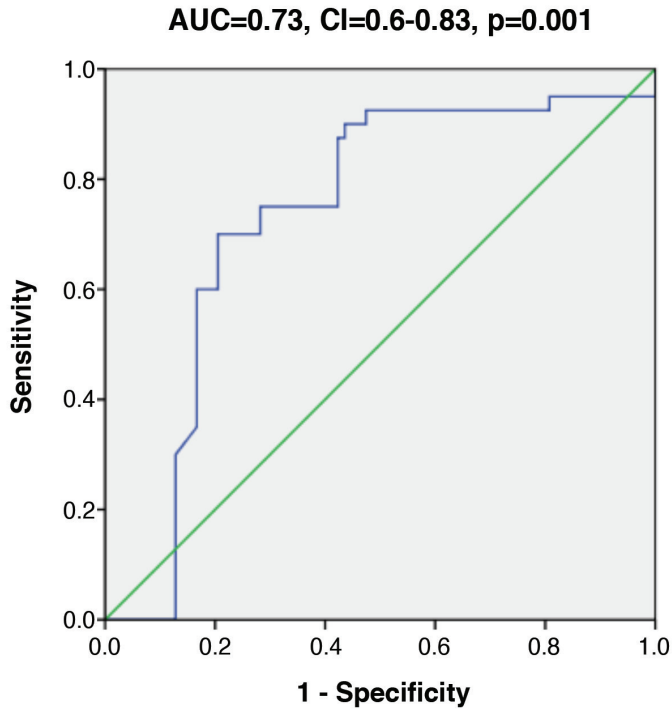


**Figure 2.** Receiver operating curve (ROC) curve of exosomal micro-RNA 34a (miR-34a) in associated dyslipidemia in type 1 diabetes mellitus. ROC curve showed the utility of miR-34a as an indicator of associated dyslipidemia. Area under the curve: 0.73 with confidence intervals: 0.63-0.83 (p = 0.001)

*AUC: area under the curve, CI: confidence interval*

**Table 3. Pearson's correlation of serum endoglin and serum intracellular adhesion molecule with different parameters in type 1 diabetes mellitus patients**

| Parameters | Serum endoglin | | Serum ICAM | |
|---|---|---|---|---|
| | r | p value | r | p value |
| **Age** (years) | -0.1 | 0.1 | -0.1 | 0.2 |
| **BMI** (kg/m$^2$) | 0.3 | 0.09 | 0.09 | 0.1 |
| **Disease duration** (years) | 0.07 | 0.4 | -0.2 | 0.6 |
| **HbA1c** (%) | 0.13 | 0.52 | -0.002 | 0.9 |
| **Total cholesterol** (mmol/L) | 0.09 | 0.1 | -0.03 | 0.73 |
| **Triglycerides** (mg/dL) | -0.06 | 0.6 | 0.03 | 0.75 |
| **HDL-C** (mg/dL) | 0.01 | 0.8 | 0.04 | 0.51 |
| **LDL-C** (mg/dL) | 0.08 | 0.1 | 0.2 | 0.2 |

r: Pearson's correlation coefficient, BMI: body mass index, HbA1c: glycated hemoglobin, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, ICAM: intracellular adhesion molecule

Dyslipidemia is a metabolic disorder commonly associated with T1DM, increasing the risk of CVD (5). In our study, the prevalence of dyslipidemia among patients with T1DM was 35%. El-Bakry et al (24) reported that 64% of Egyptian children and adolescents with T1DM showed association with dyslipidemia. In addition, the prevalence of dyslipidemia in children and adolescents with T1DM is reported to vary from 29% to 66% in cross sectional studies from different countries (25,26,27). The most common types of dyslipidemia among adolescents with T1DM also varies between hypercholesterolemia (24,28), high triglyceridemia (29,30) and high LDL-C (31,32). Differences in sample size, inclusion and exclusion criteria, degree of glycemic control and ethnicity might be the cause of this wide range of prevalences and difference in frequencies of dyslipidemia types in the previous studies.

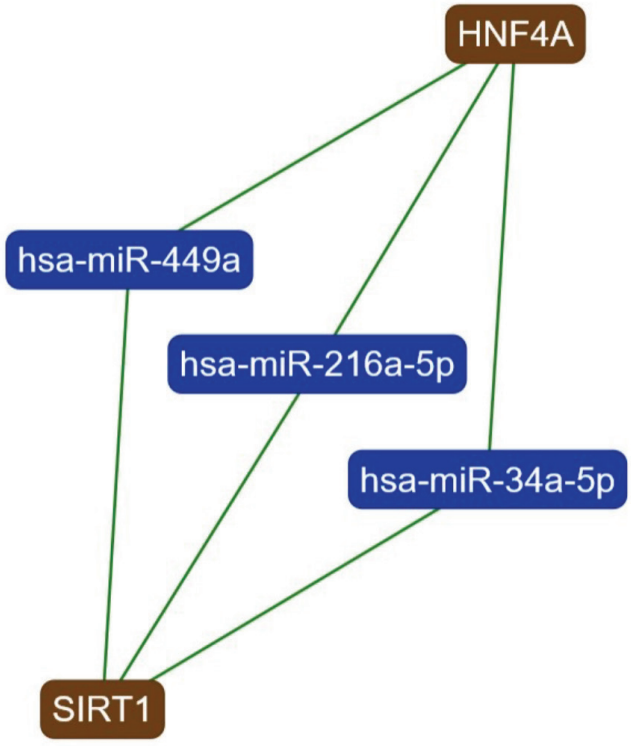


**Figure 3.** Interaction network of genes targeted by micro-RNA 34a (miR-34a) and playing a significant role in lipid metabolism. This analysis was done using (miRTargetLinkdatabase) (https://ccb-web.cs.uni-saarland.de/mirtargetlink/index.php) and retrieved that miR-34a is one of the three miRNAs that can target both *hepatocyte nuclear factor 4 (HNF4)* and the *sirtuin 1 (SIRT1)* genes (green line indicates a strong interaction)

MiR-34a is known to regulate TC and hepatic lipid metabolism through targeting and inhibition of expression of the NAD+-dependent lysine deacetylase *SIRT1*, the antiatherogenic mediator that regulates lipid metabolism and endothelial function (33). MiR-34a also down regulates *HNF4*, a gene which modulates the expression of other genes involved in lipid and glucose metabolism (34). In agreement with the previous data, we have demonstrated for the first time increased miR-34a expression in patients with dyslipidemia compared to those without dyslipidemia. Moreover, miR-34a overexpression was described in different diseases associated with dyslipidemia, particularly non-alcoholic fatty liver disease (33,35) and coronary artery disease (36) and this expression was correlated with disease severity. Linear regression analysis for our results revealed positive association between miR-34a and TC and LDL-C, while no correlation or association was detected between the miRNA and TG. Interestingly, Shen et al (20) reported positive correlation of miR-34a expression with LDL-C and negative correlation with TG in patients with T2DM. However, Xu et al (34) suggested an explanation when they showed that miR-34a regulates hepatic TGs via regulation of *HNF4* expression and its overexpression leads to accumulation of TGs in the liver and subsequently decreasing serum TGs.

Endothelial dysfunction precedes and promotes vascular inflammation and therefore atherosclerosis in T1DM and may be considered as an independent predictor of associated CVD (3). Endoglin (CD105) is a membrane

**Table 4. Pearson's correlation and linear regression analysis of micro-RNA 34a with different parameters of dyslipidemia and endothelial dysfunction**

| Parameters | Pearson's correlation | | Regression analysis | |
|---|---|---|---|---|
| | r | p value | β | p value |
| **Total cholesterol** (mmol/L) | 0.13 | 0.03 | 0.56 | 0.001 |
| **Triglycerides** (mg/dL) | 0.006 | 0.3 | 0.1 | 0.9 |
| **HDL-C** (mg/dL) | 0.12 | 0.07 | 0.1 | 0.03 |
| **LDL-C** (mg/dL) | 0.1 | 0.03 | 0.3 | 0.01 |
| **Serum endoglin** (ng/mL) | 0.6 | 0.001 | 0.65 | 0.001 |
| **Serum ICAM** (ng/mL) | 0.45 | 0.001 | 0.51 | 0.001 |

r: Pearson's correlation coefficient, β: standardized regression coefficient, HDL-C: high density lipoprotein-cholesterol, LDL-C: low density lipoprotein-cholesterol, ICAM: intracellular adhesion molecule

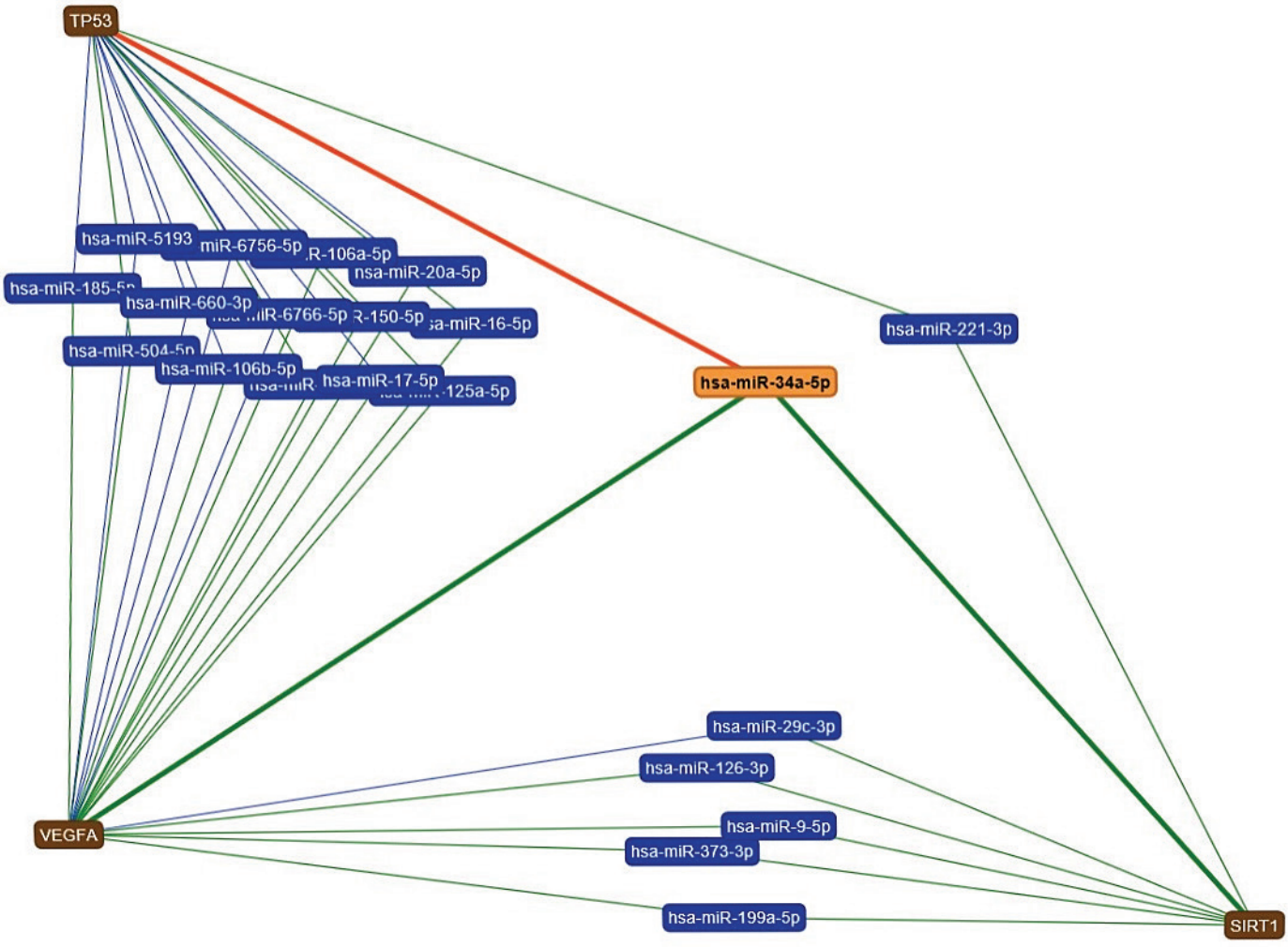


**Figure 4.** Interaction network of genes targeted by micro-RNA 34a (miR-34a) and playing a significant role in endothelial function. This analysis was done using (miRTargetLinkdatabase) (https://ccb-web.cs.uni-saarland.de/mirtargetlink/index.php) and retrieved that miR-34a is the only miRNA that targets the three major genes, vascular endothelial growth factor, *sirtuin 1 (SIRT1)* and *p53*, involved in endothelial function (green line indicates a strong interaction and red line indicates a weak interaction)

glycoprotein located on the surface of vascular endothelial cells. Endoglin, acts as a receptor for transforming growth factor-beta (TGF-β) which is an important mediator for angiogenesis and responsible for keeping the vascular endothelium healthy (37). ICAM is a surface adhesion molecule, also expressed on vascular endothelial cells and also immune cells, which facilitates cell-to-cell interaction and recruitment of leucocytes to endothelium during inflammation (38). Shedding of endoglin and ICAM1 receptors into the systemic circulation during endothelial injury renders them potential circulatory markers of endothelial dysfunction (37). In this study, levels of serum endoglin and serum ICAM were highly elevated in patients with T1DM compared to healthy controls. In agreement with our results, there have been previous reports of elevated levels of serum endoglin and serum ICAM in patients with T1DM (38,39,40). Another interesting finding was the elevation of serum endoglin and serum ICAM in patients with dyslipidemia compared to patients without dyslipidemia, in agreement to El-Kassas et al (39), who reported significant positive correlations of endoglin with TC, TG and LDL-C in children with T1DM. In addition, serum endoglin and serum ICAM showed positive correlations with exosomal miR-34a expression in our study. This data was consistent with other reports, stating that miR-34a is one of the most important endothelial miRNAs which plays a significant role in maintaining endothelial cell function by targeting many

genes including **p53**, VEGF and *SIRT1* (41). Moreover, TGF-β increases endoglin and can upregulate miR-34a, which subsequently promotes vascular inflammation by upregulating vascular cell adhesion molecule-1 and ICAM (10,42). Interestingly, it was reported that miR-34a deletion in the endothelium preserve endothelial functions in diabetic mice indicating the responsibility of miR-34a for diabetic vascular dysfunction (10).

### Study Limitations

The partial small sample size and the cross-sectional research design may be considered as limitations for this study.

## Conclusion

To the best of our knowledge, this is the first study to report the altered expression of exosomal miR-34a among children and adolescents withT1DM. Moreover, association of miR-34a with markers of dyslipidemia and endothelial dysfunction was demonstrated, suggesting a role for miR-34 in the epigenetic regulation of lipid metabolism and endothelial function in T1DM children and adolescents. More longitudinal studies with larger sample sizes and prospective design are warranted to investigate this association further. Future studies are recommended to identify the possible use of miR-34a as a therapeutic target in patients with T1DM and CVD.

### Ethics

**Ethics Committee Approval:** This study was approved by the Ethics Committee of the NRC, under approval number 16/285, in accordance with the Declaration of Helsinki 2015.

**Informed Consent:** Informed consent was obtained from each participant.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman, Concept: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman, Design: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman, Data Collection or Processing: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman, Analysis or Interpretation: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman, Literature Search: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman, Writing: Alshaymaa A. Ibrahim, Aliaa A. Wahby, Ingy Ashmawy, Rehan M. Saleh, Hend Soliman.

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# Indonesian National Growth Reference Charts Better Reflect Height and Weight of Children in West Java, Indonesia, than WHO Child Growth Standards

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### What is already known on this topic?

World Health Organization Child Growth Standards (WHOCGS) are used worldwide to interpret anthropometric measurement in children, except for those countries who have their own growth charts. In 2019, Indonesian National Growth Reference Charts (INGRC) were created, based on data from the Indonesia Basic Health Research 2013.

### What this study adds?

Growth of Indonesian children is not well represented by WHOCGS, as these standards overestimate the true prevalence of undernutrition. INGRC should be used for practical and clinical purposes.

## Abstract

**Objective:** The Indonesia Basic Health Research 2018 indicates that Indonesian children are still among the shortest in the world. When referred to World Health Organization Child Growth Standards (WHOCGS), the prevalence of stunting reaches up to 43% in several Indonesian districts. Indonesian National Growth Reference Charts (INGRC) were established in order to better distinguish between healthy short children and children with growth disorders. We analyzed height and weight measurements of healthy Indonesian children using INGRC and WHOCGS.

**Methods:** 6972 boys and 5800 girls (n = 12,772), aged 0-59 months old, from Bandung District were measured. Z-scores of length/height and body mass index were calculated based on INGRC and WHOCGS.

**Results:** Under 5-year-old Indonesian children raised in Bandung are short and slim. Mean height z-scores of boys is -2.03 [standard deviation (SD) 1.31], mean height z-scores of girls is -2.03 (SD 1.31) when referred to WHOCGS indicating that over 50% of these children are stunted. Bandung children are heterogeneous, with substantial subpopulations of tall children. Depending on the growth reference used, between 9% and 15% of them are wasted. Wasted children are on average half a SD taller than their peers.

**Conclusion:** WHOCGS seriously overestimates the true prevalence of undernutrition in Indonesian children. The present investigation fails to support evidence of undernutrition at a prevalence similar to the over 50% prevalence of stunting (WHOCGS) versus 13.3% (INGRC). We suggest refraining from using WHOCGS, and instead applying INGRC that closely mirror height and weight increments in Bandung children. INGRC appear superior for practical and clinical purposes, such as detecting growth and developmental disorders.

**Keywords:** Anthropometric measurement, Indonesian National Growth Reference Charts, World Health Organization Child Growth Standards, Bandung District children, undernutrition

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## Introduction

Stunting is considered one of the most prevalent health problems in Indonesia. Stunting is defined as the percentage of children whose height-for-age is below minus two standard deviations (SD) (moderate stunting) or minus three standard deviations (severe stunting) from the median of the World Health Organization Child Growth Standards (WHOCGS). Stunting is the impaired growth and development that children experience from poor nutrition, repeated infection, and inadequate psychosocial stimulation. The term "stunting" is commonly used to indicate chronic mal- or undernutrition during critical periods of growth and development, especially during the first 1000 days of life (1).

The Indonesia Basic Health Research 2018 indicated that Indonesian children are still among the shortest in the world. When referred to WHOCGS, the prevalence of stunting reaches as much as 43% in several Indonesian districts (2).

In 2015, Indonesia, along with other countries in United Nations, agreed on Sustainable Development Goals (SDGs) to be achieved by 2030 in order to reduce poverty, lessen the wealth gap, and protect the environment. SDGs consist of 17 core goals and 169 targets. The second goal is "to end hunger, achieve food security and improved nutrition and promote sustainable agriculture" (SDGs 2015). Stunting, since it is said to be related to nutritional status, was put on the SDGs second goal's indicator framework: to eradicate all forms of malnutrition including achieving the 2025 target on stunting and wasting, and improve nutritional needs (3,4). The WHO's Nutrition Landscape Information System defines stunting as length for age (LAZ) or height for age (HAZ) <-2 SD, and wasting as BMI for age (BAZ) <-2 SD from the median of the WHO Child Growth Standards (5). The Indonesia Basic Health Research 2018 showed that the number of stunted children is still high. While stunting is related to many factors, such as infections or psychosocial neglect, it is also associated with nutritional intake. The Indonesian government has made plans to improve the nutritional intake of Indonesian children by issuing Presidential Decree No. 42 of 2016 about the national movement for the Acceleration of Nutrition Improvement with a focus on the first 1000 days of life which prioritizes joint efforts between government and the community through coordinating stakeholder's participation and awareness towards accelerate community nutrition improvement (6).

This concept has been questioned. Stunting *per se* is not a synonym of malnutrition (7). A recent study performed in elementary school children from three Indonesian provinces, focused on the relationship between nutritional status and height and was unable to present evidence that stunting resulted from undernutrition in these children (7). This view is supported by data obtained in the Indonesia Basic Health Research 2018 (Table 1). The data illustrate the discrepancy between the large number of stunted and the comparably low number of wasted children, and question that stunting reflects undernutrition (2).

Biological and socio-economic factors are known to influence child growth. The great variety of clinical conditions associated with short stature further complicates identifying reasons for poor growth. Accurate and regular anthropometric measurements are essential, easy and inexpensive tools to help disentangling the complicated regulation of growth and to detect relevant growth and development disorders (8). As clinical practice has shown that WHOCGS seem to provide little help in this intricate matter, many countries have meanwhile constructed national growth reference charts (9,10,11,12).

Indonesia is an archipelago country formed by 17,508 islands. Its population ranks at number four in the world. There are five main islands in Indonesia: Sumatra, Java, Kalimantan, Sulawesi, and Papua (13) housing an extremely heterogeneous composition of ethnically, culturally and economically very different populations. In 2019, National Growth Reference Charts for Indonesian (INGRC) children were established, based on the Indonesia Basic Health Research 2013. The samples were taken from all Indonesian provinces, and considered representative

**Table 1. Percentage of Indonesian children under five years old below standard cut-offs for length and weight-for-height**

| Z-scores | | 2007 | 2013 | 2018 |
|---|---|---|---|---|
| LAZ/HAZ | <-2 SD -3 SD | 18.0 | 19.2 | 19.3 |
| | <-3 SD | 18.8 | 18.0 | 11.5 |
| WHZ | <-2 SD -3 SD | 7.4 | 6.8 | 6.7 |
| | <-3 SD | 6.2 | 5.3 | 3.5 |

LAZ: length-for-age z-scores for children ≤2 years old, HAZ: height-for-age z-scores for children >2 years old and WHZ: weight-for-length/height z-scores in percentage based on Indonesia Basic Health Research in 2007, 2013 and 2018, SD: standard deviation.

Adapted from Indonesia Basic Health Research, 2018.

Ref. 2.

for the Indonesian child population (9). The present study was undertaken to test the reliability of the new INGRC. The aim of this study was to compare WHOCGS with the new INGRC in under 5-year-old Indonesian children, raised in the Bandung District area. As Indonesian national references are based on healthy Indonesian children, we expected INGRC to better fit Indonesian growth patterns than WHOCGS. Yet, as short stature is commonly associated with chronic undernutrition, we focused on body mass index (BMI) as a rough indicator of the nutritional status, its association with height, and particularly, on the shape of the height and the BMI distributions. Starving and malnourished populations are on average short, but a population is never equally affected by starvation or malnutrition. Some people may receive enough food, their children grow well or almost well, others may receive too little and their children stop growing and become stunted. Children may also differ in sensitivity to food deprivation: some may stop growing early, others may grow even when food rations are very poor. Unequal food distribution and unequal biological responses do not only affect mean values of height and BMI, they will affect height and BMI variation. Unequal living conditions will raise height and BMI variance.

Indonesian's Global Hunger Index (GHI) is 20.1, which indicates that Indonesian children are considered "seriously" affected by starvation. GHI values are determined for four indicators: undernourishment (insufficient caloric intake), wasting among children under 5 years of age/low WHZ (weight-for-length) (acute undernutrition), stunting among children under 5 years of age/low HAZ (chronic undernutrition) and mortality rate of children under 5 years of age (results from undernutrition and unhealthy environment) (14). As Indonesian children, regardless of their nutritional state, are generally shorter and lighter than prescribed by WHOCGS, they will always be categorized as chronically undernourished as long as these growth charts are used.

