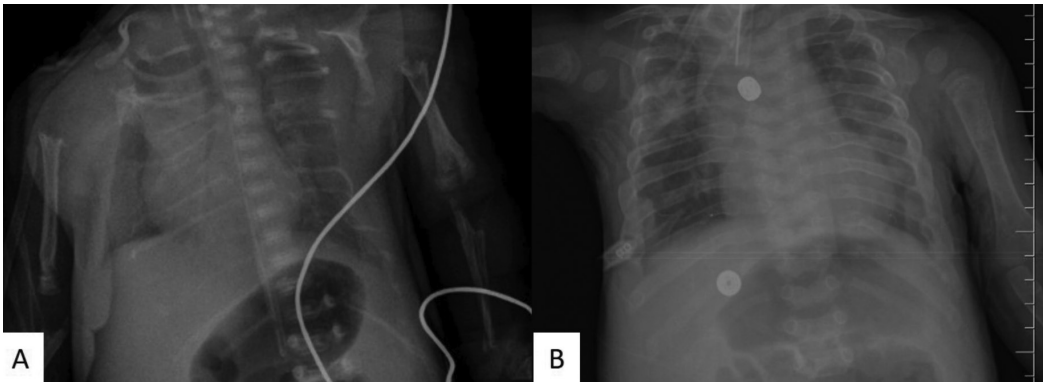
JCRPE

Journal of Clinical Research in Pediatric Endocrinology

September 2019 | volume 11 | issue 3 | www.jcrpe.org | ISSN: 1308-5727

E-ISSN: 1308-5735



General improvement before and 12 months after treatment with asfotase alfa

A Case of the Perinatal Form Hypophosphatasia Caused by a Novel Large Duplication of the *ALPL* Gene and Report of One Year Follow-up with Enzyme Replacement Therapy

Hacıhamdioğlu B et al.

DOI: 10.4274/jcrpe.galenos.2018.2018.0217

Official Journal of
Turkish Pediatric Endocrinology
and Diabetes Society

galenos yayınevi

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) - 36 IU (12 mg) enjeksiyonluk solüsyon için toz ve çözücü içeren kullanıma hazır kalem **Formül:** Rekombinant DNA teknolojisiyle Escherichia Coli hücrelerinde üretilmiş 16 IU (5,3 mg) - 36 IU (12 mg) 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 girişime 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 girişime 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:** 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. Malign bir hastalığın tedavisine sekonder büyüme hormonu yetersizliğinde malignitenin relaps belirtilerine dikkat edilmesi önerilmektedir. Çocukluk döneminde kanser sonrası sağkalımlarda, 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. 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. Kronik böbrek yetersizliğinde, tedavi başlangıcından önce böbrek fonksiyonu normalin %50 altında olmalıdır. **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:** C. **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. 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E-mail: info@galenos.com.tr
Publisher Certificate Number: 14521
www.galenos.com.tr

Printing at:
Üniform Basım San. ve Turizm Ltd. Şti.
Matbaacılar Sanayi Sitesi 1. Cad. No: 114
34204 Bağcılar, İstanbul, Türkiye
Phone: +90 212 429 10 00
Certificate Number: 42419
Date of printing: September 2019
ISSN: 1308-5727
E-ISSN: 1308-5735

JCRPE Journal of Clinical Research in Pediatric Endocrinology
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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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Tüm temel endikasyonlarda onaylı tek sıvı büyüme hormonu [1-5]

- Kullanım kolaylığı[6]
- Onaylı uzun dönem etkililik[6]
- Uygunluk[7,8]
- Kullanıma hazır sıvı formülasyon[6]
- Kanıtlanmış güvenilirlik[6]
- Yılların biyoteknoloji deneyimi[9]

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▼ **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.

OMNİTROPE® 5 mg (15 IU)/1.5 mL ve 10 mg (30 IU)/1.5 mL ve OMNİTROPE® 15 mg (45 IU)/1,5 mL SC enjeksiyon için çözelti içeren kartuş **Etkin madde:** OMNİTROPE® 5 mg kartuş 5 mg Somatropin'e eşdeğer (15 IU) 1.5 mL, OMNİTROPE® 10 mg kartuş, 10 mg Somatropin'e eşdeğer (30 IU) 1.5 mL, OMNİTROPE® 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. **Pozoloji:** 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 dozla tedaviye başlanmalıdır. Doz, IGF-1 konsantrasyonuna göre belirlenen bireysel hasta gereksinimlerine göre aşamalı olarak artırılmalıdır. **Uygulama sıklığı ve süresi:** OMNİTROPE® 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 OMNİTROPE® 5 mg (15 IU)/1.5 mL ile kullanım için özel olarak geliştirilmiş bir enjeksiyon aracı (enjeksiyon kalemi) olan SurePal™ 5; OMNİTROPE® 10 mg (30 IU)/1.5 mL ile kullanım için özel olarak geliştirilmiş bir enjeksiyon aracı (enjeksiyon kalemi) olan SurePal™ 10; OMNİTROPE® 15 mg (45 IU)/1.5 mL ile 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. 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 arttırılması 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 papillödemi için fundoskopinin yapılması tavsiye edilmektedir. Eğer papillödemi varlığı doğrulanırsa, iyi huylu intrakraniyal hipertansiyon tanısı düşünülmelidir ve eğer uygunsa büyüme hormonu tedavisi kesilmelidir. OMNİTROPE® 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 anaflaktoid reaksiyonlara sebebiyet verebilir. **Gebelik ve laktasyon Genel tavsiye Gebelik kategorisi**: C **Gebelik dönemi:** OMNİTROPE® gebelik döneminde kullanılmamalıdır. Emziren kadınlarda somatropin içeren ürünlerle yapılan klinik çalışma yoktur. Emzirmenin durdurulup durdurulmayacağına ya da OMNİTROPE® tedavisinin durdurulup durdurulmayacağına/ tedaviden kaçınılıp kaçınılmayacağına ilişkin karar verilirken, emzirenin çocuk açısından faydası ve OMNİTROPE® 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 lösemi vakaları meydana geldiği rapor edilmiştir ve bu durum pazarlama sonrası deneyimde yer almıştır. Beyin ve kafaya 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 insulin duyarlılığı nedeniyle hiperglisemi somatropine ait sınıf etkisi olduğu düşünülebilir. Ayrıntılı bilgi için TITCK onaylı KUB 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 OMNİTROPE®'un subkütan enjeksiyonundan sonra Cmaks ve tmaks değerleri sırasıyla 72 ± 28 mikrogram/L ve 4 ± 2 saattir. Sağlıklı yetişkenlerde 10 mg/1,5 mL OMNİTROPE®'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 OMNİTROPE®'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, OMNİTROPE® 5 mg/1,5 mL ve 10 mg/1,5 mL'nin subkütan uygulamasından sonra 3 saatlik, OMNİTROPE® 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:** OMNİTROPE® 5 mg (15 IU)/1.5 mL için 24 ay, OMNİTROPE® 10 mg (30 IU)/1.5 mL için 18 ay, OMNİTROPE® 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. 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DOI: 10.4274/jcrpe.galenos.2019.2019.0007

J Clin Res Pediatr Endocrinol 2019;11(3):220-226

Pitfalls with Vitamin D Research in Musculoskeletal Disorders and Recommendations on How to Avoid Them

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Abstract

Reports suggesting that vitamin D may have extraskeletal roles have renewed interest in vitamin D research and stimulated publication of an increasing number of new studies each year. These studies typically assess vitamin D status by measuring the blood concentration of 25-hydroxyvitamin D [25(OH)D], the principal circulating metabolite of vitamin D. Unfortunately, variations in assay format, inconsistency in interpreting 25(OH)D concentrations, cohort bias (age, body mass index, race, season of measurements etc.) and failure to measure critical variables needed to interpret study results, makes interpreting results and comparing studies difficult. Further, variation in reporting results (reporting mean values vs. percent of the cohort that is deficient, no clear statement as to clinical relevance of effect size, etc.) further limits interstudy analyses. In this paper, we discuss many common pitfalls in vitamin D research. We also provide recommendations on avoiding these pitfalls and suggest guidelines to enhance consistency in reporting results.
Keywords: Vitamin D, 25-hydroxyvitamin D, deficiency

Introduction

Interest in nonconventional actions of vitamin D remains high many years after the first reports linking vitamin D to a variety of extraskeletal actions. According to a Pubmed.gov search on the term "vitamin D" (December 2018) there were 4497 publications in 2018, and that does not include books, symposium proceedings or media articles on vitamin D. This interest naturally extends to pediatric orthopedic conditions, where studies have attempted to identify associations between vitamin D status and fracture risk, fracture severity, fracture healing, and skeletal disorders such as adolescent idiopathic scoliosis (AIS). Studies that assess vitamin D status do so by measuring the concentration of 25-hydroxyvitamin D [25(OH)D] in blood. This is because 25(OH)D is the principal circulating form of vitamin D, and is the precursor to the biologically active metabolite, 1,25-dihydroxyvitamin D [1,25(OH)D] (1,2). The concentration of 25(OH)D in blood reflects both the amount of parent vitamin D_3 that is generated in the skin upon exposure to ultraviolet B radiation as well as the amounts of vitamin D_2 and D_3 that are obtained from the diet and vitamin supplements. Despite enormous interest in vitamin D research, there are many pitfalls that cloud interpretation of study results. This is due in part to the many variables that affect 25(OH)D concentration.

Our objective was to identify and discuss common design and data presentation problems in vitamin D study results. Reviews of the pediatric vitamin D literature, namely studies associating vitamin D status to fracture risk and studies assessing vitamin D status in AIS, are used as examples of the lack of clarity in presentation of study results. Finally, we will make recommendations about how these common pitfalls can be avoided.

Categories of Pitfalls

Common problems that confound vitamin D research studies can be divided into three categories: 1) design issues, 2)

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Conflict of interest: None declared
Received: 07.01.2019
Accepted: 08.02.2019

inconsistent presentation of results, and 3) failure to account for variables that are known to influence serum 25(OH)D concentrations. In addition, a lack of assay standardization further confuses the picture, particularly when reports do not provide adequate information about how 25(OH)D was assayed. When such problems are present, they can make it difficult to generate direct comparisons with other studies and can confound interpretation of results.

As an example, we reviewed all known papers reporting 25(OH)D in patients with AIS (n = 5) (3,4,5,6,7). Only two papers indicated the race/ethnic distributions in the cohort. All five papers reported the mean ± standard deviation (SD) for 25(OH)D but only two reported percent deficiency. Between these two reports, each used a different definition of deficiency. None of the papers accounted for use of vitamin supplements nor the use of sunscreen. Only two studies had the same inclusion criteria for the Cobb angle (thus, the severity of scoliosis varied). One paper did not report the type of assay used. Of the other four papers, three different assay systems were used. Individually, all the studies are interesting and make legitimate contributions, however, they are not directly comparable nor could they be used in a meta-analysis, in part because of the variability in study design and data presentation.

Design Problems

Research studies should be hypothesis driven and not simply reporting measurement results. Stating *a priori* whether vitamin D status is hypothesized as a causative factor in the condition being studied, is secondary to the condition itself (i.e., the condition impacts vitamin D status), or has no relation to the condition, would help frame interpretation of results. Also, it should be stated *a priori* what difference in the magnitude of percent deficiency or 25(OH)D concentration between two comparative groups is considered to be clinically meaningful. For example, "We hypothesize that low 25(OH)D is a causative factor in spinal curve progression in AIS manifest by at least a 30% difference in 25(OH)D concentration compared to controls". Such a statement would temper overinterpretation of clinically insignificant differences (despite statistically significant differences).

The blood level of 25(OH)D can change substantially in a very short period of time (literally within minutes) after exposure to sunlight, or over a longer time period with the use of oral supplements (2,8,9). Therefore, studies must be designed so that measurement of 25(OH)D occurs at time points that are relevant to the development of the study endpoints. For example, if attempting to correlate fracture risk with vitamin D status, the 25(OH)D should be measured within a very short time period after the patient presents with the fracture. For studies in which an effect might take place over a protracted period of time such as bone mineral density or muscle mass changes, or the effect of a treatment or procedure that may last many months, it would be appropriate to verify *chronic* vitamin D status. This might require repetitive determination of 25(OH)D levels over an interval of time that corresponds to the duration of time necessary for the outcome to be realized. This is because 25(OH)D levels over time are susceptible to the phenomenon of regression to the mean, whereby a patient may be deficient on one measurement, but insufficient or normal at a subsequent measurement (due perhaps as a consequence of season, lifestyle factors, assay variability, etc.). For example, if assessing the effect of vitamin D status on height over three years, it would be inappropriate to measure a single 25(OH)D concentration at the beginning of the study and then assume that this is a valid reflection of vitamin D status over the entire three year period of study.

Failure to measure all critical variables may confound interpretation of results (Table 1; variables affecting 25(OH)D concentration will be discussed further below). For example, if a study hypothesis states that vitamin D status impacts spinal curve progression in scoliosis or is a factor in fracture risk, then it is important to not only measure known variables that affect 25(OH)D concentration [such as body mass index (BMI), use of vitamin supplements, etc., see Table 1] but also known variables that affect curve progression (such as bone mineral density, growth stage, and menarchal status). A major problem with the concept that poor vitamin D status [i.e., low concentration of 25(OH)D] is clinically meaningful is that it is unusual to observe signs or symptoms that can be directly attributed to the "low" 25(OH)D concentrations. In fact, patients frequently have 25(OH)D levels in the deficient range without any obvious symptoms or abnormalities in standard serum chemistries. In addition to the well known detrimental effect of vitamin D deficiency on bone, vitamin D deficiency can also have a detrimental effect on skeletal muscle; these include type 2 muscle fiber atrophy and metabolic changes manifest as muscle weakness which has been associated with increased risk of falling (1,2,10,11). Thus, we suggest all studies should attempt to collect bone mineral density, and weight- and age-adjusted measures of muscle strength, which could include grip strength or proximal muscle strength (ability to rise from a sitting position, stair climbing or speed walking) (11). If such measures are normal in the presence of vitamin D insufficiency then that could argue against the clinical relevance of an observation of suboptimal vitamin D status.

A measurement of parathyroid hormone (PTH) may be useful in interpreting the 25(OH)D values (12,13,14,15). A feedback relationship between serum ionized calcium, PTH and vitamin D metabolism is well established [as blood calcium levels drop, PTH levels rise and among other effects, stimulate synthesis of 1,25(OH)D] (1,2). Hence, PTH will increase if 25(OH)D is low enough to impact calcium metabolism, although the set point for this may vary from patient to patient (12,13,14,15). Theoretically, if a compensatory increase in PTH is not observed in conjunction with a "low" 25(OH)D, then the clinical significance of the "low" 25(OH)D value may be questionable. While these relationships are well established in adults, a compensatory increase in PTH as 25(OH)D falls below a critical threshold may occur at different thresholds in children or elderly adults (14).

Investigators must carefully select the control group used to compare 25(OH)D concentrations or prevalence of deficiency. This is not easily accomplished but is often directly related to the study hypothesis. For example, if the study hypothesis is that low 25(OH)D is a risk factor for severe pediatric forearm fractures requiring surgical reduction, then a logical control group would be patients with less severe fractures that can be treated conservatively, who are matched for age, sex, BMI, activity level, sun exposure, multivitamin use, etc. It would not be appropriate, for example, to use hospitalized children who may have illnesses that could impact vitamin D status.

Inconsistent Presentation of Results

Most papers present serum concentrations of 25(OH)D as the group mean ± SD. However, perhaps more important is the distribution of values within the cohort (the percent that are deficient, insufficient and sufficient). Some papers do not report this distribution, so it is not possible to fully interpret the mean 25(OH)D value (see discussion of subgroup analyses below). Box and whisker plots would be an effective way to present these data.

One issue related to the presentation of a distribution of values is the definition of cutoffs defining deficiency. Unfortunately, these vary according to which guidelines are followed, but most are trending toward defining vitamin D deficiency as 25(OH)D <20 ng/mL (Table 2) (16,17,18,19,20,21).

Table 1. Variables known to affect serum 25(OH)D concentration

Variable	Impact on vitamin D metabolism
Age	Limited evidence that ***25(OH)D decreases*** going into teen years. Many reports of ***decreased*** levels in institutionalized frail elderly men and women (22,23,24)
BMI or age-adjusted classifications as overweight, obese	Well established that ***25(OH)D is lower*** in obese subjects compared to normal weight subjects (25,26,27,28)
Race	Well established that nonwhite subjects (African Americans, Hispanics, Asians) have ***lower 25(OH)D*** compared to Caucasian subjects (22,29,30,31)
Sun exposure	***25(OH)D increases*** with increasing unprotected sun exposure (the response can very rapid). However, because of the difficulty in obtaining reliable estimates of sun exposure, investigators should evaluate data for possible impact of season of 25(OH)D measurement. With respect to study design in studies with multiple comparison groups, ensure that samples are collected equally by season [i.e., if a study has two groups of patients, and one group had most samples collected in summer and the other had most samples collected in winter, then there could be bias in the 25(OH)D concentrations] (2,8,9,32)
Sunscreen use or skin protected by clothing	Well established that ***25(OH)D decreases*** with excessive use of sunscreen or clothing protection (which may be cultural) (2,8,9,32)
Dietary intake of vitamin D	***25(OH)D decreased*** if diet is devoid of foods that contain vitamin D. But the effect of diet on 25(OH) D can rarely be effectively isolated, and patient self-reporting of dietary intake is generally unreliable. However, study enrollment criteria can exclude those patients with complete avoidance of dairy products or fatty fish (1,2)
Use of vitamin supplement	***Higher 25(OH)D*** compared to subjects that do not use a vitamin D supplement (2,33)
Use of medications known to affect vitamin D metabolism	Various medications affect vitamin D metabolism directly or indirectly (via effects on calcium balance). For example, use of seizure drugs phenobarbital and phenytoin, and anti-tuberculosis drugs (isoniazid) result in ***decreased 25(OH)D*** synthesis in the liver. Study exclusion criteria should include current use of such medications (2,34)
Hospitalization or medical conditions limiting sun exposure, associated with poor diet, leading to frailty, wasting etc.	Patients that are ill or frail with reduced exposure to sunlight and/or have vitamin D deficient diets will have ***decreased 25(OH)D*** compared to healthy subjects. This is especially true if there are impairments in liver of kidney function, which should be an exclusion criterion in a study (2,33,35)

BMI: body mass index

Table 2. Variation in guidelines defining vitamin D status

Organization/Society Guidelines for Defining Vitamin D Status	Vitamin D status (values are ng/mL)					
	Severe deficiency	Deficiency	Insufficiency	Sufficiency	No added benefit	Possible harm (toxicity)
Institute of Medicine, 2011 (16)		< 12	12-20	21-30	31-50	> 50
Endocrine Society, 2011 (17)		< 20	20-29	30-100		> 100
American Academy of Pediatrics (Pediatric Endocrine Society), 2008 (18,19)	< 5	5-15	16-20	21-100	101-149 (excess)	> 150
Kidney Disease Outcomes Quality Initiative (20)	< 5	5-15	16-30	> 30		
Mayo Medical Laboratories (21)	< 10	10-24		25-80		> 80
Quest Diagnostics (commercial lab)		< 20	20-29	> 30		

Failure to Account for Variables Known to Affect the Serum Concentration of 25(OH)D

Interpretation of study results can be influenced by subgroup analysis, and failure to report subgroup results can distort the true findings in a study. It is critically important to carefully characterize the study cohort because many factors can affect the 25(OH)D level in blood (either increasing or decreasing the concentration) (Table 1). Lifestyle factors known to affect 25(OH)D include sun exposure practices such exuberant use of sunscreen, diets low in dairy products and various supplements and medications that influence vitamin D metabolism (multivitamins, calcium supplements with vitamin D, anticonvulsants, etc.). Perhaps the most important patient characteristic that affects 25(OH)D is race/ethnicity. Studies consistently report that nonwhite cohorts (African Americans, Hispanics, Asians) have significantly lower 25(OH)D concentrations than Caucasians. However, the relative contributions of genetics (including skin pigmentation and body fat profiles), socioeconomic status and culture (including diet low in vitamin D, avoidance of sun exposure, etc.) on these findings remains unclear. Another important demographic variable is BMI, as 25(OH)D is lower in obese subjects compared to normal weight subjects. Failure to carefully match controls, or failure to present subgroup analyses can lead to bias in results and misinterpretation. The implications are obvious. For example, if one cohort has a high percentage of patients who take multivitamins that contain a form of vitamin D and the comparative group does not, then the difference in 25(OH)D between them could be affected by the use multivitamins.

Table 3 shows results of a study conducted to document vitamin D status in children with radius fractures (mean age, 9.8 ± 3.4 years; 65% were boys). These previously unpublished data show the potential for misleading reporting of study findings. The total cohort mean is less than the cuttoff of 30 ng/mL that many labs and some guidelines define as vitamin D sufficiency. However, the subgroup of Caucasian subjects had a mean 25(OH)D that was 25% higher (and in the "sufficient" range, > 30 ng/mL), compared to all nonwhites [despite no significant difference in BMI, a variable that can affect 25(OH)D concentration]. Thus, we suggest it would be misleading to report only the total group mean 25(OH)D value given this significant subgroup difference. Table 4 shows the covariable confounder of BMI on the risk for severe fracture, in which both a high BMI (classifying patients as obese or overweight) and 25(OH)D deficiency were independent risk factors for having a severe distal radius fractures requiring surgical management. Failure to report and discuss the impact of BMI would be misleading, possibly overweighting the impact of the vitamin D status as risk factor for severe fractures.

25(OH)D Assay Issues

Multiple assay systems are available for the measurement of 25(OH)D in blood. These assays can be grouped into two general categories: 1) immune based and 2) chromatography based (ultraviolet or mass spectrometric detection). The mass spectroscopy systems are currently favored as the standard. In fact, the liquid chromatography tandem mass spectroscopy method is used by the Center for Disease Control and Prevention as the reference measurement system. However, these systems require sophisticated equipment and technical expertise and are difficult to automate. Hence, immunoassays are more commonly available.

Unfortunately, there are well described problems with variation and a lack of congruity between different assays systems, with considerable differences in the 25(OH)D concentration reported when the same sample is assayed by different systems (36,37,38,39,40,41,42). Further, there

Table 3. Subgroup effects on reported mean 25(OH) D concentration in 100 children with radius fractures (previously unpublished data)

	All patients	All African Americans and Hispanics (n = 77)	Caucasian (n = 23)
Body mass index	19.5 ± 4.2	19.8 ± 3.85	18.5 ± 4.92
25(OH)D, ng/mL	27.5 ± 8.28	26.0 ± 7.17*	32.5 ± 3.89

Data are mean ± standard deviation.

25(OH)D was assayed within 30 days of the forearm fracture using a blood-spot card technique (ZRT Laboratory, Beaverton, OR). Samples were assayed using a liquid chromatography-mass spectrometry method.

*Unpaired t-tests compared to Caucasian group, $p < 0.0001$

Table 4. Body mass index as a significant confounder in study results (previously unpublished data)

	No reduction or closed reduction (n = 88)	Surgical reduction (n = 12)
Age, years	9.52 ± 3.2	12.07 ± 1.91 p = 0.008*
Body mass index	18.9 ± 4.0	23.9 ± 4.5 p = 0.0001*
25(OH)D, ng/mL	28.1 ± 8.08	23.3 ± 8.83 NS, p = 0.057*
Percent deficient [25(OH)D < 20 ng/mL]	17	50 p = 0.0172**

Both body mass index (and status as overweight and obese) and vitamin D deficiency were significant independent risk factors for surgical reduction.

*Compared to non-operative management group, unpaired t-test.

**Compared to non-operative management group, Fisher's exact test.

Data are mean ± standard deviation.

25(OH)D was assayed within 30 days of the forearm fracture using a blood-spot card technique (ZRT Laboratory, Beaverton, OR). Samples were assayed using a liquid chromatography-mass spectrometry method)

are differences between assays with respect to their ability to measure both 25(OH)D_2 and 25(OH)D_3 and some assays may cross-react and measure other metabolites. There can be a clinically important difference if one assay system measures 25(OH)D 10-15% lower than another, as it could impact the number of patients reported as deficient. These assay differences may be problematic when attempting to compare studies or do meta-analyses. Thus, it is important to know in detail about the assay system used when evaluating the results of a study (some papers do not report the type of assays used). Efforts are in progress to standardize 25(OH) D assays and to have lab certification of assay systems to mitigate the various issues associated with multiple assays types. However, at the time of writing, such a solution has not been implemented.

Solutions and Recommendations

Professional societies or journals that publish vitamin D-related studies could help standardize reporting of results by providing guidelines and checklists for ensuring some degree of study standardization. This especially applies to standardizing the presentation of cutoff values for defining deficiency.

Design

- Investigators should state *a priori* what difference between the main study cohort and the comparative group is hypothesized to be clinically relevant to frame later interpretation of obtained results.
- Investigators should measure and report all variables known to affect 25(OH)D and make adjustments in data, or discuss the absence of such information in a paragraph regarding study limitations and the possible impact on interpretation.
- Measurements of 25(OH)D should occur over the time interval during which an effect or outcome is expected, not merely at the beginning or end of a study.
- While it is important for the sake of generalizability to have diversity in study cohorts (and institutional review boards may require it), it may be problematic for vitamin D studies. Study designs should either consider enrolling homogeneous patient groups or anticipate the need for subgroups analyses. The latter would require a randomized block design for prospective studies or matched patient selection in retrospective studies, with large enough sample size to allow for subgroup analyses.

Presentation of Data

- The mean 25(OH)D alone does not provide enough information about the true vitamin D status in a cohort. All vitamin D studies should report not only the mean 25(OH) D but also the percent deficiency (the range of values can be eloquently presented using box and whisker plots). However, guidelines defining deficiency are not consistent. We suggest that a solution for minimal standardization is for journals to require that results be presented for a sliding scale of cutoff points, reporting percent deficiency at < 10, < 20 and < 30 ng/mL (as simple bar graphs). Not only would that allow readers to judge for themselves what is true deficiency, but would be beneficial for comparing results between papers and documenting important detail in the literature in the likely event of future adjustments of cutoff values.

25(OH)D Assay

• Details of the assay must be presented in publications, and if possible, whether the lab and assay have been certified per the National Institute of Standards and Technology (an agency of the U.S. Department of Commerce, Gaithersburg, MD), or the Vitamin D Standardization Program of the National Institutes of Health Office of Dietary Supplement.

• If investigators plan to do long-term data collection or multiple vitamin D studies, they should consider preparing an internal standard that can be remeasured over time or in serial studies to account for assay drift. This can be done by collecting a large sample of blood and then freezing aliquots that can be serially measured. This would be particularly important in multisite studies if a central assay lab could not be used. Reference samples of 25(OH)D can also be purchased commercially. Alternatively, samples can be held until the end of the study and run together as a single batch to avoid issues with assay drift [serum 25(OH)D is generally stable when samples are frozen at -20 degrees centigrade and not affected by multiple freeze-thaw cycles].

Conclusion

We suggest that guidelines should be available to standardize studies of vitamin D and data presentation to allow for direct comparisons and for high-quality meta-analyses. The authors believe that the suggestions made here are relatively easy to implement. Professional societies and especially journal editorial boards should consider checklists to ensure critical data elements are presented in published vitamin D studies.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Gary M. Kiebzak, Kevin M. Neal, Design: Gary M. Kiebzak, Kevin M. Neal, Pooya Hosseinzadeh, Robert C. Olney, Michael A. Levine, Data Collection or Processing: Gary M. Kiebzak, Analysis or Interpretation: Gary M. Kiebzak, Kevin M. Neal, Pooya Hosseinzadeh, Robert C. Olney, Michael A. Levine, Literature Search: Gary M. Kiebzak, Kevin M. Neal, Writing: Gary M. Kiebzak, Kevin M. Neal, Pooya Hosseinzadeh, Robert C. Olney, Michael A. Levine.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2018.2018.0213

J Clin Res Pediatr Endocrinol 2019;11(3):227-233

Elevated Random Luteinizing Hormone is an Unreliable Indicator for Pubertal Suppression in Girls Treated with Monthly Leuprolide for Idiopathic Central Precocious Puberty

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What is already known on this topic?

To date, there has been no consensus method for biochemical monitoring for idiopathic central precocious puberty (ICPP). Previous studies have shown that girls with CPP commonly have pubertal luteinizing hormone (LH) on random measurement, particularly in those treated with long-acting gonadotropin-releasing hormone analog implant. However, data in girls treated with monthly leuprolide acetate are limited. Moreover, there has been no study examining random LH or its association with growth rates and final adult height (FAH) in a longitudinal fashion.

What this study adds?

This study demonstrated that random LH was elevated in ~60% of ICPP girls treated with monthly leuprolide with a magnitude of rising LH higher than previously reported. In respect to fact that elevated random LH was not associated with pubertal progression, or decreased FAH, it does not appear to be a reliable method for ICPP monitoring.

Abstract

Objective: Longitudinal data regarding random luteinizing hormone (LH) concentrations in patients with idiopathic central precocious puberty (ICPP) during treatment are limited. Therefore, we sought to evaluate random LH and estradiol concentrations during monthly leuprolide injection and their associations with pubertal progression and final adult height (FAH) in girls with ICPP.

Methods: Medical records of 27 girls with ICPP who had attained FAH were reviewed. Patients' height, weight, Tanner stage, growth rate (GR), bone age, random LH measured by both immunoradiometric and immunochemiluminescent methods, follicular-stimulating hormone (FSH) and estradiol levels were monitored until FAH.

Results: Treatment was started at a mean (±standard deviation) age of 8.1±0.6 years with mean duration of 3.9±0.2 years. At six months of follow-up, random LH ($p=0.048$), FSH ($p<0.001$) and estradiol ($p=0.023$) concentrations were decreased compared with baseline. Thereafter, random LHs were well suppressed. GRs gradually decreased to prepubertal norm by month 12. Seventeen patients (63%) exhibited pubertal LH concentrations at least once during treatment visits. Furthermore, 43 of a total 116 (37%) LH measurements were found elevated. However, those patients with elevated random LH did not show signs of pubertal progression. After treatment, mean FAH was greater than predicted adult height ($p<0.0001$) and target height ($p=0.03$). At no time points of treatment did random LH, FSH and estradiol correlate with GRs or FAH.

Conclusion: Elevated random LH is commonly found in ICPP girls during monthly leuprolide treatment. However, these elevations were not associated with clinical progression of puberty or decreased FAH, suggesting that it is not a reliable method for CPP monitoring.

Keywords: Central precocious puberty, final adult height, leuprolide acetate, monitoring, pubertal progression, random luteinizing hormone

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Conflict of interest: None declared

Received: 31.08.2018

Accepted: 25.12.2018

J Clin Res Pediatr Endocrinol
2019;11(3):227-233

Introduction

Idiopathic central precocious puberty (ICPP) is one of the most common endocrine disorders in girls. Gonadotropin-releasing hormone analog (GnRHa) is the mainstay of therapy for pubertal suppression. This treatment also improves final adult height (FAH) (1,2) and reduces negative psychosocial consequences (3,4). However, the biochemical monitoring for ICPP treatment remains challenging since there are no robust data demonstrating universal cut-off levels of random or stimulated LH for defining pubertal suppression (1,5,6,7). Moreover, the association between a lack of biochemical suppression with clinical progression and its influence on growth rates (GR) or FAH have not been evaluated.

To date, suppressed stimulated LH has been widely used in several research projects to determine treatment efficacy in ICPP (5,8,9). Nevertheless, some hospitals, including ours, have employed random (basal/unstimulated) LH for treatment monitoring because this approach is less invasive, less time-consuming and more convenient for resource-limited settings (7,10). The limited number of short-term studies (10,11) have shown that random LH was commonly found elevated during 3-monthly and annual regimens for GnRHa treatment. However, these data are limited when using a monthly preparation of leuprolide treatment (8). Further, longitudinal data from this monitoring are needed to better understand the changes of random LH over time. Therefore, we sought to longitudinally assess the serum concentrations of random LH, follicular-stimulating hormone (FSH) and estradiol in patients receiving monthly leuprolide injection and to assess associations with pubertal progression and FAH in girls with ICPP.

Methods

Patients

This study protocol was approved by the Khon Kaen University Ethical Committee (approval number; HE591411). Since this is a retrospective study and our patient data were anonymised, informed consent was not required by our Ethical Committee. During the period between January 2002 and December 2016, 420 girls were referred for precocious puberty evaluation to our Pediatric Endocrine Clinic, Khon Kaen University Hospital.

Inclusion criteria were: 1) breast development (Tanner stage ≥2) under the age of 8 years; 2) advanced bone age (BA) ≥1 year; 3) GnRHa-stimulated LH ≥6.9 IU/L (12,13,14); 4) normal brain and pituitary magnetic resonance imaging; 5) receiving monthly GnRHa treatment.

There were 142 girls diagnosed as ICPP. One hundred and ten girls received monthly GnRHa treatment. Forty-seven girls reached FAH with complete medical records. Of these, 20 girls were excluded due to markedly advanced BA (≥13 years) at initial presentation, as this BA represents ~95% of FAH, and increased random LH concentrations may have less effect on the FAH compared to patients with lesser bone maturation. Therefore, there were 27 girls included in the analysis.

Treatment

Treatment was started in patients with 1) rapid progression, 2) BA advancement ≥2 years, or 3) differences in predicted adult height (PAH) and target height (TH) of ≥1.5 standard deviation (SD) score at initial or during follow-up. All patients received a monthly 3.75 mg depot leuprolide acetate injection until the chronological age (CA) of 12 years. During treatment, no patient showed any clinical characteristics of pubertal progression (a change in breast Tanner stage, increased GR or vaginal bleeding), therefore, none received treatment modification.

Follow up Examination and Hormonal Measurement

Height, weight and Tanner stage of breast and pubic hair were assessed every 3-6 months until the patients attained FAH. Height was measured to the nearest 0.1 cm using a stadiometer; and body weight to the nearest 0.1 kg. GR was calculated as a change in height during a 1-year interval. BA was assessed at the initial visit and before treatment discontinuation. Pre-treatment PAH was estimated using the accelerated table of the Bayley and Pinneau method (15). All clinical parameters were assessed by the same pediatric endocrinologist. FAH was defined as a GR ≤0.5 cm/year or a BA ≥16 years.

Morning blood samples for serum random (basal) LH, FSH and estradiol were collected at 7-8 am, before the regular scheduled leuprolide injection, every 3-6 months at the pediatric endocrine clinic in the first year of treatment, and then every 6 or 12 months if no clinical progression was evident. During 2002-2013, serum LH and FSH were measured using a sensitive IRMA (RIA-gnost®, monoclonal mouse antibodies, hLH and hFSH; CIS Bio International, Gif-Sur-Yvette, France) with a lower detection limit of 0.1 and 0.15 IU/L with inter-assay coefficient variations of 9.4% and 8.7%, respectively. After 2011, LH and FSH were analyzed using a more sensitive immunocemiluminescent assay (ICMA) from Esoterix Laboratories (Calabasas Hills, CA, USA) with a detection limit of 0.02 IU/L for both hormones and inter-assay coefficients of variation were 10.7% for LH and were 9.0% for FSH. In summary, 27/27, 25/25, 25/25,

17/22 and 9/17 random LH levels were analyzed using IRMA at 6, 12, 24, 36 and 48 months of treatment time points, respectively, and the rest were analyzed on ICMA. Estradiol levels were measured by an ICMA (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) with a detection limit of 5 pg/mL. Hormonal values falling below the lower detection levels were taken as the lower detection levels. The random pubertal LH concentrations were defined as ≥0.6 IU/L for IRMA (12,13) and ≥0.3 IU/L for ICMA (10).

Statistical Analysis

Statistical analysis was performed using SPSS v18 (IBM Inc., Chicago, Ill., USA). Data were presented as number, percentages, mean ± SD and median (Q1, Q3). A t-test, paired t-test or chi-square test were used to test the differences between groups as appropriate. Repeated measures ANOVA was performed to compare two repeated measurements in the same individuals. Correlations were evaluated using Spearman correlation coefficients. Non-parametric tests were used for non-normally distributed data. P value < 0.05 was considered significant.

Results

Patient Characteristics

Baseline patient characteristics are shown in Table 1. Patients reported their mean ± SD age of breast onset at 6.9 ± 0.1 years, however, treatment was started at mean age of 8.1 ± 0.2 years with a mean duration of 3.9 ± 0.2 years. The reason for a delay in treatment initiation was mainly due to lack of knowledge about their disease. All patients reported good treatment compliance, and none had missed or delayed the scheduled leuprolide injection. As expected, the patients had typical features of ICPP including breast development, tall stature, advanced BA and elevated random or stimulated LH concentrations.

GRs and FAH

Mean GR ± SD at pre-treatment and 6, 12, 24, 36 and 48 months during treatment were 8.5 ± 2.5, 6 ± 1.2, 5.6 ± 1.1, 4.1 ± 1.5, 4.1 ± 1.3 and 2.6 ± 1.4 cm/year, respectively. After treatment, mean FAH was greater than initial PAH ($p < 0.0001$) and TH ($p = 0.03$, Table 2).

Serum LH, FSH and Estradiol Concentrations

Figure 1 shows random LH and estradiol concentrations during treatment. At six months of follow-up, the median serum LH [1.7 (0.3, 3.5) *vs* 0.46 (0.02, 1.14) IU/L; $p = 0.048$], FSH [4.2 (2.6, 5.8) *vs* 1.6 (1.3, 2.1) IU/L; $p < 0.001$) and estradiol [36 (11, 53) *vs* 9 (4, 12) pg/mL; $p = 0.023$)] significantly declined from pre-treatment concentrations. Thereafter, the overall LH concentrations remained suppressed and tended to rise again at 36 and 48 months of follow-up. The median (Q1, Q3) random LH concentrations measured on IRMA were 0.4 (0.1, 1.02) at 6 months, 0.11 (0.1, 0.64) at 12 months, 0.24 (0.1, 0.62) at 24 months, 0.34 (0.07, 0.82) at 36 months and 0.6 (0.22, 0.85) at 48 months of treatment. The LH concentrations measured on ICMA at 36 and 48 months of treatment were 0.2 (0.09, 0.52) and 0.24 (0.23, 0.61), respectively. Median FSH values were 1.6 (0.82, 2.7), 1.8 (1.2, 2.6), 1.6 (1.2, 2.5), 1.4 (0.6, 1.7) and 0.6 (0.54, 1.7) at 12, 24, 36 and 48 months of treatment, respectively.

During treatment, 37% of a total of 116 LH measurements were within the pubertal range (≥0.6 IU/L for IRMA and ≥0.3 IU/L for ICMA). Among these, 10/27 (37%), 7/25 (30%), 7/25 (28%), 9/22 (41%) and 10/17 (61%) of the patients had elevated random LH at month six (range, 0.85-2.92 IU/L), month 12 (range, 0.6-1.8 IU/L), month 24 (range, 0.61-1.8 IU/L), month 36 (range: 0.30-0.60

Table 1. Baseline patient clinical and hormonal characteristics

Characteristics	Patients (n = 27)
Age of onset, years	6.9 ± 0.5
Age of start of treatment, years	8.1 ± 0.5
BA, years	11 ± 0.9
HT, cm	136.3 ± 6.3
HT, SDS	2.3 ± 1.3
WT, kg	35.8 ± 7
WT, SDS	3.3 ± 2
BMI, kg/m^2	19.1 ± 2.8
BMI, SDS	1.3 ± 1.1
TH, cm	156.0 ± 4.3
TH, SDS	-0.2 ± 0.9
PAH, cm	152.0 ± 6
PAH, SDS	-1 ± 1.3
Breast Tanner stage	3 (3, 4)
Pubic Tanner stage	1 (1, 2)
Basal / post-stimulated LH (IU/L)	1.74 (0.33, 3.47) / 27 (13, 64)
Basal / post-stimulated FSH (IU/L)	4.2 (2.65, 5.5) / 15 (12.8, 18.7)
Estradiol (pg/mL)	36 (11, 53)

HT-SDS: height-standard deviation score, WT-SDS: weight-SDS, BMI: body mass index, TH-SDS: target height-SDS, PAH-SDS: Predicted adult height-SDS, LH: luteinizing hormone, FSH: follicular stimulating hormone, BA: bone age

Data are presented as mean ± SD or median and interquartile range (Q1, Q3)

IU/L for ICMA and 0.60-2.5 IU/L for IRMA) and month 48 (range: 0.3-0.83 for ICMA and 0.6-2 IU/L for IRMA), respectively. Moreover, 17 out of 27 individuals (63%) exhibited pubertal LH at least once at some point during treatment. However, these patients showed no signs of pubertal progression and thus none received treatment modification. Of a total of 112 estradiol measurements, 29% were above the lower detection limit (5 pg/mL). However, all values were in the prepubertal range of < 20 pg/mL (16) throughout the treatment duration.

Correlation

Random LH did not correlate at any of the time points with GR (Table 3) or FAH (data not shown). Also, there was no correlation between random LH, FSH, estradiol and body mass index (BMI) during treatment (data not shown). Neither FSH nor estradiol correlated with BMI, GRs and FAHs at any time points during treatment (data not shown).

Patients with Frequently Elevated Random LH

Table 4 shows the clinical characteristics of three patients who presented with markedly advanced BA (BA-CA ~ 4.7-5.6 years), compromised PAH and frequent elevations in random LH during treatment. However, they did not show any signs of pubertal advancement. They each attained FAH which was higher than their PAH without any therapeutic modification.