We hypothesized that:

1) height and BMI of under 5-year-old Indonesian children raised in the Bandung District area would be smaller than suggested by WHO Child Growth Standards.

2) the variance of height and BMI would be broader than suggested by WHO Child Growth Standards.

In addition, we hypothesized:

3) that wasted children (BMI $<$-2 SD, using WHOCGS) are shortest.

## Methods

Length/height and weight of 12,772 healthy children, 6,972 (54.6%) boys and 5800 girls aged 0-59 months, from Bandung District area, were measured. The sample was taken from 31 sub-districts and included both urban and rural children from the whole spectrum of economic provenance, including children both from impoverished and affluent backgrounds. Length/height and weight measurements were performed according to a standard procedure (15). The weight was measured to the nearest 100 grams using Indonesian Dacin scale, which is the most commonly used scale for children in Indonesia Primary Maternal and Child Health Care. The length of children ≤2 years old was measured using an infantometer in a supine position. In children $>$2 years old, height was measured using microtoise stadiometer to the nearest millimeter (Dacin scale was manufactured by Sanes Sumber Makmur, both infantometer dan microtoise were manufactured by GEA Medical).

The data were obtained from the Health Office of Bandung District's Nutritional Status Monitoring for Children Under 5 Years Old. Measurements were done in March 2019 by healthcare providers using standardized tools. Written consent for Nutritional Status Monitoring was obtained from parents according to the policy of Bandung District Health Office. Weight, height and BMI were compared to WHO Child Growth Standards and INGRC (9,16).

This study was approved by the Ethics Committee of Faculty of Medicine, Universitas Padjadjaran, Ethical Approval no 1170/UN6.KEP/EC/2019, and conformed to the ethical guidelines of the Declaration of Helsinki.

### Statistical Analysis

Statistical analyses were performed using SPSS, version 24.0 (IBM Inc., Armonk, NY, USA). All data were plotted on charts using The R project for statistical computing version 3.5.0 (17). The F-test was used to compare variances.

Children were anonymized and de-identified before analysis.

## Results

Under 5-year-old Indonesian children raised in the Bandung District area are short (Figure 1) and slim (Figure 2). Mean height z-scores of boys was -2.03 (SD 1.31), mean height z-scores of girls was -2.03 (SD 1.31) when referred to WHOCGS, indicating that more than 50% of these children are stunted. When referred to INGRC, the percentage of stunted children declined to 13.3%. Depending on the growth reference used, between 9% (WHOCGS) and 15%

(INGRC) of the Bandung District area children are wasted (Table 2).

Table 2 illustrates to what extent the choice of the growth reference chart influences the apparent percentage of stunted and wasted children. Whereas INGRC identifies 10.8% moderately, and 2.5% severely stunted children in Bandung District, reference to WHOCGS suggested that 34.72% were moderately, and 21.59% were severely stunted.

Figures 3 and 4 illustrate the frequency distributions of LAZ/HAZ and BAZ based on WHOCGS and on INGRC, and a virtual cohort with random z-scores defined by mean values of zero, and standard deviations of one. Neither LAZ/HAZ nor BAZ are normally distributed. Bandung children are shorter and slimmer than suggested by WHOCGS and by INGRC. True LAZ/HAZ and BAZ distributions are broader than the virtual random distributions, and the LAZ/HAZ distributions are significantly skewed ($p < 0.001$). Table 3 and Figures 3 and 4 indicate that Bandung District children are heterogeneous, with substantial subpopulations of tall children as indicated by the elongated right leg of the height z-score curves.

The broadened and skewed distributions of height and weight z-scores suggest inequality among Bandung District children. In view of the common perception that short stature is considered an indicator of chronic undernutrition, and BMI an indicator of the nutritional status, we also studied the association between height and BMI (Figure 5, 6).

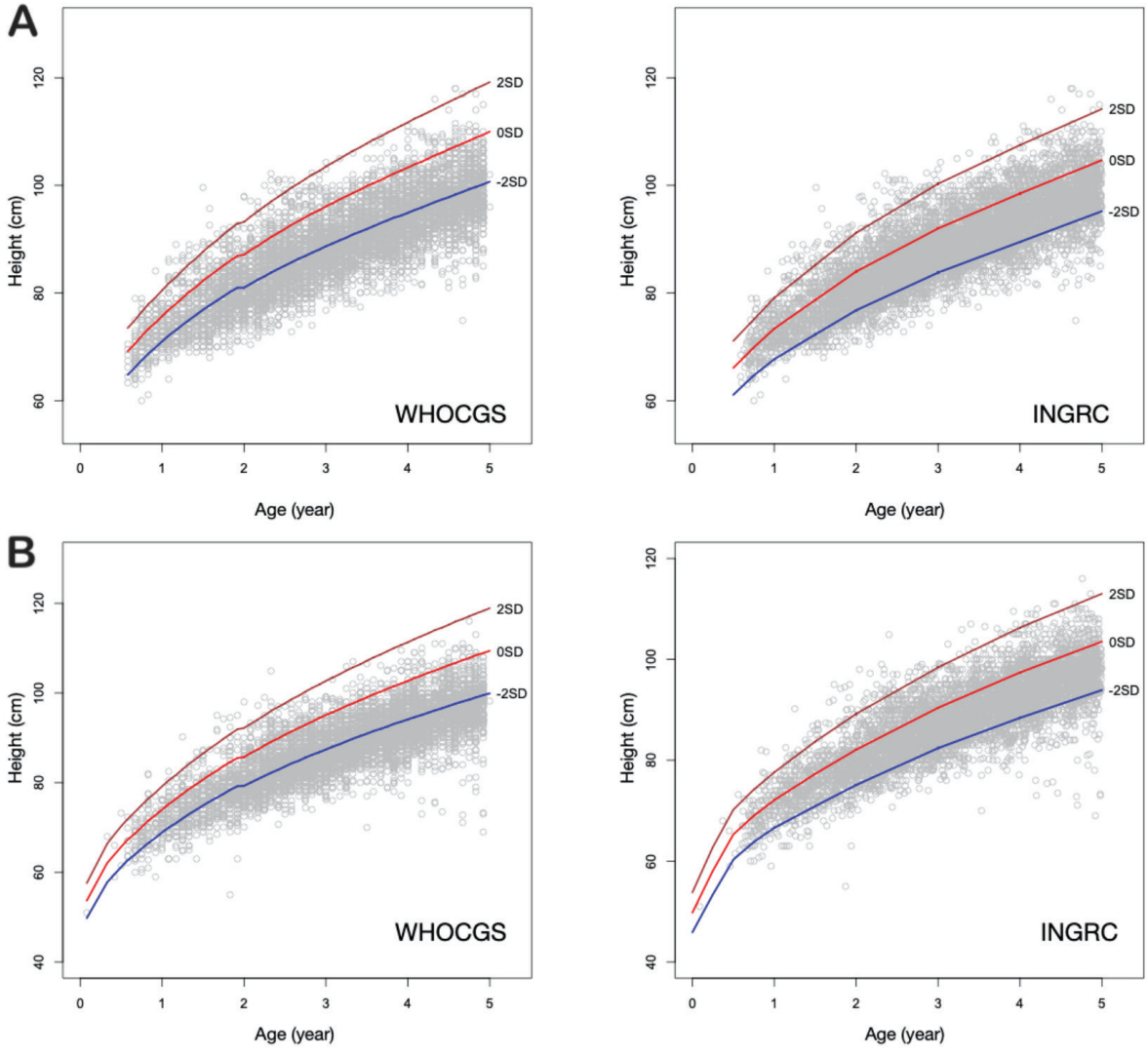


**Figure 1.** Height of Bandung District Boys (A) and Girls (B) plotted on WHOCGS and INGRC. The children from Bandung District are short. More than 50% must be considered stunted according to WHOCGS.

*WHOCGS: World Health Organization Child Growth Standards, INGRC: Indonesian National Growth Reference Charts*

We found the opposite of what was expected, that is being thin is not associated with being short. The association between LAZ/HAZ and BAZ is negative. Slim children are taller. In order to further scrutinize this association, we investigated LAZ/HAZ of those children who are by definition considered wasted (BAZ < -2 SD, WHOCGS). LAZ/HAZ of wasted boys was -1.3 (SD 1.48), LAZ/HAZ of wasted girls was -1.34 (SD 1.44). Wasted Bandung District children are on average 0.7 SD taller than their peers (Table 3: all boys: -2.03 SD, all girls -2.05 SD; $p < 0.001$ for both sexes).

## Discussion

The Indonesia Basic Health Research 2018 indicated that Indonesian children are short. This also applied to children raised in the Bandung District area. When referred to WHO Child Growth Standards (WHOCGS), more than 50 % of these children are stunted. Under 5-year-old Bandung District children are also slim. This confirms our first hypothesis.

Bandung District children are heterogeneous. The variance of height and BMI is broader than suggested by WHOCGS, confirming our second hypothesis. The combination of being on average short and slim and the heterogeneity of the population of Bandung children might, at first view, support the general perception that these children suffer from malnutrition, and that length-for-age may indeed, serve as an appropriate indicator for chronic nutritional deficiency (18). However, this impression is deceptive. The present study illustrated that being slim is not associated with being short. The present analysis clearly rejects the third hypothesis. The very slim (wasted) children with BMI < -2 SD (WHOCGS) are not the shortest. On the contrary,

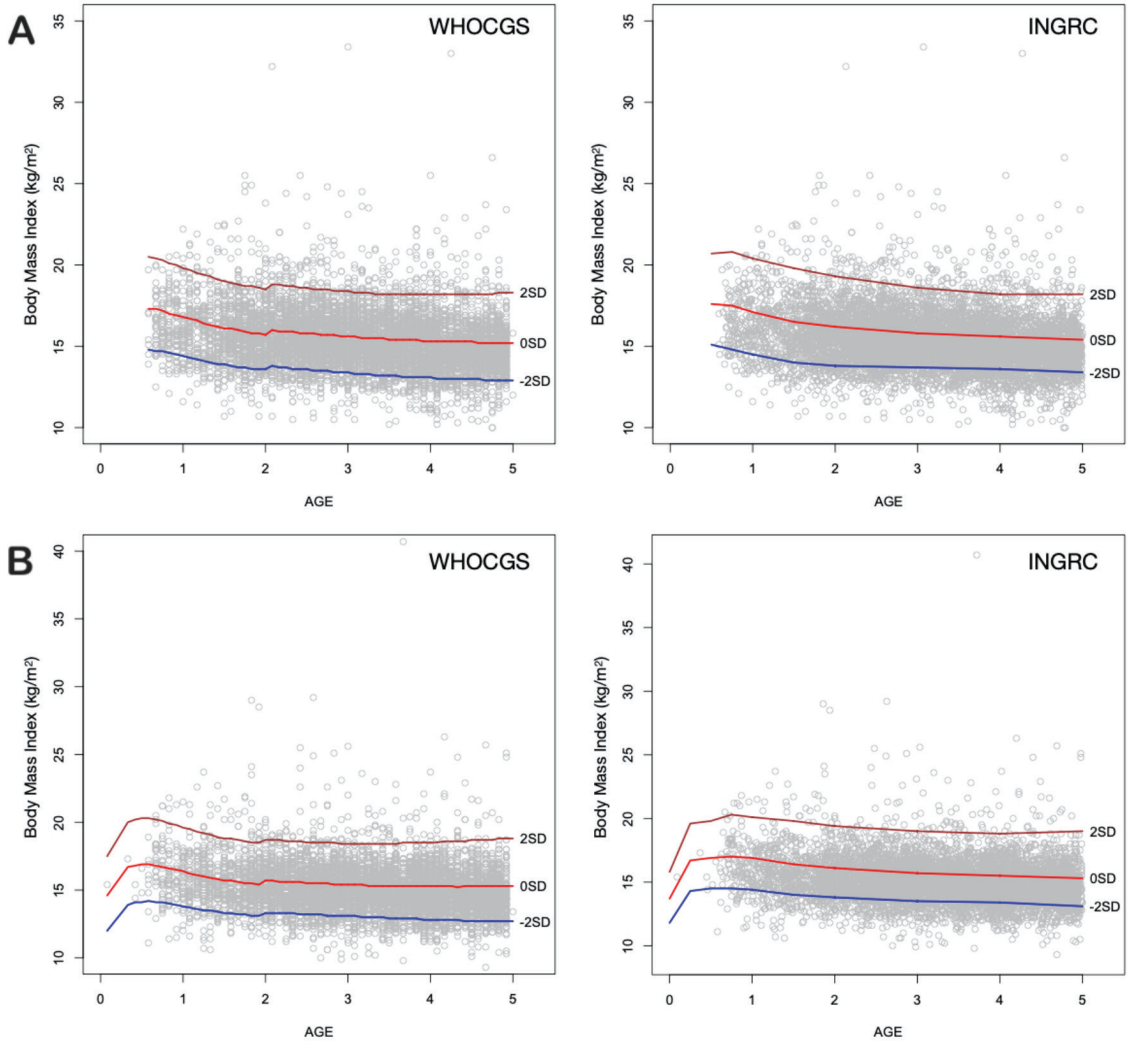


**Figure 2.** BMI of Bandung District Boys (A) and Girls (B) plotted on WHOCGS and INGRC. The number of obese children is very small.

*WHOCGS: World Health Organization Child Growth Standards, INGRC: Indonesian National Growth Reference Charts, BMI: body mass index*

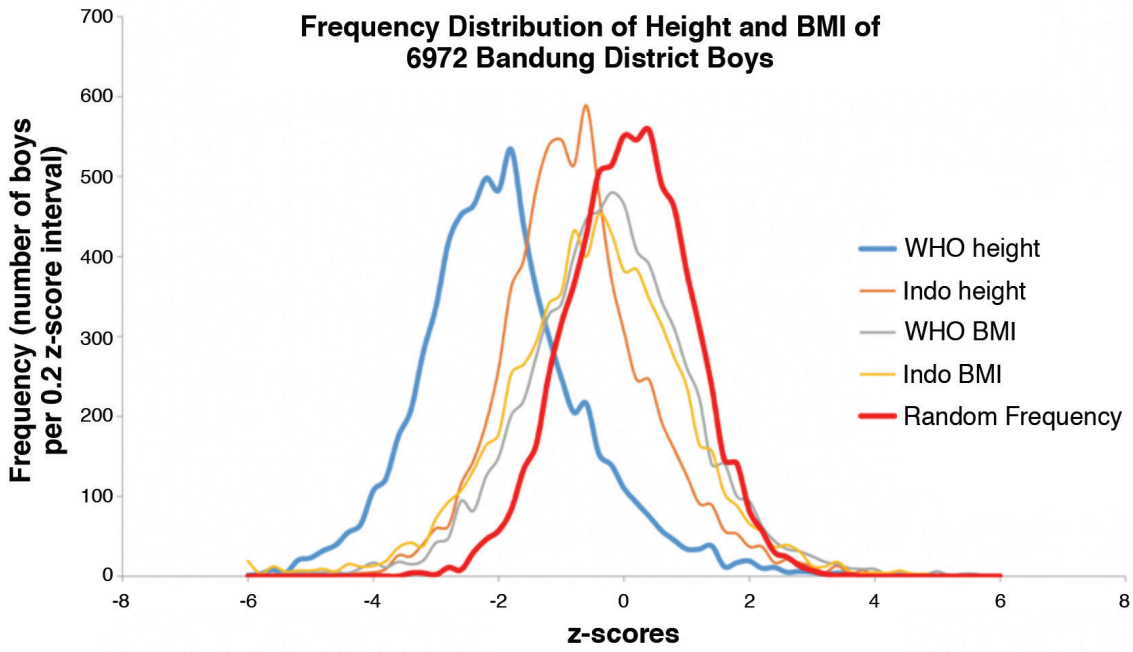


**Figure 3.** Frequency distribution of height and body mass index of 6,972 Bandung District boys

*WHO BMI: World Health Organization Child Growth Standards body mass index, Indo BMI: Indonesian National Growth Reference Charts body mass index*

**Table 2. Number and percentage of severely stunted [length/height-for-age z-scores (LAZ/HAZ) <-3 SD], moderately stunted LAZ/HAZ <-2 SD, normal, and tall LAZ/HAZ > 3 standard deviation (SD) children; and severely wasted [body mass index for-age z-scores (BAZ) <-3 SD], moderately wasted BAZ <-2 SD, normal, overweight BAZ >2 SD, and obese children BAZ >3 SD. P values (Mann-Whitney U test) refer to the difference between World Health Organization Child Growth Standards and Indonesian National Growth Reference Chart**

| | Z-scores | WHOCGS n (%) | INGRC n (%) | p value |
|---|---|---|---|---|
| LAZ/HAZ | >3 SD | 75 (0.59) | 246 (1.93) | <0.001 |
| | ≤3 SD ≥-2 SD | 5504 (43.09) | 10828 (84.78) | |
| | <-2 SD ≥-3 SD | 4435 (34.72) | 1379 (10.80) | |
| | <-3 SD | 2758 (21.59) | 319 (2.50) | |
| BAZ | >3 SD | 147 (1.15) | 99 (0.78) | <0.001 |
| | ≤3 SD >2 SD | 320 (2.51) | 266 (2.08) | |
| | ≤2 SD ≥-2 SD | 11164 (87.41) | 10505 (82.25) | |
| | <-2 SD ≥-3 SD | 873 (6.48) | 1227 (9.61) | |
| | <-3 SD | 268 (2.10) | 675 (5.28) | |

WHOCGS: World Health Organization Child Growth Standards, INGRC: Indonesian National Growth Reference Charts, LAZ/HAZ: length/height-for-age z-scores, BAZ: body mass index-for-age z-scores, SD: standard deviation

**Table 3. Mean z-scores for length/height-for-age and body mass index-for-age**

| | WHOCGS | | INGRC | |
|---|---|---|---|---|
| | LAZ/HAZ (SD) | BAZ (SD) | LAZ/HAZ (SD) | BAZ (SD) |
| Boys | -2.03 (1.31*) | -0.33 (1.36*) | -0.84 (1.17*) | -0.57 (1.46*) |
| Girls | -2.05 (1.25*) | -0.36 (1.27*) | -0.83 (1.17*) | -0.70 (1.44*) |

Z-scores for length/height-for-age and BAZ of 6972 boys and 5800 girls aged 0-59 months, from Bandung District area, referred to WHOCGS and to INGRC. Asterisks indicate that SD are greater than 1.0 ($p < 0.001$).

LAZ/HAZ: length/height-for-age z-scores, BAZ: body mass index-for-age z-scores, SD: standard deviation, WHOCGS: World Health Organization Child Growth Standards, INGRC: Indonesian National Growth Reference Charts

children who are by definition "wasted" are on average 0.7 standard deviations taller than their peers. Stunting is not a synonym of malnutrition (7).

The observation that the thinnest children are tallest questions the current concept of nutrition-dependent growth regulation. An estimated 50 percent prevalence of stunting when using WHOCGS can by no means, plausibly suggest that half of the Bandung District children suffer from chronic undernutrition, repeated infections or child neglect and lack of psychosocial stimulation.

The 2019 Food Security and Vulnerability Atlas (FSVA) classified districts in Indonesia into one to six priority groups, from the most food-insecure to the most food-secure. FSVA exhibits Districts in West Java as priority 5 (22%) and 6 (78%) which indicating appropriate food-security (19). Studies in respect of availability of major food products, including fruits, vegetables, livestock and fisheries in West Java, especially Bandung District, revealed steady and secure food diversification policies, which are able to cover production, distribution, access and demand among the community (20,21). Based on Central Bureau of Statistics Republic of Indonesia, the Gross Regional Product Nominal (GRP Nominal) per capita of West Java Province on 2019 ranked the third highest of 34 provinces

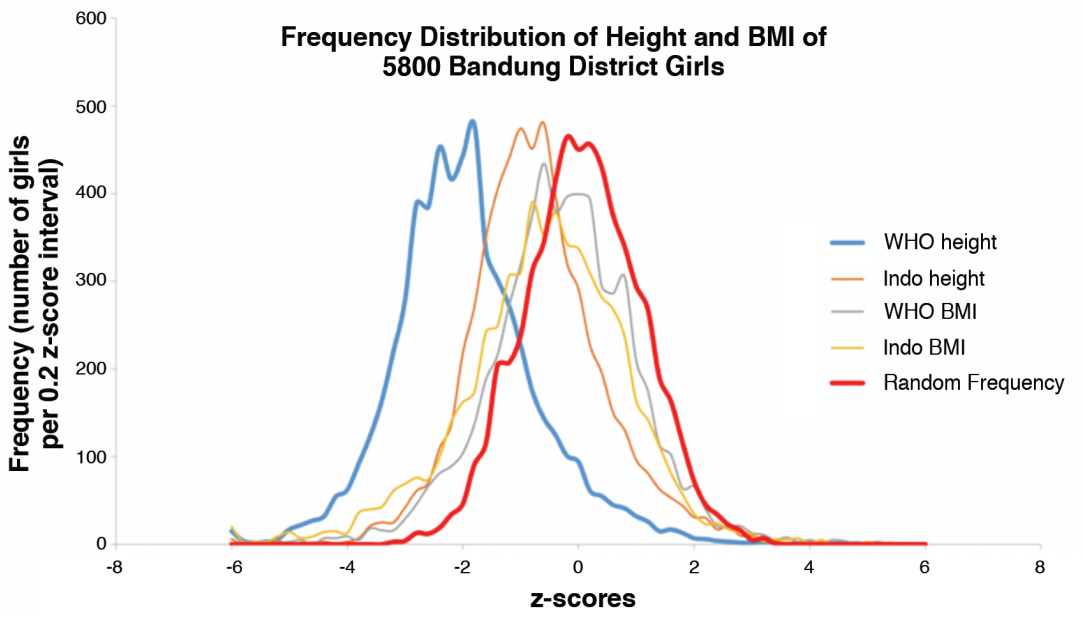


**Figure 4.** Frequency distribution of height and body mass index of 5,800 Bandung District girls

*WHO BMI: World Health Organization Child Growth Standards body mass index, Indo BMI: Indonesian National Growth Reference Charts body mass index*

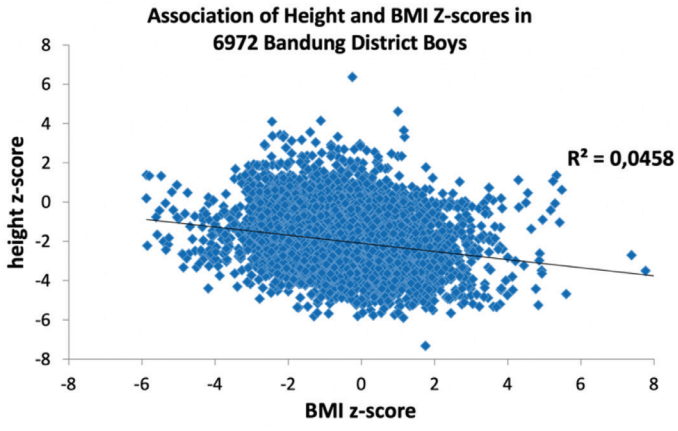


**Figure 5.** Association of height and body mass index z-scores in 6,972 Bandung District boys

*BMI: body mass index*

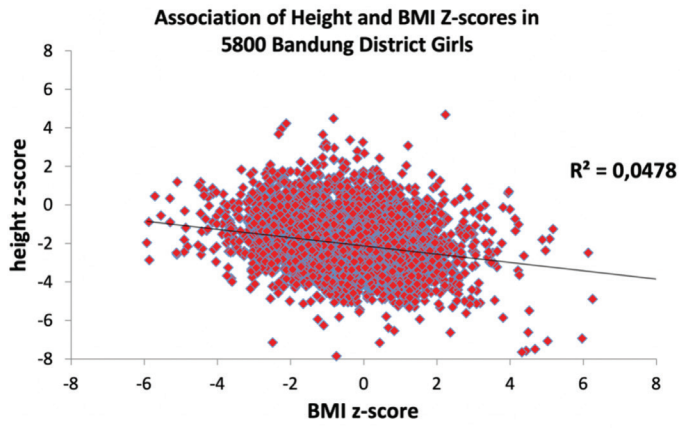


**Figure 6.** Association of height and body mass index z-scores in 5,800 Bandung District girls

*BMI: body mass index*

and the Gross Regional Domestic Revenue of Bandung District on 2016 ranked 27 out of 514 districts and cities in Indonesia. Bandung also ranked 114 of 120 in Global City Competitiveness Index in 2012 (22,23,24). Children in Bandung grew in supportive atmosphere. The Bandung city government provides comprehensive attention on children's health, education and psychosocial needs. This leads Bandung won the Child-Friendly City award from the Ministry of Women's Empowerment and Child Protection for the third time in a row on 2019 (25).