Table 2. Final height outcome in 27 girls with idiopathic central precocious puberty

Pre-treatment			Post-treatment		
Height, cm (SDS)	**TH, cm (SDS)**	**PAH, cm (SDS)**	**FAH, cm (SDS)**	**FAH-PAH, cm (SDS)**	**FAH-TH, cm (SDS)**
136.3 ± 6.3	156.0 ± 4.3	152.0 ± 6	159.5 ± 5.2[a,b]	5.9 ± 6.2	3.9 ± 0.9
(2.3 ± 1.3)	(-0.2 ± 0.9)	(-1 ± 1.3)	(0.3 ± 1)	(1.1 ± 1.3)	(0.7 ± 0.9)

SDS: standard deviation score, TH: target height, PAH: predicted adult height, FAH: final adult height.

Data are presented as mean ± SD, [a]p = 0.03 (FAH *vs* TH), [b]p < 0.0001 (FAH *vs* PAH)

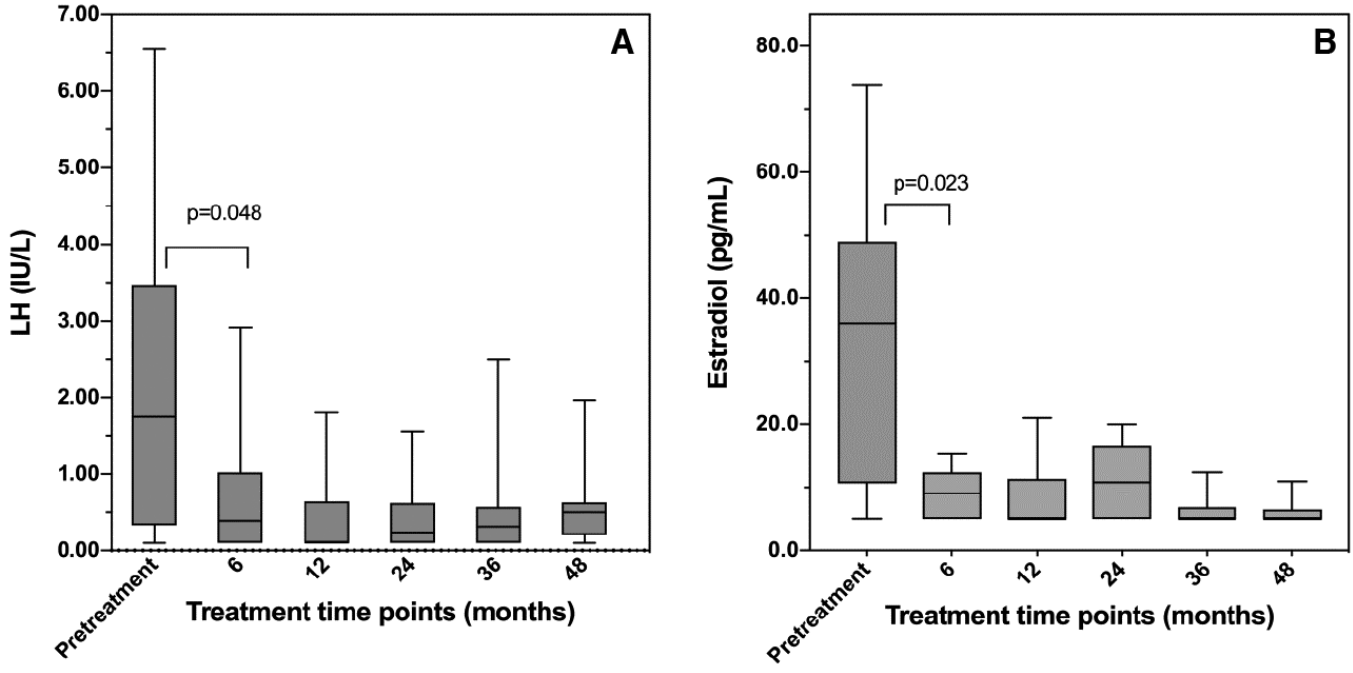

Figure 1. Random LH (A) and estradiol (B) concentrations before and during treatment.

A) At month six, random LH concentrations were significantly lower than pre-treatment values (p = 0.048). Thereafter, there were no statistical differences in random LH concentrations between each consecutive time point (6, 12, 24, 36 and 48).

B) At month six, serum estradiol concentrations were significantly lower than pre-treatment values (p = 0.023). There were no statistical differences in serum estradiol concentrations between each consecutive time point (month 6, 12, 24, 36 and 48).

Data are presented as box and whisker plots. The center lines, lower edges and upper edges of the boxes indicate median, 25th- and 75th percentiles, respectively. The whiskers indicate 5th-95th percentile.

LH: luteinizing hormone

Discussion

Monitoring patients with ICPP is a clinical challenge since there is no consensus in method for monitoring. Failure HPG-axis suppression can cause short FAH (1). In this study, we demonstrated longitudinal serum random LH concentrations during treatment and their relationship with pubertal progression and FAH.

Our study demonstrated that random LH was elevated in 37% of total measurements in ICPP patients during monthly leuprolide treatment with the magnitude of increase as high as 2.9 IU/L for IRMA and 0.62 IU/L for ICMA. Moreover, two-thirds of our patients exhibited pubertal LH levels at least once at some time points during follow-up and 30-60% of patients had random LH concentrations in the pubertal range during each year of treatment. Yet, none had pubertal progression by clinical examination. There are only three studies evaluating random LH concentrations or prevalence of increased random LH in CPP patients during GnRHa treatment as the main outcome [one in patients with trimonthly GnRHa injection (7) and the others in patients receiving annual GnRHa implant (10,11)]. These studies showed similar proportions of patients with elevated random LH compared to our study results. Several findings in random LH concentrations in ICPP patients during various forms of GnRH treatment were reported (5,8,10,11,17). Neely et al (8) found that their subjects treated with a higher dose (7.5-15 mg) of monthly leuprolide had a satisfactory clinical and biochemical (random LH) suppression. Their patients' maximal random LH concentrations in each follow-up visit were also lower than what was found in our patients (immunofluorometric assay, IFMA; 0.5-0.8 IU/L *vs* 1.8-2.9 IU/L). However, our patients were treated with a relatively lower dose of leuprolide (3.75 mg, 90-140 µg/kg) which is a commonly used dose in most Asian countries (18,19). Elevated random LH was also reported in 50-60% of ICPP patients treated with histrelin implant, without pubertal progression (10,11). However, stimulated LH was well suppressed in these patients. Similar findings were also demonstrated in a study using monthly leuprolide (20) and another study using 3-monthly leuprolide (17). In contrast, Brito et al (5) reported that two out of 18 patients had pubertal breakthrough, although there was no specific report regarding "pubertal progression", which was confirmed by a modest increase in random (0.9-1.1 IU/L, IFMA) and stimulated LH (4.3-5.7 IU/L). Therefore, these data indicated that elevated random LH is an unreliable marker for pubertal suppression.

Several studies have shown a correlation between random (basal) and stimulated LH concentrations, including Lee et al's (7) study, which is the only work suggesting the random (basal) LH cut-off value. This study showed that basal LH (ICMA) ≥0.6 IU/L had an optimal sensitivity (80%) and specificity (70%) for predicting stimulated LH levels of >4 IU/L. Again, this study did not demonstrate the relationship between increased basal LH or increased stimulated LH with clinical parameters, such as BA or clinical progression.

Our data also demonstrated that elevated random LH was not associated with GR nor FAH. There has been only one study (21) showing a similar result of lacking relationship

Table 3. Correlation analysis between random luteinizing hormone and estradiol concentrations with growth rates at corresponding time points

Parameters	Spearman's correlation (r) with GRs	p value
LH (month 12)	0.286	0.175
LH (month 24)	-0.181	0.419
LH (month 36)	-0.266	0.319
LH (month 48)	-0.734	0.158
Estradiol (month 12)	-0.102	0.801
Estradiol (month 24)	-0.113	0.887
Estradiol (month 36)	0.323	0.280

LH: luteinizing hormone, GR: growth rate

Data are shown as Spearman's correlation coefficient (r)

Table 4. Clinical, hormonal characteristics and final adult height in three idiopathic central precocious puberty patients with persistent elevation of random luteinizing hormone levels during treatment

No	CA (yr)	BA (yr)	TH (cm)	PAH (cm)	FAH (cm)	Treatment duration (yr)	Random LH levels (IU/L)				
							6 m	1 y	2 y	3 y	4 y
1	7.8	12.5	158.0	150.4	158	4.6	2.9	0.1	0.2	1.06	2.0
2	6.9	12.5	156.0	151.5	162	4.9	0.1	0.1	1.1	2.5	0.62
3	7	12	156.0	153.7	162	4.8	0.9	-	0.98	2.4	0.60*

CA: chronological age, BA: bone age, TH: target height, PAH: predicted adult height, FAH: final adult height, LH: luteinizing hormone, yr: year

Dash line (-) indicates missing data

*Indicates random luteinizing hormone from immuno chemiluminescent assay. Other values were measured using IRMA

between random LH and GR. That study also found that random LH had a negative association with the changes in PAH ($r = -0.309$; $p < 0.05$), assessed by regular BA examination. To our knowledge, there have been no studies demonstrating the association between either random or stimulated LH and FAH. Our study showed that random LH was not correlated with FAH. Although two thirds of patients experienced pubertal LH, mean FAH was higher than PAH and at the upper range of TH. Further, the FAH and net height gain after GnRHa treatment in our patients with elevated random LH were similar to what have been previously reported in patients with suppressed stimulated LH (6,18,19,22,23). It is also worth mentioning that our three patients with frequently elevated random LH did not exhibit any signs of pubertal progression, and eventually attained FAH which was higher than their PAH and TH without treatment modification. Taken together, our results indicated that elevated random LH, in the context of no clinical progression being observed, may not have a negative effect on FAH.

In animal study, random LH was elevated during a continuous buserelin infusion, yet the pulsatile fashion was abolished (24,25) that resulted in ineffective sex hormone production. This finding may explain why our patients did not exhibit clinical signs of pubertal progression in response to increased random LH. Moreover, an alteration in the ratio of immunoreactive to bioactive gonadotropins (26) has also been described in patients receiving GnRHa. However, these hypotheses have never been tested in ICPP patients. Further research is needed.

In addition to random LH, we examined the association between estradiol concentration and pubertal advancement. We found that ~30% of the patients had slightly elevated estradiol concentrations above the lower detection limit (5-20 pg/mL) which are, however, considered prepubertal concentrations. In correlation analysis, we did not find associations between estradiol and random LH, BMI, GR nor FAH. These findings are incongruent with previous reports (21,27,28,29). Other studies also demonstrated that estradiol did not relate to BA maturation (21,25).

Study Limitations

Our study has some weaknesses. First, we did not regularly assess BA, therefore, we could not evaluate the influence of increased random LH on skeletal maturation. Second, this is a retrospective study, in which we did not evaluate the stimulated LH concentrations at the time when elevated random LH was found. This led to an inability to assess the unknown effect of stimulated LH on GR and FAH. Our study also has a relatively small sample size. However, to our knowledge, this research is the first to demonstrate the longitudinal data of random LH concentrations throughout the treatment duration of ICPP, as well as the association with clinical markers of pubertal progression and FAH.

Conclusion

In conclusion, we reported the longitudinal evidence that elevated random LH is commonly found in ICPP girls during monthly leuprolide treatment. However, the increase in random LH is not associated with clinical progression of puberty nor with FAH, suggesting that this method is unreliable and may not be useful for ICPP monitoring. More studies are needed to evaluate the relationship between elevated basal or stimulated LH and BA maturation or FAH.

Acknowledgements

We gratefully acknowledge the patients and their families. This research was supported by Faculty of Medicine Research Fund, KKU.

Ethics

Ethics Committee Approval: This study protocol was approved by the Khon Kaen University Ethical Committee (approval number: HE591411).

Informed Consent: Since this is a retrospective study and our patient data were anonymised, informed consent was not required by our Ethical Committee.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Pattara Wiromrat, Design: Pattara Wiromrat, Data Collection or Processing: Pattara Wiromrat, Analysis or Interpretation: Pattara Wiromrat, Literature Search: Pattara Wiromrat, Ouyporn Panamonta, Writing: Pattara Wiromrat, Ouyporn Panamonta.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2018.2018.0274

J Clin Res Pediatr Endocrinol 2019;11(3):234-239

Early Menarche is a Risk Factor for Short Stature in Young Korean Females: An Epidemiologic Study

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What is already known on this topic?

In European countries, age at menarche was positively associated with final adult height and negatively associated with body mass index.

What this study adds?

This is the first study to show that early menarche is a risk factor for short adult stature in Korean females. The odds ratio for short stature in females with early menarche was 2.62 after adjusting for mother's height.

Abstract

Objective: To assess the association between age at menarche and adult height [and body mass index (BMI)] in young Korean females and also to investigate whether early menarche (< 12 years) is a risk factor for short stature and obesity in young Korean females.

Methods: Data on 1148 females aged 18-30 years and 612 mother (612 pairs of mothers and daughters) from the 6th Korea National Health and Nutrition Examination Survey (2013-2015) were analyzed.

Results: Among 1148 females, 256 (22.3%) had early menarche. Their stature was approximately 0.445 cm shorter when menarche had occurred one year earlier. The prevalence of short stature (≤153 cm) and obesity (BMI ≥25) was higher in females with early menarche compared to those with later menarche (short stature: 10.5% *vs* 6.4%, obesity; 20.7% *vs* 13.1%, all $p < 0.001$). In multivariate regression, the odds ratio (OR) for short stature was 2.62 [95% confidence interval (CI): 1.26-5.44] after adjusting for current age and mother's height. OR for obesity was 1.74 (95% CI: 0.98-3.07) after adjusting for age and maternal BMI.

Conclusion: Final height in girls is influenced by age of menarche. Early menarche increased the risk for adult short stature in young Korean females.

Keywords: Early menarche, short stature, adult height, obesity, KNHANES

Introduction

Age of menarche is known to be influenced by several factors, such as genetics, ethnicity, geography, socioeconomic status and especially nutritional status (1,2,3,4). Several studies including this one have reported a relationship between early menarche age (< 12 years) and the risk of obesity, insulin resistance, metabolic syndrome, nonalcoholic fatty liver disease, diabetes and cardiovascular disease in adulthood (5,6,7,8,9). Thus, management of risk factors for early menarche in the pediatric population may reduce the risk of adult metabolic disease.

Short stature is typically defined as an adult height that is more than two standard deviations (SD) below the mean for age and sex (10). In developed countries, this typically includes adult men who are shorter than 166 cm and adult women who are shorter than 153 cm. Several factors might cause short adult height. The main factors appear to be the effects of multiple familial genes and environment, and the complex interplay between these. In addition, short adult height can be caused by pathological states, including genetic disease such as Turner syndrome, prolonged chronic disease, malnutrition, prolonged treatment with certain drugs (steroids) and hormone deficiency states such

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Conflict of interest: None declared
Received: 19.11.2018
Accepted: 30.12.2018

as growth hormone deficiency. Short adult height may also occur because of early fusion of growth plates as a result of precocious puberty (11).

In Europe and the USA, women with earlier menarche were reported to have reached shorter adult height compared to women who had menarche at a later age (12,13,14). Furthermore, in Asian countries including Korea, female age of menarche has recently shown a downward trend to younger age (5,15,16).

The aim of this study was to investigate whether final adult height is associated with age at menarche in young Korean females. We also assessed whether an independent association exists between early menarche and short adult stature or obesity in Korean females. For this purpose, we investigated the data of 12,537 women who participated in the Korea National Health and Nutrition Examination Survey (KNHANES-VI).

Methods

The data of the 6th KNHANES-VI (2013-2015) data were used in the study. KNHANES-VI is a cross-sectional survey with multi-staged, stratified sampling design and offers nationally representative data conducted by the Division of Chronic Disease Surveillance, Korea Centers for Disease Control and Prevention (17). Written informed consent was secured by all of the participants before the study had begun, and the KNHANES was conducted following ethical approval by the Institutional Review Board of the Korea Centre for Disease Control and Prevention (No: 2013-07CON-03-4C, 2013-12EXP-03-5C).

Among the 12,537 females who participated in KNHANES-VI, we selected data on menarcheal age and anthropometric variables. There were 1148 young females aged 18 to 30 years and mothers' height and weight data were available in 612 of the 1148 subjects. Weight was determined to the nearest 0.1 kg on a medical balance (GL-6000-20, CAS, Seoul, Korea) and height was measured to the nearest 0.1 cm with a wall-mounted stadiometer (Seca 220, Seca, Hamburg, Germany). Body mass index (BMI) was calculated by dividing the weight by the height squared (kg/m^2). Height in Korean females reaches a near plateau at age 16 according to 2017 Korean National Growth Charts (18).

"Age of menarche" is defined as age of the first menstrual period and the data was collected using the questionnaire method. The question was open-ended: "At what age did you have your first menstrual period (menarche)?" Age of years represents age between 11.00-11.99 years. We defined early menarche as <12 years. Short stature was defined as a height less than ≤153 cm (≤5th percentile of a female Korean population) and obesity as a BMI ≥25, using Asian criteria (19). Household income as a surrogate marker of socioeconomic status was assessed according to the following categorical variables: low (1Q), lower middle (2Q), upper middle (3Q), and high (4Q) in KNHANES.

Statistical Analysis

Data related to anthropometric measurements and other covariates were stratified by early menarche and later menarche. The Student's t-test and chi-square test were used in the comparison of early menarche and later menarche. Continuous variables are reported as means ± SD, and categorical variables are reported as percentages (%). Linear regression analysis was used to evaluate the predictors of the subject's height as a dependent variable using heights at menarche as predictive variables, controlling for current age. For the assessment of odds ratios (ORs) of short stature or obesity according to early menarche, multivariable logistic regression was used. The ORs including 95% confidence interval (CI), between early menarche and short stature (or obesity) were calculated before and after adjusting for age, and other confounders. In the final analysis, household income was excluded as there was no significant difference of prevalence of short stature from quartile to quartile. All statistical analyses were performed by using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). P values <0.05 were considered significant.

Results

The characteristics of the study subjects were divided according to early menarche or later menarche and are summarized in Table 1. At the time of the study, the mean ± SD current age of all subjects was 23.5 ± 3.5 years. Mean ± SD age of menarche was 12.7 ± 1.6 years. Mean ± SD (range) height was 161.6 ± 5.8 (138-179) cm and mean ± SD (range) BMI was 21.6 ± 3.7 (15-49) kg/m^2.

Among the 1148 female subjects, 256 (22.3%) had early menarche and 892 had later menarche. Mean ± SD current age was significantly younger in the early menarche group (22.9 ± 3.4 *vs* 23.7 ± 3.5 years; $p=0.001$). This group was also significantly shorter (160.4 ± 5.1 *vs* 161.9 ± 6.0 cm; $p<0.001$) and had a higher BMI (22.4 ± 3.8 *vs* 21.3 ± 3.5; $p<0.001$) than the later menarche group, The early menarche group also had a higher prevalence of short stature (10.5% *vs* 6.4%) and obesity (20.7% *vs* 13.1%) (see Figure 1). However, there was no difference in prevalence affected by household income.

In the subgroup of subjects with available maternal anthropometric data (n = 612), the mean ± SD age of the mothers was 50.3 ± 4.6 years and maternal mean ± SD age at menarche was 14.3 ± 1.7 years. Differences in age, age at menarche, height and BMI between mother and daughter were 27.5 ± 3.5 years, 1.5 ± 2.0 (-4 to 8) years, 4.4 ± 5.9 (-20 to 23) cm and 2.5 ± 3.9 kg/m^2, respectively.

There was no difference in mother's age, height, weight and BMI values between the early menarche and later menarche groups. However, the mothers of subjects with early menarche had earlier menarche than mothers of subjects with later menarche (13.8 ± 1.5 *vs* 14.4 ± 1.7 years; p < 0.001). There was also no difference in prevalence of short stature and obesity in the mothers.

Table 1. Characteristics of the subjects stratified by age at menarche

	Age at menarche		
Characteristics	**Early menarche (< 12 years)**	**Later menarche (≥12 years)**	**p value**
Subjects			
Number	256	892	
Age of menarche (years)	10.7 ± 0.6	13.2 ± 1.2	< 0.001
Age at time of survey (years)	22.9 ± 3.4	23.7 ± 3.5	0.001
Height (cm)	160.4 ± 5.1	161.9 ± 6.0	< 0.001
Weight (kg)	57.6 ± 10.5	56.0 ± 9.9	0.026
Body mass index (kg/m^2)	22.4 ± 3.8	21.3 ± 3.5	< 0.001
Household income			
Low (1Q)	0.9 %	8.7 %	0.077 (for all SES)
Lower middle (2Q)	22.6 %	28.3 %	
Upper middle (3Q)	29.9 %	32.0 %	
High (4Q)	38.9 %	30.9 %	
Mothers			
Number	131	481	
Age of menarche (years)	13.8 ± 1.5	14.4 ± 1.7	< 0.001
Age at time of survey (years)	50.0 ± 3.4	50.3 ± 3.5	0.479
Height (cm)	157.6 ± 4.4	157.6 ± 5.5	0.933
Weight (kg)	58.6 ± 7.2	58.6 ± 8.2	0.968
Body mass index (kg/m^2)	23.6 ± 2.7	23.6 ± 3.2	0.921
Daughter-mother height difference (cm)	3.3 ± 5.3	4.7 ± 6.0	0.009

Differences between subjects with early menarche (< 12 years) and later menarche (≥12 years) were compared using Student's t-test for continuous variables and chi-square test for %.

SES: socioeconomic status

Height and BMI according to age at menarche are depicted in Figure 2. In linear regression, female grew approximately 0.445 cm shorter when menarche occurred one year earlier calculated as: subject's height (cm) = subject's age at menarche (years) x 0.445 - subject's age (years) x 0.03 + 156.56 (R^2 = 0.014; p < 0.001). The ORs for short stature and obesity in females with early menarche compared to later menarche are summarized in Table 2. The crude OR for short stature in a female with early menarche

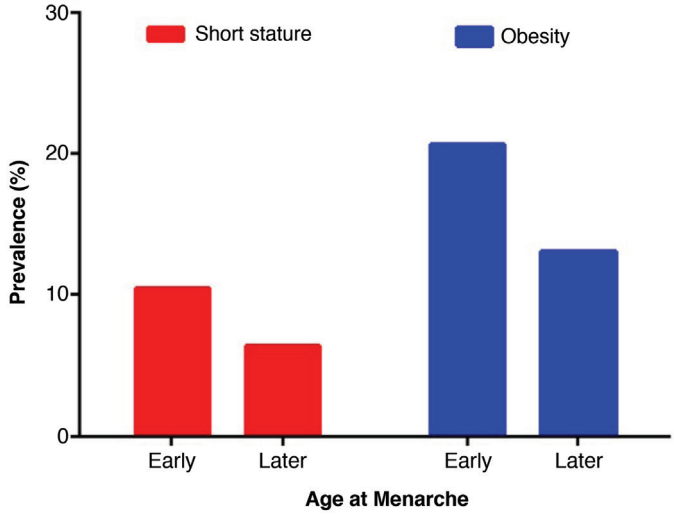

Figure 1. Prevalence of short stature and obesity according to early and later menarche. Females with early menarche had higher prevalence of short stature (10.5 *vs* 6.4 %) and obesity (20.7 *vs* 13.1 %)

Table 2. Odds ratios for short stature and obesity between subjects with early menarche and the reference group

	Early menarche (< 12 years)		Later menarche (≥12 years)
	OR	**95% CI**	
Short stature*	1.73	1.07-2.79	1.00
Model 1 age	1.71	1.06-2.78	1.00
Model 2 age + mother's height	2.62	1.26-5.44	1.00
Obesity‡	1.73	1.21-2.45	1.00
Model 1 age	1.79	1.25-2.58	1.00
Model 2 age + mother's BMI	1.74	0.98-3.07	1.00

*Short stature is defined as below the 5th percentile of Korean females aged 20-30 (16)

‡Obesity was defined as BMI ≥25 kg/m^2

*Odds ratios of short stature and obesity of early menarche compared with later menarche, multivariate logistic regression was used.

BMI: body mass index, CI: confidence interval, OR: odds ratio, Model 1: Results were adjusted for current age (in years), Model 2: Results were adjusted for age (in years), mother's height (or BMI)

was 1.73. The OR decreased to 1.71 after adjusting for current age (Model 1) and increased to 2.62 after further adjusting for mother's height (Model 2). Here, the Exp(B) of mother's height was 0.799 (95% CI: 0.742-0.860). The OR for obesity in females with early menarche was 1.73. The OR increased to 1.79 after adjusting for current age (Model 1) and subsequently decreased to 1.74 (95% CI: 0.98-3.07) after adjusting for age and mother' BMI (Model 2).

Discussion

In this study, we found that for each year earlier that menarche occurred in young Korean females final height was 0.445 cm less. We also found that females with early menarche had a 10.5% chance of having short adult stature, the rate of which was 2.62-fold higher than those with later menarche. In addition, the OR for obesity in females with early menarche was 1.73 compared to those with later menarche.

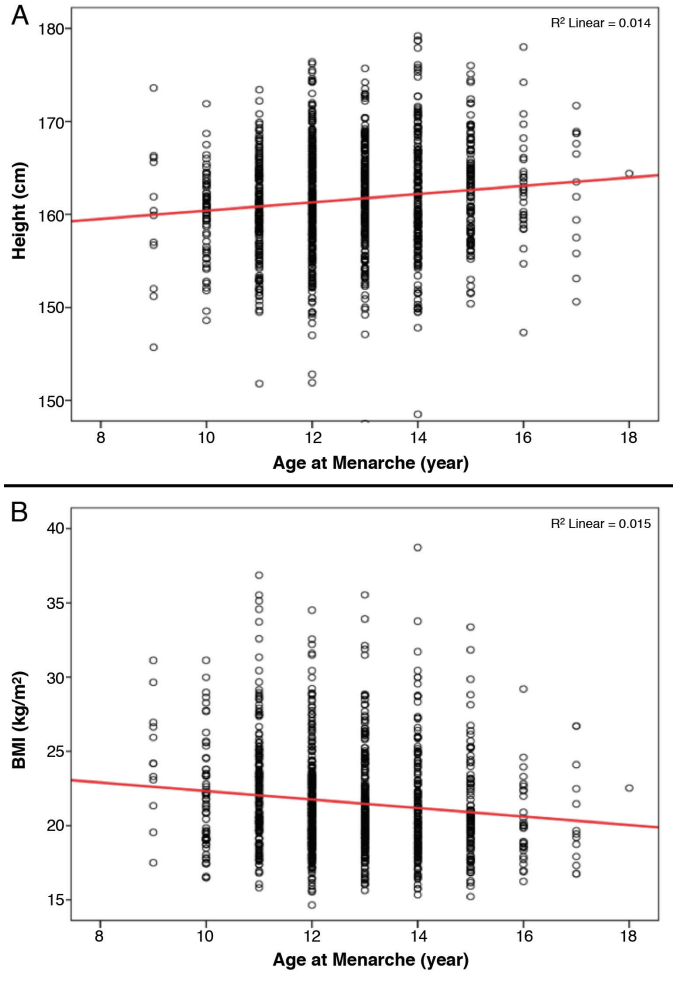

Figure 2. A, B) Height and body mass index according to age at menarche. Females were 0.445 cm shorter when menarche occurred 1 year earlier

The prevalence of early menarche has increased dramatically in Korea based on our study (5). In this study, the percentage of subjects who experienced menarche before the age of 12 years was 22.3%, while this percentage in the mothers of the subjects was 2.5%. The prevalence in the subjects was nine times higher than that of their mothers. Similar to many other countries, South Korea has shown rapid decrease of female age in menarche from mid-20th century to the present, and this is probably due to improvements in nutrition and living conditions (13,20). In our previous study, we reported the rapid decrease of mean age of menarche over time, from 15.62±1.88 years for females born between 1950 and 1954, to 13.11±1.52 years for those born between 1980 and 1984, to 12.60±1.14 years for those born between 1990 and 1994 (5). This trend might be due to changes in the socioeconomic environment, the most important of which is probably improvement in nutritional status (13). The nutritional status of South Korea rapidly improved after the Korean Civil War. South Koreans rapidly accepted western culture, especially in terms of dietary habits, after the 1988 Olympic Games, while North Korean refugees still show an age of menarche around 16.0±2.1 years (20). Nutrition, in particular, appears to play an important role in the time of onset of menarche. There are great many reports indicating that girls with higher body weight, higher BMI and more body fat reach menarche at an earlier age (13,21,22). It has been suggested that a 'critical weight' is needed for menarche to occur (23,24). Furthermore, other factors such as birth weight, prenatal nutrition, type of diet and exposure to endocrine disruptors, have been suggested as likely contributory factors for earlier pubertal development and early menarche (25,26,27). Another suggested reason for the increasing prevalence of early menarche in Korean girls is a rapid increase in the prevalence of central precocious puberty (CPP). CPP can cause early menarche in girls and result in short adult stature due to early epiphyseal fusion (11). The annual incidence of CPP in girls has significantly increased from 3.3 to 50.4 per 100,000 girls during 2004 to 2010 in Korea (28). The incidence of girls diagnosed with CPP has markedly increased in the 21st century. In Denmark for instance, it is much higher compared to 40 years ago (29).

In this study, 10.5% of females with early menarche had a short stature. The OR for short stature was 2.62-fold after adjusting for current age and mother's height in females with early menarche compared to those with later menarche. In basic terms, for each year of delay in age at menarche, a Korean female will grow to be be approximately 0.445 cm taller in her final height. This finding agrees studies from other countries (12,21,30,31). According to the European

Prospective Investigation into Cancer and Nutrition study, based on 286,205 women from nine European countries, women grew approximately 0.35 cm taller when menarche occurred one year later (range by country: 0.13-0.50 cm) (12). Furthermore, a 1-year increase in age of menarche caused an increase in standing height, leg length and trunk height of 0.76, 0.41 and 0.35 cm, respectively, in a USA birth cohort (31).

The pathogenesis of this trend in age of menarche may be explained by the earlier closure of epiphyseal growth plates due to an increase in ovarian estrogens (32,33). A low dose of estrogen will induce stimulation of the growth hormone-insulin-like growth factor 1 axis and a pubertal growth spurt in early puberty. However, increase in estrogen binding to its receptors in the growth plate cartilage might cause early epiphyseal fusion by advancing growth plate senescence (33). A delay in menarche allows continued growth of long bones before epiphyseal fusion, leading to an increase in adult height. Gonadotropin releasing hormone analog (GnRHa) treatment in girls with CPP could improve final adult height and increase the age of menarche close to that of the general population. The height gain achieved after GnRHa treatment in children with CPP, depends on the age of onset of puberty and onset of treatment (34).

In this study, the OR for obesity in females with early menarche was 1.73. However, after adjusting for age and maternal BMI, we failed to find an association between early menarche and obesity ($p = 0.058$). The most plausible reason for this might be the relatively small number of subjects analyzed. Additionally, socioeconomic factors assessed in our study failed to demonstrate an association with adult short height. This finding agrees with a previous Korean study and supports the hypothesis that socioeconomic status is not an independent predictor of age at menarche or final height in well-developed countries (35,36).

Study Limitations

The major strength of the present study is that it is based on a national representative study population. However, this study has some limitations. First, the cross-sectional nature of the study prohibits making conclusions regarding the existence of a causal relationship between age of menarche and adult height. Second, we could not adjust for other confounders such as the fathers' height and birth weight, which might influence the subjects' adult height. Recent studies showed an association between lower birth weight and early menarche (26). Finally, the age at menarche was self-reported. It is known that age at menarche based on recall data is not very accurate, especially when the time between menarche and current age is more than three years (37). However, other studies have shown high correlations ($R = 0.67$ to 0.79) between age at menarche by recall during middle-age and the original childhood data (38).

Conclusion

In conclusion, we found that early menarche is a risk factor for shorter adult stature and obesity in young Korean females. To the best of our knowledge, this is the first study to show that early menarche is a risk factor of short adult stature in Korean females. Some girls, despite being of normal height during childhood and during the pubertal years might grow to short adult stature due to earlier fusion of the growth plates. In light of the rapidly increasing prevalence of early menarche, knowledge concerning onset of puberty, progression tempo and age at menarche is important in identifying females at risk. Further long term cohort investigations are needed to fully explain these causal relationships.

Ethics

Ethics Committee Approval: The Research Ethics Committee of the Korea Centers for Disease Control approved the study protocol (no: 2013-07CON-03-4C, 2013-12EXP-03-5C).

Informed Consent: All participants or their parents signed a written informed consent form.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Jung Sub Lim, Design: Sol Kang, Jung Sub Lim, Data Collection or Processing: Yoon Mo Kim, Jun Ah Lee, Dong Ho Kim, Analysis or Interpretation: Sol Kang, Jung Sub Lim, Literature Search: Sol Kang, Jung Sub Lim, Writing: Sol Kang, Jung Sub Lim, Jun Ah Lee.

Financial Disclosure: No financial disclosure. This study was partially supported by a grant of the Korea Institute of Radiological and Medical Sciences (KIRAMS), funded by Ministry of Science and ICT (MSIT), Republic of Korea. (No. 50541-2018).

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2018.0255

J Clin Res Pediatr Endocrinol 2019;11(3):240-246

Cases Referred from the Turkish National Screening Program: Frequency of Congenital Hypothyroidism and Etiological Distribution

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What is already known on this topic?

Congenital hypothyroidism (CH) is the most commonly seen endocrinological disorder of childhood. An increase in the prevalence of CH has been reported worldwide in the last 20 years.

What this study adds?

The rate of elevated neonatal thyroid stimulating hormone (TSH) was found to be 55% and approximately one of every two cases who were referred from national screening program was diagnosed with elevated neonatal TSH. Diagnosis was made in the first month in 87% of all cases. Dysgenesis and dyshormonogenesis rates were equal at 33.3%.

Abstract

Objective: The aim of this study was to evaluate cases referred from the congenital hypothyroidism (CH) newborn screening program.
Methods: Infants referred to Pediatric Endocrinology between 30.09.2015 - 01.04.2018 because of suspected CH identified by National Neonatal Screening Program were prospectively evaluated.
Results: Of the 109 newborns referred to our clinic, 60 (55%) were diagnosed with elevated neonatal thyroid stimulating hormone (TSH). The diagnosis of elevated neonatal TSH was made in 52 (47.7%) and eight (7.3%) infants at initial evaluation and after follow up, respectively of all referrals with 86.7% (52/60) diagnosed at initial visit. The median first and second heel prick times were 1.8 (0-7) and 8.72 (4-30) days. The median age at starting treatment of the infants diagnosed as a result of initial evaluation was 22.13 (7-53) days. Clinical findings associated with CH were present in 19 (36%) of patients. Etiology in patients diagnosed with elevated neonatal TSH on admission was: agenesis in one (2.08%); ectopia in one (2.08%); hypoplasia in 14 (29.16%); normal gland *in situ* 16 (33.3%); and hyperplasia in 16 (33.3%). The median time to normalization of TSH and free thyroxine concentrations after treatment initiation was 11.02 (4-30) and 9.03 (3-30) days, respectively.
Conclusion: The rate of diagnosis in the first month was found to be 87%. The etiological incidence of both dysgenesis and dyshormonogenesis was equal at 33.3%. The majority of cases with normal thyroid gland will be diagnosed with transient hypothyroidism but some of them may be diagnosed with thyroid dyshormonogenesis so the rate of dyshormonogenesis will increase later after final diagnosis.
Keywords: Congenital hypothyroidism, neonatal screening program, newborn, thyroid hormones

Introduction

Congenital hypothyroidism (CH) is the most commonly encountered endocrinological disorder of childhood worldwide. Its incidence was reported as approximately 1/4000 in the 1970s when the screening program was first used. In the last 20 years, an increase in the prevalence of CH has been reported worldwide, possibly as a result of a gradual reduction in referral screening cut-off values (1).

In a study conducted in Turkey in 2003, the incidence of permanent CH was reported as 1/2512 (2). In an evaluation of data between the years 2008 and 2010, the incidence of CH was reported as 1/888 in 2008, 1/592 in 2009, and 1/650 in 2010 (3).

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Conflict of interest: None declared
Received: 23.10.2018
Accepted: 05.01.2019

The absence of disease-specific clinical manifestation in CH at birth and the fact that complications can be prevented with early initiation of treatment within the first few weeks after delivery, plus the fact that treatment is effective and inexpensive, led to the establishment of national screening programs. A CH screening program was first implemented in 1974 in Quebec, Canada, and in Pittsburgh and Pennsylvania in the US (4,5). In Turkey, although local screening was conducted before, nationwide CH screening in conjunction with phenylketonuria screening was introduced for the first time on 25 December 2006 in Turkey (6,7).

In Turkey, a thyroid stimulating hormone (TSH) bloodspot threshold value of 20 mIU/L was used in the first years of screening and subsequently this value was decreased to 15 mIU/L in January 2009, upon detection of cases of missed diagnosis. However, in 2016, the screening cut-off TSH threshold value was again increased to 20 mIU/L (3,8). According to the guidelines of the Turkish CH national screening program, an infant is considered to have passed the screening test when the blood spot TSH concentration is < 5.5 mIU/L. Values between 5.5 mU/L and 20 mIU/L, are reported for a second evaluation. All cases with a capillary TSH concentration above 5.5 mIU/L in repeat blood samples or above 20 mIU/L in the first sample are referred to the appropriate center for evaluation by venous thyroid function testing, including thyroxine (T4) and TSH.

In this present study, we aimed to investigate the rate of diagnosis and the time of diagnosis in cases with elevated TSH referred from the screening program; to evaluate the clinical and laboratory findings in these cases; and also to determine the etiological distribution among these patients.

Materials and Methods

All infants referred to the Outpatient Clinic of Dr. Sami Ulus Obstetrics and Gynecology, Child Health and Disease Department of Ankara University, between the dates of September 30th 2015 and April 1st, 2018, because of suspected CH following newborn screening were prospectively evaluated. The study was approved by the Local Ethics Committee (no: 45/2015). Prior to inclusion in the study, informed consent was obtained from all parents.

In our study, serum T_4 and TSH levels were measured on the day of referral. The diagnosis and treatment plan was based on the Lawson Wilkins Pediatric Endocrine Society Guideliness for Congenital Hypothyroidism (9). Date of birth, date of admission, postnatal age, gender, gestational age in weeks, type of delivery and birth weight were recorded in all cases included in the study. Number, dates and results of heel prick tests were recorded. All cases were investigated for family and maternal history of thyroid disease, including a family history of CH. Consanguinity between parents, presence of iodine exposure in the mother and the baby and the iodine content of the salt used during pregnancy were recorded.

Body weight, height and head circumference were measured on admission. All patients were examined for clinical signs and symptoms associated with CH which included: lethargy, inactivity, hypotonia, difficulty in feeding, excessive sleepiness, constipation, abdominal distention, umbilical hernia, prolonged jaundice, galactorrhea, weak cry, hypothermia, cutis marmoratus, nasal congestion and dry and coarse skin. Physical examination was made for both additional congenital abnormalities and goiter. Serum thyroglobulin, TSH receptor binding antibody (TRB-Ab) and spot urinary iodine levels were measured in patients diagnosed with CH. The localization, volume and parenchymal echogenicity of the thyroid gland were evaluated by thyroid ultrasonography (USG). Thyroid gland localization and activity were evaluated by thyroid scintigraphy.

Serum TSH, free T_4 (fT_4), and free tri-iodothyronine (fT_3) concentrations were measured by chemiluminometric method using an Advia Centaur XP (Siemens Healthcare limited-Oakville) device. Thyroglobulin concentration was measured by immunoassay method on the Siemens Immulite 2000 analyzer device with the immulite 2000-thyroglobulin kit (Siemens Healthineers-United States). TRB-Ab level was measured on the Berthold 1B2111 device (Berthold, USA) by radioimmunassay with a Beckmann Coulter RRA Anti-R TSH kit (Beckman Coulter Company-Czech Republic). Spot urinary iodine level was measured on Agilent 7500 ICP-MS device (Agilent Technologies, USA) by using ICP-MS analysis technique.

Thyroid USG was performed by a radiologist using a 7.5-MHz linear probe with a Toshiba Aplio 500 US device (Toshiba Medical Systems Co. Ltd, Otawara, Japan). Thyroid volume was calculated for each lobe using the following formula: (D1xD2xD3/1000) x 0.523 where D1 is the longest longitudinal, D2 is the anteroposterior and D3 is the largest transverse diameters, in cm, for each lobe and the total volume was calculated as the sum of two lobes in mL. Thyroid volumes for the neonatal period with a value of less than 0.64 mL (10th percentile) in patients were considered to constitute hypoplasia, those with a volume greater than the 95th percentile value of 1.15 mL were considered to constitute hyperplasia and total volumes between 0.64 mL and 1.15 mL were considered normal (10). Thyroid scintigraphy was performed in the Nuclear Medicine

Department of our hospital using General Electric Millenium device (General Electric; Elgems, Tirat Carmel, Israel) and gamma camera with technetium 99 pertechnetate.