Bandung District is wealthy, with no evidence of food shortage or clinical signs of malnutrition in the children raised in this area. The claim that 50% of the healthy Bandung District children as mal- or undernourished, is unsubstantiated. The observation that the slimmest children of Bandung District grew best, further challenges the prevalent concept of length-for-age being the indicator of choice in monitoring chronic nutritional deficiency.

Yet, the question remains: why are these children short, and why do the slimmest children grow best? Modern nutrition studies do not throw light on this matter, but numerous historic observations match our findings.

Most Europeans of the 19th century were shorter than modern Indonesians. In spite of the tremendous wealth of the European nations at that time, European children grew poorly. Even upper-class urban adolescents grew less than modern Indonesians (26). This almost ubiquitous pattern of historic European growth changed after World War I. In 1919, the German pediatrician Schlesinger wrote: "In the second year of the war (World War I), there were more than a few groups of boys from the public citizens' and advanced educational schools who were 1-2 cm taller than in the year 1913 (before the war). This difference in the second year of the war was even more conspicuous, as at the same time there was a very clear and not very small weight loss". And based on measurements of the loss in body fat, both in absolute terms and related to body height, Schlesinger wrote in 1924 that: "even more regular is the deficit in weight in 1916 versus 1913, when taking into account the length of the body, which in this period has partly shifted in the opposite direction" (27). Even though the children became slimmer, they nevertheless grew taller. The rapid secular height trends after World War I coincided with the political transition from feudalism in the Imperial period to socialist or democratic structures. The adolescents raised at that time, anticipated rapid political changes, liberation and equal opportunities, and closely coinciding with the political changes, increased in height by one to two millimeters per annual cohort (28).

Human growth is regulated by biological factors such as nutrition, genetics, and general health (26), but recent evidence also suggests social, economic, political and emotional (SEPE) factors (29). Political transition appears to be one of the most distinguished promotors of human growth factors. Absence of political oppression is a growth stimulus. Hermanussen and Scheffler (28) (2016) discussed community effects on body height, and considered stature as a social signal. The data of the present investigation indirectly support this vision. Indonesia is still a developing country but it shows advancements in democratisation, personal freedom and equal opportunities. The recent political achievements provide possible explanations for the negative association between height and BMI. Bandung children are slim, but not because of nutrition deficits. The subpopulation of the very slim and taller than average children of Bandung, mirrors the SEPE situation that was prevalent in central Europe after World War I, at the dawn of political modernization (29).

Use of the WHOCGS categorizes more than 50% of the healthy Bandung District children as "stunted", thereby alleging chronic mal-and undernutrition (1) of these children. The present investigation fails to support evidence for this concept. We suggest refraining from using global growth charts, and instead strongly support applying the new INGRC. These charts are based on data from Indonesia Basic Health Research 2013. They also closely mirror height and weight increases of Bandung children, and appear superior for practical and clinical purposes, such as detecting growth and developmental disorders.

In view of body height as a mirror of the SEPE situation of a country (29), we consider frequent updating the INGRC essential. We are convinced that coinciding with the political modernization, Indonesian children will in the near future follow the same global growth standards for height and weight as suggested by the WHO. Child health care and prevention require relevant national references for height, weight and BMI.

### Study Limitations

The study was performed in a cross-sectional sample of infants and children, with no detailed information on individual nutrition, individual health, individual repeated infection and individual socio-economic background. Thus, the data do not allow direct inferences between growth, nutritional situation, morbidities, psychosocial status, and socio-economic circumstances. Instead we used data of the Indonesia FSVA with verified local information. Considering the GRP Nominal per capita of West Java Province, the Gross Regional Domestic Revenue of Bandung District and

Bandung children's health, education and social services, it seems that Bandung District is in good economy condition, adequate psychosocial stimulation status, with good food security and absence of child poverty.

## Conclusion

The WHOCGS seriously overestimates the true prevalence of undernutrition in Indonesian children. The present investigation fails to support evidence of undernutrition. We suggest refraining from using WHOCGS, and instead applying INGRC. These latter charts closely mirror height and weight increments in Bandung children. They appear superior to currently used WHO Child Growth Standards for practical and clinical purposes, such as detecting growth and developmental disorders.

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### Ethics

**Ethics Committee Approval:** This study was approved by the Ethics Committee on Faculty of Medicine, Universitas Padjajaran, Ethical Approval No 1170/UN6.KEP/EC/2019 and had been conformed to the ethical guidelines of the Declaration of Helsinki.

**Informed Consent:** This data is obtained from the Health Office of Bandung District's Nutritional Status Monitoring for Children Under 5 Years Old.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Novina Novina, Budi Setiabudiawan, Concept: Novina Novina, Michael Hermanussen, Christiane Scheffler, Aman B. Pulungan, Budi Setiabudiawan, Design: Novina Novina, Budi Setiabudiawan, Michael Hermanussen, Christiane Scheffler, Aman B. Pulungan, Data Collection or Processing: Novina Novina, Budi Setiabudiawan, Vitriana Biben, Yoyos Dias Ismiarto, Analysis or Interpretation: Novina Novina, Michael Hermanussen, Christiane Scheffler, Yudhie Andriyana, Literature Search: Novina Novina, Michael Hermanussen, Christiane Scheffler, Aman B. Pulungan, Budi Setiabudiawan, Writing: Novina Novina, Budi Setiabudiawan, Michael Hermanussen, Christiane Scheffler.

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CASE REPORT

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# Heterozygous Insulin Receptor *(INSR)* Mutation Associated with Neonatal Hyperinsulinemic Hypoglycaemia and Familial Diabetes Mellitus: Case Series

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### What is already known on this topic?

Homozygous or compound heterozygous mutations in the *insulin receptor (INSR)* gene, linked with Rabson-Mendenhall and Donohue syndromes, have been described to cause neonatal hypoglycaemia.

### What this study adds?

We report the first case-series of neonatal hyperinsulinemic hypoglycaemia associated with heterozygous mutations in *INSR* leading to variable phenotype among the family members, ranging from neonatal hypoglycaemia to adult-onset diabetes mellitus. This highlights the importance of genetic analysis and long-term follow up of these patients.

## Abstract

Mutations in the *insulin receptor (INSR)* gene are associated with insulin resistance and hyperglycaemia. Various autosomal dominant heterozygous *INSR* mutations leading to hyperinsulinemic hypoglycaemia (HH) have been described in adults and children (more than 3 years of age) but not in the neonatal period. Family 1: A small for gestational age (SGA) child born to a mother with gestational diabetes presented with persistent hypoglycaemia, was diagnosed with HH and responded well to diazoxide treatment. Diazoxide was gradually weaned and discontinued by 8 months of age. Later, the younger sibling had a similar course of illness. On genetic analysis a heterozygous *INSR* missense variant p.(Met1180Lys) was found in the siblings, mother and grandfather but not in the father. Family 2: A twin preterm and SGA baby presented with persistent hypoglycaemia, which was confirmed as HH. He responded to diazoxide, which was subsequently discontinued by 10 weeks of life. Genetic analysis revealed a novel heterozygous *INSR* missense variant p.(Arg1119Gln) in the affected twin and the mother. Family 3: An SGA child presented with diazoxide responsive HH. Diazoxide was gradually weaned and discontinued by 9 weeks of age. Genetic analysis revealed a novel heterozygous *INSR* p.(Arg1191Gln) variant in the proband and her father. We report, for the first time, an association of *INSR* mutation with neonatal HH responsive to diazoxide therapy that resolved subsequently. Our case series emphasizes the need for genetic analysis and long-term follow up of these patients.

**Keywords:** *INSR* mutation, congenital hyperinsulinism, neonatal hyperinsulinemic hypoglycemia, familial diabetes mellitus

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## Introduction

*Insulin receptor (INSR)* is a trans-membrane receptor from the tyrosine kinase family where insulin binds to two distinct sites on each subunit of the receptor (1,2). *INSR* mutations are associated with severe insulin resistance (IR) phenotypes, such as Rabson-Mendenhall, Donohue and type A IR syndrome (3,4). Rabson-Mendenhall and Donohue syndromes are recessively inherited conditions leading to decreased expression of receptor, blockage of receptor transport to the plasma membrane or decreased insulin binding, associated with extreme IR. Type A IR is associated with mono allelic *INSR* missense mutations, which cause a less severe phenotype and are most likely to occur within the tyrosine kinase domain (4).

Insulin is an anabolic peptide hormone secreted by the beta cells of pancreatic islets. Inappropriate secretion of insulin leads to persistent/recurrent hypoglycaemia (hyperinsulinemic hypoglycaemia, HH), which can result from a mutation in a number of different genes (5,6).

There are several reports of autosomal dominant heterozygous *INSR* mutations causing hypoglycaemia in adults but to the best of our knowledge there is no data about heterozygous *INSR* mutations causing neonatal HH (7,8,9,10,11,12).

In this study, we report three different families presenting with variable clinical manifestations, ranging from neonatal HH to adult onset type 2 diabetes mellitus, associated with heterozygous *INSR* mutation.

## Case Reports

### Family 1

A 13-day-old female baby (proband) was referred to the tertiary endocrine unit with persistent neonatal hypoglycaemia since three hours of life (capillary blood glucose: 1.9 mmol/L). She was born by emergency caesarean section due to breech presentation at 37 weeks gestation, weighing 2.41 kg [-2.42 standard deviation score (SDS)] and measuring 47 cm in length (-1.15 SDS). There was a history of gestational diabetes in the mother requiring insulin treatment. The hypoglycaemia was initially managed with high concentration dextrose containing fluids [glucose infusion rate (GIR): 18.1 mg/kg/min] and intravenous glucagon (10 mcg/kg/hr) infusion. HH was confirmed on hypoglycaemia screen (Table 1). The echocardiogram was normal and she was subsequently commenced on oral diazoxide at 5 mg/kg/day and chlorothiazide at 7 mg/kg/day. She responded well to diazoxide which enabled weaning off the intravenous fluids and intravenous glucagon. She was fully established on oral feeds prior to discharge. During subsequent follow up, the diazoxide and chlorothiazide were slowly weaned and fully discontinued by eight months of age following which a 16-hour controlled fast showed complete resolution of HH (Table 2).

The second child was born at 38 week of gestation by elective caesarean, weighing 2.43 kg (-2.35 SDS) and measuring 48 cm in length (-0.62 SDS). There was no history of birth asphyxia. The mother once again developed gestational diabetes but did not require insulin treatment unlike the previous pregnancy. At 10 hours of age she had symptomatic hypoglycaemia (capillary blood glucose: 1.9 mmol/L) and was managed with intravenous dextrose containing fluids (GIR of 11.5 mg/kg/min). At 48 hours of age, a hypoglycaemia screen (Table 1) was undertaken which confirmed HH. Echocardiogram did not reveal any underlying cardiac abnormality. Diazoxide was started at 3 mg/kg/day to achieve normoglycaemia. Oral chlorothiazide (7 mg/kg/day) was added in conjunction with diazoxide. The intravenous fluids and glucagon were gradually weaned and the patient was established on full enteral feeds prior to discharge. During subsequent follow up, the diazoxide and chlorothiazide were slowly weaned and fully discontinued by 11 months of age, following which she underwent a controlled fast appropriate for age which confirmed the resolution of HH (Table 2).

The proband's mother had gestational diabetes mellitus during both pregnancies, requiring insulin treatment during the first pregnancy. She had normal body mass index

**Table 1. Biochemical parameters during the hypoglycemic episode**

| Hypo screen | Family 1 | | Family 2 | Family 3 |
|---|---|---|---|---|
| | Proband | Sibling | Proband | Proband |
| Lab glucose (3.5-5.5 mmol/L) | 1.4 | 1.8 | 2.01 | 1.9 |
| Insulin (pmol/L) | > 644.4 | 408 | 315 | 56 |
| C-peptide (pmol/L) | 1463 | 663 | 523 | 503 |
| Plasma free fatty acid (umol/L) | 382 | 207 | - | - |
| 3-hydroxybutyrate (umol/L) | 80 | 24 | - | - |
| Cortisol (nmol/L) | 364 | 416 | 415 | - |

(BMI) (BMI 24 kg/m$^2$) and had no features of IR, such as acanthosis nigricans (AN), previous menstrual abnormalities or hirsutism (HR). The proband's maternal grandfather (BMI 25 kg/m$^2$) was diagnosed with type 2 diabetes mellitus at the age of 45 years and was treated with metformin. On examination he did not have any signs of IR or any history of hypoglycaemia.

Targeted next generation sequencing of the known hyperinsulinism genes identified a novel heterozygous *INSR* variant p.(Met1180Lys) (c.3539T > A) in the proband, her sister, mother and maternal grandfather. No further disease-causing variants were identified (Figure 1).

## Family 2

The proband was a preterm (36 weeks gestation) twin, born to non-consanguineous parents by elective LSCS with a birth weight of 2.025 kg (-3.75 SDS). Hypoglycaemia was recorded on the first day of life (capillary blood glucose 1.1 mmol/L). There was no history of birth asphyxia. The second twin had a birth weight of 2.56 kg (-1.95 SDS) and did not have any hypoglycaemia during the neonatal period. There was a maternal history of hypothyroidism, which was well controlled on thyroxine. The hypoglycaemia was initially managed with high concentration dextrose containing fluids (GIR: 15.2 mg/kg/min) and intravenous glucagon (10 mcg/kg/hr) infusion. The hypoglycaemia screen (Table 1) confirmed HH. Echocardiography revealed moderate pulmonary stenosis and small patent foramen ovale. The child was subsequently commenced on diazoxide at 7.5 mg/kg/day and spironolactone at 2 mg/kg/day. He responded well to diazoxide which enabled weaning off the intravenous fluids and intravenous glucagon. He was fully established on formula feeds prior to discharge. During subsequent follow up, the diazoxide and spironolactone were slowly weaned and fully discontinued by 10 weeks of age.

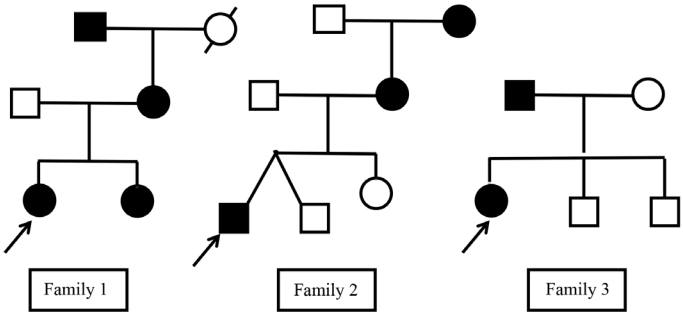


**Figure 1.** Pedigree chart showing autosomal dominant inheritance in the families; circle denotes females, square males. The proband is indicated by arrow mark in each family, dotted symbol denote person with diabetes with unknown mutation status and solid symbols denote affected subjects with (Family 1) *INSR* gene p.(Met1180Lys) or (Family 2) *INSR* gene p.(Arg1119Gln) or (Family 3) *INSR* gene c.3572G > A, p.(Arg1191Gln) mutation

**Table 2. Biochemical evaluation at the end of 16 hour controlled fast**

| Hypo screen | Family 1 | |
|---|---|---|
| | Proband | Sibling |
| Lab glucose (3.5-5.5 mmol/L) | 4.4 | 3.4 |
| Insulin (pmol/L) | 74 | 74 |
| C-peptide (pmol/L) | 189 | 283 |
| Plasma free fatty acid (umol/L) | 1362 | 714 |
| 3-hydroxybutyrate (umol/L) | 845 | 348 |

The proband's elder sibling is a 3-year-old healthy girl with no history of neonatal hypoglycaemia. The proband's mother did not have any symptoms related to hypoglycaemia and was able to fast for prolonged hours during Ramadan. She had a normal BMI, no signs of IR, such as AN, and there was no previous history of menstrual abnormalities or HR. There was a history of type 2 diabetes in the maternal grandfather and maternal great grandmother.

Targeted next generation sequencing of the known hyperinsulinism genes identified a novel heterozygous *INSR* variant p.(Arg1119Gln) (c.3356G > A) in the proband and her mother. No further disease-causing variants were identified. Samples from the twin and older sister were not available for testing (Figure 1).

## Family 3

The proband was born was born at 37 week of gestation, weighing 2.20 kg (-1.51 SDS). There was no history of birth asphyxia. At a few hours of age she developed symptomatic hypoglycaemia (capillary blood glucose: 1.1 mmol/L) and was managed with intravenous dextrose containing fluids. At 48 hours of age, a hypoglycaemia screen (Table 1) was undertaken which confirmed HH. Echocardiogram did not reveal any underlying cardiac abnormality. Diazoxide (5 mg/kg/day) in conjunction with chlorothiazide (7 mg/kg/day) was started. The intravenous fluids and glucagon were gradually weaned and the patient was established on full enteral feeds prior to discharge. During subsequent follow up, the diazoxide and chlorothiazide were slowly weaned and fully discontinued by nine weeks of age.

The proband's father had a normal BMI and did not have any symptoms related to IR, such as AN, or glucose variability (HbA1c: 36 mmol/L, fasting glucose: 4.6 mmol/l). The proband has two elder siblings who did not have any hypoglycaemia or symptoms related to IR.

Targeted next generation sequencing of the known hyperinsulinism genes identified a novel heterozygous *INSR* variant p.(Arg1191Gln) (c.3572G > A) in the proband and her father. No further disease-causing variants were identified (Figure 1).

## Discussion

The human *INSR* is a heterotetramer composed of two α and two β subunits. The α subunit is entirely extracellular and the β subunit has extracellular, transcellular and intracellular domains that expresses tyrosine kinase activity. Insulin binds to α subunits and stimulates β subunit auto phosphorylation and kinase activity (1,2). A single gene, *INSR* located at 19p13.2 of 22 exon length, codes for both α and β subunits (13). Homozygous and compound heterozygous mutations in *INSR* lead to severe IR (Donohue syndrome, Rabson-Mendenhall syndrome), whereas the heterozygous mutations in *INSR* cause the milder phenotype of IR syndrome (14,15).

**Table 3. List of reported patients with hypoglycemia associated with heterozygous *INSR* mutation**

| Reference | *INSR* location | Amino acid change (HGVS nomenclature) | Age of presentation (years) | Reported fasting hypoglycemia | Reported post-prandial Hypoglycemia | Plasma glucose (mmol/L) | Corresponding serum insulin (pmol/L) | Other clinical features | Treatment | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| Our patient (Family 1) | Exon 20 | p.(Met1180Lys) | Newborn (proband) | Yes | No | 1.4 (F) | > 694.4 | SGA | Diazoxide | Resolution of hypoglycemia (medication stopped) |
| | | | Newborn (sibling) | Yes | No | 1.8 (F) | 408 | SGA | Diazoxide | |
| Family 2 | | p.(Arg1119Gln) | Newborn | Yes | No | 2.0 (F) | 315.2 | SGA | Diazoxide | |
| Family 3 | | p.(Arg1191Gln) | Newborn | Yes | No | 1.9 (F) | 56.2 | SGA | Diazoxide | |
| Innaurato et al (8) | | p.(Phe1213Leu) | 11 | No | Yes | 3.0 (PP) | 598.6 | AN | - | - |
| | | | 36 (mother) | No | No | 1.9 (PP) | 295.8 | - | | |
| | | | 19 (brother) | No | No | 2.2 (PP) | 588.2 | - | | |
| Preumont et al (10) | | p.(Met1180Val) | 16 | No | Yes | 3.4 (F) | 604 | AN, PA, HR | Metformin | Reduction in hypoglycemia |
| | | | 48 (mother) | - | - | 2.4 (?) | 355 | OG | Metformin | Reduction in hypoglycemia |
| | | p.(Arg1201Gln) | 43 | No | Yes | 3.2 (PP) | 1337 | No s/o IR | Metformin | Reduction in hypoglycemia |
| Krishnamurthy and Pingul (11) | | p.(Arg1131Trp) | 12 | Yes | No | 5.8 (F) | 698 | AN, HR, CL | Metformin | Reduction in hypoglycemia |
| Huang et al (9) | Exon 20 | p.(Arg1174Trp) | 16 | Yes | No | 2.6 (PP) | 2090 | AN, OG | - | - |
| | | | 8 (brother) | - | - | 1.9 (PP) | 467 | SGA (2.4 kg) | | |
| *Højlund et al (7) | Exon 20 | p.(Arg1174Gln) | 12 | No | Yes | 3.3 (F) | 360 | OG | - | - |
| Enkhtuvshin et al (18) | Exon 17 | Δ Leu999 | Newborn | - | - | 1.1 | - | SGA (2.2 kg) | - | Resolution at 10 hours of life |
| | | | Newborn (sibling) | - | - | 1.2 | - | Birth weight: 2.5 kg | - | Resolution at 3 hours of life |

*Another 8 family members were also reported with hypoglycemia and same mutation.

AN: acanthosis nigricans, PA: primary amenorrhea, OG: oligomenorrhea, HR: hirsutism, CL: clitoromegaly, s/o: signs of, IR: insulin resistance, F: fasting, PP: post-prandial, SGA: small for gestational age

Heterozygous mutations in *INSR* cause IR in coexistence with hypoglycaemia, which may be due to selective impairment of *INSR* function in skeletal muscle causing defective peripheral glycogen formation and IR whilst the preserved *INSR* function in the liver leads to suppressed hepatic glucose production causing hypoglycaemia (10,16). This is possibly due to the differential effect of insulin on the phosphorylation of the *INSR* substrates (IRS) -1 and -2 in the skeletal muscle and liver (16). IRS-2 is constitutively phosphorylated due to the increased binding of the kinase regulatory loop binding domain of IRS-2 to the mutated receptor, preventing further activation by insulin or insulin-like growth factor (IGF) 1 in the muscles, whereas IRS-1 phosphorylation is normally activated by basal as well as stimulated levels of insulin (17). This would explain the relative IR in the skeletal muscle in contrast to the insulin sensitivity in the liver.

Only a few patients with a heterozygous *INSR* mutation associated with episodes of hypoglycaemia have been reported (Table 3), mainly demonstrating post-prandial hypoglycaemia following oral glucose tolerance test (8,9). Symptomatic fasting hypoglycaemia has been reported in a few patients; however, age appropriate controlled fasts were not undertaken (8,9,10,11). Symptomatic hypoglycaemia is reported to have occurred as young as three years of age in one patient, however HH was not confirmed (7). The signs of IR were noted in most of these patients while there is only minimal information provided about the individual family members. Enkhtuvshin et al (18) reported a heterozygous *INSR* mutation associated with type 2 diabetes mellitus in a mother and transient hypoglycaemia in both children at birth with the same mutation, however hyperinsulinemia was not documented and hypoglycaemia resolved within 10 hours in both the siblings.

There have been several reported cases associated with homozygous or compound heterozygous mutations causing Donohue syndrome (complete absence of functional insulin receptors) and leading to fasting hypoglycaemia which has been suggested as the effect of insulin on type 1 IGF receptors (19). However type 1 IGF receptors disappear from the liver in adult life (20), which doesn't explain the several reported cases of hypoglycaemia in adult life associated with heterozygous *INSR* mutations (Table 3).

Neonatal HH has not been previously reported in association with heterozygous *INSR* mutations (7,8,9,10,11). The precise mechanism by which these mutations lead to diazoxide responsive neonatal HH, which resolves in infancy, is not clear. One of the possible explanations could be the effect of high levels of insulin on type 1 IGF receptors in the neonatal period with subsequent resolution of hypoglycaemia due to β-cell exhaustion and potential developmental changes in the expression of IGF1 receptor (21,22). In adults, the deposition of amyloid in the pancreas has been observed following chronic IR, which subsequently leads to type 2 diabetes mellitus (23).

In our study, among all three families, affected children were small for gestational age similar to the previously reported patients (9,18). Since insulin has a central role in controlling foetal growth, genetic factors, which impair insulin secretion or action, would be expected to reduce foetal growth. This has been demonstrated experimentally in transgenic mice lacking key intermediates of the insulin-signalling pathway (24). In our study, the affected adult family members were constitutionally lean, which suggests that peripheral IR might not only prevent glycogen storage in muscles, but also reduce lipogenesis and increase lipolysis in fat cells (9).

Although signs of IR, such as AN, and symptoms of androgen excess, such as menstrual abnormalities and hirsutism, are common in patients with *INSR* mutation, the families we describe did not have any such signs or symptoms, supporting the notion that different mutations in *INSR* lead to different phenotypes. Unfortunately we did not have access to insulin level data in the adult members of the families. Moreover, a common heterozygous mutation, p.Arg1174Gln, has been described to be associated with variable clinical phenotypes within the members of the same family (7).

Apart from the genetic background, the phenotype could also be influenced by environmental factors. Patients with IR and compensatory increase in insulin secretion may develop diabetes mellitus in later life when the ability to secrete insulin declines. This leads to postprandial glucose intolerance, followed by fasting hyperglycaemia and diabetes mellitus, which might be prevented by early inventions that include dietary and behavioural modifications. These observations suggest that patients with *INSR* mutation need to be followed up long term for other manifestations in later life.

Diazoxide is the first line treatment for congenital hyperinsulinism, which works as an agonist at KATP channel leading to termination of Ca+ dependent insulin release (25), however there is no data regarding the use of diazoxide in individuals with an *INSR* mutation causing neonatal HH. *INSR* mutations lead to high insulin levels due to IR and metformin has been used effectively in the adult population (10,11) although the mechanism is not clear. Avoidance of high glycaemic index foods may improve symptoms in the patients with post-prandial hypoglycaemia (11).

## Conclusion

We report the first series of heterozygous *INSR* gene mutations causing neonatal HH. This study also highlights that the same gene mutation can lead to variable phenotype within the same family members. Hence, a detailed genetic testing in the family is essential for long-term follow up. All our patients followed a relatively benign clinical course during infancy and the hyperinsulinism resolved in the first year of life, but further monitoring will inform us of the clinical course through to adult life. There is no consensus about the treatment of children with *INSR* mutations due to its rarity, but lifestyle modification could play a key role in long-term management.

### Ethics

**Informed Consent:** Informed consent was obtained from the parents of the patients for publication of this case series.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: Aashish Sethi, Senthil Senniappan, Design: Aashish Sethi, Senthil Senniappan, Syed Haris Ahmed, Data Collection or Processing: Aashish Sethi, Senthil Senniappan, Nicola Foulds, Sarah Ehtisham, Mohammed Didi, Jayne Houghton, Kevin Colclough, Sarah E. Flanagan, Analysis or Interpretation: Mohammed Didi, Jayne Houghton, Kevin Colclough, Sarah E. Flanagan, Literature Search: Aashish Sethi, Senthil Senniappan, Jayne Houghton, Kevin Colclough, Syed Haris Ahmed, Sarah E. Flanagan, Writing: Aashish Sethi, Syed Haris Ahmed, Senthil Senniappan.

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CASE REPORT

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# Novel *MTTP* Gene Mutation in a Case of Abetalipoproteinemia with Central Hypothyroidism

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### What is already known on this topic?

The coexistence of abetalipoproteinaemia (ABL) and peripheral hypothyroidism has been previously reported.

### What this study adds?

The coexistence of ABL and central hypothyroidism has not been reported previously. A homozygous novel mutation [c.506A > T (p.D169V)] was detected in the *MTTP* gene. Our patient had dysmorphic features which is very rare in cases of ABL.

## Abstract

Abetalipoproteinaemia (ABL) is an autosomal recessive disorder characterized by very low plasma concentrations of total cholesterol and triglyceride (TG). It results from mutations in the gene encoding microsomal TG transfer protein (MTTP). A nine-month-old girl was admitted to hospital because of fever, cough, diarrhea and failure to thrive. She had low cholesterol and TG levels according to her age. The peripheral blood smear revealed acanthocytosis. Thyroid function test showed central hypothyroidism. Cranial magnetic resonance imaging revealed the retardation of myelination and pituitary gland height was 1.7 mm. A homozygous novel mutation [c.506A > T (p.D169V)] was detected in the *MTTP* gene. Vitamins A, D, E, and K and levothyroxine were started. The coexistence of ABL and central hypothyroidism has not previously been reported. A homozygous novel mutation [c.506A > T (p.D169V)] was detected in the *MTTP* gene.
**Keywords:** Abetalipoproteinaemia, central hypothyroidism, *MTTP* gene, novel mutation

## Introduction

Abetalipoproteinaemia (ABL) (ABL; OMIM 200100) is an autosomal recessive disorder characterized by very low plasma concentrations of total cholesterol (TC) and triglyceride (TG). The disorder was first described in 1950 by Bassen and Kornzweig (1) in a patient with atypical retinitis pigmentosa. ABL results from mutations in the gene encoding microsomal TG transfer protein (MTTP). Patients with ABL often present with a range of symptoms such as failure to thrive, steatorrhea, hepatomegaly, loss of night and/or color vision, acquired atypical pigmentation of the retina, spinocerebellar ataxia, coagulopathy and myopathy, including fat malabsorption and manifestations of fat soluble vitamin deficiencies (2,3). Early detection and a low fat diet with fat soluble vitamin supplementation can prevent the neurological and ophthalmological complications (4).

Here, we report the coexistence of ABL and central hypothyroidism. This has not been previously reported. In addition, this patient had dysmorphic features. Coexistence of ABL and dysmorphic features is very rare. We detected a novel homozygous mutation in the *MTTP* gene.

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**Conflict of interest:** None declared
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## Case Report

A nine-month old girl was admitted to our hospital because of fever, cough, diarrhea (11 or 12 episodes a day), and failure to thrive. She was born by normal delivery at term with a birth weight of 3200 grams after an uneventful pregnancy. The patient was the second child of a non-consanguineous Turkish couple, who also had a 5-year-old healthy daughter.

At presentation her body weight was 4.7 kg [standard deviation (SD): -3.5], height was 62.5 cm (SD: -1.7), head circumference was 39 cm (3% percentile). Relative index was 71.2.

She was pale, her hair was thin and weak, her subcutaneous adipose tissue was decreased. She had rales in the middle zone of her left lung. Her abdomen was distended and bowel loops were prominent. She also had umbilical and bilateral inguinal hernia. She had dysmorphic features including hypertelorism, frontal bossing, triangular face and retromicrognathia (Figure 1).

Her head control was complete but she could not sit without support. Deep tendon reflexes were normoactive.

Laboratory investigations revealed: hemoglobin level was 9.8 g/dL; leukocyte count was 14820/mm$^3$; and platelet count was 363000/mm$^3$. Serum transaminases were mildly elevated with aspartate aminotransferase of 105 U/L, (normal range: 0-33) and alanine aminotransferase of 112 U/L (normal range: 0-32).

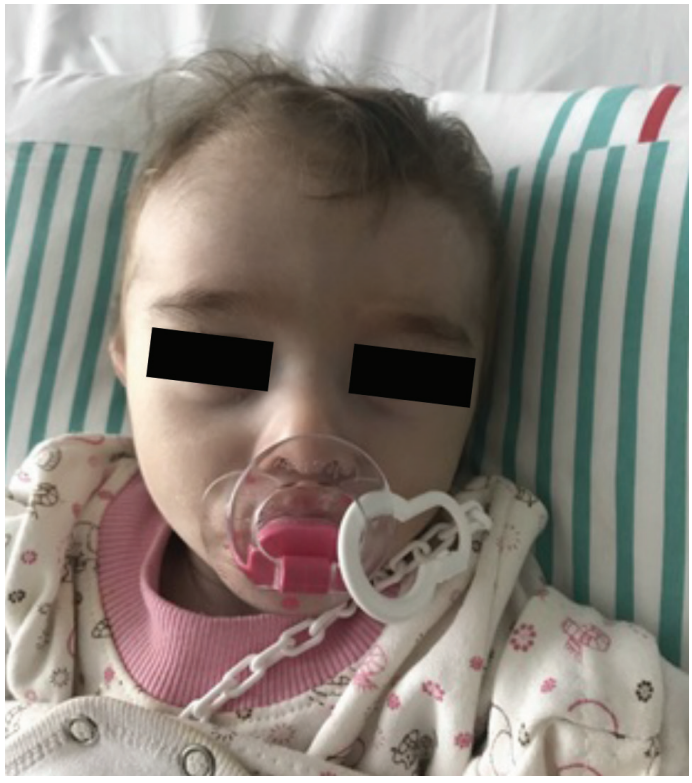

**Figure 1.** Dysmorphic features including hypertelorism, frontal bossing, triangular face and retromicrognaty

Vitamin D concentration was 11.6 μg/L (normal >30), alkaline phosphatase concentration was 99 u/L (normal range: 142-335), calcium concentration was 9.03 mg/dL (normal range: 8.6-10.2), phosphorus concentration was 3.15 mg/dL (normal range: 2.45-4.5) and parathyroid hormone concentration was 60 mg/L (normal range: 15-65).

TORCH screen was negative and other infections due to hepatotropic viruses or human immunodeficiency virus were ruled out. The results of coagulation tests, renal functions and electrolytes were also normal. She was hospitalized three times for bronchiolitis. Cystic fibrosis was considered in this patient due to malnutrition, recurrent bronchiolitis and elevation of liver function tests. Molecular genetic analysis of the *CFTR* gene was normal. Stool examination revealed no reducing substances and showed fat droplets. The search for pathogenic bacteria or parasites was negative. Normal levels of anti-endomysial antibodies ruled out celiac disease and basic metabolic tests including ammonia, lactate, pyruvate, blood acyl carnitine profile and amino acid analysis, urinary organic acid analysis, homocysteine and biotinidase activity were all normal. Congenital immune deficiency was ruled out. Immunoglobulin (Ig) profile (quantitative measurement of IgA, IgM, IgG and IgE), CD markers (CD3, CD19, CD56) and fagotest were normal. She had low cholesterol and TG concentrations with TC of 26 mg/dL (normal range: 3-200) and TG of 9 mg/dL (normal range: 0-200) according to her age. The peripheral blood smear revealed acanthocytosis (Figure 2).

The concentrations of vitamin E at 0.87 mg/L (normal range: 6.6-14.3), vitamin A at 71 ug/L (normal range: 316-820) and vitamin D at 11.6 ug/L (normal values >30) were

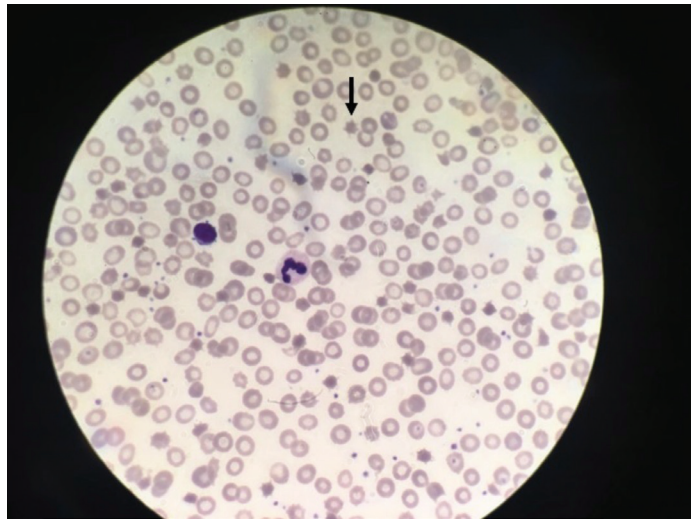

**Figure 2.** Acanthocytosis in the peripheral blood smear

very low. ABL was considered in this patient with these clinical and laboratory findings. Abdominal ultrasonography revealed multiple small stones (<3 mm) in both kidneys and ophthalmologic examination was normal.

Vitamin A (200 IU/kg/day), D (1200 IU/day), E (100 IU/kg/day), and K (5 mg/week) were started. High caloric (150 kcal/kg/day), low fat diet (15 %) with medium chain TG and Basic F® formula were also started.

Thyroid stimulating hormone (TSH) was 1.4 mU/L (normal range: 0.73-8.35) and thyroxine (T4) level was 8.7 ng/L (normal range: 9.2-19.9). Control TSH level was 3 mU/L, and T4 level was 7.4 ng/L. Therefore, levothyroxine (12.5 mcg/day) was started. After the treatment, thyroid function tests were studied intermittently and levothyroxine dose was increased to 37.5 mcg/day. Free triiodothyronine (fT3) level was 3.55 ng/L (normal range: 2.15-5.83).

Follicle-stimulating hormone, luteinizing hormone, adrenocorticotropic hormone (ACTH), cortisol and prolactin levels were evaluated for multiple pituitary insufficiency in addition to central hypothyroidism. Low dose ACTH stimulation test was performed due to a basal cortisol concentration of 10.1 μg/dL (normal >15). The peak cortisol values on low dose ACTH stimulation test were 35.8 μg/dL and evaluated as an adequate cortisol response. Prolactin concentration was 15.12 μg/L and accepted as normal. Insulin-like growth factor-1 (IGF-1) concentration was 8.53 ng/mL (SD -4.28). This low IGF-1 level was attributed to malnutrition. IGF-1 evaluation was planned according to anthropometric follow-up. Cranial magnetic resonance imaging (MRI) revealed the retardation of myelination and pituitary gland height was 1.7 mm (Figure 3). A further sagittal MRI view of the pituitary gland is shown in Figure 4.

Endoscopy was performed to support the diagnosis due to delay in obtaining the molecular genetic analysis. Macroscopic findings of endoscopy included normal mucosa of esophagus and stomach but "snow-like" appearance and pathologic findings were found in duodenum. Microscopic study showed widespread intracytoplasmic vacuolized degeneration of the villi.

*MTTP* gene analysis revealed a novel homozygous pathogenic variant [c.506A>T (p. D169V)]. *In silico* analysis indicated that the D169V substitution in *MTTP* was probably damaging. She is now 15 months old, her body weight was 8.9 kilograms (10-25% percentile) and height was 72.5 cm (3% percentile). She is receiving vitamin A (250 IU/kg/day), vitamin D (1200 IU/day), vitamin E (150 IU/kg/day), vitamin K (5 mg/week) and levothyroxine (37.5 mcg/day). She continues with a low-fat (15 %) and high-calorie diet. Vitamin A and E concentrations, together with thyroid function (TSH=3.9 mU/L, T4=14 ng/L) and coagulation tests, were normal. Stool number decreased significantly (2 or 3 episodes a day). Neurological examination is improved and she is standing and walking with support. This case report was written after receiving informed consent from the family.

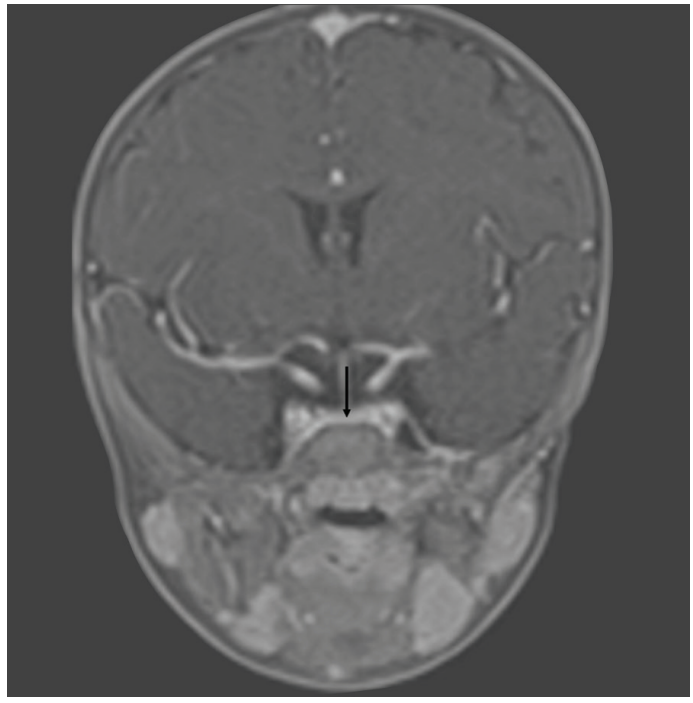

**Figure 3.** The appearance of pituitary gland

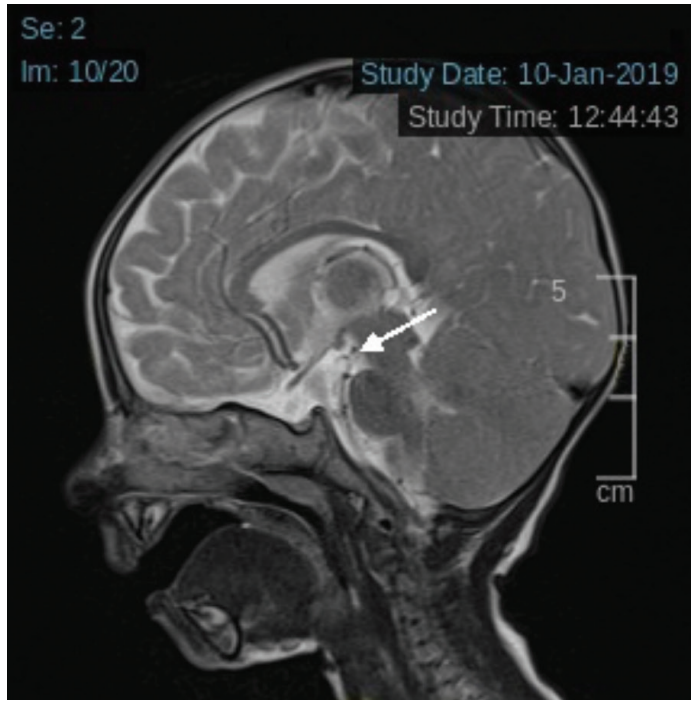


**Figure 4.** Sagittal magnetic resonance imaging image of the pituitary gland

## Discussion

Homozygous hypolipoproteinemia, and chylomicron retention disease have similar clinical findings with ABL. The diagnosis of ABL appeared to be the most likely in view of the normal plasma levels of TC and TG levels found in the parents, which suggested an autosomal recessive transmission. Our patient had also low levels of TG. For this reason, we sequenced the *MTTP* gene. *MTTP* gene analysis revealed a homozygous novel mutation [c.506A > T (p. D169V)].

More than 30 mutations in *MTTP* have been identified. The majority are point mutations resulting in either splicing errors or premature truncations (5). Our case had a point mutation in *MTTP*.

There were some clinical features rarely associated with ABL in the present case. Facial dimorphism and psychomotor retardation have not often been described. It has been suggested that psychomotor retardation occurs due to hypothyroidism. Hasosah et al (6) reported dysmorphic features including hypertelorism, short nose, long philtrum, and thin upper lip in an 18-month-old male ABL patient.

Fat malabsorption causes a combination of unabsorbed fatty acids with calcium ions in the intestinal lumen leading to excessive absorption of oxalate. Rashtian et al (2) reported nephrolithiasis in a 12-months-old male infant, similar to the findings in our patient.

Hypothyroidism can be associated with ABL. Al-Mahdili et al (7) reported a mild case of ABL in association with subclinical hypothyroidism in a 32-year-old female. However, coexistence of ABL and central hypothyroidism has not been previously reported.

Euthyroid sick syndrome (ESS) is characterized by modification of thyroid hormone homeostasis due to non-thyroidal diseases. ESS has been described in liver disease, renal failure, after stress or surgery, in malnutrition or in malignancies. ESS is present if free fT3 was below the lower limit and free T4 was within the normal or low limits, while TSH was in the normal range (8). fT3 level of our patient was 3.55 ng/L (normal range: 2.15-5.83). Therefore, ESS was ruled out.

Krysiak and Okopie (9) reported that untreated or poorly managed ABL can impair the production of steroid hormones and cause some endocrine disorders such as chronic adrenal failure and hypergonadotropic hypogonadism.

Illingworth and Orwoll (10) reported that suboptimal response to corticotrophin stimulation maintained stable levels of plasma cortisol and showed no evidence of adrenal insufficiency with prolonged corticotrophin stimulation in ABL and hypobetalipoproteinaemia. The same group also showed that (11) a total absence of low density lipoprotein (LDL) does not impair adrenal steroidogenesis in the basal state and highlighted that plasma LDL serves as an important source of cholesterol for adrenal corticosteroid synthesis under conditions of sustained stimulation with ACTH (12).

Triantafillidis et al (13) and Illingworth et al (14) reported that patients with ABL have reduced levels of progesterone. This was attributed to low levels of serum LDL cholesterol. Reduced levels of leptin and IGF-1 are probably attributed to the impairment of nutritional status. Arem et al (15) reported that severe LDL cholesterol insufficiency impairs the initial glucocorticoid response to ACTH stimulation, but not overall cortisol production during sustained ACTH stimulation. Severe LDL cholesterol insufficiency may also contribute to the reduction of testosterone in chronically ill patients.

Ocular manifestations are variable, retinitis pigmentosa, ophthalmoplegia, ptosis, nystagmus, peripapillary chorioretinal degeneration, macular atrophy have been reported (16,17). The absence of ocular manifestations in our patient was attributed to the fact that they may appear at any time during the first two decades of life.

Hepatic involvement may include steatosis and elevated serum transaminase levels. In a few cases of ABL, hepatic injury progressed to fibrosis and cirrhosis, requiring transplantation (4,18). The hepatic manifestation in our patient was limited to elevated levels of serum transaminases.

## Conclusion

ABL is a rare disease of lipoprotein metabolism. Symptoms can be debilitating in most patients. Life expectancy is reduced without treatment. The coexistence of the disorder and central hypothyroidism has not been previously reported. ABL in this case was due to a novel homozygous mutation in the *MTTP* gene.