Statistical Analysis

In the presentation of descriptive statistics; the data obtained by measurement were expressed as mean ± standard deviation and (minimum-maximum) and categorical data as number (percentage). Cross-table analyzes, Pearson and Fisher's Exact chi-square tests were used to compare the qualitative characteristics of the groups. Compatibility to normal distribution of the numerical measurements in the groups was examined by independent groups for those with normal distribution in numerical measurements and with Mann-Whitney U test for those without normal distribution. IBM Statistical Package for the Social Science (SPSS; IBM Inc., Chicago, IL., USA) version 22 was used for all statistical analyzes. A p value of <0.05 was considered statistically significant.

Results

Of the 109 newborns referred from the neonatal screening program a total of 60 (55%) infants were diagnosed; 52 (47.7%) at initial evaluation and eight (7.3%) at follow up. Twenty nine (48.3%) of patients with elevated neonatal TSH were female and female to male ratio was 1:1.07. Gender, gestational age, term/preterm rate, type of delivery and birth weight were similar in patients with elevated neonatal TSH compared to babies referred but who were not diagnosed with elevated neonatal TSH (non-CH group) (Table 1). Parental consanguinity rate was significantly higher ($p=0.004$) at 31.7% (19/52) in patients with elevated neonatal TSH compared to 8.16% (4/49) in the non-elevated neonatal TSH group. The family history of thyroid disease, family history of CH, the rate of iodized salt consumption and iodine exposure were similar in patients with elevated neonatal TSH compared to the non-elevated neonatal TSH group (Table 2).

One hundred patients (91.74%) required a second heel prick test. The mean number of heel prick test was

Table 1. Clinical features of all referrals

	Total (n = 109)	CH + (n = 60)	CH- (n = 49)	CH + /CH- p value
Gender				
Female	49 (45%)	29 (48.3%)	20 (40.8%)	0.433
Male	60 (55%)	31 (51.7%)	29 (59.2%)	
Birth week	38.07 ± 2.30	38.3 ± 1.65	37.6 ± 2.88	0.215
Maturity				
Term	92 (84.4%)	51 (85%)	41 (83.7%)	0.850
Preterm	17 (15.6%)	9 (15%)	8 (16.3%)	
Type of delivery				
Vaginal	53 (48.6%)	30 (50%)	23 (46%)	0.750
C/S	56 (51.4%)	30 (5%)	26 (54%)	
Birth weight (gram)	3042.06 ± 593.2	3064.3 ± 510.47	3014 ± 685	0.751

Mean ± standard deviation or %'s are given.
CH: congenital hypothyroidism, C/S: cesarean

Table 2. Demographic characteristics of congenital hypothyroidism (CH) group and non-CH group

	CH + (n = 60)	CH- (n = 49)	CH + /CH- p value
Consanguinity	19 (31.7%)	4 (8.16%)	0.004
Thyroid disease in family	26 (43.3%)	22 (44.8%)	0.870
CH in family	7 (11.22%)	5 (10.20%)	1.00
Iodized salt consumption in pregnancy	50 (83.3%)	34 (69.4%)	0.135
Iodine exposure	11 (15.6%)	6 (12.2%)	
Umblical care	10 (90.9%)	5 (83.3%)	0.544
Other	1 (9.1%)	1 (16.7%)	

Mean ± standard deviation or %'s are given.
CH: congenital hypothyroidism

similar in patients with elevated neonatal TSH and the non-elevated neonatal TSH group. For all referrals the first heel prick time was 1.97 ± 1.58 days, the second heel prick time was 8.5 ± 4.62 days and the third heel prick time was 15.9 ± 5.43 days. The mean age at diagnosis in the elevated neonatal TSH group was 30.2 ± 24.8 days; 22.13 ± 10.35 days in 52 (86.7%) patients diagnosed as a result of initial evaluation and 82.62 ± 28.53 days in eight (13.3%) patients diagnosed at follow up. Of the patients diagnosed on initial evaluation, two (4%) patients were diagnosed within the first seven days, three (6%) patients between eight and 14 days, 40 (76%) patients between 15 and 28 days and seven (13%) patients later than 28 days. Thus 87% of patients with elevated neonatal TSH were diagnosed and treatment initiated in the first month. Duration between the second heel prick time and the diagnosis was 13.98 ± 9.97 days.

Clinical findings which are associated with hypothyroidism were detected in 19 (36%) of 52 infants diagnosed as a result of initial evaluation. There was lethargy in two (10.5%) patients, feeding difficulty in three (16.78%), constipation in six (31.5%), constipation and umblical hernia in one (5.26%), prolonged jaundice in six (31.5%) and weak cry in one (5.26%) patient. None of the patients diagnosed at follow up exhibited signs or symptoms associated with CH. Goiter was detected on physical examination in two patients diagnosed as a result of initial evaluation. There were concomitant abnormalities in 11 (18.2%) of all CH cases: four atrial septal defect (ASD), one PS, one hydrocephaly and meningomyelocele, two developmental dysplasia of hip and three patients had Down syndrome). The babies with Down syndrome all had cardiac abnormalities commonly associated with this condition; ASD, patent ductus arteriosus and ventricular septal defect.

Body weight, height and head circumference were similar on admission in patient diagnosed with elevated neonatal TSH and non-elevated neonatal TSH referrals. The mean TSH level was 79.5 (2-150) μIU/mL, mean fT_4 level was 0.76 (0.12-1.68) ng/dL and mean fT_3 level was 3.36 (0.36-5.01) pg/mL in patients diagnosed with elevated neonatal TSH. The fT_4 level was low in 33 (55%) patients (< 0.9 ng/dL) and normal in 27 (45%) patients (> 0.9 ng/dL) on admission (Table 3).

Urinary iodine levels were measured in 29 patients and thyroglobulin concentration in 35 patients diagnosed as a result of initial evaluation. Urinary iodine level was normal in nine (31%) patients and high in 20 (69%) patient; thyroglobulin level was normal in five (14.3%) patients and high in 30 (85.7%) patients. TRB-Ab level was measured in 36 patients and it was negative in 32 (88.8%) patients, borderline positive in two (5.6%) patients and positive in two (5.6%) patients. Only one of the two patients who were TRB-Ab positive had autoimmune thyroid disease in maternal history.

Thyroid USG was performed in 49 (81.7%) infants diagnosed with elevated neonatal TSH at initial evaluation. The mean thyroid gland volume was 1.06 ± 0.95 (0-3.74) mL. In three (6.12%) cases, the thyroid gland was not visualized in the normal location. One of these patients found to have sublingual ectopic thyroid gland and one of these patients found to have agenesis on thyroid scintigraphy performed before replacement treatment. Thyroid scintigraphy was not performed in the third baby but the thyroglobulin value was normal (16 ng/mL) in this case, so it was thought that there might be an inactivating mutation at TSH receptor. There was hypoplasia in 14 (29.16%) patients, normal gland in 16 (33.3%) patients and hyperplasia in 16 (33.3%) patients. Ectopic thyroid

Table 3. Postnatal age, anthropometric measurements and laboratory findings in all referrals at first assessment

	Total (n = 109)	CH + (n = 60)	CH- (n = 49)	CH + /CH- p value
Postnatal age (day)	27.47 ± 14.2	23.9 ± 13.01	31.8 ± 14.4	< 0.001
Weight (gr)	3960 ± 590	3850 ± 800	4100 ± 920	0.058
Height (cm)	52.1 ± 2.42	51.5 ± 1.99	52.9 ± 2.68	0.052
Head circumference (cm)	36.3 ± 1.87	35.9 ± 1.66	36.9 ± 1.99	0.053
TSH (μIU/mL)	42.2 ± 54.3	71.05 ± 59.2 79.5 (2-150)	6.95 ± 4.49 6.34 (1.53-27.8)	< 0.001
fT_4 (ng/dL)	1.04 ± 0.37	0.83 ± 0.35	1.30 ± 0.17	
Low	33 (30.2%)	33 (55%)	-	< 0.001
Normal	27 (69.8%)	27 (45%)	49 (100%)	
fT3 (pg/mL)	3.79 ± 0.93	3.44 ± 1.03	4.2 ± 0.56	0.001

Mean ± standard deviation or %'s are given. Only for TSH median and range is given as well.

TSH: thyroid stimulating hormone, CH: congenital hypothyroidism, fT_4: free thyroxine, fT_3: free tri-iodothyronine

gland rate was found to be 2.08% (1/48), agenesis rate was 2.08% (1/48), hypoplasia rate was 29.1% (14/48) and total of thyroid dysgenesis rate was found to be 33.3% (16/48). Sixteen patients (33.3%) with hyperplastic thyroid gland were diagnosed as dyshormonogenesis, 16 patients (33.3%) with normal thyroid gland were diagnosed as transient hypothyroidism and/or possible dyshormonogenesis. The time of diagnosis, consanguinity rate, TSH, fT_4, thyroglobulin and spot urinary iodine levels were similar in these three groups (Table 4).

Levothroxine was started at a mean dose of 9.34 (2.10-15.0) µg/kg/d in patients diagnosed with elevated neonatal TSH as a result of initial evaluation. TSH level normalization time after the treatment was 11.02 (4-30) days which equates to a postnatal age of 33.83 (13-70) days. Normalization of fT_4 in those patients with low fT_4 at diagnosis was a little faster at 9.03 (3-30) days after treatment initiation and by the postanatal age of 31.4 (19-56) days.

Discussion

In our study, only 60 (55%) of 109 cases referred from the national screening program were diagnosed with elevated neonatal TSH. In a study conducted in our country, it was reported that 114 (44.5%) of 256 cases referred from national screening program were diagnosed with CH (11). In our study, female to male ratio was calculated as 1/1.07 in patients with elevated neonatal TSH. While previous studies have shown that female preponderance in female to male ratio in patients with CH was 1.8/1, 1.4/1 (12), it has been pointed out that the dominance of gender in recent years has shifted to male direction as 1:1.14, 1/1.16 (8,13). Dysgenesis is more common in female sex, whereas dyshormonogenesis is much lower in girls (13). In our study, low rate of cases diagnosed with dysgenesis and milder cases due to lowering TSH screening cut-offs may be the cause of variability in gender distribution.

On admission to hospital, the mean age of the cases was found 27.47 ± 14.2 (7-70) days in our study and 24.54 ± 13.46 (4-168) days in the study of Kor and Kor (8). The mean age at diagnosis was 30.2 ± 24.8 days in all CH cases; 22.13 ± 10.35 in patients diagnosed as a result of initial evaluation and 82.62 ± 28.53 days in patients diagnosed at follow up in our study. Kor and Kor (8) reported in their study that 223 (96%) of all 233 patients were diagnosed as a result of initial evaluation and the mean age of diagnosis was 19.87 ± 7.63 (4-51); 10 (4%) of patients were diagnosed at follow up and the mean age of diagnosis was 43.71 ± 14.02 (29-65) days. The mean age of diagnosis was found as 19.7 ± 8.30 (5-60) days in the study of Peltek Kendirci et al (11), 38.1 ± 58 (4-342) days in the study of Kuşdal et al (14) and 23 ± 14 days in the study of Simsek et al (15). Early diagnosis is the most important aim of the screening program. In Turkey, in a study comparing the pre-screening and post-screening diagnosis time, prior to the introduction of screening diagnosis occurred at a mean of 292 days whereas it decreased to 35.2 days after screening was introduced (16). Bongers-Schokking et al (17) reported that there was no neurodevelopmental difference in the patients with CH whose treatment was initiated with appropriate dose in the first 13 days compared to a healthy group. In our country, despite the screening program having been implemented for 12 years, diagnosis is still occuring later than in other national programmes, although there is marked variability from center to center. This is still a major improvement over the diagnosis times reported prior to the introduction of screening in Turkey. However, there is the delay between second heel prick test and first clinic visit is nearly 19 days. Further analysis of the causes of delay between referral and initial diagnostics visits would enable some remedial organisation to be put in place.

The mean TSH level was 54.8 (7.81-150) µIU/mL in patients diagnosed as a result of initial evaluation and 11.23 (2.02-12.9) µIU/mL in patients diagnosed at follow up in our study.

Table 4. Comparison of three groups with dysgenesis, dyshormonogenesis and transient hypothyroidism or possible dyshormonogenesis

	Dysgenesis	Normal Possible dyshormonogenesis or transient hypothyroidism	Hiperplasia Dyshormonogenesis	p
The time of diagnosis (day)	18.88 ± 10.33	25.25 ± 12.54	22.75 ± 8.1	0.103
Consanguinity rate	%41	%25	%25	0.53
TSH (µIU/mL)	78.83 ± 60.1	75.44 ± 58.3	95.40 ± 59.67	0.78
fT_4 (ng/dL)	0.82 ± 0.28	0.79 ± 0.35	0.61 ± 0.31	0.22
Thyroglobulin (ng/mL)	203.47 ± 120	227.1 ± 105	293 ± 21	0.284
Spot urinary iodine (µg/L)	5034.6 ± 47	4337 ± 42	2121 ± 2229	0.486

TSH: thyroid stimulating hormone, fT_4: free thyroxine

The mean TSH level was found as 55.2 ± 33.85 μIU/mL in the study of Peltek Kendirci et al (11), 15.8 ± 28.69 μIU/mL in the study of Kor and Kor (8). Variability in individual TSH levels may be the cause.

In our study, the rate of presence of symptoms was 36.5% in patients with elevated neonatal TSH and the most common symptoms were constipation and prolonged jaundice. The rate of presence of concomitant congenital abnormality in patients with elevated neonatal TSH was found to be 18.3% (11/60). The most common abnormalities were cardiac malformations detected in seven cases (11.7%). Three patients (5%) were diagnosed with Down syndrome (diagnosed with CH subsequently). In the study of Razavi et al (18), the rate of accompanying abnormality was found to be 19.1% (30/157) which is similar to the rate we report. Razavi et al (18) also reported 4.6% of abnormalities being cardiac malformations and the rate of Down syndrome was 7.6% (12/157).

In our study, 16 patients (33.3%) with elevated neonatal TSH were diagnosed as dysgenesis. Hyperplasia was detected in 16 (33.3%) patients who were assigned a diagnosis of dyshormonogenesis. The majority of cases with normal thyroid gland will be diagnosed with transient hypothyroidism but some of them may later be diagnosed with thyroid dyshormonogenesis, so that it was concluded that the exact ratio can only be given when the patients are old enough to undertake a trial off T_4 replacement therapy. Kor and Kor (8) reported that the etiological distrubition of CH was evaluated by thyroid USG and thyroid dysgenesis rate was found to be 28.6% of patients (24.5% hypoplasia, 4.1% agenesis), 71% of patients were found to be normal thyroid gland and 0.4% of patients were found to have hyperplasia. In the study of Kuşdal et al (14), 28.2% of patients had thyroid dysgenesis (10.2% agenesis, 10.2% hypoplasia, 5.2% ectopia, 2.6% hemiagenesis) and all the remaining cases had normal thyroid gland by USG evaluation (14). Özgelen et al (13) reported that the rate of dysgenesis was found to be 83.7% of the patients with CH (51.7% hypoplasia, 21.7% agenesis, 10.3% ectopia) and rate of dyshormonogenesis was found to be 10.3% in patients; etiological distribution in this study was made only among the cases with permanent CH and both USG and scintigraphy were used for etiological evaluation. Similarly, in the study of Bezen et al (19), etiological distribution was evaluated among the cases with permanent CH; rate of dysgenesis was found to be 52.2% (34.8% hypoplasia, 17.4% ectopia) and rate of dyshormonogenesis was found to be 47.8% in patients. Although there are many studies which have reported that the rate of dysgenesis is high (20,21), there has been an increase in the rate of dyshormonegenesis in recent studies in accord with our findings (19,22). An Italian national study conducted by Olivieri et al (12) reported the rate of dysgenesis in CH cases was 82% between years 1987 and 1998 although it decreased significantly compared to the previous period with a rate of 58% between years 1999 and 2006 due to significant chnages in the screening protocols used in Italy and, the authors speculate, the increased survival of many more premature infants. In the second period of the study, it was stated that the rate of detection of normal and hyperplastic thyroid gland increased compared to the first period, which again may be due to the changes in the Italian screning system and the greater proportion of unwell premature infants. In addition, it was noted that the rate of dyshormonogenesis was significantly higher in patients who had parental consanguinity (23). The consanguinity rate in our study was higher in patients with elevated neonatal TSH but there was no difference in consanguinity rate according to the etiology of elevated neonatal TSH In the group with dysgenesis, while consanguinity rate was 41.2%, it was 50% in the non-dysgenesis group (25% of whom had normal thyroid gland and 25% with hyperplasia). However, we think that the exact effect of consangunity in the etiological distribution of elevated neonatal TSH may be resolved by the evaluation of patients with permanent CH at follow up.

Study Limitations

Thyroglobulin and urine iodine levels were not evaluated at diagnosis in all patients with elevated neonatal TSH. Imaging was not performed in all patients with elevated neonatal TSH.

Conclusion

The rate of elevated neonatal TSH in the first month was 87%. The mean time of initiation of treatment was 22 (7-53) days. Dysgenesis rate was 33.3% and dyshormonogenesis rate was the same at 33.3%. The majority of cases with normal thyroid gland will be diagnosed with transient hypothyroidism but some of them may be diagnosed with thyroid dyshormonogenesis.

Ethics

Ethics Committee Approval: The ethics committee approval of this study was received on 29.09.2015 from Zekai Tahir Burak Women's Health, Education and Research Hospital Clinical Research Ethics Committee (45/2015).

Informed Consent: Written consent was obtained from patients's parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Zeynep Donbaloğlu, Şenay Savaş-Erdeve, Concept: Şenay Savaş-Erdeve, Semra Çetinkaya, Zehra Aycan, Design: Şenay Savaş-Erdeve, Zeynep Donbaloğlu, Data Collection or Processing: Zeynep Donbaloğlu, Şenay Savaş-Erdeve, Analysis or Interpretation: Zeynep Donbaloğlu, Şenay Savaş-Erdeve, Semra Çetinkaya, Zehra Aycan, Literature Search: Zeynep Donbaloğlu, Şenay Savaş-Erdeve, Writing: Zeynep Donbaloğlu, Şenay Savaş-Erdeve.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2018.0157

J Clin Res Pediatr Endocrinol 2019;11(3):247-252

Risk Factors for Childhood Overweight and Obesity in Ukraine and Germany

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What is already known on this topic?

Obesity in children and adolescents has become an increasing and widely-distributed health problem, especially in industrialized countries. Risk factors include both genetic predisposition and socioeconomic factors. Only a few studies have compared the distribution of socioeconomic risk factors for childhood obesity across different countries.

What this study adds?

The current study is the first one to analyze the distribution of risk factors for obesity in Ukraine and Germany. Similar risk factors for obesity were observed in both countries although the prevalence of these risk factors varied between the two populations.

Abstract

Objective: The prevalence of overweight and obesity in childhood and adolescence are rapidly increasing and influenced by genetic, familial, environmental, socioeconomic and cultural factors. The aim of the study was to compare risk factors for childhood obesity in Ukraine (UA) and Germany (DE) using comparable investigative tools.
Methods: Two groups of children, aged 8 to 18 years, from DE (93 children) and UA (95 children) were divided into overweight and obese groups. Anthropometric data and detailed medical history were collected.
Results: Risk factors in pregnancy (prematurity, weight gain > 20 kg, early contractions) were equally frequent in both groups. Positive correlations of body mass index (BMI)-standard deviation score (SDS) between children and mothers were noted. The proportion of family members with diabetes mellitus was lower in the UA group. Obesity was more frequent at one year of age in DE children. The DE group also became overweight at an earlier age and remained overweight over a longer period of time compared to UA. The mean BMI-SDS of obese children was lower in the UA group. In both groups waist circumference to height ratio was > 0.5, indicating presence of a cardiometabolic risk factor. About half of the patients in both groups had blood pressure values exceeding the 95th percentile.
Conclusion: Similar risk factors for obesity were observed among two groups of children in UA and DE. Differences were observed regarding the prevalence of specific risk factors for childhood obesity. Population-specific distribution of risk factors needs to be considered in order to optimize prevention and treatment strategies.
Keywords: Obesity, risk factors, childhood, adolescent, overweight

Introduction

Obesity in children and adolescents has become an increasing and widespread health problem, especially in industrialized countries. According to the World Health Organization (WHO) more than 41 million children under the age of five suffered from overweight or obesity in 2016 (1). In Germany (DE), according to two large national studies (KIGGS and Crescnet), 15-17% of children aged three to 17 years were overweight and 6.2-7.6% were obese (2). In Ukraine (UA), the incidence of childhood obesity until

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Conflict of interest: None declared
Received: 17.06.2018
Accepted: 05.01.2019

recently was much lower but has shown a significant rise over the last decade, increasing from 0.083% among age groups 0-18 years in 2003, to 1.23% in 2009 and 1.34 in 2016 (3,4).