### Ethics

**Informed Consent:** Written consent form was obtained from the parents.

**Peer-review:** Externally and internally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Pembe Soylu Ustkoyuncu, Esra Eren, Durmus Dogan, Concept: Pembe Soylu Ustkoyuncu, Aysegul Yilmaz, Esra Eren, Design: Pembe Soylu Ustkoyuncu, Durmus Dogan, Data Collection or

Processing: Songul Gokay, Fatma Turkan Mutlu, Gokce Yildiz, Analysis or Interpretation: Aysegul Yilmaz, Durmus Dogan, Literature Search: Aysegul Yilmaz, Songul Gokay, Fatma Turkan Mutlu, Writing: Pembe Soylu Ustkoyuncu, Gokce Yildiz.

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CASE REPORT

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# Primary Hyperparathyroidism Presenting as Posterior Reversible Encephalopathy Syndrome: A Report of Two Cases

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### What is already known on this topic?

Hypercalcemia, mostly severe hypercalcemia secondary to malignancies, has occasionally been implicated in the causation of posterior reversible encephalopathy syndrome (PRES). Primary hyperparathyroidism (PHPT) is usually associated with mild-moderate hypercalcemia and has rarely been implicated in PRES.

### What this study adds?

Herein, we report two cases of adolescent PHPT presenting as PRES. We propose that serum calcium levels should be checked in all patients with PRES and that PHPT be regarded as a differential diagnosis in those with underlying hypercalcemia.

## Abstract

Posterior reversible encephalopathy syndrome (PRES) is a clinico-radiological entity characterized by subcortical vasogenic edema presenting with acute neurological symptoms. Common precipitating causes include renal failure, pre-eclampsia/eclampsia, post-organ transplant, and cytotoxic drugs. Hypercalcemia is a rare cause of PRES; most cases occur in the setting of severe hypercalcemia secondary to malignancy or iatrogenic vitamin D/calcium overdose. Primary hyperparathyroidism (PHPT), as a cause of PRES, is an oddity. We report two cases of adolescent PHPT presenting with generalized tonic-clonic seizures and altered sensorium. On evaluation, both had hypertension, severe hypercalcemia (serum calcium 14.1 mg/dL and 14.5 mg/dL, respectively) and elevated parathyroid hormone levels. Magnetic resonance imaging (MRI) revealed T2/fluid-attenuated inversion recovery hyperintensities located predominantly in the parieto-occipital regions, suggestive of PRES. Identification and excision of parathyroid adenoma led to the restoration of normocalcemia. Neurological symptoms and MRI changes improved subsequently. An extensive literature search revealed only four cases of PHPT-associated PRES; none of them being in the pediatric/adolescent age group. The predominant clinical manifestations were seizures and altered sensorium. All had severe hypercalcemia; three had hypertension at presentation, while one was normotensive. Parathyroid adenomectomy led to normalization of serum calcium and resolution of neurological symptoms and radiological changes. Thus, severe hypercalcemia, although rare in PHPT, can lead to hypercalcemic crisis precipitating acute hypertension that can result in cerebral endothelial dysfunction with the breakdown of the blood-brain barrier, culminating in PRES. We therefore recommend that serum calcium levels should be checked in all patients with PRES and that PHPT be regarded as a differential diagnosis in those with underlying hypercalcemia.

**Keywords:** Hypercalcemia, posterior reversible encephalopathy syndrome, primary hyperparathyroidism

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## Introduction

Posterior reversible encephalopathy syndrome (PRES) refers to a disorder of subcortical vasogenic edema in patients presenting with acute neurological symptoms, namely altered sensorium, seizures, headache, visual disturbances, and rarely, focal neurological deficits (1). Radiologically, it is characterized by the presence of bilateral hemispheric edema predominantly involving, but not solely restricted to, the parieto-occipital lobes. PRES has a good prognosis; the clinical and radiological features are reversible over days to weeks. The basic underlying pathophysiology is brain endothelial injury resulting from abrupt changes in blood pressure (BP) or direct toxic effects of cytokines on the endothelium that leads to the breakdown of the blood-brain barrier culminating in brain edema (1). Accordingly, PRES is well recognized in the settings of renal failure, preeclampsia/eclampsia, allogenic bone marrow transplantation, solid-organ transplantation, cytotoxic drugs, and autoimmune disorders (2). Hypercalcemia has rarely been implicated as a cause of PRES (3,4,5,6,7,8,9,10,11). However, all the reported cases had severe hypercalcemia, either secondary to malignancy (3,4,5,6,7,8), vitamin D toxicity (9), iatrogenic calcium infusion (10) or granulomatous infection (11). Primary hyperparathyroidism (PHPT) as a cause of PRES is an oddity with only a few cases reported in world literature (12,13,14,15). Herein, we report two cases of PHPT presenting as PRES and thereafter all the anecdotal cases hitherto reported in world literature are summarized.

## Case Reports

### Case 1

A 12-year-old boy presented with upper abdominal pain and recurrent episodes of vomiting for four days. The day before admission he had one episode of generalized tonic-clonic convulsion (GTCS) lasting for about 30 seconds that was followed by altered sensorium. At presentation to the emergency department (ED), he had impaired mentation, irrelevant talk, and a Glasgow Coma Scale (GCS) of 12/15 ($E_4V_3M_5$). Pupils were bilaterally reacting to light. Vitals recorded were: pulse rate-96/min; BP-140/100 mmHg (mean arterial pressure-120 mmHg; $>99^{th}$ centile for age); respiratory rate 28/min; and capillary refill time $<2$ seconds. He had an upper abdominal tenderness; the rest of the physical examination was unremarkable. While at the ED, he had another episode of GTCS and was immediately started on phenytoin. BP control required labetalol infusion. Preliminary investigations revealed an elevated total leukocyte count of 18000/µL, increased serum calcium [corrected serum calcium 14.1 mg/dL (range: 8.8-10.4)], low serum phosphorous [2 mg/dL (range: 3.7-5.4)], and high serum amylase [1917 IU/L (range: 19-86)] and lipase [641 IU/L (range: 12-70)]. Serum creatinine was normal. The cerebrospinal fluid analysis was unremarkable. Ultrasonography of the abdomen showed a bulky pancreas with peri-pancreatic fat stranding (suggestive of pancreatitis) and bilateral nephrolithiasis. Non-contrast computerized tomography (CT) of the head was non-contributory; hence contrast-enhanced magnetic resonance imaging (CEMRI) was performed which was suggestive of T2/fluid-attenuated inversion recovery (T2/FLAIR) hyperintensities, with diffusion restriction involving the parieto-occipital areas (predominantly left-sided), indicative of PRES (Figure 1A, 1B). Hypercalcemia was managed with parenteral

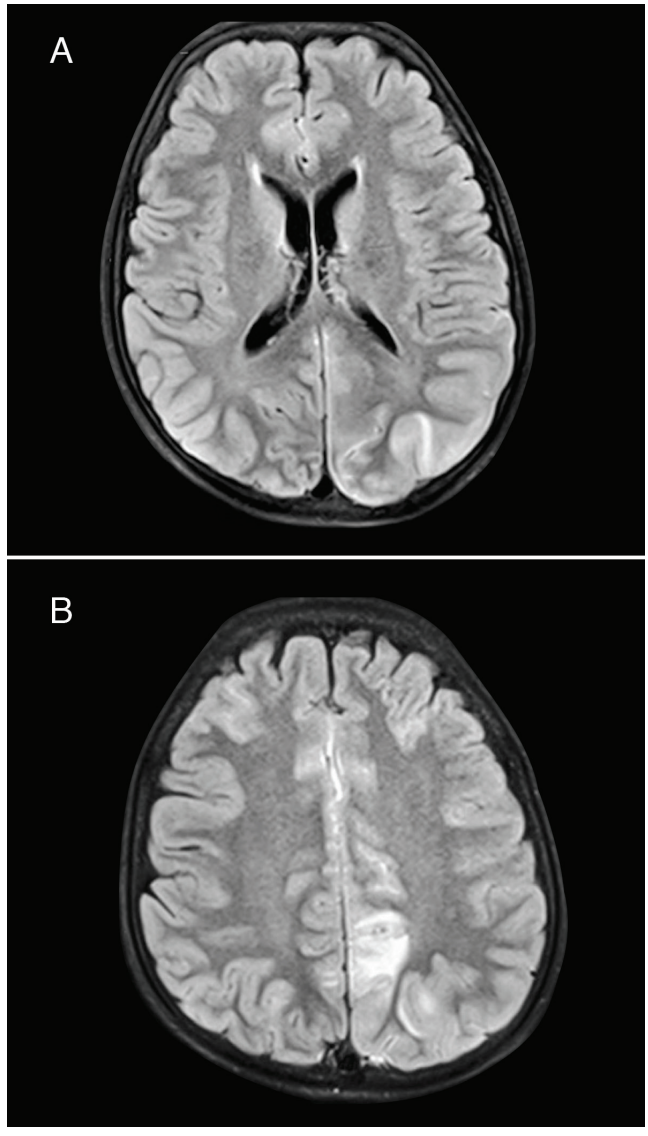


**Figure 1. A, B)** Magnetic resonance imaging of brain (first case) with T2/fluid-attenuated inversion recovery images showing hyperintensities in the parieto-occipital regions, predominantly on the left side

hydration and furosemide. Thereafter he was moved to the pediatric intensive care unit (ICU). Labetalol infusion, phenytoin, and parenteral fluids were continued. On day 3, his serum calcium came down to 11.8 mg/dL and BP was under control. His sensorium improved, however, he remained somewhat drowsy. Detailed work-up at ICU showed elevated serum intact parathyroid hormone (iPTH) [iPTH 203 pg/mL (range: 15-65)] and 25-hydroxyvitamin D of 22.6 ng/mL. Contrast-enhanced CT of the abdomen done on day 6 reconfirmed the finding of acute pancreatitis along with cholelithiasis and bilateral nephrolithiasis. There were no supra-renal masses. Ultrasound of the neck was normal. $^{99m}$Tc-sestamibi scan revealed a 1.0x0.9 cm tracer-avid lesion suggestive of left inferior parathyroid adenoma. A diagnosis of hypercalcemic crisis secondary to PHPT was made. Suspecting multiple endocrine neoplasia syndrome, relevant investigations revealed normal serum prolactin, normal serum insulin-like growth factor 1 (IGF-1) (age and pubertal status matched), normal sella (on CEMRI), and non-elevated 24-hours urinary metanephrine and nor-metanephrine. Sanger sequencing for the *MEN1* gene did not reveal any mutation. Work-up for secondary causes of hypertension including renal artery Doppler, plasma aldosterone concentration/plasma renin ratio, and urinalysis were unremarkable. Hence hypertension was attributed to PHPT. He underwent open surgical excision of the left parathyroid mass. The remaining three parathyroid glands were explored and, in view of normal morphology, they were left *in situ*. Histopathology of the excised tissue showed parathyroid adenoma. Post-operatively his calcium and iPTH levels came down to 8.8 mg/dL and 28 pg/mL, respectively. His sensorium completely improved on day 1 post-surgery. He was taken off antihypertensive medications and discharged on day 4 post-surgery. At follow-up, he remained normotensive and normocalcemic. MRI brain repeated after three months post-surgery showed complete resolution of prior changes.

Informed written consent was obtained from the patient's father.

## Case 2

A 16-year-old boy presented with a two day history of altered sensorium following an episode of GTCS. His parents had noticed an alteration in his behavior over the past two weeks, in the form of apathy, irritability, and decreased alertness. Past medical history was relevant in that he had suffered fractures of his right humerus and right neck of femur following a fall from a motorcycle one month previously and was immobilized in plaster casts at a local hospital. At presentation to the ED, he had altered mentation with a GCS of 11/15 ($E_3V_3M_5$). The right lateral margin of his tongue was lacerated suggestive of tongue-bite. He was found to have hypertension (BP = 180/100 mmHg). The fundus examination was unremarkable with no evidence of papilledema. Neck rigidity was absent. Preliminary investigations revealed hypercalcemia (corrected serum calcium 14.5 mg/dL), hypophosphatemia (serum phosphate 1.1 mg/dL), normonatremia, normokalemia and normal renal function. The cerebrospinal fluid analysis was normal. Non-contrast CT of the head showed multiple lytic lesions in the calvarium, while brain parenchyma appeared grossly normal. Hence, a CEMRI brain was performed which showed areas of cortical and subcortical white matter hyperintensities on T2/FLAIR-weighted images with diffusion restriction involving the parietal, occipital, and frontal regions (predominantly left-sided), suggestive of PRES (Figure 2A, 2B). Hypercalcemia was managed

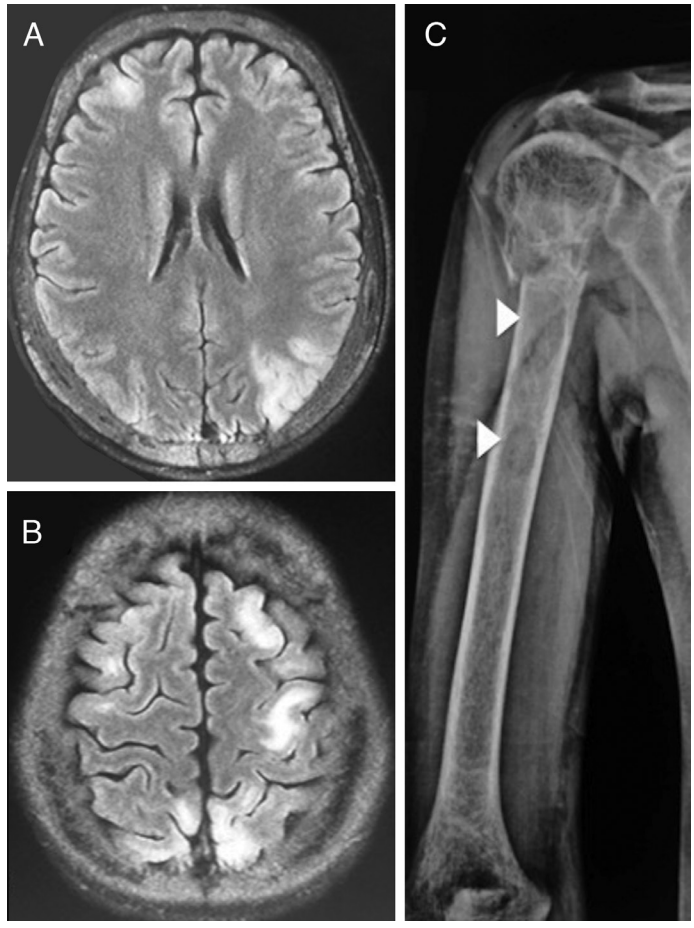


**Figure 2. A, B)** Magnetic resonance imaging of brain (second case) with T2/fluid-attenuated inversion recovery images showing hyperintensities in the parietal, occipital and frontal regions, more marked on the left side. **C)** Radiograph of the right shoulder and arm showing a displaced fracture of the surgical neck of the right humerus. Two lytic lesions are seen in the shaft of the right humerus suggestive of brown tumors of hyperparathyroidism (marked in white arrow heads)

with parenteral fluids and parenteral zoledronic acid while hypertension control required labetalol infusion. Detailed workup for the cause of hypercalcemia revealed iPTH of 2491 pg/mL and 25-hydroxyvitamin D of 18.38 ng/mL. Ultrasonography of the neck was non-contributory, however, $^{99m}$Tc-sestamibi scan revealed a 2.5x1.6 cm left inferior parathyroid adenoma. Radiographs showed fractures of the neck of right femur and surgical neck of the right humerus, multiple lytic lesions (suggestive of brown tumors) (Figure 2C), diffuse cortical thinning of long bones, and sub-periosteal resorption of the phalanges of fingers. Dual-energy X-ray absorptiometry was suggestive of low bone mineral density. Abdominal ultrasonography revealed a small right renal calculus. Secondary causes of hypertension, such as renal parenchymal disease, renal artery stenosis, pheochromocytoma, primary aldosteronism, Cushing's syndrome, and hyperthyroidism were diligently ruled out. Hence, a clinical diagnosis of PHPT was made; hypertension and PRES were attributed to the hypercalcemic crisis. In view of severe hypercalcemia, iPTH level more than 10 times upper limit of normal, young age, male gender, concomitant bone, and renal involvement, a possibility of parathyroid carcinoma was considered (16,17,18). Lack of similar family history, normal serum prolactin, and age/pubertal status matched serum IGF-1 levels, normal sella (on CEMRI), absence of any thyroid nodule and non-visualization of any jaw lesion on imaging made syndromic causes of PHPT less likely. Sanger sequencing for the *MEN1* gene did not reveal any mutation. By day 3 of admission, his serum calcium came down to 12.3 mg/dL and there was a marked improvement in his sensorium. On day 5 he underwent excision of the left inferior parathyroid mass; the mass was not infiltrating the surrounding tissues and could easily be dissected out. The remaining three parathyroid glands were explored, however, they appeared morphologically normal to the operating surgeon. His calcium and iPTH levels came down to 9.4 mg/dL and 43 pg/mL, respectively on day 1 post-surgery. Histopathology of the excised lesion was suggestive of parathyroid adenoma with no features of parathyroid carcinoma. His sensorium completely improved; his anti-hypertensive requirement came down and he was discharged on 5 mg of amlodipine. At one month follow-up, he was normocalcemic. His BP was in the low-normal range; hence, amlodipine was stopped. When reviewed at three months, he was normotensive and normocalcemic. A repeat MRI brain showed a complete resolution of the T2/FLAIR hyperintensities.

Informed written consent was obtained from the patient's father.

## Discussion

Herein we have reported two cases of PHPT presenting with predominantly neurological complaints and diagnosed as having PRES. Both of them had hypertension and severe hypercalcemia (serum calcium > 14 mg/dL) at presentation. Neurological manifestations, hypertension, and MRI changes resolved following parathyroid adenomectomy and restoration of normocalcemia. These two cases add to the small list of reports of PHPT presenting as PRES. These cases are however very unusual as both of them were young. PHPT *per se* is an uncommon endocrine disease in the pediatric population with a prevalence of 2-3 cases per 100,000 (19). Similarly, PRES in the pediatric/adolescent population is even less common with mostly anecdotal case reports and a few small case series (20,21). No case of PRES in young PHPT has hitherto been reported.

PRES, also known as reversible posterior leukoencephalopathy syndrome, is a clinico-radiological entity characterized by acute neurological symptoms. First described in 1996, the entity remains rare with its global incidence being unknown. Most cases occur in young-to-middle aged adults with a female preponderance. PRES is commonly seen in the setting of renal failure, pre-eclampsia/eclampsia, and accelerated hypertension. Acute hypertension, more precisely abrupt fluctuations in BP cause endothelial dysfunction, breakdown of the blood-brain barrier, and subsequently vasogenic edema, leading to PRES (1). The predominant involvement of the posterior regions of the brain in PRES is primarily believed to be due to lower density of sympathetic innervation of the vertebrobasilar system, a factor that maintains cerebral autoregulation and protects the brain from severe hypertension (22). Hypertension in PRES is however not universal; 15-20% of patients are normotensive or even hypotensive (23). Endothelial dysfunction and subsequently interstitial brain edema in such cases is mediated by excessive circulating cytokines. PRES occurring in the setting of underlying autoimmune diseases, post-organ transplant, cytotoxic/immunosuppressive drug use, and sepsis appears to be mediated predominantly by cytokines (1).

Hypercalcemia is rarely cited as a cause of PRES. Multiple mechanisms have been proposed for hypercalcemia-induced PRES. Vasospasm of the cerebral vessels being one of them (5,8). Hypercalcemia leads to augmented actin-myosin coupling, resulting in vascular smooth muscle contraction and subsequent vasospasm in the cerebral circulation (8). The subsequent perturbations in cerebral blood flow lead to endothelial cell injury, culminating in PRES (6). In addition, a sudden rise in BP induced by acute hypercalcemia can

precipitate PRES. Hypertension in such settings is mediated not only by a direct effect of calcium on vascular smooth muscle but also by an indirect effect of calcium-mediated hypercatecholaminemia (24). High levels of circulating calcium can directly lead to endothelial dysfunction. Rats with diet-induced hypercalcemia exhibit a transformation of their endothelial cells to a predominantly pro-inflammatory phenotype (25). Hypercalcemia has been shown to increase the expression of renal endothelin-1, inducible nitric oxide synthase, and other pro-inflammatory cytokines in rats (26,27). Lastly, hypercalcemia-induced hypomagnesemia has been proposed as one of the underlying mechanisms in the failure of cerebral autoregulation (3). Thus, cerebral vasospasm, acute hypertension, endothelial dysfunction, and hypomagnesemia provide an optimum milieu for precipitating PRES.

Amidst the plethora of patients with hypercalcemia encountered in routine clinical practice, the rarity of occurrence of PRES needs to be explained. The extent of hypercalcemia perhaps dictates the pathogenesis of PRES. In all the hitherto reported cases of hypercalcemia and PRES, the corrected serum calcium levels were more than 13 mg/dL (3,4,5,6,7,8,9,10,11,12,13,14,15). Accordingly, hypercalcemia-induced PRES has mostly been recognized either in the setting of malignancy or iatrogenic vitamin D/calcium overdose (3,4,5,6,7,8,9,10). PRES in the setting of PHPT is extremely rare. This probably reflects the relatively mild-moderate levels of serum calcium seen in PHPT patients. In a registry of 464 patients with histologically proven PHPT, the mean calcium level was 11.9 mg/dL (28). In addition to absolute calcium levels, the rate of rise in serum calcium is probably equally important. Hypercalcemia is of more rapid onset in malignancy when compared to PHPT (29). The relatively rapid rise in serum calcium in association with underlying malignancies perhaps causes sudden perturbations in cerebral blood flow, leading to PRES. Alternatively, the rarity of association can also be explained on the basis that most patients with hypercalcemia presenting with altered sensorium do not undergo neuro-imaging, thereby leading to under-diagnosis of PRES.

After an extensive literature search, thee were only four cases of PHPT associated with PRES (Table 1) (12,13,14,15). The case reported by Popkirov et al (15) cannot strictly be labeled as PHPT; rather it was a case of tertiary hyperparathyroidism developing in a patient with hereditary hypophosphatemic rickets following long-term phosphate supplementation. All but one patient was male; all were above 50 years of age. The predominant clinical manifestations were seizures and altered sensorium; other associated symptoms included headache, visual hallucinations, visual field defects, aggressive outbursts (12) and worsening of extrapyramidal disease (14). Hypertension at presentation was seen in all but one patient. All had severe hypercalcemia, ranging from 14.3 mg/dL to 21.2 mg/dL. Consistent with the diagnosis, all had elevated serum PTH levels. A parathyroid adenoma was located in all four cases. Parathyroid adenomectomy led to normalization of serum calcium along with a rapid and complete resolution of the neurological symptoms. The case reported by Au et al (13) however had residual neurological deficits in the form of persistent left-sided homonymous hemianopsia and neglect, likely because of underlying hypoxic brain damage as the patient had been in status epilepticus for six days. The resolution of MRI changes had been documented in only two cases with normalization occurring as early as seven days following the restoration of normocalcemia. Okaygün et al (30) reported a case of PHPT with severe hypercalcemia, pancreatitis, and encephalopathy; cranial CT revealed periventricular ischemia, however, the diagnosis of PRES is debatable.