Numerous risk factors for childhood obesity have been discussed in the literature. Parental overweight is considered to be a particularly important indicator for overweight and obesity among their children (5). Further risk factors include characteristics of the maternal medical history during pregnancy and the perinatal period. Thus, excessive maternal weight gain during pregnancy and maternal smoking during pregnancy contribute to the development of obesity and its incidence (6). In addition, high birth weight constitutes a significant risk factor for the development of childhood obesity (7). Moreover, children with rapid weight gain during the first four to six months of life have been shown to have an increased risk of developing obesity by the age of seven years. Breastfeeding over a period of at least six months reduces the risk of obesity by 40% (8). Children who have become overweight by the age of six years often remain overweight at age 14 years (9).

Since obviously both genetic and socioeconomic factors have an influence on the development of childhood obesity, we investigated similarities and differences regarding these risk factors in two cohorts from two different European countries (UA and DE).

Methods

The study was conducted at two university children hospital outpatient centers, in Simferopol, UA (2010-2011) and in Heidelberg, DE (2012-2013). At both centers WHO standards were used for definition of overweight and obesity. Inclusion criteria were: age 8-18 years, BMI 85^{th}-95^{th} percentile (overweight) and $>95^{th}$ percentile (obesity), informed consent. Exclusion criteria were: age <8 years or >18 years, chronic endocrinopathies (diabetes mellitus ect.), genetic disorders, disabled children, chronic inflammatory diseases (e.g. M. Crohn), lack of informed consent. Inclusion and exclusion criteria were identical. Comparable questionnaires for risk factors and for both patient and family medical history were used at both centers.

The study population in the UA consisted of 95 (35 girls and 60 boys) otherwise healthy children aged 10 to 18 [mean ± standard deviation (SD) = 13.5 ± 0.4] years, divided into an overweight (36 children) and an obesity (59 children) group. The study population in DE consisted of 93 (46 girl, 47 boys) otherwise healthy children aged 8 to 18 years (mean ± SD = 12.5 ± 2.9) years divided into an overweight (24 children) and an obesity (69 children) group.

The populations were comparable according to sex, Tanner stages and age and did not show any statistically significant difference. Physical examination in outpatient departments included measurement of height, weight (in underwear), waist and hip circumference, blood pressure and examination of the skin for acanthosis nigricans. BMI-SDS, waist/hip circumference ratio (WHR), and waist circumference/height ratio (WHtR) were calculated. Standardized patient history included pregnancy history (maternal obesity, weight gain of more than 20 kg, arterial hypertension, premature (before 37^{th} gestational week) or postmature (after 42^{nd} gestational week) delivery, perinatal asphyxia, duration of breastfeeding and family history (overweight or obesity, diabetes mellitus, arterial hypertension in first degree relatives). All risk factors were recorded with regard to their presence or absence. The children's history of weight gain was also evaluated (reported by parents and according to medical reports).

The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg (approval number: S-337/2013, approval date: 22/07/2013). Written consent was taken from the parents at the beginning of the study in accordance with the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS), version 20.0.0 for Windows (SPSS Inc., Chicago, IL., USA). As part of the descriptive analysis, sample size, arithmetic mean, median, maximum (max) and minimum (min), and SD were determined. To test the variables for normality of the distribution, the Kolmogorov-Smirnov test with an error probability of 0.05 was used. The t-test and the Mann-Whitney U test were used to test for significant differences between groups. Gender differences in categorical variables were tested with the chi-square test. A statistical parameter of $p<0.05$ was considered significant.

Results

The risk factors during pregnancy occurred with an approximately equal frequency among the DE and UA populations (Table 1). Premature birth was reported in 8-12% of all children. In the DE population 21% of mothers of overweight children and 33% of mothers of obese children gained more than 20 kg during pregnancy. In the UA population the incidence of excessive weight gain during pregnancy among mothers of obese children was 25%. Early contractions were reported by 13-17% (DE) and 20-28% (UA) of mothers, respectively (Table 1).

The prevalence of obesity was significantly higher in first-degree relatives of obese children compared to relatives of overweight children ($p < 0.05$). A highly significant positive correlation was found between the children's and the mothers' BMI-SDS (1.03 ± 1.26 for the overweight and 1.96 ± 1.20 for the obese group) in the DE population ($r = 0.46$, $p < 0.0001$).

Birth weight (3295 ± 474 g in UA population, 3352 ± 517 g in DE population) and birth length (50.6 ± 2.4 cm in UA population, 51.1 ± 2.1 in DE population) as well as their relation to BMI-SDS did not differ among the two groups. In both populations, obese children were more frequently obese at the age of one year (33.3% in UA population, 27.3% in DE population) compared to overweight children (14.3% in UA population, 17.4% in DE population). On average, the children in the UA population were breastfed for a longer period of time (6.8 ± 6.7 months in the obese, 7.1 ± 7.2 months in the overweight group) compared to the children in the DE population (4.6 ± 6.2 months in the obese, 6.1 ± 7.3 months in the overweight group). The DE population became overweight significantly earlier and therefore remained overweight over a significantly longer period. The mean duration of obesity in the DE population was 7.6 ± 4.3 years (min 1.2, max 18.0), the mean duration of overweight 7.2 ± 5.0 years (min 1.1, max 18.0) compared to 5.7 ± 3.5 years (min 1.0, max 13.7) and 4.71 ± 3.5 years (min 0.8, max 14.0), respectively in the UA population. In both populations, the duration of the overweight period significantly influenced the BMI-SDS. The mean BMI-SDS of obese children was lower in the UA population than in those in the DE population [BMI-SDS (UA) 2.31 ± 0.49 *vs* BMI-SDS (DE) 2.52 ± 0.55 ($p < 0.05$)]. There were no significant differences in BMI-SDS in overweight children. In both populations, the BMI-SDS was significantly influenced by the Tanner stage ($p < 0.05$) (Table 2).

There were significant differences between the DE and UA populations regarding WHR and WHtR ($p < 0.05$)

Table 1. Prenatal and familial risk factors in the overweight and obese groups

	DE ($n_{total} = 93$)		UA ($n_{total} = 95$)	
Risk factors	**n**	**%**	**n**	**%**
Overweight group				
During pregnancy				
Maternal obesity	5	21%	1	3%
Arterial hypertension	2	8%	1	3%
Early contractions	4	17%	10	28%
Gestational age < 37 weeks	2	8%	4	11%
Perinatal asphyxia	2	8%	1	3%
Family history				
Diabetes mellitus	14	58%	6	17%
Arterial hypertension	12	50%	14	39%
Obesity	14	58%	14	39%
Obese group				
During pregnancy				
Maternal obesity	22	33%	15	25%
Arterial hypertension	11	17%	4	7%
Early contractions	9	13%	12	20%
Gestational age < 37 weeks	7	10%	7	12%
Perinatal asphyxia	5	8%	2	3%
Family history				
Diabetes mellitus	47	68%	17	29%
Arterial hypertension	47	68%	28	47%
Obesity	55	80%	37	63%

Statistical analyses were not suitable in some parameter, due to small mumbers, and therefore this table is presented in a descriptive manner only.

DE: Germany, UA: Ukraine

Table 2. Anthropometric data

		DE-Group		UA-Group	
		Mean	**SD**	**Mean**	**SD**
BMI-SDS	Overweight	1.44	0.20	1.42	0.19
	Obese	2.52*	0.55	2.35*	0.49
Tanner 1-2	Overweight	1.41	0.21	1.52	0.11
	Obese	2.32**	0.27	2.51**	0.49
Tanner 3-5	Overweight	1.47	0.19	1.35	0.19
	Obese	2.62**	0.63	2.19**	0.45

*$p < 0.05$ between DE and UA.

**$p < 0.05$ between Tanner stages.

DE: Germany, UA: Ukraine, BMI-SDS: body mass index-standard deviation score, SD: standard deviation

Table 3. Risk factors for metabolic syndrome

		DE-Group		UA-Group	
Prevalence		**Mean**	**SD**	**Mean**	**SD**
Waist/hip circumference ratio	Overweight	1.09*	±0.13	0.86*	±0.09
	Obese	1.11*	±0.10	0.84*	±0.08
Waist circumference/ height ratio	Overweight	0.51	±0.04	0.48	±0.03
	Obese	0.58	±0.07	0.55	±0.05
		n	**%**	**n**	**%**
Blood pressure > 95th percentile	Obese	37	54%	26	22%
	Overweight	8	33%	12	33%
Acanthosis nigricans	Obese	36	52%	13	22%
	Overweight	2	8%	5	14%

*$p < 0.05$ between DE and UA.

DE: Germany, UA: Ukraine, SD: standard deviation

(Table 3). The children in the UA population showed no significant differences in the WHR according to sex or age compared to the DE population. In the DE population the WHtR was significantly influenced by gender ($p < 0.05$). In both populations children showed central trunk obesity (predominantly the girls in the UA population). In obese children the WHtR exceeded 0.5. In the DE population 54% of obese patients had blood pressure values above the 95th percentile compared to 22% in the UA population (Table 3). Acanthosis nigricans was observed twice as often among patients in the DE population. In both populations, acanthosis nigricans was observed significantly more frequently in obese patients compared to the overweight patients (Table 3).

Discussion

The greater the frequency of risk factors identified in mothers during pregnancy, the greater was the likelihood that the child's BMI-SDS would be increased (10). Mothers with a normal BMI usually gain 11 to 16 kg during pregnancy (11). In the present study we found in both countries that an excessive weight gain of mothers during pregnancy was associated with the risk for childhood obesity (12,13).

The prevalence of birth before 37 or after 42 gestational weeks in the DE study population was within the expected range (14). These figures showed a somewhat higher prevalence in the UA group. Gestational hypertension was found to occur in 5-10% of all pregnancies (15). The same prevalence was observed in the mothers of overweight children in our study. By contrast, the mothers of children with obesity were twice as likely to have developed gestational hypertension (17%). However, this finding was limited to the DE population. There are no exact data on the prevalence of early contractions with estimates ranging from 5% up to 35% of pregnancies (16). If early contractions are considered as a potential threat of premature birth, the increased incidence may be considered as a relevant risk factor for childhood overweight and obesity. Perinatal asphyxia may be equally considered a risk factor. In our patients, the prevalence of perinatal asphyxia both in the UA and in the DE populations exceeded the prevalence of 0.5-1% (5-10:1000 births) observed in the general population (17).

Several studies have shown that the BMI-SDS of the parents plays an important role influencing the BMI-SDS of a child (18). In the current study, maternal BMI-SDS was significantly higher in obese children as compared to the values of mothers of overweight children. A highly significant positive correlation was found between the BMI-SDS of the children and the BMI-SDS of their parents. There was a significant positive correlation between the number of familial risk factors (diabetes mellitus and arterial hypertension) and the BMI-SDS values of the children .

The prevalence of arterial hypertension ranges from 32.3% in developed to 40.8% in developing countries (19). The present study indicates that first degree relatives of obese children have a higher prevalence of arterial hypertension and furthermore have a significantly higher prevalence of diabetes mellitus. According to the atlas of the International Diabetes Federation, the prevalence of diabetes in DE was 10.6% in 2015, with a proportion of undetected diabetes of 38.2%. In UA during the same year, the respective numbers were 8% and 43.2% (20). We suspect that in the UA population there may be a greater deficit in the diagnosis of DM and arterial hypertension in adults, and that the actual frequency is most likely significantly higher. This suspicion is supported by the increased incidence of obesity in families in the UA population.

Currently there are no studies showing the prevalence of obesity in one-year-old children. In the US, the prevalence among children aged 0-2 years was reported as high as 8.1% (21). In our study, the prevalence of overweight and obesity at the age of one year was much higher with 17-27% in the DE population and 14-44% for children in UA. The results support the potential importance of BMI in children under two years of age to identify an increased risk of later obesity (22). Furthermore, breastfeeding plays an important role in the prevention of obesity. Studies have shown that formula-fed infants have a higher chance of becoming obese later in life compared to breastfed infants (23).

So far only a small number of studies have investigated the onset of obesity. Most of these studies have identified the preschool age of 5-7 years as a risk period (24). In our study, children and parents reported significant weight gain starting from the age of 5-6 years in the DE population and 7-10 years in the UA population. This information might be important to identify the right timing for intervention, investigation and prevention. The increase in BMI-SDS is influenced by the duration of overweight and/or obesity in childhood. Therefore, initiating intervention and therapy as early as possible is important. The children of the UA population had lower BMI-SDS compared to the DE population. To our knowledge this is the first study comparing these two countries.

The data on which parameter is the better one to describe abdominal fat distribution in children is controversial. While American sources showed that BMI and WHtR did not differ

in identifying children with cardiovascular risk factors, other studies found that waist circumference and WHtR were better predictors of cardiovascular risks compared to BMI (25). Recent research has shown that WHR may not be an informative parameter for cardiometabolic risk. On the other hand, WHtR is associated with cardiometabolic risk compared to BMI-SDS in both adults and children. In both populations children showed trunkal obesity. In German children aged 12-18 years WHR was reported as 0.83 ± 0.05 in boys and as 0.78 ± 0.06 in girls (26).

The normal value for WHtR has been reported to be below 0.5 (27). In our study, WHtR was >0.5 in children with obesity from the UA population and in children with overweight and with obesity from the DE population which may indirectly be interpreted as a cardiometabolic risk factor.

The prevalence of high blood pressure in obesity varies: from 21-35%, up to 46% in children and 40% in adults (28). According to other studies the prevalence of hypertension in overweight children increased from 6.6% in boys aged 2-5 years to 13.3% in adolescents 16-19 years; and in girls 4.4% and 16.3% respectively (29). In our study, we found a prevalence of 44.1% in the UA obese group while in the DE group the prevalence of hypertension was even higher, at 56.1%.

Acanthosis nigricans is associated with diabetes mellitus type 2 and insulin resistance and correlates strongly with obesity, although it has been reported to be present in 17% of healthy children (30). Acanthosis nigricans was observed twice as often in the DE patients compared to the UA population with a moderately higher prevalence compared to healthy children. In both populations, acanthosis nigricans was significantly more common in obese patients than in patients who were overweight.

Study Limitations

The main limitation of our study is that we relied on self-reports of the parents for pregnancy history and history of weight gain of their children.

Conclusion

The anamnestic risk factors for overweight and obesity in children were very similar in the DE and UA subjects, except for the number of familial risk factors which did not correlate with the BMI-SDS values in the UA population. We assume that in the UA population there is a greater deficit in the diagnosis of diabetes mellitus and arterial hypertension in adults and that a substantial fraction of adult cases of diabetes and arterial hypertension remain undiagnosed in the UA. The actual prevalence of these conditions is likely to be significantly higher. Relevant risk factors for the development of obesity include family and pregnancy history, as well as neonatal and infant medical history. Further important risk factors include anthropometric parameters. Since BMI normal values are age-dependend and increase during adolescents, BMI-SDS should be used for the evaluation of the degree of childhood obesity. WHtR, blood pressure and presence of acanthosis nigricans are important prognostic indicators for the risk of obesity related diseases, and should be determined in children and adolescents with overweight/obesity. The children from the DE population became overweight significantly earlier compared to the UA population. DE patients with obesity also had higher BMI SDS.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg (approval number: S-337/2013, approval date: 22/07/2013).

Informed Consent: Written consent was taken from the parents at the beginning of the study in accordance with the Declaration of Helsinki.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2018.0277

J Clin Res Pediatr Endocrinol 2019;11(3):253-261

Evaluation of IGF1/IGFBP3 Molar Ratio as an Effective Tool for Assessing the Safety of Growth Hormone Therapy in Small-for-gestational-age, Growth Hormone-Deficient and Prader-Willi Children

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What is already known on this topic?

Growth hormone (GH) therapy is widely used, but concerns have been raised that it might increase cancer and cardiovascular risks.

What this study adds?

This study provides support for the use of IGF1/IGFBP3 molar ratio as a tool for assessing the safety of the therapeutic adaptation of GH therapy in children.

Abstract

Objective: IGF1 concentration is the most widely used parameter for the monitoring and therapeutic adaptation of recombinant human growth hormone (rGH) treatment. However, more than half the variation of the therapeutic response is accounted for by variability in the serum concentrations of IGF1 and IGFBP3. We therefore compared the use of IGF1/IGFBP3 molar ratio with that of IGF1 concentration alone.

Methods: We selected 92 children on rGH for this study and assigned them to three groups on the basis of growth deficiency etiology: small for gestational age (SGA), GH deficiency (GHD) and Prader-Willi syndrome (PWS). Plasma IGF1 and IGFBP3 concentrations and their molar ratio were determined.

Results: Before rGH treatment, mean IGF1/IGFBP3 molar ratio in the SGA, GHD and PWS groups was 0.14 ± 0.04, 0.07 ± 0.01 and 0.12 ± 0.02, respectively. After the initiation of rGH treatment, these averages were 0.19 ± 0.07, 0.20 ± 0.08 and 0.19 ± 0.09, within the normal range for most children, even at puberty and despite some significant increases in serum IGF1 levels.

Conclusion: We consider IGF1/IGFBP3 molar ratio to be a useful additional parameter for assessing therapeutic safety in patients on rGH, and for maintaning the values within the normal range for age and pubertal stage.

Keywords: GH therapy, IGF1/IGFBP3 molar ratio, growth hormone deficiency, small for gestational age, Prader-Willi syndrome

Introduction

IGF1 serum concentration remains the most widely used parameter for the monitoring and adjustment of recombinant human growth hormone (rGH) treatment (1). However, more than 58% of the variation in the therapeutic response to rGH over the first year of treatment in children can be explained by the variability of serum concentrations of IGF1 and IGFBP3 (2).

In current practice, due to technical difficulties,total IGF1 concentration is usually measured without determination of free IGF1 levels (2). Free IGF1 can be assumed as the

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Conflict of interest: None declared
Received: 28.11.2018
Accepted: 10.02.2019

bioactive form, but it is in equilibrium with bound IGF1 engaged in large and small complexes, according to the mass action law; almost 99% of the serum IGF1 is engaged in such large and small complexes (1).

It is therefore impossible to determine tissue bioavailability and, thus, therapeutic efficacy or safety of IGF1 from IGF1 or IGFBP3 assays alone. Furthermore, a lack of correlation between daily GH secretion and total IGF1 concentration has been reported in some cases (1). This situation may be due to the presence of various polymorphisms affecting sensitivity to GH, which may differ for IGF1 and IGFBP3. It should also be noted that IGF1 concentrations vary considerably among individuals (3,4).

Finally, the dynamics of serum concentrations of IGF1 and its carrier protein, IGFBP3, remain unclear in patients on rGH treatment, and conflicting results have often been obtained (5). Moreover, high IGF1 concentrations have been implicated in cancer, whereas IGFBP3 has a protective effect (6). We therefore believe that determining the bioavailability of IGF1 by calculating the IGF1/IGFBP3 molar ratio would provide more information about the safety of rGH treatment than the use of total IGF1 assays alone.

Methods

This retrospective study was performed at the Hormonology and Functional Endocrine Explorations Laboratory of Armand Trousseau Hospital in Paris. We selected, from our database, 92 children on rGH treatment followed at our outpatient clinics. These children were assigned to three groups on the basis of the etiology of their growth deficiency: 20 children who were small for gestational age (SGA group), 61 children with GH deficiency (GHD group), and 11 children with Prader-Willi syndrome (PWS group) (see Table 1A). Retrospective study of the patients' files covered the period 2011-2017 and the clinical and biological data under treatment corresponded to the period March 2016-March 2017. The duration of the treatments and the follow-up of the patients varied from two to seven years.

The doses of rGH administered were as follows: 34.96 ± 14.35 µg/kg/day for the SGA group, 25.56 ± 10.01 µg/kg/day for the GHD group and 21.67 ± 8.45 µg/kg/day for the PWS group. No adverse events attributable to rGH were reported in these children during follow-up.

Clinical [etiological diagnosis, height, weight, body mass index (BMI), rGH dose] and biological (IGF1, IGFBP3, insulin, fasting glycemia, lipid status, HbA1c) data were collected by consulting the patient's medical records. Clinical parameters (height, weight and BMI) were standardized relative to the corresponding national reference ranges (7). IGF1 and IGFBP3 are expressed as Z-standard deviation (SD) scores (SDS) adjusted for age, sex and pubertal stage. Baseline clinical and biological characteristics were obtained for each of the etiological groups (Table 1A). National ethics and confidentiality rules were respected, in accordance with the legislation in force for retrospective studies of cohort files.

The IGF1 and IGFBP3 assays were performed with the IDS-Isys system (Immunodiagnostic Systems, 153 Avenue d'Italie, 75013 Paris, France), in an automated procedure based on ELISA with detection by chemiluminescence. The IDS-Isys device was calibrated according to the new World Health Organization international standard for IGF1 NIBSC 02/254, in accordance with the recommendations of the Growth Hormone Research Society and the International IGF Research Society (8,9,10,11).

Serum IGF1 and IGFBP3 concentrations were interpreted by comparison with the reference intervals established specifically for this iSYS device by Bidlingmaier et al (10) for IGF1 and by Friedrich et al (11) for IGFBP3.