The sequence of events leading to PRES in our two cases needs clear explanation. Severe hypercalcemia (serum calcium >14 mg/dL) had precipitated the hypercalcemic crisis. However, as has already been said, the occurrence of severe hypercalcemia in PHPT is a rarity; only 6% of PHPT patients treated at the Surgical Service at the University of Michigan Hospital during a 16-year period had severe hypercalcemia. Hypercalcemia would have subsequently led to hypertension, although, high BP has been recorded in normocalcemic PHPT as well. Higher levels of pressor hormones and increased cardiovascular reactivity to catecholamines have been implicated as the cause of hypertension in PHPT. Hypercalcemia and hypertension would have subsequently worked in tandem to precipitate PRES. Acute pancreatitis, as present in our first case, might also have contributed to the occurrence of PRES. Hypercalcemia in the second case might have been further aggravated following immobilization after sustaining a fracture of the right neck of femur. Besides, PTH *per se* has been implicated in directly causing endothelial dysfunction. Although PTH-induced endothelial dysfunction can lead to PRES in PHPT, the same does not hold true in PTH-independent causes of hypercalcemia as PTH levels are suppressed. This implies that hypercalcemia is more important in the causation of PRES than elevated PTH levels.

## Conclusion

In conclusion, we have presented two cases of young PHPT presenting as PRES. Severe hypercalcemia and hypertension were common to both; MRI was suggestive

**Table 1. Four cases of primary hyperparathyroidism-associated posterior reversible encephalopathy syndrome hitherto reported in the world literature. The two index cases described herein have also been included in the table**

| Serial no. (reference) | Age (years)/sex | Presenting neurological symptoms | BP at presentation | Corrected serum calcium | Serum PTH | Parathyroid adenoma localization | Following parathyroidectomy and hypercalcemia correction |
|---|---|---|---|---|---|---|---|
| 1 (12) | 51 / F | Headache, nausea, vomiting, visual hallucinations, aggressive outbursts, later somnolence | Normotension | 15.5 mg/dL | 465 pg/mL | Right parathyroid adenoma | Resolution of symptoms and MRI changes |
| 2 (13) | 58 / M | Acute cognitive decline, status epilepticus | Hypertension | 21.2 mg/dL | 844 pg/mL | Left parathyroid adenoma | Resolution of symptoms with persistence of left homonymous hemianopsia and neglect. No mention about resolution of MRI changes. |
| 3 (14) | 78 / M | Two episodes of GTCS. Fluctuating alterations in alertness in the previous month. | Hypertension | 14.3 mg/dL | 256 pg/mL | Left inferior parathyroid adenoma | Resolution of symptoms and MRI changes |
| 4 (15) | NA / M | Seizures and coma | Hypertension | 15.3 mg/dL | 1119 pg/mL | NA | Resolution of symptoms. No mention about resolution of MRI changes. |
| 5 (index case 1) | 12 / M | One episode of GTCS followed by altered sensorium | Hypertension | 14.1 mg/dL | 203 pg/mL | Left inferior parathyroid adenoma | Resolution of symptoms and MRI changes |
| 6 (index case 2) | 16 / M | One episode of GTCS followed by altered sensorium. Altered behavior over past two weeks. | Hypertension | 14.5 mg/dL | 2491 pg/mL | Left inferior parathyroid adenoma | Resolution of symptoms and MRI changes |

BP: blood pressure; PTH: parathyroid hormone; GTCS: generalized tonic-clonic seizures; MRI: magnetic resonance imaging, NA: not applicable, M: male, F: female

of T2/FLAIR hyperintensities predominantly affecting the occipito-parietal regions. Neurological symptoms and MRI changes resolved with the restoration of normocalcemia following parathyroid adenomectomy. We therefore propose that serum calcium levels should be checked in all patients with PRES and that PHPT be considered as a differential diagnosis in those with underlying hypercalcemia.

### Ethics

**Informed Consent:** Obtained from patients' father.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Arunanshu Behera, Concept: Rimesh Pal, Sanjay Kumar Bhadada, Design: Rimesh Pal, Data Collection or Processing: Aditya Dutta, Kanhaiya Agrawal, Nimisha Jain, Pinaki Dutta, Analysis or Interpretation: Rimesh Pal, Literature Search: Nimisha Jain, Pinaki Dutta, Anil Bhansali, Writing: Rimesh Pal.

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CASE REPORT

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# The Effectiveness of Sirolimus Treatment in Two Rare Disorders with Nonketotic Hypoinsulinemic Hypoglycemia: The Role of mTOR Pathway

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### What is already known on this topic?

Nonketotic-hypoinsulinemic hypoglycemia (NkHH) is a very rare problem of glucose consumption increase without hyperinsulinism. In these cases, there is no effective therapy other than frequent feeding to counter hypoglycemia.

### What this study adds?

Sirolimus treatment may function as a further therapeutic option in NkHH. It could be a lifesaving tool for those kind of disorders as sirolimus appears to control the peristent hypoglycemia effectively in patients with NkHH, by inhibition of mammalian target of rapamicin.

## Abstract

Nonketotic-hypoinsulinemic hypoglycemia (NkHH) is a very rare problem charcterized by increase in glucose consumption without hyperinsulinism. This disorder has mainly been reported in cases with *AKT2* mutation and rarely in cases with *PTEN* mutation. In cases with *PTEN* or *AKT2* mutation, there is no effective therapy other than frequent feeding to counter hypoglycemia. The mammalian target of rapamicin (mTOR) inhibitor, sirolimus, has been used in hyperinsulinemic hypoglycemia that was unresponsive to other medical treatment. In the insulin signaling pathway, both AKT2 and PTEN function upstream of mTOR. However, the role of Sirolimus on hypoglycemia in *AKT2* and *PTEN* mutations is unknown. Case 1: Six month-old female with *AKT2* mutation [c.49G > A (p.E17K)] and evidence of NkHH. Frequent feeding was unsuccesful in correcting hypoglycemia and her proptosis continued to worsen. Sirolimus treatment was started at three years of age. Subsequently, blood glucose (BG) levels increased to normal levels. Case 2: In a male with *PTEN* mutation (p.G132V (c.395G > T), persistent NkHH started at 16 years of age (fasting BG: 27 mg/dL, fasting insulin 1.5 mmol/L, while ketone negative). Sirolimus treatment was started and hypoglycemia was succesfully controlled. NkHH is a very rare and serious disorder which is challenging, both for diagnosis and treatment. Additionally, *AKT2* and *PTEN* mutations may result in NkHH. Sirolimus treatment, through mTOR inhibition, appeared to be effectively controlling the peristent hypoglycemia and may be a life-saving therapy in this NkHH due to *AKT2* and *PTEN* mutations.

**Keywords:** *AKT2*, *PTEN*, sirolimus, hypoglycemia, treatment

## Introduction

Recurrent/persistent fasting hypoglycema is a life-threatening condition in childhood and is frequently related to either hyperinsulinism or inborn errors of metabolism impairing hepatic glucose production (1). Hyperinsulinism is the most common cause of persistent hypoketotic, hypofattyacidaemic, hypoglycaemia in infancy and childhood. In this situation, excessive insulin secretion suppresses the mobilisation of fatty acids from adipose tissue, preventing ketone body synthesis in the liver (2,3). Another well known cause of nonketotic hypoglycemia are fatty acid oxidation defects (4).

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Recently, a few cases with unexplained, recurrent and severe fasting hypoglycemia without hyperinsulinism or fatty acid oxidation defects have been reported (1,5,6,7). We prefered to use the term of "nonketotic-hypoinsulinemic hypoglycemia (NkHH)" in these cases.

NkHH is a very rare problem of glucose consumption increase without hyperinsulinism. In 2011, the first case with genetic defects of *AKT2* leading to NkHH was published. *AKT2* is a serine/threonine kinase that plays an important role in insulin signal transduction (7). Normally, when insulin combines with its receptor at target tissue, it requires phosphatidylinositol-3,4,5-trisphosphate (PIP3) to accumulate at the plasma membrane to facilitate insulin transmission within the cell. Gain of function mutation of *AKT2* cause PIP3 accumulation without the need for insulin. The biochemical profile of *AKT2* activating mutation is very similar to hyperinsulinism (7).

Similar to *AKT2* activating mutation, a defect in other molecules that have a role in insulin signalling are expected to be a cause of NkHH. Rarely, NkHH can also develop with mutation of the tumor suppressor gene *PTEN* (8).

Treatment of hypoglycemia in NkHH can be very difficult, because there is no beneficial medical therapy to counteract insulin synthesis or secretion. In these cases, no effective therapy is available other than frequent feeding to prevent hypoglycemia. Thus, there is no available therapy at all in patients who cannot be fed for any reason, including vomiting, gastrointestinal problems, or anorexia.

The mammalian target of rapamycin (mTOR) inhibitor, sirolimus, has been used in hyperinsulinemic hypoglycemia which was unresponsive to other medical treatment (3,9). In the insulin signaling pathway, both *AKT2* and *PTEN* function upstream of mTOR. Thus inhibition of mTOR should counter activating mutations in *AKT2* and *PTEN*. However, the effect of sirolimus in hypoglycemia due to *AKT2* and *PTEN* mutations is unknown.

In this paper, clinical and biochemical characteristics of two rare cases with NkHH are presented. Additionally, the effect of sirolimus on hypoglycemia is reported in these cases.

## Case Reports

### Case 1

A seven month-old female patient was brought to clinic by her family because she had recurrent hypoglycemia for one month. She was born at term with a history of polyhydramnios. On physical examination there was bilateral proptosis, hypertrichosis, hypertelorism, a flat nasal bridge, macroglossia and acanthosis nigricans. At the time of admission her height was 68 cm (50$^{th}$ percentile) with a weight of 7700 g (25-50$^{th}$ percentile) and a head circumference of 44 cm (75$^{th}$ percentile). She had hypoinsulinemia (< 0.2 mIU/mL, C-peptide < 0.1 ng/mL) and was nonketotic during hypoglycemia when blood glucose (BG) was 27 mg/dL. Other biochemical and hormonal analysis showed normal results. Due to hypoglycemia occuring during fasting, frequent feeding and addition of cornstarch to foods was implemented. Whenever severe hypoglycemia occured, intravenous glucose infusion was also given.

Genetic analysis revealed a *de novo AKT2* mutation [c.49G > A (p.E17K)] in the patient (5). During follow-up, frequent feeding was unsuccesful in treating all the hypoglycemic episodes. Clinically, acanthosis nigricans and proptosis continued to worsen (Figure 1). After informed consent was given by her parents, sirolimus treatment was started at three years of age. On sirolimus treatment BG levels increased to normal levels (mean BG before treatment: 48-52 mg/dL/day, after treatment 77-108 mg/dL/day). Prior to starting sirolimus treatment, it was observed that she could not fast longer than 3 hours although this increased to 4 to 5 hours with the treatment. Neurological evaluation revealed normal language, cognitive, social, and fine motor development with a slight delay in gross motor development.

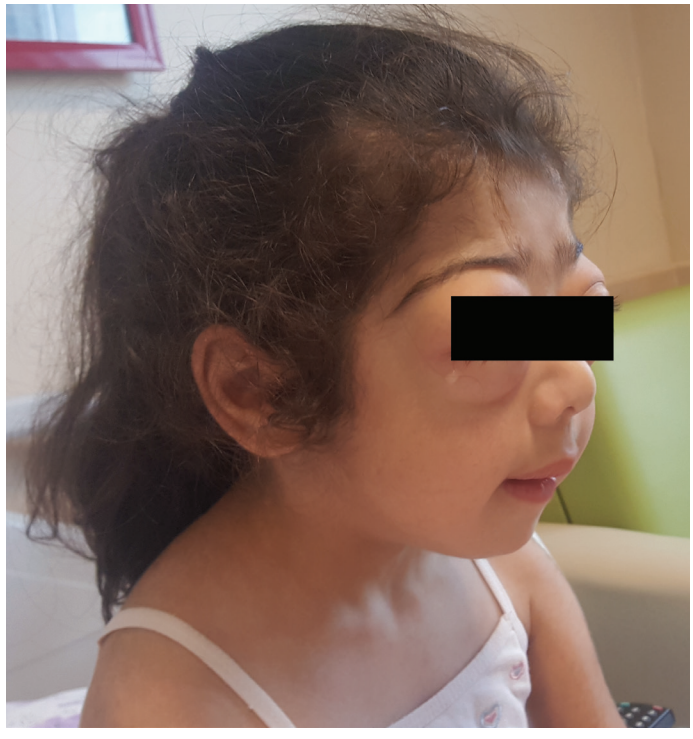

**Figure 1.** Case 1 with *AKT2* mutation

### Case 2

A male with multiple systemic involvement was diagnosed with hamartoma-tumor syndrome before his admission to the endocrinology department. He had verrucous epidermal nevus and adrenal hemorrhage at birth, and at five month of age renal vein and inferior vena cava thrombosis with hypertension. At 16 months of age, he developed pelvic and retroperitoneal lipomatosis, multiple polyps of the colon and focal segmental glomerulosclerosis. Total colectomy for polyps was caried out due to the recurrent bleeding. He also had macrocephaly, delayed motor mental development and epileptic seizures (Figure 2). These symptoms suggested *PTEN* hamartoma-tumor syndrome (PHTS), so mutation analysis was conducted. Results revealed a *PTEN* mutation [p.G132V (c.395G>T)] (10). Due to the *PTEN* mutation, he carried a high risk of thyroid malignancy. Due to this risk prophylactic thyroidectomy was performed and thyroxine replacement was started.

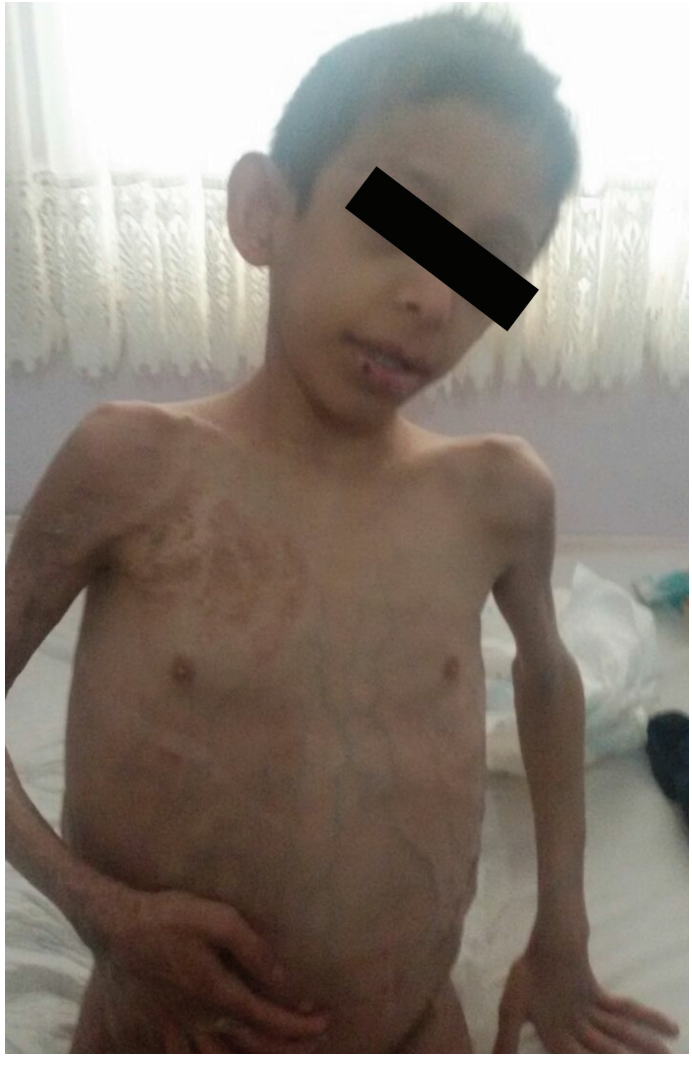

**Figure 2.** Case 2 with *PTEN* mutation

During his follow-up, at 16 years of age, severe recurrent hypoglycemia (fasting BG: 6 to 27 mg/dL) was noted. Fasting insulin was low (1.5 mmol/L), while ketone was negative. Hypoglycemia was persistent even though there was no known causes of hypoglycemia, with no detection of congenital metabolic disorders.

A tentative diagnosis of NkHH was reached and frequent feeding was offered, but this was ineffective in resolving the hypoglycemic attacks. In addition, feeding was not always possible due to occasional anorectic episodes.

We were aware that *PTEN* may have a role in the insulin signalling pathway and there were reports suggesting that a few patients with *PTEN* mutation also had hypoyglycemic events (7,8).

After informed consent was given, sirolimus treatment was started. Follow-up examinations and evaluations were done at three-monthly intervals. These included complete blood count, serum BUN, creatinine, electrolytes, aminotransferase measurement (aspartate aminotransferase and alanine aminotransferase), lipid profile and hemoglobin A1c. Sirolimus treatment also resulted in an improvement in duration of fasting time in this patient, although the effect was not as marked. Fasting time for case 2 increased to 3 to 4 hours, up from no more than 2 hours, prior to sirolimus. Case 2 required an increased dose of sirolimus during the first month of treatment. With increased dosage, the frequency and severity of hypoglycemia reduced: mean BG before treatment was 46-64 mg/dL/day; after treatment this was 62-92 mg/dL/day. The lowest fasting glucose level of 32 mg/dL was experienced by case 2.

### Sirolimus Dosing

For both patients, initial sirolimus dose was 0.5 mg/m$^2$/day. The dose of sirolimus was then titrated according to the serum level for both patients (between 4 and 12 mg/dL). Final sirolimus dose was 1 mg/m$^2$/day in case 1 and 4 mg/m$^2$/day in case 2. Of note, transient leucocytosis was observed in case 1 without any other obvious cause. Duration of sirolimus treatment wase 42 months in case 1 and nine months in case 2.

Informed consent for the publication of these cases was obtained from both sets of parents.

## Discussion

It is accepted that glucose homeostasis is maintained by the action of insulin on muscle, adipose tissue and liver (5). Insulin stimulate energy storage and growth through effects on glucose, lipid and amino acid metabolism. At the cellular

level, insulin effects are mediated by a transmembrane tyrosine kinase receptor that phosphorylates insulin receptor substrate (IRS) and other adaptor proteins. Further downstream, insulin signaling leads to activation of AKT serine/threonine kinases (1).

In recent times, NkHH cases due to activation of the insulin signaling pathway have begun to be published. *AKT2* mutation has been shown to be directly causative for this specific condition (1,8,11). *AKT2* is critical for the control of glucose and lipid metabolism. It is recruited to the cell surface by phosphoinositide 3-kinase (PI3K) and phosphorylated by pyruvate dehydrogenase kinase 1 and mTOR c2 kinases. It has a transducer effect during insulin signaling to GLUT4 (12). The causes of hypoglycemia in *AKT2* mutation are related to the activation of the insulin signalling pathway. The first case with a gain of function of *AKT2* causing hypoinsulinemic hypoglycemia was reported in 2011 (1). Since then, a few cases have been reported, including the two presented herein (5,6,7).

*AKT2* is a signal transducer in both glucose metabolism and lipid homeostasis (12). In our case, extra-ocular adipose tissue expansion leading to proptosis was prominent. Some deposits of adipose tissue may be more responsive to *AKT2* mutation than others. The cause of this difference is unknown although one plausible explanation may be different levels of expression of *AKT2* in metabolic tissues (12).

*PTEN* is one the most important tumour suppressors. Deactivation of *PTEN* causes the activation of mTOR c1, which in turn leads to the augmented translocation of specific mRNAs which is crucial for cell growth and proliferation (13). The *PTEN*–PI3K–AKT–mTOR pathway has a central role in the regulation of glucose metabolism. This pathway has downstream effects on the insulin receptor and IRS adaptor molecules. It is known that the PI3K–AKT pathway enhances insulin-mediated glucose uptake and membrane translocation of the glucose transporter GLUT4, and inhibits gluconeogenesis (13). *PTEN* deficiency results in enhanced activation of the AKT signaling pathway. A mutation of *PTEN* may augment PI3K signaling to AKT, which then affects the mTOR pathway (14).

Usually, hypoglycemia in a patient with *PTEN* gene mutation is not noticed. Schmid et al (8) reported a case with PHTS, which was caused by germ line mutations in the *PTEN* gene. The case was treated with sirolimus for uncontrolled tumor cell proliferation. In this case a fasting glucose level of 1.9 mmol/L (reference: 3.6-5.6 mmol/L; equivalent to 35 mg/dL) was detected at the age of 42 months, although there was no extra information about the course of hypoglycemia. In our PTHS patient (case 2), hypoglycemia was detected at 16 years old. Blood sugar levels were very low at times and frequent feeding was ineffective to resolve hypoglycemia. In particular, the management of hypoglycemia in anorectic periods in this patient were very challenging. This situation prompted the search for alternative or additional treatment modalities.

*PTEN* loss induces adipogenic-like transformation in hepatocytes and transcription of genes involved in lipogenesis and β-oxidation (13). Additionally, mTOR has a central role in the regulation of cell cycle and initiation of transcription by translating the signalling for growth and proliferation. The administration of rapamycin (mTOR inhibitor) in patients with *PTEN* mutation has been found to be effective in reducing hamartomatous masses, lipomatous lesions, and thymus hyperplasia with clinical recovery (8,15,16). Hence, both hypoglycemia and uncontrolled cell proliferation in various tissues may be controlled with mTOR inhibition. With sirolimus treatment, hypoglycemia in case 2 was controlled more succesfully.

A dysregulated *PTEN*–PI3K–AKT–mTOR signaling pathway may result not only in extensive tumor cell proliferation, but also deregulation of glucose metabolism. Increased glucose utilization is consistent with hyperactivation of the PI3K/AKT pathway, being one of the key mediators of increased glucose utilization observed in many cancer cells (17). Kinross et al (18) developed a murine model with Pik3ca H1047R mutation. Pik3ca is the gene encoding p110, a catalytic subunit of PI3K. In this model, a dramatic increase in body weight, which was associated with increased organ size, a reduction in BG levels and undetectable insulin levels, were observed. In the human, mosaic activating mutations in PI3K are known to cause segmental overgrowth. In a study which evaluated the metabolic phenotype of 22 patients with mosaic activating mutations affecting PI3K, three patients were found to have early onset, severe, nonketotic hypoglycemia (11).

With all of these findings, it seems probable that dsyregulation of the insulin signalling pathway affecting *AKT2*, *PTEN*, or PI3K could cause NkHH with syndromic features. Unexplained hypoglycemia mimicking that of hyperinsulinism with no detectable insulin should alert the physician to a possible insulin transducing defect. An effective treatment option for hypoglycemia in these patients could be to block insulin signalling. The only known, widely-used agent to impact this pathway is the mTOR inhibitor, sirolimus. Until now, sirolimus has been successfully used for the severe, diffuse form of congenital hyperinsulinism (9). In our cases, we decided to give sirolimus to improve hypoglycemia in a patient with *AKT2* mutation and another patient with PTHS as sirolimus will inhibit mTOR, the next

downstream step in *PTEN*/AKT signalling. In both patients hypoglycemia was controlled with sirolimus treatment.

The reported side effects of sirolimus treatment include immunosuppressive effects, oral mucositis, renal dysfunction, pneumonitis, increased serum aminotransferase levels, hepatitis, and dyslipidemia (9,19). Of these, only mild leucocytosis was observed in case 1 without any additional symptoms.