The IGF1/IGFBP3 molar ratio was calculated according to the formula as previously described (11,12): [IGF1 (ng/mL) x 0.13] / [IGFBP-3 (ng/mL) x 0.035]

The reference values used for the interpretation of IGF1/IGFBP3 molar ratios were based on the data collected in our pediatric functional endocrine exploration department at Trousseau Hospital (see Table 1B). These data come from a control population made up of children who came to consult for follow-up of unrelated pathologies: moderate well-

Table 1. A) Clinical and hormonal characteristics of the children

	SGA group	GHD group	PWS group
Clinical data			
Number of children	20	61	11
Age (years)	12.27 ± 3.50 (5.9-18.9)	12.02 ± 4.09 (1.4-18.9)	10.88 ± 5.23 (1.4-16.4)
Boys/girls	15/5	41/20	6/5
Weight (SDS)	-0.71 ± 2.52	0.83 ± 2.46	7.97 ± 3.20
Height (SDS)	-1.46 ± 0.87	-0.97 ± 1.16	-0.76 ± 1.53
BMI (SDS)	0.57 ± 2.44	0.58 ± 1.88	9.44 ± 2.87
Biological data			
IGF1/IGFBP3 ratio before rGH	0.14 ± 0.04	0.07 ± 0.01	0.12 ± 0.02
IGF1/IGFBP3 ratio after rGH	0.19 ± 0.07	0.20 ± 0.08	0.19 ± 0.09

SDS: standard deviation scores, rGH: recombinant human growth hormone, SGA: small for gestational age, GHD: growth hormone deficiency, PWS: Prader-Willi syndrome, BMI: body mass index

balanced asthma follow-up under inhaled corticosteroid, isolated hypospadias or testicular ectopy, moderate growth retardation of around -1 SDS and treated hypothyroid children stably euthyroid. These data were obtained from control children of different ages and pubertal stages and were comparable, as described by Friedrich et al (11), for IGF1/IGFBP3 molar ratio determinations.

The study design (retrospective analysis of the data) was approved by the Ethical Committee of Trousseau Hospital without an approval number. Informed consent has been obtained from the parents after full explanation of the purpose and nature of all procedures used.

Statistical Analysis

Prism 6 Software (GraphPad Software, La Jolla, CA, USA) was used for all statistical analyses. ANOVA was performed to compare quantitative and qualitative variables between groups. A paired t-test was performed to compare qualitative variables between groups. Differences were considered significant if the p value was < 0.05. A correlation analysis (Spearman) was carried out between the individual values of the three ratios and those of quantitative parameters.

Results

Before rGH treatment, the mean ± SD molar ratio was 0.14 ± 0.04 (range: 0.09-0.23) in the SGA group, 0.07 ± 0.01 (range: 0.06-0.08) in the GHD group and 0.12 ± 0.02 (range: 0.10-0.14) in the PWS group (Figure 1A).

The IGF1/IGFBP3 molar ratio values of the various groups in this study improved on rGH treatment, reaching values in the normal range for healthy children of the same age (Table 1A, 1B). Mean ± SD IGF1/IGFBP3 molar ratio on rGH treatment was 0.19 ± 0.07 (range: 0.12-0.35) for the SGA group, 0.20 ± 0.08 (range 0.04-0.36) for the GHD group and 0.19 ± 0.09 (range: 0.04-0.32) for the PWS group (Figure 1B). There was a positive correlation between the individual dose values of rGH and the individual values of the whole ratio ($p = 0.03$). However the correlation between low-normal and high ratio groups and growth increments was not significant ($p = 0.15$), probably due to the heterogeneity of age and duration of treatment.

In the SGA group (Figure 2), two children with high serum IGF1 concentrations (> +2 SDS) had normal IGF1/IGFBP3 molar ratios, because their IGFBP3 levels were also high (> +2 SDS).

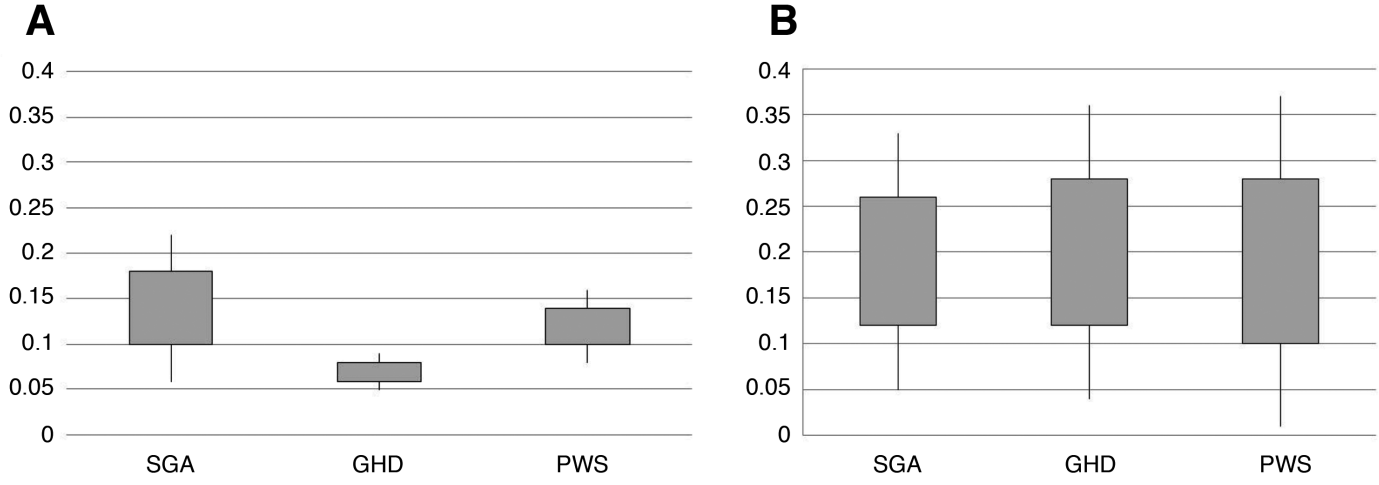

Figure 1. Distribution of IGF1/IGFBP3 ratio in the three groups before (A) and after (B) recombinant human growth hormone treatment. Rectangles represent values between +1 and -1 standard deviation scores (SDS) and bars represent SDS values

SGA: small for gestational age, GHD: growth hormone deficiency, PWS: Prader-Willi syndrome

Table 1. B) Change in IGF1/IGFBP3 molar ratio in control children by age and pubertal stage

Samples, n	23	18	15	16	18
Puberty stage				P1	P2-P5
Age (years)	0-4	> 4-8	> 8-10	9-13	11-17
Mean IGF1/IGFBP3 molar ratio	0.10	0.12	0.14	0.16	0.27
Standard deviation	0.05	0.03	0.03	0.05	0.06
Range	0.03-0.24	0.08-0.18	0.10-0.21	0.10-0.20	0.19-0.39

In the GHD group, an analysis of IGF1 concentration according to age and pubertal stage after treatment revealed that 13% of children had IGF1 concentrations > +2 SDS, particularly during the pubertal period. However, when IGFBP3 levels were also taken into account, the IGF1/IGFBP3 molar ratios of these children were found to be in the normal range for age and pubertal stage (Figure 3, Table 1B). We also found one five-year-old child with a normal IGF1 concentration but a very high molar ratio (> +2 SDS), because of a very low IGFBP3 concentration (<-2 SDS), requiring therapeutic adaptation (Figure 3).

In the group of treated children with PWS, we identified one case in which serum IGF1 concentration was normal but the IGF1/IGFBP3 molar ratio was low due to a high

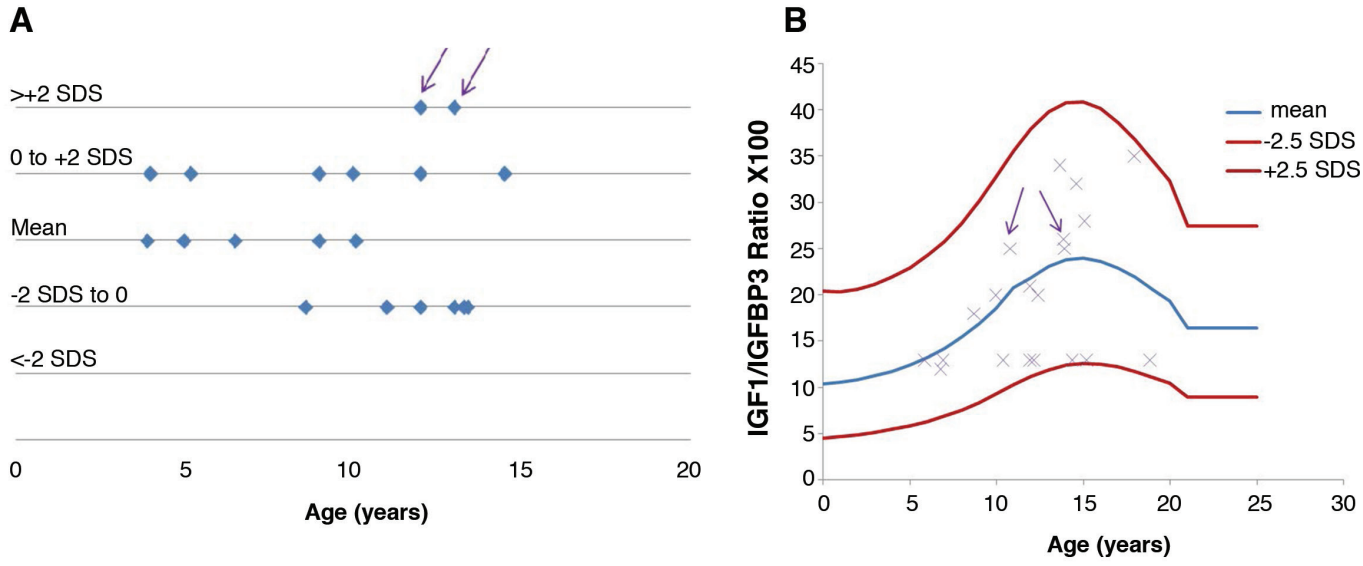

Figure 2. Distribution of IGF1 concentration (A) and IGF1/IGFBP3 molar ratio (B), in small for gestational age (SGA) children during growth hormone treatment. The IGF1 values have been distributed according to the standard deviation scores (SDS) intervals established by Bidlingmaier et al (10). In the SGA group, two children had IGF1 concentrations > +2 SDS, but IGF1/IGFBP3 molar ratios in the normal range (arrows)

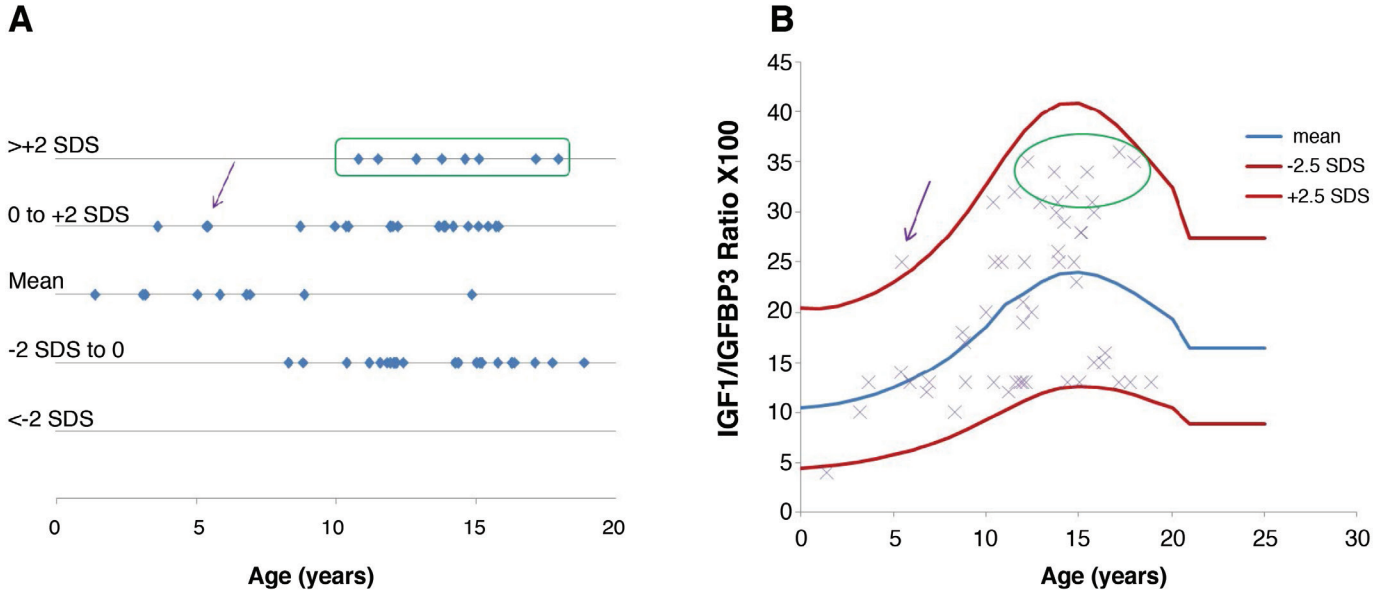

Figure 3. Distribution of IGF1 (A) and IGF1/IGFBP3 molar ratio (B), in children with growth hormone deficiency (GHD) during GH treatment. In the GHD group, eight children had IGF1 concentrations > +2 SDS (circled cases), but IGF1/IGFBP3 molar ratios in the reference range. Conversely, one five-year-old child had an IGF1 concentrations in the reference range but a very high molar ratio due to a very low IGFBP3 concentration (<-2 SDS; arrow)

IGFBP3 concentration (+2 SDS), one case in which serum IGF1 concentration was normal but the molar ratio was high due to a very low IGFBP3 concentration (<-2 SDS) and one case in which serum IGF1 concentration was high but the molar ratio was low, due to a high IGFBP3 concentration (Figure 4).

We then assessed the clinical, biochemical and metabolic characteristics of children on rGH with IGF1/IGFBP3 molar

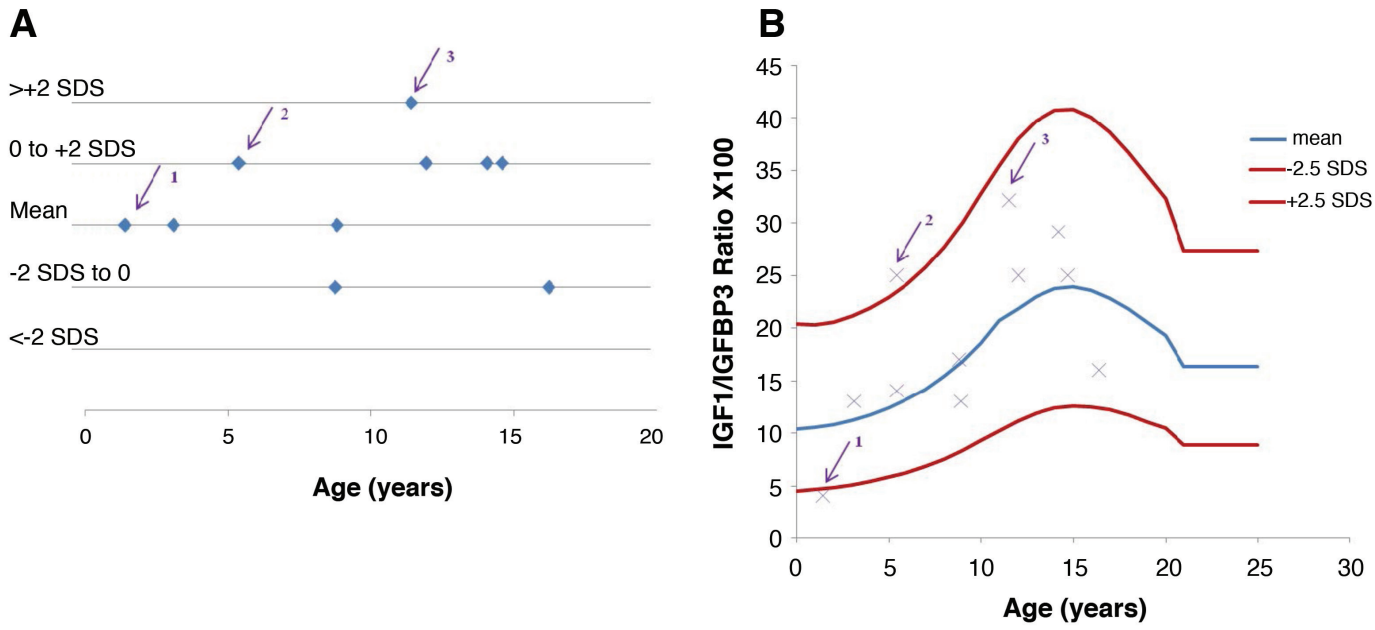

Figure 4. Distribution of IGF1 (A) and IGF1/IGFBP3 molar ratio (B), in children with Prader-Willi syndrome (PWS) during growth hormone (GH) treatment. In the PWS group, three children had discrepancies between serum IGF1 levels [expressed in standard deviation scores (SDS)] and IGF1/IGFBP3 molar ratio. 1st case (arrow 1): 1.4-year-old child with PWS and an IGF1 concentration in the normal range (expressed in SDS) but a low molar ratio (due to very high IGFBP3 concentration > +2 SDS). 2nd case (arrow 2): 5.4-year-old child with PWS and an IGF1 concentration in the normal range (expressed in SDS) but a high IGF1/IGFBP3 molar ratio (due to low IGFBP3 concentration). 3rd case (arrow 3): 11.4-year-old child with a high IGF1 concentration (expressed in SDS) and an IGF1/IGFBP3 molar ratio in the reference range (IGFBP3 concentration towards the upper end of the reference range)

Table 2. Clinical and metabolic characteristics of children on recombinant human growth hormone with molar ratios higher or lower than the normal range

				ANOVA		Post-hoc ANOVA p values		
	Low ratio (n = 41)	Normal ratio (n = 33)	High ratio (n = 18)	F-value	p value	Low *vs* normal	Low *vs* high	Normal *vs* high
Ratio (post rGH)	0.12 ± 0.02	0.24 ± 0.03	0.32 ± 0.02	467.56	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Age (years)	9.92 ± 4.77	12.61 ± 2.85	14.19 ± 2.01	10.00	0.0001	0.0051	0.0004	0.0379
BMI (SDS)	0.41 ± 1.63	0.31 ± 1.01	0.18 ± 1.30	0.16	0.84	0.7661	0.6139	0.7267
Height (SDS)	-0.85 ± 1.10	-0.95 ± 1.41	-1.38 ± 0.99	1.19	0.30	0.7228	0.0933	0.2828
rGH dose (µg/kg/day)	24.99 ± 9.46	28.33 ± 9.82	39.67 ± 17.20	8.82	0.0003	0.1989	0.0002	0.0153
Insulin (mIU/L)	5.47 ± 3.30	5.85 ± 3.15	9.77 ± 4.17	7.25	0.0017	0.7450	0.0011	0.0125
HbA1c (%)	5.33 ± 0.36	5.07 ± 0.28	5.40 ± 0.34	1.87	0.17	0.0976	0.7801	0.1334
Fasting glycemia (mmol/L)	4.72 ± 1.30	4.77 ± 0.25	4.80 ± 0.29	0.03	0.96	0.8974	0.8152	0.7744
Total cholesterol (mmol/L)	4.00 ± 0.64	4.18 ± 1.13	3.60 ± 0.59	1.57	0.22	0.6327	0.1188	0.1296
HDL-cholesterol (mmol/L)	1.63 ± 0.38	1.66 ± 0.56	1.46 ± 0.17	0.65	0.52	0.9131	0.2256	0.3279
LDL-cholesterol (mmol/L)	1.96 ± 0.49	2.06 ± 0.75	1.9 ± 0.47	0.19	0.82	0.6973	0.7734	0.5844
Triglycerides (mmol/L)	0.61 ± 0.29	0.73 ± 0.38	0.77 ± 0.30	0.92	0.40	0.3828	0.1715	0.7808

SDS: standard deviation scores, rGH: recombinant human growth hormone, BMI: body mass index, HDL: high-density lipoprotein, LDL: low-density lipoprotein

ratios inappropriate for age, either higher (> +2SDS) or lower (< -2 SDS) than the normal range (Table 2).

The three study groups were heterogeneous, differing significantly in terms of age and IGF/IGFBP3 molar ratio increases with age. We also noted that both the doses of rGH administered and insulinemia were significantly higher in children with a high molar ratio than in those with a low or normal molar ratio (Table 2). Moreover there was a positive correlation between individual values of insulinemia and the individual values of the whole ratio ($p = 0.01$).

For the other metabolic parameters considered (fasting glycemia, lipid status, HbA1c), we found no significant differences between children with low, normal and high IGF1/IGFBP3 molar ratios (Table 2).