## Conclusion

NkHH is a very rare but significant disorder which presented some challenges in both diagnosis and treatment. Activating mutations in *AKT2* or *PTEN*, upstream from mTOR in the insulin signalling pathway, may lead to NkHH. Sirolimus treatment, resulting in mTOR inhibition, appeared to be effective in controlling the persistent hypoglycemia in two cases. Sirolimus may be a life-saving therapeutic option for some of these rare diseases caused by increased activation of insulin signalling.

### Ethics

**Informed Consent:** Informed consent for the publication of these cases was obtained from both sets of parents.

**Peer-review:** Externally peer-reviewed.

### Authorship Contribution

Concept: Zeynep Şıklar, Merih Berberoğlu, Design: Zeynep Şıklar, Merih Berberoğlu, Data Collection or Processing: Zeynep Şıklar, Tuğba Çetin, Nilgün Çakar, Analysis or Interpretation: Zeynep Şıklar, Merih Berberoğlu, Literature Search: Zeynep Şıklar, Writing: Zeynep Şıklar.

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CASE REPORT

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# A Case Report of Pycnodysostosis Associated with Multiple Pituitary Hormone Deficiencies and Response to Treatment

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### What is already known on this topic?

Pycnodysostosis is a rare autosomal recessive osteosclerotic bone disorder caused by a mutation in the *Cathepsin-K* gene. Growth hormone (GH) deficiency is associated with nearly half of the patients suffering from pycnodysostosis. The treatment is mainly conservative and supportive. Recombinant human GH (rhGH) has been used in a selected group of patients.

### What this study adds?

We report a female with pycnodysostosis with associated with GH deficiency who was managed with rhGH. She had a favourable response with improvement in height standard deviation score of 1 over 18 months of treatment. rhGH unmasked central hypothyroidism requiring l-thyroxine replacement. A patient with pycnodysostosis requires monitoring for central hypothyroidism and other pituitary hormone deficiencies, especially if rhGH treatment is being offered.

## Abstract

Pycnodysostosis is a rare autosomal recessive osteosclerotic bone disorder associated with short stature and multiple bony abnormalities. Growth hormone (GH) deficiency may contribute to short stature in about 50% of patients. Available literature has rarely reported other pituitary hormone deficiencies in pyknodysostosis. Though the management remains conservative, recombinant human GH (rhGH) has been tried in selected patients. Here we present a case of pycnodysostosis which was evaluated for associated co-morbidities and found to have multiple pituitary hormone deficiencies. A 7-year-old girl was referred to our centre for evaluation of short stature. On examination, she had frontal and occipital bossing, limited mouth opening, hyperdontia with multiple carries, short and stubby digits and short stature. Investigation revealed dense sclerotic bones with frontal and occipital bossing, non-fusion of sutures with obtuse mandibular angle, non-pneumatised sinuses, small 'J' shaped sella turcica, acro-osteolysis of digits and absent medullary cavities. *Cathepsin-K* gene mutation analysis confirmed the diagnosis of pycnodysostosis. She was screened for associated co-morbidities and was found to have concomitant GH deficiency. Treatment with rhGH brought about an increase of 1 standard deviation score in height over 2 years and also unmasked central hypothyroidism at three months necessitating thyroxine replacement.

**Keywords:** Pycnodysostosis, short stature, multiple pituitary hormone deficiencies, *Cathepsin-K* gene mutation

## Introduction

Pycnodysostosis is a rare autosomal recessive osteosclerotic bone disorder (1). It is caused by homozygous or compound heterozygous mutation in the *Cathepsin-K (CTSK)* gene, which maps to chromosome 1q21 (2,3,4). The disease is characterised by specific bony abnormalities, facial features and short stature (5,6). Growth hormone (GH) deficiency is present in about half the patients with pycnodysostosis (5). However, other pituitary hormone deficiencies have not been reported to date.

Presently, there is no established therapy for pycnodysostosis (7,8). The management is primarily symptomatic and preventive (9). In a case series of eight patients with pycnodysostosis, four patients had associated GH deficiency. These four responded well to GH treatment with normalisation of insulin-like growth factor-1 (IGF-

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1) and acceleration in growth velocity (5). Karamizadeh et al (10) in a cohort of a further eight patients with pycnodysostosis reported a positive impact on linear growth with GH treatment. GH treatment, based on an IGF-1-based dosing regimen, when offered to three children with pycnodysostosis and 16 children of idiopathic short stature, resulted in near-normal stature and body proportion (11). Genetic testing and counselling is often a neglected aspect in pycnodysostosis and should be offered to all patients and relatives.

Here we present a case of pycnodysostosis associated with GH deficiency. The patient responded favourably to recombinant human GH (rhGH) treatment. However, rhGH treatment unmasked central hypothyroidism, necessitating l-thyroxine replacement.

## Case Report

A 7-year-old girl was referred to our centre for evaluation of short stature. She was the product of a second-degree consanguineous marriage. She had two elder sisters, aged 16 and 18 years, who had similar morphological features and a brother aged 14 who was healthy (Figure 1). The sisters had attained menarche at 14 years of age. Anti-natal and peri-natal periods were uneventful. Her birth weight was 2.1 Kg. The parents reported a poor gain in height since birth. She had a history of two fractures on trivial trauma; one involving the right tibia and one involving the left radius at 4 and 5 ½ years of age respectively. The fractures took a long time to heal. Height and weight were plotted on the Indian Association of Paediatrics growth chart for Indian girls aged 5-18 years (12). On physical examination, her height was 84 cm [-5.6 standard deviation score (SDS)], and the head circumference was 68.5 cm (Figure 2). The mother's height was 158 cm, and the father's height was 172 cm, thus her target height was 158.5 cm. The affected sisters at 16 and 18 years of age were 110.5 and 114 cm, respectively and both were below the 5$^{th}$ centile. The height of the unaffected elder brother was 158 cm, approximating to the 50$^{th}$ centile. The brother had not reached a final height. The sibling sisters have attained final height, as the epiphyses were fused. Notably, the final heights of the sibling sisters was less than expected, even for patients with Pycnodystosis. The medical records of the index case showed that her height velocity had been 1.2 cm/year for the last two years. She was pre-pubertal. She had facial dysmorphism with frontal, occipital bossing and limited mouth opening (Figure 3). She had short hands with short and stubby fingers with dystrophic nails (Figure 4). Her anterior fontanelle had not closed and measured 1.8 cm. The fontanelles of the affected sisters had closed. Examination of the oral cavity revealed hyperdontia, multiple caries and a grooved palate. Hepatosplenomegaly was present.

Laboratory examination revealed normal haematological parameters. The serum calcium, urea, creatinine, phosphorus, alkaline phosphatase, intact parathyroid

**Figure 1.** The index case (extreme right) and the sibling sister aged 16 years (centre) and 18 years (extreme left) share the same phenotypic features

hormone and 25 (OH) vitamin D levels were normal. Her IGF-1 level was 56 µg/L (normal range: 58-367 µg/L for a seven-year-old girl). A GH stimulation test was carried out with clonidine and revealed a peak value of 1.1 ng/mL, indicating GH deficiency. Her basal and adrenocorticotropin hormone stimulated cortisol levels were within normal limits. Serum thyroxine (T4) was 6.5 µg/dL (normal value: 4.6-12 µg/dL), and TSH was 3.2 mIU/mL. Serum IGF-1 for the affected sister aged 16 years was 182.6 µg/L (normal range: 127-541 µg/L) and of the sister aged 18 years was 201.2 µg/L (normal range: 121-486 µg/L); both had normal thyroid function profiles. Radiographic examination revealed generalised osteosclerosis. The medullary cavities were absent in the long bones. Skull radiography revealed frontal and occipital bossing, non-fusion of sutures with obtuse mandibular angle, absence of mastoid air cells and small 'J' shaped sella turcica (Figure 5). Terminal phalanges of hands showed acro-osteolysis (Figure 4). Magnetic resonance imaging of the sella revealed a hypoplastic anterior pituitary with a volume of 121 $mm^3$ ( < $5^{th}$ centile for age) (13) and a typical posterior pituitary bright spot. Arterial blood gas analysis did not reveal any hypoxia. Polysomnography did not show any evidence of obstructive sleep apnoea. Audiometry and ophthalmic examination were normal.

Molecular testing of the *CTSK* gene showed a homozygous missense variant CTSK:C.890G > C. The parents were found to be carriers of the same variant. Consent could not be obtained from the affected sisters and the unaffected brother for genetic testing due to the financial constraints of the family. The parents were counselled regarding their carrier status, and the siblings were advised regarding the benefits of genetic testing.

The index case was offered conservative and symptomatic management. Orthodontic and endodontic treatment was provided for caries and malposition of teeth. Counselling regarding oral hygiene, fracture prevention and other psychiatric aspects of the disease were undertaken.

In light of the GH deficiency, rhGH was administered at 0.16 mg/kg/week. An IGF-1 level was repeated after four weeks, which was still low. Based on the IGF-1 response, the dose was gradually increased to 0.48 mg/kg/week. An incremental

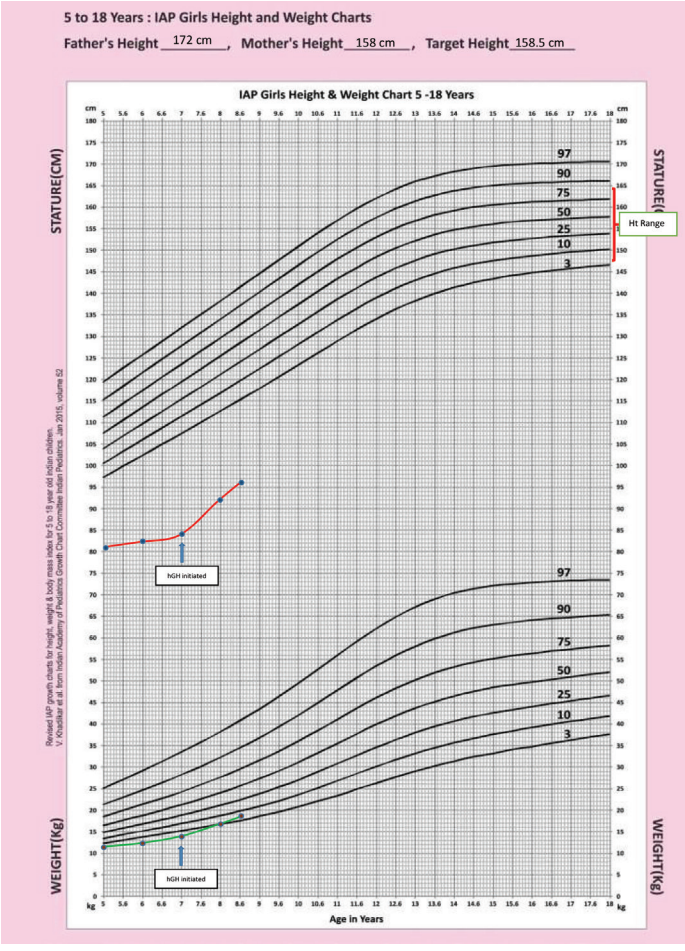


**Figure 2.** Growth chart of the index patient showing increase in height velocity on starting treatment

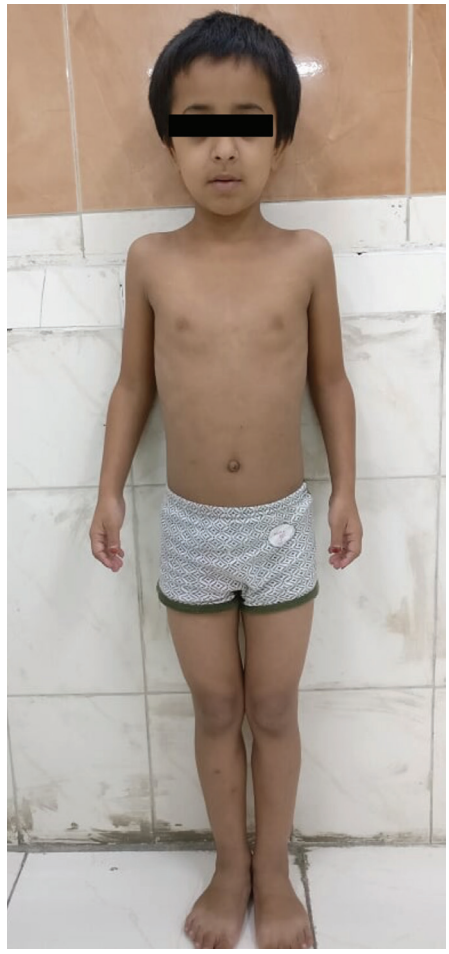

**Figure 3.** Clinical image of the index patient. Frontal bossing and typical facies are present. The carrying angle is wide

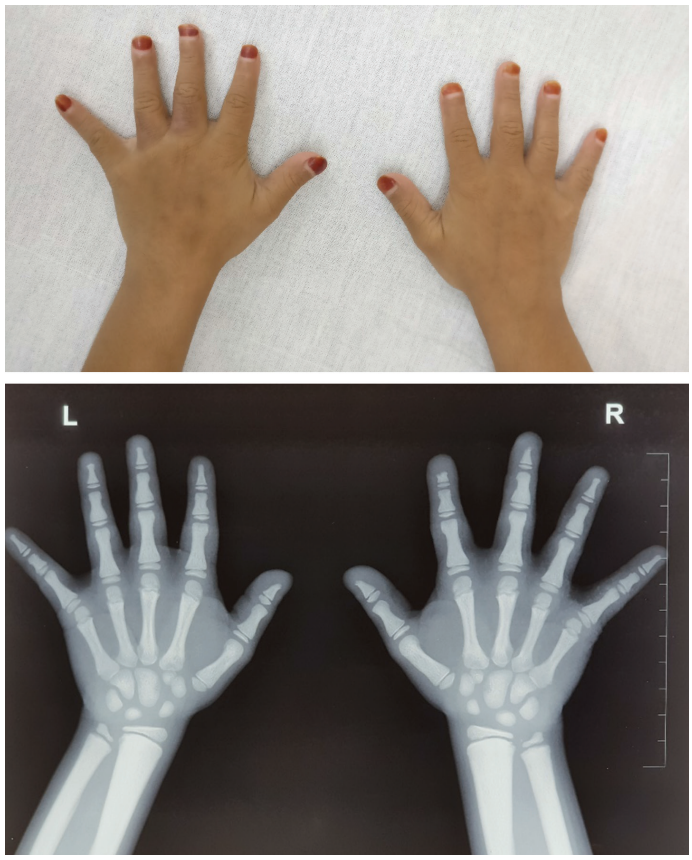


**Figure 4.** The image shows short and stubby digits. X-ray reveals acro-osteolysis of the terminal phalanges. The medullary cavity is absent in the long bones of the hand

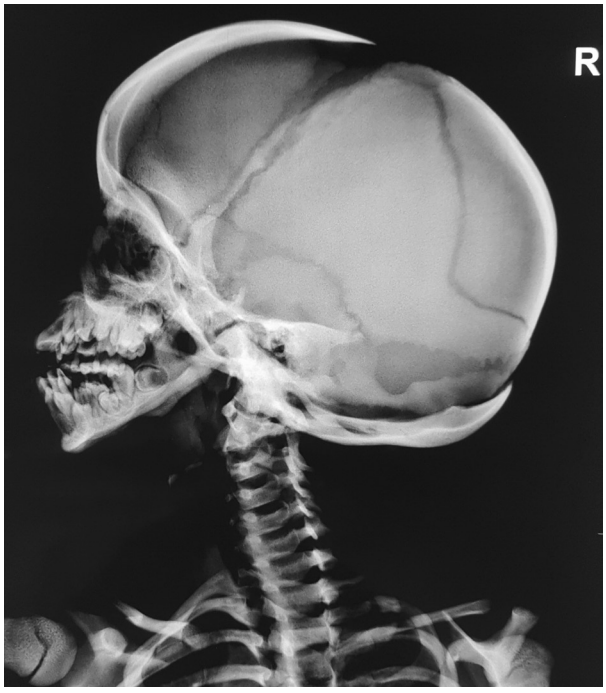


**Figure 5.** X-ray skull lateral view showing open fontanelles, obtuse mandibular angle and non-pneumatised sinuses. Also noted is acro-osteolysis of the clavicle

IGF-1 response and increase in height velocity ruled out GH resistance. Compliance with treatment was good on regular monitoring. On follow-up, a repeat evaluation of her thyroid axis after three months of treatment revealed a T4 of 3.1 µg/dL and TSH of 9.2 mIU/mL. She was started on levothyroxine replacement of 50 µg/day and after two months of replacement therapy the T4 had normalised to 10.4 µg /dL and the TSH was 4.9 mIU/mL. At the end of 18 months of treatment, her height was 97 cms with a 9 cm increase in stature over the first year of treatment and four cms over the next six months. Her height velocity was 8.7 cm/year over 18 months, and there was a gain of 1 SDS in height over this treatment period. There was no adverse effect on GH treatment.

Informed consent was obtained from the guardian.

## Discussion

Pycnodysostosis remains a rare cause of short stature. However, in patients suffering from the disease, short stature is a constant feature, afflicting 90.32% of cases ranging from -1.5 to -6 SDS, with our case having a height SDS of -5.6 at presentation (13). The short height is primarily due to impaired bone remodelling and subsequent sclerosis of the bones. Other contributors to short stature in pycnodysostosis are malnutrition, chronic airway obstruction and hypoxemia (5). Our patient had a healthy body mass index and arterial blood gas analysis, and so these abnormalities were ruled out in our patient. Pycnodysostosis may be associated with hypopituitarism (4). In our patient, the pituitary-adrenal axis was intact. However, she had a low IGF-1. As alteration in the GH-IGF-1 axis has not been studied in pycnodysostosis, we decided to confirm GH secretory defect with a GH stimulation test, with an inadequate secretory response indicating GH deficiency. GH deficiency has been demonstrated in 50% of the patients of pycnodysostosis. The deficiency may be due to pituitary hypoplasia caused by an increased bone volume of the sella and increased intrasellar pressure (5). Bone age in pycnodysostosis cannot be accurately assessed as disease-specific nomograms are not available making bone age determination difficult to assess in these patients.

The diagnosis is clinical and is based on relevant history, clinical features and radiological examination, as was in this case. If available, a genetic analysis should be carried out. We could demonstrate a *CTSK* gene homozygous missense variant CTSK:C.890G>C on molecular genetic testing. The mutation has been previously reported in humans (HGMD CS072172). The mutation results in a change in protein structure with threonine replacing serine at position 297 of

the Cathepsin-K protein (S297T) (14). Polyphen-2 predicts the mutation to be "Possibly damaging" and Mutationtaster predicts the mutation to be "Damaging" (15,16). Genetic analysis may reveal novel *CTSK* gene mutation in a homologous or a compound heterozygous pattern (2). Genetic testing, and counselling is often a neglected aspect of the disease. Genetic testing was offered to the index patient and the parents. However, due to financial constraints, this offer could not be extended to the siblings.

The treatment remains conservative and primarily targets preventive counselling. The patients can live a near-normal life. GH and IGF-1 have an anabolic role in bone metabolism (17). Only a few studies have demonstrated the efficacy of rhGH treatment for pycnodysostosis. Rothenbühler et al (11) initiated GH treatment in three children with pycnodysostosis at a dose of 29 µg/kg/day, 67 µg/kg/day and 120 µg/kg/day and observed near-normal adult stature and normalisation of skeletal proportions. GH treatment, at a dose of 18 U/m2/week, significantly improved growth velocity from $3.3 \pm 0.8$ cm/year to $9.4 \pm 2.1$ cm/year during the first year and $7.5 \pm 1$ cm/year in the second year (5). Height SDS and growth velocity increased on rhGH treatment at a dose of 50 µg/kg/day, when compared to pre-treatment levels and after stopping GH therapy in patients manifesting with GH deficiency in pycnodysostosis (10). The dose used by our case was initially 23 µg/kg/week, which was gradually increased to 68 µg/kg/week based on IGF-1 levels. The doses of rhGH required for pycnodysostosis are reported to be higher than those used in idiopathic short stature and GH deficiency (18), and this was true in our patient (5,10,11). Our patient had an increase in growth velocity to 8.7 cm/year. The SDS also improved from -5.6 to -4.59, a gain of 1 SDS. rhGH treatment may unmask central hypothyroidism in between 36% and 47% of patients who appear euthyroid before initiation of rhGH treatment (19). Central hypothyroidism, though rarely reported in pycnodysostosis, was exposed by rhGH therapy in our patient, requiring levothyroxine replacement.

## Conclusion

Pycnodysostosis remains a rare cause of short stature and is associated with various skeletal and dental abnormalities. GH deficiency may be present in around half of these patients. Other contributors to short height including malnutrition, chronic hypoxemia, hypopituitarism and intrinsic short stature should be evaluated in all patients. Though the primary management remains conservative, GH treatment is effective in some patients. Other pituitary hormone deficiencies may be associated with pycnodysostosis, and rhGH treatment may unmask underlying central hypothyroidism in this group of patients. Genetic counselling should be offered to all patients and their family.

### Ethics

**Informed Consent:** Informed consent was obtained from the guardian.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: Vishesh Verma, RK Singh, Concept: Vishesh Verma, Design: Vishesh Verma, Data Collection or Processing: RK Singh, Analysis or Interpretation: Vishesh Verma, Literature Search: Vishesh Verma, RK Singh, Writing: Vishesh Verma.

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# Hypophosphatasia: The Unusual Presentation

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**Keywords:** Hypophosphatasia, corneal opacity, band keratopathy, neonatal presentation

**Dear Editor,**

Esmel-Vilomara et al (1) describe the unique case of an infant with an extraordinary presentation of hypophosphatasia with corneal opacity diagnosed shortly after birth during clinical investigation. The authors found a previously unreported mutation, found in the *ALPL* gene encoding TNAP. It was a heterozygous variant, c.1292T > A found in exon 11 of the *ALPL* gene, producing an amino acid change p.(Val431Asp). The authors suggest that the underlying etiology may explain the mild phenotype of this case of hypophosphatasia using bioinformatics tools. The newborn presented with a transient band keratopathy, which can occur in the setting of hypercalcemia (2,3). Hypercalcemia can trigger band keratopathy, and this disorder can be transient. Band keratopathy is a corneal disease that originated from the appearance of calcium deposits on the central cornea. This aspect is a notable example of metastatic calcification, which occurs in hypercalcemia. Transient band keratopathy has been described in patients with systemic hypercalcemia. The etiology includes pituitary disturbances, renal failure, and sarcoidosis. Nevertheless, neonatologists should consider keeping in mind that another presentation may be more common in hypophosphatasia, i.e., the Vitamin B6 dependent seizure. Vitamin B6-dependent seizures include a group of treatable diseases (*ALDH7A1* deficiency, *PNPO* deficiency, *PLP* binding protein deficiency, hyperprolinemia type II, hypophosphatasia, and glycosylphosphatidylinositol anchor synthesis defects) responding to pyridoxine or pyridoxal-5I-phosphate (4). Baumgartner-Sigl et al (4) presented a 7-month-old girl, who presented as a neonate with pyridoxine-responsive seizures but without bone abnormalities. She had initial normal cognitive milestones but later failed to thrive. Nearly undetectable serum ALP activity, elevated plasma PLP and urinary phosphoethanolamine and inorganic pyrophosphate levels, hypercalcemia, hypercalciuria, and nephrocalcinosis were consistent with infantile hypophosphatasia. Sequence analysis of the *TNAP* gene revealed missense mutations in exon 7 (c.677T > C, p.M226T) and exon 10 (c.1112C > T, p.T371I). Overall, the clinical presentation of hypophosphatasia remains highly variable, ranging from perinatal death to adult osteopenia and dental problems. There are six subtypes of hypophosphatasia, including lethal perinatal, benign perinatal, infantile, childhood, adult, and odontoid-hypophosphatasia with the lethal perinatal hypophosphatasia being the most severe (5). Babies born with this condition show rapidly worsening alterations of calcium/phosphate metabolism (hypercalcemia), apneic spells, seizures, and progressive encephalopathy that may occasionally mimic a hypoxic-ischemic encephalopathy. Despite considered rare, hypophosphatasia affects all races around the world, with a highly variable incidence and a particularly high prevalence in the United States and Canada. Thus, Esmel-Vilomara et al's (1) patient addresses the important aspect of the clinical screening for metabolic disorders in routine clinical examination of babies.

## Ethics

**Peer-review:** Internally peer-reviewed.

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NOTES

NOTES

# Norditropin NordiFlex®

## BİLDİKLERİNİZİN **BİR KALEM ÜSTÜNDE!**

**Norditropin NordiFlex®**
ilk kullanımdan sonra
**21 gün** oda sıcaklığında
**saklanabilen tek**
**büyüme hormonudur.**[1]*

Sadece
**Norditropin NordiFlex®** ile
Kolay Saklama
Koşulları

**21 GÜN**
**25°C**



**Gigi,** 4 yaşında, gebelik yaşına göre küçük ağırlıkta doğmuş, gebelik yaşına uygun ağırlıkta doğmuş olan ikizi ile birlikte

*Norditropin® NordiFlex® 5, 10 ve 15 mg kullanımdan sonra oda sıcaklığında (25°C'ye kadar) 21 gün süreyle saklanabilir.

**Referanslar: 1.** Norditropin® NordiFlex® ürün bilgisi.

**Norditropin® NordiFlex®**
**Bileşimi:** 5 mg/1.5 mL kullanıma hazır kalem ml'sinde 3.3 mg, 10 mg/1.5 mL kullanıma hazır kalem ml'sinde 6.7 mg ve 15 mg/1.5 mL kullanıma hazır kalem ml'sinde 10 mg somatropin (rekombinant büyüme hormonu) içerir. **Farmasötik Form:** Enjeksiyonluk çözelti içeren kullanıma hazır kalem. **Endikasyonları:** ***Çocuklarda:*** Büyüme hormonu eksikliğine (BHE) bağlı büyüme geriliği, kızlarda gonadal disgeneziye bağlı büyüme geriliği (Turner Sendromu), puberte öncesi çocuklarda kronik böbrek hastalığına bağlı büyüme gecikmesi, doğum boyu ve/veya ağırlığı -2 SS'nın altında olan ve 4 yaşına veya daha sonrasına kadar büyümeyi yakalayamamış (son yıl süresince büyüme hızı SSS < 0) gebelik yaşına göre küçük (SGA) doğmuş kısa boylu çocuklarda büyüme geriliği (şu anki boy SSS <-2.5 ve parental düzeltilmiş boy SSS < -1). ***Erişkinlerde:*** Çocukluk döneminde başlayan BHE: Üçten fazla hipofiz hormonu eksikliği olanlarda, tanımlanmış bir genetik sebebe, yapısal hipotalamo-hipofizer anomalilere, santral sinir sistemi tümörlerine veya yüksek doz kraniyal ışınlamaya bağlı şiddetli BHE olan kişilerde ya da hipotalamo-hipofizer hastalık veya yetmezliğine sekonder BHE'li kişilerde, eğer büyüme hormonu tedavisini bıraktıktan en az 4 hafta sonra IGF-I < -2 SSS ise test gerekli değildir. Diğer tüm hastalarda IGF-I ölçümü ve bir büyüme hormonu stimülasyon testi gereklidir. Erişkinlik döneminde başlayan BHE: Bilinen hipotalamo-hipofizer hastalıkta, kraniyal ışınlama ve travmatik beyin hasarında belirgin BHE (hipotalamo-hipofizer aksta prolaktin dışında başka bir eksiklik). Akstaki diğer eksiklikler için yeterli replasman tedavisinin başlatılmasından sonra bir provokatif test ile BHE gösterilmelidir. **Kontrendikasyonlar:** Tümör aktivitesi bulgu varlığında; açık kalp cerrahisi, abdominal cerrahi, kazaya bağlı çoklu travma, akut solunum yetmezliği veya benzer durumları takiben akut kritik hastalık komplikasyonları olan hastalarda; somatropine ya da bileşimindeki maddelerden herhangi birisine aşırı duyarlılık durumlarında; kronik böbrek yetmezliği olan çocuklarda renal transplantasyon yapılırken; epifizleri kapanmış çocuklarda kullanılmamalıdır. **Kullanım şekil ve dozu:** Cilt altına enjeksiyon ile (s.c.) kullanılır. Doz hastaya göre ve hastanın tedaviye verdiği yanıt göz önüne alınarak düzenlenmelidir. Genellikle, her gün akşamları ve enjeksiyon yeri değiştirilerek uygulama önerilmektedir. **Genel olarak önerilen doz:** ***Çocuklarda:*** Büyüme hormonu yetersizliği: 0.025-0.035 mg/kg/gün veya 0.7-1.0 mg/m²/gün. Turner Sendromu: 0.045-0.067 mg/kg/gün veya 1.3-2 mg/m²/gün. Kronik böbrek hastalığı: 0.050 mg/kg/gün veya 1.4 mg/m²/gün. Gebelik yaşına göre küçük: 0.035 mg/kg/gün veya 1 mg/m²/gün. ***Erişkinlerde:*** Erişkinlerde replasman tedavisi: Doz, hastanın gereksinimine göre belirlenmelidir. Çocukluk döneminde başlayan BHE'si olan hastalarda tedaviye 0.2-0.5 mg/gün dozla başlanması ve sonrasında IGF-I konsantrasyonlarına göre dozun ayarlanması önerilmektedir. Erişkinlikte başlayan BHE hastalarında tedaviye düşük dozla başlanması önerilir: 0.1-0.3 mg/gün. Dozun, hastanın tedaviye verdiği yanıt ve hastanın advers etkiler ile ilgili deneyimleri göz önüne alınarak birer aylık aralıklar ile artırılması önerilmektedir. Serum İnsülin Benzeri Büyüme Faktörü I (IGF-I), doz titrasyonu için rehber olarak kullanılabilir. Doz ihtiyacı yaşa bağlı olarak azalır. İdame dozu kişisel farklılıklar göstermekle birlikte, nadiren 1.0 mg/gün değerinin üzerine çıkar. **Uyarılar/Önlemler:** Tedavisi, her zaman bu konuda bilgi ve deneyimi olan uzman hekimler tarafından yapılmalıdır. Önerilen maksimum günlük doz aşılmamalıdır. Turner Sendromlu hastalarda el ve ayaklarda büyüme artışı gözlenirse, dozun, doz aralığındaki daha düşük bir doza düşürülmesi düşünülmelidir. Kronik böbrek hastalığı olan hastalarda, böbrek fonksiyonları takip edilmelidir. Turner Sendromlu ve SGA'lı çocuklarda tedaviye başlamadan önce ve daha sonra yılda bir kez açlık insülin ve kan glukoz değerlerinin ölçülmesi ve insülin tedavisi almakta olanlarda dozun izlenmesi önerilir. Belirgin diyabet ortaya çıkarsa büyüme hormonu tedavisi uygulanmamalıdır. Aşırı obezite, üst solunum yolu obstrüksiyonu, uyku apnesi öyküsü veya tanımlanamamış solunum enfeksiyonu gibi risk faktörlerinden biri ya da birden fazlası olan Prader-Willi sendromlu hastalarda somatropin tedavisinin başlanması ile ani ölümler bildirilmiştir. İlerleyen hipofiz hastalığı olan hastalarda hipotiroidizm gelişebilir. Şiddetli ve tekrarlayıcı baş ağrısı, görme bozuklukları, bulantı varlığında hasta papil ödemi açısından incelenmelidir. Somatropin tedavisi gören yetişkinlerde veya çocuklarda yeni primer kanser riskinin arttığına dair bir kanıt yoktur. Malign hastalığı tamamen remisyonda olan hastalarda, somatropin tedavisi, relaps oranının artması ile ilişkili bulunmamıştır, ancak bu hastalar relaps açısından somatropin tedavisinin başlangıcından itibaren yakından izlenmelidir. Somatropin uygulanan hastalarda daha önce teşhis edilmemiş olan santral hipoadrenalizm aşikar hale gelebilir ve glukokortikoid replasmanı gerekli olabilir, daha önce teşhis edilen hastada ise hastada doz artımı gerekebilir. Somatropin almakta olan bir kadın oral östrojen tedavisine başlarsa somatropin dozunun artırılması veya aksi şekilde östrojen tedavisini bıraktığı takdirde büyüme hormonu fazlalığının ve/veya yan etkilerin önlenmesi için somatropin dozunun azalması gerekebilir gerekebilir. ***Gebelik kategorisi:*** C. Gebelik döneminde somatropin tedavisinin güvenilirliği açısından yeterli kanıt bulunmamaktadır. Somatropinin insan sütüne geçip geçmediği bilinmediğinden emziren kadınlara verileceği zaman dikkat edilmelidir. **Yan Etkiler/Advers Etkiler:** Erişkinlerde periferik ödem, baş ağrısı, parestezi, artralji eklem sertliği ve miyalji görülebilir. Çocuklarda döküntü, artralji, miyalji ve periferik ödem seyrek olarak ve baş ağrısı yaygın olmayan şekilde görülebilir. Lokal enjeksiyon yeri reaksiyonları oluşabilir. Bazı nadir vakalarda benign intrakraniyal hipertansiyon bildirilmiştir. Turner Sendromlu çocuklarda büyüme hormonu tedavisi sırasında el ve ayaklarda büyümenin arttığı bildirilmiştir. **Etkileşimler:** Glukokortikoidler ile birlikte kullanılması büyümeyi inhibe edebilir. Büyüme; gonadotropin, anabolik steroidler, östrojen ve tiroid hormonu gibi diğer tedavilerden de etkilenebilir. **Saklamaya Yönelik Özel Tedbirler:** Açıldıktan sonra: Buzdolabında (2°C-8°C) maksimum 4 hafta saklayınız. Işıktan koruyunuz. Dondurmayınız. Ürün, alternatif olarak, 25°C'nin altında maksimum 3 hafta saklanabilir. **Ruhsat Sahibi:** Novo Nordisk Sağlık Ürünleri Tic. Ltd. Şti. Nispetiye Cad. Akmerkez E3 Blok Kat 7 34335 Etiler – İstanbul. **Ruhsat Tarihi ve No:** Norditropin® NordiFlex® 5mg; 07.01.2002-111/56, Norditropin® NordiFlex® 10mg; 25.12.2001-111/45, Norditropin® NordiFlex® 15mg; 25.12.2001-111/44 **Yalnız reçete ile kullanılmalıdır. Perakende Satış Fiyatı:** Ürünün güncel fiyatı için lütfen firmamıza başvurunuz. **Kısa Ürün Bilgisi Yenilenme Tarihi:** 06.01.2020. Norditropin® NordiFlex® Novo Nordisk'in ticari markasıdır. Daha geniş bilgi için firmamıza başvurunuz.

TR20NX00013

novo nordisk®

norditropin
nordiflex®
somatropin (rDNA orijinli) enjeksiyon


**Referanslar: 1.** Hey-Hadavi J et al. Clin Ther. 2010;32:2036-47. **2.** Genotropin® GoQuick™ 16 IU (5.3 mg) Kısa Ürün Bilgisi. **3.** Genotropin® GoQuick™ 36 IU (12 mg) Kısa Ürün Bilgisi.

**Genotropin® Kısa Ürün Bilgisi Özeti:**
GENOTROPİN GOQUİCK® 16 IU (5,3 mg/ml) - 36 IU (12 mg/ml) enjeksiyonluk çözelti için toz ve çözücü içeren kullanıma hazır kalem **Formül:** Rekombinant DNA teknolojisiyle Escherichia Coli hücrelerinde üretilmiş 16 IU (5,3 mg/ml) - 36 IU (12 mg/ml) somatropin içerir. **Endikasyonları:** Büyüme hormonunun yetersiz salgılanmasına bağlı çocuklardaki büyüme bozukluklarında; gonadal disgenezi (Turner Sendromu) ile birlikte bulunan büyüme bozukluklarında; kronik böbrek yetersizliği olan prepubertal çocuklardaki büyüme bozukluklarında; SGA tedavisinde – doğum ağırlığı ve/veya uzunluğu -2 SD olan ve 4 yaşı ve sonrasında gerekli büyümeyi yakalayamamış (son 1 yılda yıllık boy kazanımı SDS<0) çocuklarda veya gestasyonel yaşına göre küçük doğmuş olan (SGA) kısa çocuklardaki büyüme bozukluklarında (uzunluk SDS<-2.5 ve ebeveyne uyarlanmış uzunluk SDS<-1) – ; hipotalamus-hipofizer hastalığı saptanan hipofizer cerrahi girişim geçirmiş, kraniyal radyoterapi görmüş veya çocuklukta başlamış büyüme hormonu yetmezliği olan erişkinler ile hipofizde adenomu olan hastalarda büyüme hormonu eksikliği varsa veya büyüme hormonu yetersizliğini düşündüren bulguların bulunması durumunda biyokimyasal tanı testleri ile büyüme hormonu eksikliği kesin olarak saptanan yetişkinlerde, özetle: konjenital veya idiopatik hipofiz hastalıkları, hipotalamus hipofiz tümörleri ve tedavileri sonunda, kraniofarenjioma tedavisinden sonra, cerrahi girişim hasarlarında, Sheehan sendromu ve vasküler sebeple gelişen iskemik sebepli büyüme hormonu yetersizlikleri, radyasyon, travma, kronik otoimmun, bakteriyel veya viral enflamasyonlar ile hemokromatozis ve amiloidoziste görülen hipofizer yetmezliklerde, septo-optik displazide meydana gelebilen aşikâr büyüme hormonu eksikliğinin replasmanı için büyüme hormonu replasman tedavisi endikasyonu vardır. **Pozoloji:** Çocuklardaki büyüme hormonu salgılanma yetersizliğine bağlı büyüme bozukluğunda: Genellikle 0,025 – 0,035 mg/kg veya 0,7 –1,0 mg/m² önerilmektedir. Turner Sendromuna bağlı büyüme bozukluğu: 0,045–0,050 mg/kg veya 1,4 mg/m² önerilir. Kronik böbrek yetmezliğine bağlı büyüme bozukluğu: 0,045–0,050 mg/kg (1,4 mg/m²) önerilir. Büyüme hızı çok düşükse daha yüksek dozlar gerekebilir. Gestasyonel yaşa göre küçük doğmuş (SGA) olan kısa boylu çocukların büyüme bozukluklarında: Final uzunluğa erişinceye kadar genellikle vücut ağırlığına göre günlük 0,035 mg/kg (1,0 mg/m²) önerilmektedir. Yetişkinlerdeki büyüme hormonu eksikliği: Çocukluk çağı BHY sonrasında büyüme hormonu tedavisine devam eden hastalarda önerilen yeniden başlangıç dozu 0,2-0,5 mg/gün' dür. Yetişkin başlangıçlı BHY olan hastalarda tedavi düşük doz (0,15-0,3 mg/gün) ile başlamalıdır. **Uygulama şekli:** Dozlama ve uygulama sıklığı bireyselleştirilmelidir. Enjeksiyonlar subkütan enjeksiyon şeklinde ve lipoatrofi gelişmesini önleyebilmek için her seferinde yeri değiştirilerek uygulanır. **Kontrendikasyonlar:** Etkin madde veya yardımcı maddelerden herhangi birine karşı aşırı duyarlılık durumunda kullanılmamalıdır. Somatropin, tümör aktivitesini gösteren herhangi bir bulgunun bulunması durumunda kullanılmamalıdır. Büyüme hormonu tedavisine başlanmadan önce intrakraniyal tümörler inaktif olmalı ve antitümör tedavi tamamlanmış olmalıdır. Tümör büyümesine ilişkin kanıt olması halinde tedavi sonlandırılmalıdır. GENOTROPİN GOQUİCK® epifizleri kapanmış çocuklarda büyümenin uyarılması için kullanılmamalıdır. Açık kalp ameliyatı, abdominal cerrahi, kazaya bağlı multipl travma, akut solunum yetmezliği veya benzeri durumları izleyen komplikasyonların bulunduğu akut kritik hastalığı olan hastalara GENOTROPİN GOQUİCK® uygulanmamalıdır. **Özel kullanım uyarıları ve önlemleri:** Hastalığın tanısı ve GENOTROPİN GOQUİCK® tedavisi, terapötik kullanım endikasyonunda; hastaların tanı ve tedavisinde yeterli nitelikte ve tecrübeli doktorlar tarafından başlatılmalı ve takip edilmelidir. Maksimum önerilen günlük doz aşılmamalıdır. Miyozit çok nadir bir advers olaydır ve koruyucu madde metakrezol ile ilişkili olabilir. Somatropin insülin hassasiyetini azaltabilir. Diabetes mellitus olan hastalarda somatropin tedavisine başlandıktan sonra insülin dozunun ayarlanması gerekebilir. Büyüme hormonu, T4'ün T3'e tiroit dışı dönüşümünü artırabilir ve bu durum serum T4'ünün azalmasına ve serum T3'ünün artmasına yol açabilir. Tiroit fonksiyonu tüm hastalarda takip edilmelidir. Malign bir hastalığın tedavisine sekonder büyüme hormonu yetersizliğinde malignitenin relaps belirtilerine dikkat edilmesi önerilmektedir. Çocukluk döneminde kanser sonrası sağ kalımlarda, somatropin ile tedavi edilen hastalarda ilk neoplazma sonrası ikinci bir neoplazma gelişiminde risk artışı bildirilmiştir. Büyüme hormonu yetersizliği dahil, endokrin bozukluğu olan hastalarda kalça ekleminde epifiz kayması genel popülasyondan daha sık görülebilir. Şiddetli veya tekrarlayan baş ağrısı, görme sorunları, bulantı ve/veya kusma gelişmesi halinde papilla ödemi için fundoskopi yapılması önerilmektedir. Büyüme hormonu eksikliği olan az sayıda hastada lösemi bildirilmiştir ve bu hastalardan bazıları somatropin ile tedavi edilmiştir. Somatropin içeren ürünlerin hepsinde olduğu gibi, hastaların düşük bir yüzdesinde GENOTROPİN GOQUİCK®'e karşı antikorlar gelişebilir. Seyrek görülmekle birlikte; somatropin ile tedavi edilen hastalarda; özellikle karın ağrısı gelişen çocuklarda pankreatit dikkate alınmalıdır. SGA olarak doğan kısa boylu çocuklarda tedaviye başlamadan önce büyüme bozukluğuna neden olacak diğer tıbbi nedenler veya tedaviler ekarte edilmelidir. SGA çocuklarda tedaviye başlamadan önce ve daha sonra yılda bir kez, açlık insülin ve kan glukozu düzeyleri ölçülmelidir. SGA çocuklarda tedaviye başlamadan önce ve daha sonra yılda iki kez, IGF-I değerleri ölçülmelidir. Kronik böbrek yetersizliğinde, tedavi başlangıcından önce böbrek fonksiyonu normalin %50 altında olmalıdır. Böbrek transplantasyonunda tedaviye devam edilmemelidir. **İlaç Etkileşimleri:** Glukokortikoidlerle eş zamanlı tedavi somatropin içeren ürünlerin büyümeyi tetikleyici etkilerini engelleyebilir. Büyüme hormonu eksikliği olan yetişkinlerde yapılan bir etkileşim çalışmasında somatropin uygulamasının sitokrom P450 izoenzimleriyle metabolize olduğu bilinen bileşiklerin klirensini artırdığı belirtilmektedir. **Gebelik kategorisi:** Gebelik kategorisi C'dir. Kontrasepsiyon kullanmayan çocuk doğurma potansiyeline sahip kadınlarda somatropin içeren ürünler önerilmemektedir. Emziren kadınlarda somatropin içeren ürünlerle ilgili klinik çalışmalar yapılmamıştır. Somatropinin anne sütüne geçip geçmediği bilinmemektedir, ancak yeni doğanlarda intakt proteinin gastrointestinal kanaldan emilme olasılığı oldukça düşüktür. Bu yüzden emziren kadınlara somatropin içeren ürünler verilirken dikkatli olunmalıdır. **Araç ve makine kullanımı üzerindeki etkiler:** GENOTROPİN GOQUİCK®'in araç ve makine kullanımı üzerinde etkisi bulunmamaktadır. **İstenmeyen etkiler:** Enjeksiyon bölgesi reaksiyonları, artralji, periferik ödem, parestezi, karpal tünel sendromu, miyalji, kas-iskelet sertliği çok yaygın ve yaygın görünen istenmeyen etkilerdir. **Doz aşımı ve tedavisi:** Akut doz aşımı başlangıçta hipoglisemi ve takiben hiperglisemiye neden olabilir. Uzun süreli doz aşımı fazla miktardaki insan büyüme hormonunun bilinen etkilerine benzer belirti ve bulgulara neden olabilir. **Saklama koşulları:** Sulandırılmadan önce: Buzdolabında (2°C - 8°C'de) veya 25°C'nin altında maksimum 1 ay boyunca saklayınız. İki kompartımanlı kartuşu/önceden doldurulmuş kalemi ışıktan korumak için dış kutusunda saklayınız. Sulandırıldıktan sonra: Buzdolabında (2°C - 8°C'de) saklayınız. Dondurmayınız. İki kompartımanlı kartuşu/önceden doldurulmuş kalemi ışıktan korumak için dış kutusunda saklayınız. **Raf Ömrü:** Sulandırılmadan önce: 25°C'nin altında oda sıcaklığında 1 ay, (2°C – 8°C)'de buzdolabında 36 ay. Sulandırıldıktan sonra: (2°C – 8°C) 'de buzdolabında 28 gün. Ürün, ışıktan ve donmaktan korunarak saklanmalıdır. **Ticari Takdim Şekli ve Ambalaj Muhtevası:** 16 IU, 36 IU GoQuick enjeksiyonluk çözelti için toz ve çözücü içeren 1 adet kullanıma hazır kalem. Reçete ile satılır. **Satış Fiyatı:** Genotropin GoQuick® 16 IU 288,96 TL (19.02.2020), Genotropin GoQuick® 36 IU 652,48 TL (19.02.2020). Ödeme koşulları ile ilgili detaylı bilgi için Sağlık Uygulamaları Tebliği'ne bakınız. **Kısa ürün bilgisi/ kullanma talimatı onay tarihi:** Genotropin GoQuick® 16 IU: **KUB Onay Tarihi:** 18.06.2019, **Ruhsat No:** 103/41, **İlk Ruhsat tarihi:** 18.12.1997, **Ruhsat yenileme tarihi:** 02.08.2012 Genotropin GoQuick® 36 IU: **KÜB Onay Tarihi:** 18.06.2019, Ruhsat No: 128/74, İlk Ruhsat tarihi: 13.08.2009, Ruhsat yenileme tarihi: 14.05.2015 **Ruhsat sahibi:** Pfizer PFE İlaçları A.Ş. 34347 Ortaköy/ İstanbul. Tel: 0212 310 70 00. Daha geniş bilgi için firmamıza başvurunuz. www.pfizer.com.tr

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