Discussion

During rGH treatment, increases in the serum concentrations of IGF1 and IGFBP3 are expected because these factors are known to be GH-dependent and to have low basal levels in patients with GHD (13,14).

rGH modifies the distribution of circulating IGF1 between ternary complexes (IGF1/IGFBP3/ALS), binary complexes (IGF1/IGFBP3) and free forms (13). It simultaneously stimulates the production of all three forms, but this effect is stronger for ternary and binary complexes than for free forms (13). The concentration of the bioactive free form of IGF1 does not increase on treatment, even if the total amount of IGF1 increases (13). Thus, rGH increases the levels of IGF1 and IGFBP3 in a heterogeneous, dose-dependent (5,15) manner that differs between individuals (12,13).

In this context, IGFBP3 has been reported to be less sensitive to rGH than IGF1 in adults, with the increase in the concentrations of the former responding less quickly than the latter following rGH therapy (15). This difference results from the hepatic synthesis of IGFBP3s being under the control not only of GH, but also of IGF1s (15). Ranke et al (5) showed that, on rGH treatment, the IGF1-dependent increase in IGFBP3 concentration occurs in two phases: an initial linear phase, followed by a saturation phase in which IGFBP3 concentrations reach a plateau, despite further increases in IGF1 concentration. This would account for the increase in IGF1/IGFBP3 ratio at high doses of rGH. This phenomenon also highlights inequalities in the ability of the liver to synthesize IGF1 (synthesized by hepatocytes) and IGFBP3 (synthesized by Kupffer cells) (16). Thus, at high doses of rGH, serum IGF1 concentration may increase more strongly and more rapidly than IGFBP3 concentration, resulting in an increase in tissue-bioavailable IGF1 concentration (15).

Serum IGF1 and IGFBP3 disorders are known to be associated with a shorter lifespan in humans (17) due to the associated high risk of cardiovascular (18,19,20) and neoplastic comorbidity (21). Indeed, high concentrations of IGF1, a mitogen and anti-apoptotic hormone, have been implicated in the occurrence of various types of cancer (22,23), particularly when associated with low levels of IGFBP3 (24,25,26). In contrast, high serum IGFBP3 concentrations appear to have protective effects against cancer (6) but have been shown to be associated with diabetes, high triglyceride levels and hypertension, whereas low IGFBP3 levels are associated with a large waist circumference and low levels of HDL cholesterol (27).

These findings reflect the U-shaped correlation curve obtained for the relationship between serum IGF1 and IGFBP3 concentrations initially described by the histograms of Juul et al (28) and subsequently confirmed by the "quartiles" of Park and Cohen (29). Indeed, these representations of the risk of cardiovascular ischemia reported by Juul et al (28) and of cardiovascular and neoplastic risks reported by Park and Cohen (29), highlight the need to take both IGF1 and IGFBP3 into account, because these two parameters have "opposite" biological actions (28) and a dynamic "yin-yang" relationship (29).

We investigated the potential utility of the IGF1/IGFBP3 molar ratio for use in the adaptation of rGH treatment in three different etiological groups (children SGA, or with GHD or PWS). Indeed, SGA children are characterized by "GH resistance", resulting in a need for higher rGH doses than are used in other children matched for age, sex and puberty stage (5,30). In children, rGH dose is generally adjusted progressively, on a case-by-case basis (31). There are currently no clear guidelines on the mode of therapeutic adaptation according to the results obtained (12,29). This complicates therapeutic management, because it is considered important to keep IGF1 concentration below +2 SDS, because of the increased risk of cardiovascular diseases and neoplasm (31).

By contrast, almost all PWS children have a somatotropic deficit (32,33), justifying the systematic (34) early initiation of rGH treatment, within the first year of life (32,33,35). Unlike SGA children, children with PWS are particularly "rGH-sensitive" and have very high serum IGF1 concentrations (towards and above the upper limit of the normal range) (33,34,36).

For the three pathological entities studied (GHD, SGA and PWS), we found that the determination of IGF1/IGFBP3

molar ratio was a useful additional tool for the adaptation of rGH treatment with a view to improving safety.

The safety of rGH treatment is generally ensured by monitoring to keep serum IGF1 concentration at values below the +2 SDS threshold for age and pubertal stage (12). Our results indicate that there may be discrepancies between total IGF1 concentration and IGF1/IGFBP3 molar ratio with a potential impact on the safety of rGH treatment. The use of this ratio made it possible to optimize rGH administration so as to minimize the risk of adverse effects, particular those of a metabolic, cardiovascular or neoplastic nature (17,28,37).

Calculation of the IGF1/IGFBP3 molar ratio is, thus, a potentially useful additional tool because this ratio does not necessarily vary with increases in IGF1 concentration and it takes into account the variation of serum IGFBP3 levels (12).

We were able to determine approximate values for IGF1/IGFBP3 molar ratio before and after the initiation of rGH treatment in children SGA or with GHD or PWS. We found that the mean ratio increased after the initiation of rGH treatment in all groups, but that it remained within the reference range for age and pubertal stage in most children. Our values were consistent with those reported in previous studies. Romer et al (38) reported a low ratio in patients with GHD (0.13 ± 0.07) before rGH, with a sharp increase to more than 0.32 ± 0.07 after treatment initiation. Our results are also consistent with those of Cabrol et al (12), who reported a mean molar ratio before treatment of 0.14 (range: 0.10 to 0.27) in children SGA, increasing to 0.19 (range: 0.15 to 0.23) after treatment.

Finally, on rGH treatment at the doses currently recommended, the IGF1/IGFBP3 molar ratio remained within the normal range for age and sex, even during puberty. We also found that, in children treated with rGH, significant increases in serum IGF1 levels were sometimes associated with IGF1/IGFBP3 molar ratios within the normal range for age and pubertal stage. This suggests that, even in cases of high IGF1 concentration, the action of this molecule is counteracted by an adaptation of IGFBP3 levels, decreasing IGF1 bioavailability.

Study Limitations

This study included a small number of subjects and our results therefore require confirmation in a larger cohort.

Conclusion

We consider IGF1/IGFBP3 molar ratio to be a useful additional parameter for assessments of treatment safety and for the adjustment of rGH treatment. The goal would be to maintain this ratio within the normal reference range for age and pubertal stage.

Ethics

Ethics Committee Approval: All procedures involving human participants in this study were performed in accordance with the French national rules for ethics and confidentiality, also in accordance with current legislation for retrospective non-interventional studies of cohort files.

Informed Consent: All clinical data and biological analyses were performed as part of routine follow-up, without the collection of additional samples. Under French law, no specific consent for this anonymous study was therefore required. Concerning the ratio reference values of the control children, the families gave their consent to use the remains of the blood samples to this purpose.

Peer-review: Externally and internally peer-reviewed.

Authorship Contribution

Medical Practices: Yves Le Bouc, Concept: Yves Le Bouc, Meriem Gaddas, Laurence Périn, Design: Yves Le Bouc, Meriem Gaddas, Laurence Périn, Data Collection or Processing: Yves Le Bouc, Laurence Périn, Analysis or Interpretation: Yves Le Bouc, Meriem Gaddas, Laurence Périn, Literature Search: Meriem Gaddas, Yves Le Bouc, Writing: Meriem Gaddas, Yves Le Bouc.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2018.2019.0241

J Clin Res Pediatr Endocrinol 2019;11(3):262-269

Associations Between Serum Uric Acid Concentrations and Cardiometabolic Risk and Renal Injury in Obese and Overweight Children

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What is already known on this topic?

In obese (OB) adults and adolescents, higher uric acid (UA) concentrations are associated with the risk factors characterizing the metabolic syndrome (MetS) and also with fasting insulin level and insulin resistance (IR). All these factors are predictive for both cardiovascular diseases and type 2 diabetes. Despite the knowledge that UA is associated with obesity-related comorbidities such as MetS, cardiovascular risk factors and kidney diseases, studies in overweight (OW) and OB children are rare and the results are still controversial.

What this study adds?

This study confirms associations of elevated serum UA with greater waist-to-hip ratio, lower HDL-cholesterol and hypertriglyceridemia, as well as with the presence of MetS and IR in OB and OW children. Moreover, the number of criteria related to MetS is significantly associated with the elevation of UA.

Abstract

Objective: The aim of this study was to assess the association between serum uric acid concentration (SUAC) and the parameters of the metabolic syndrome (MetS) and insulin resistance (IR). The secondary aim was to evaluate whether hyperuricemia is associated with renal injury and cardiovascular risk in obese (OB) and overweight (OW) children.

Methods: The subjects of this study consisted of OB/OW children and adolescents (ages: 8-18 years). Sex and age specific serum uric acid (SUA) olarak değiştirilecek percentiles were used and a SUA $>75^{th}$ percentile was accepted as hyperuricemia. Anthropometric data, blood pressure (BP) measurements and biochemical parameters, including fasting blood glucose, insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol, triglycerides (TG), aspartate aminotransferase, alanine aminotransferase, homeostatic model assessments of IR (HOMA-IR) and SUAC were recorded. Oral glucose tolerance tests (OGTT) were performed in all patients. MetS was defined according to the International Diabetes Federation criteria. Total cholesterol/HDL-c ratio >4 and TG/HDL-c ratio >2.2 were used as the atherogenic index (AI) indicating cardiovascular risk. Urinary albumin excretion in a 24-hour and also in a first-morning urine sample were measured. Renal injury was assessed by microalbuminuria according to the National Kidney Foundation criteria.

Results: There were 128 participants; 52 (40%) had elevated (SUA $>75^{th}$ percentile) and 76 had (60%) normal SUAC. The mean ± SD age was 13.1 ± 2.6 years and 87 (67.4%) were female. The mean ± SD weight was 73 ± 18.97 kg and mean ± SD height was 155.4 ± 12.11 cm. There was no statistical difference between the groups with and without hyperuricemia in terms of age, sex, puberty stage and degree of obesity. Increased SUAC were significantly associated with higher waist-to-hip ratio (WHR), fasting insulin levels and insulin at 30 and 60 minutes during OGTT, HOMA-IR, lower HDL-c and presence of hypertriglyceridemia as well as with decreased HDL-c, increased AI, presence of IR and MetS. BP and microalbuminuria were not associated with SUAC. SUAC showed significant positive correlations with waist circumference, WHR, post-challenge glucose level at 60 minutes, with fasting insulin, post-challenge insulin levels at 30, 60, 90 and 120 minutes and also with HOMA-IR, total cholesterol/HDL-c ratio, TG/HDL-c ratio and a number of other criteria related to MetS. Also, an inverse correlation with HDL-c was noted.

Conclusion: In OB/OW children frequency of MetS, IR and dislipidemia increases with increased SUAC, a finding independent of age, puberty, gender and body mass index. Patients meeting all of the MetS criteria had the highest SUAC. These results demonstrate that the association between UA and metabolic and cardiovascular risk factors can be detected early in childhood. Thus, we recommend monitoring SUAC in OB children and we believe that prevention of SUAC elevation in early life has a potential protective effect on metabolic impairment and subsequent comorbidities.

Keywords: Serum uric acid concentration, obesity, metabolic syndrome, insulin resistance, renal injury, cardiovascular risk, child

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Conflict of interest: None declared

Received: 08.11.2018

Accepted: 10.02.2019

Introduction

Uric acid (UA) is the end-product of purine metabolism, produced by the liver and excreted by the kidneys (1). Serum UA concentration (SUAC) increases progressively with body growth from early childhood until the ages of 15-17 years (2). Obese (OB) individuals have higher SUAC than in normal-weight peers. Hyperuricemia and obesity probably influence one another in many ways, depending on multiple mechanisms. Hyperuricemia may contribute to development of obesity by accelerating hepatic and peripheral lipogenesis (3). On the other hand, obesity may lead to serum UA (SUA) elevation due to several factors, such as OB subjects having reduced renal clearance of UA and obesity being associated with elevated activity of xanthine oxidase and increased production of UA by adipose tissue (4).

The increase in SUAC is an independent risk factor for lifestyle related diseases such as hypertension, renal diseases, cardiovascular diseases and also has a potential role in the development of the metabolic syndrome (MetS), hyperinsulinemia and IR measured by the homeostatic model assessment of insulin resistance (HOMA-IR) (5). However, the relationship between obesity-related metabolic risk factors and SUAC in childhood is still controversial. While some studies report a strong association between these variables (6,7), others did not confirm an independent association (8,9).

In this study we aimed to investigate, whether increased SUAC is related to MetS risk factors, using standard methodology (10). We also aimed to evaluate the association between hyperuricemia with renal injury or cardiovascular risk factors in OB and overweight (OW) children.

Methods

Study Population

Children who visited the Pediatric Endocrinology Outpatient Clinic for general obesity screening were enrolled in the study. Ethics committee approval was obtained from Manisa Celal Bayar University (20.478.486). A total of 128 OB and OW children of ages 8 to 18, with a body mass index (BMI) greater than the 85th percentile for age and gender according to the Center for Disease Control and Prevention (CDC-2000) data (11), were included in the study. The patients were divided into two groups according to their SUAC.

Children with type 1 or type 2 diabetes or whose obesity was related to a syndrome (Prader-Willi syndrome, Laurence-Moon Biedl syndrome, etc.) or to an endocrinologic condition such as Cushing's syndrome or hypothyroidism were excluded. Subjects referred to our clinic for conditions related to obesity (e.g. alterations in bloodglucose levels, arterial hypertension, dyslipidemia, liver steatosis, hyperuricemia etc.), children who have received or are currently receiving treatments such as glucose or lipid-lowering drugs and/or anti-hypertensive medication, children with liver, kidney or other systemic diseases and family history of symptomatic hyperuricemia were also excluded from the study.

Procedures

Physical examination and laboratory results of all subjects were recorded. All of the evaluations were conducted by specially trained clinical research staff.

Anthropometric and Clinical Measurements

Height and weight were measured by a wall-mounted stadiometer for height and a calibrated scale for weight. The weight of each subject was measured with all clothing and shoes removed except undergarments. Waist circumference (WC) was measured with a non-stretchable tape to the nearest 0.1 cm midway between the lowest rib and the highest point of the iliac crest parallel to the floor, in a standing and relaxed position and during expiration. Hip circumference was measured at the widest portion of the buttocks. Waist-to hip ratio (WHR) was calculated. Pubertal development stage was recorded according to Tanner classification. Blood pressure (BP) was measured with the right arm in the supine position after a five-minute rest, using a mercury sphygmomanometer with an appropriately sized cuff, and a stethoscope placed over the brachial artery pulse; three systolic and diastolic BP (SBP, DBP) measurements were taken two minutes apart and the mean of the two last values was used in data analyses.

Laboratory Tests

Results of assessment of biochemical analytes including serum glucose, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), triglycerides (TG) and SUA were recorded. Each child underwent an oral glucose tolerance test (OGTT) following an overnight fasting of 12-14 hours. After subjects ingested a glucose solution containing 1.75 g/kg glucose (maximum 75 g), blood samples were obtained every 30 min for 120 min, for measurement of plasma glucose and insulin. In all subjects, the first-morning urine specimen was analyzed for albumin and creatinine. Urine was collected for 24 hours, and urinary albumin was measured. Samples showing pyuria and hematuria were excluded.

Total body obesity was estimated by BMI, central obesity measured by WHR or WC, atherogenic dyslipidemia by increased TG, decreased HDL-c and increased ratio of total cholesterol/HDL-c and TG/HDL-c. Presence of systolic and diastolic hypertension and hyperglycemia including fasting blood glucose level and/or abnormal glucose responses on OGTT, hyperinsulinemia and IR measured by the homeostatic model assessments of IR (HOMA-IR) were estimated in all subjects (10).

BMI was calculated by the standard formula (weight in kg divided by the square of height in metres). BMI standard deviation score (BMI SDS) and BMI percentiles were calculated using age and gender specific norms published by the CDC (11). Obesity was defined as BMI ≥95th percentile and OW as BMI ≥85th percentile for age and sex. The extent of obesity was quantified using Cole's LMS method which stratifies obesity on the basis of a threshold BMI Z-score of 2.0 or more, namely, moderate obesity as a Z-score of 2.0-2.5, and severe obesity as a Z-score above 2.5 (12). WC percentiles were stratified according to sex and age, identifying abdominal obesity as a WC ≥90th percentile as previously described (13). WHR was used as an index of fat distribution. A testicular volume of ≥4 mL in males, and breast development of Tanner stage 2 and over in females, were considered as findings of puberty (14).

IR was evaluated with the aid of HOMA-IR index using a standard formula: fasting insulin (μU/mL) x fasting glucose (mmol/L) divided by 22.5. IR criteria were HOMA-IR >2.5 for prepubertal children and HOMA-IR >4.0 for adolescents (15). Impaired fasting glucose was defined as a fasting plasma glucose level between 100 and 125 mg/dL without a history of diabetes mellitus (16). Impaired glucose tolerance was defined according to World Health Organization criteria, a condition in which fasting blood glucose levels in venous plasma drop to <140 mg/dL and the 120 minute post challenge blood glucose is between 140 and 200 mg/dL (16).

Hyperinsulinemia was defined as a fasting insulin ≥15 μU/mL, or an insulin level during the OGTT test of ≥150 μU/mL and/or ≥75 μU/mL at 120 minutes following the start of the OGTT (17).

MetS was defined according to the International Diabetes Federation criteria (17). MetS can be diagnosed in children 10 to 16 years old when the following criteria are fulfilled: a WC ≥90th percentile (sex and age specific), together with two or more risk factors. These risk factors are: 1) fasting blood glucose levels ≥100 mg/dL (5.6 mmol/L); 2) serum TG concentration ≥150 mg/dL (1.7 mmol/L) or treatment for elevated TG; 3) a low HDL-c <40 mg/dL (1.03 mmol/L) or treatment for low HDL-c; 4) either SBP ≥130 mmHg or DBP ≥85 mmHg, or treatment for hypertension, or a SBP level of at least 95th percentile for sex, age and height (18).

For children 16 years and older, the adult criteria can be used (19). MetS can not be diagnosed in children younger than 10 years of age, but vigilance is recommended if the WC is ≥90th percentile (20). Total cholesterol/HDL-c ratio is defined as the atherogenic index (AI), according to which a ratio of >4 (normal = 2.5) is considered as a cardiovascular risk (20). The TG to HDL-ratio >2.2 was also considered as a marker of atherogenic risk (21). Hypertension was defined as a value above the 95th percentile for age and height according to the National Health and Nutrition Examination Survey (22). Microalbuminuria in children and adolescents was defined as a urinary albumin excretion rate of 30-300 mg/24 hours and 3-30 mg/mmoL creatinine (30-300 mg/g creatinine) in a first-morning urine sample (23). Hyperuricemia was defined as SUA value ≥75th percentile, adjusted for age and sex (24).

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as numbers and percentages. Normal distribution was tested using the Kolmogorov-Smirnov test. Between-group comparison for categorical variables was performed by using the χ^2 test or Fisher's exact tests. Student's t-tests and Mann-Whitney U test were used for comparison of continuous variables. Correlations were investigated using Pearson's correlation test. Statistical analyses were performed using the Statistical Package for Social Sciences 15.0 program (SPSS 15.0; IBM Inc., Chicago, Ill., USA). P values <0.05 were considered statistically significant.

Results

In this study, 128 OB and OW children/adolescents were evaluated. Of these 52 (40%) had elevated SUA defined as SUAC ≥75th percentile, adjusted for age and sex and 76 (60%) had normal SUAC. The mean age of the participants was 13.1 ± 2.6 (range 8-18) years and 87 (67.4%) were female. Clinical and laboratory variables were compared in children with and without hyperuricemia and the results are presented in Table 1. The group with hyperuricemia was not statistically different from the group without hyperuricemia in terms of age, sex, puberty stage and degree of obesity. Subjects with hyperuricemia had higher WHR and lower HDL-c compared with those with normal SUAC. Moreover, subjects with hyperuricemia who showed higher insulin

levels either at fasting or as responses to OGTT at 30 and 60 minutes and also tended to have higher IR values than those without hyperuricemia but this latter parameter did not reach significance.

Increased SUAC was significantly associated with the criteria related to MetS. Table 2 shows that elevated SUAC were significantly associated with hypertriglyceridemia, decreased HDL-c, increased AI, presence of IR and MetS.

Table 3 shows the results of the correlation analysis between the variables with SUAC in all subjects. SUAC showed a significant positive correlation with WC, WHR, post-challenge glucose level at 60 minutes, fasting insulin, post-challenge insulin levels at 30, 60, 90 and 120 minutes, HOMA-IR, total cholesterol to HDL-c ratio, TG to HDL-c ratio, criteria related to MetS and an inverse correlation with HDL-c.

Table 1. Clinical and laboratory characteristics of the study groups

Variables	Serum uric acid concentration ≥75th percentile (n = 52)	Serum uric acid concentration < 75th percentile (n = 76)	p
Age (years)	13.2 ± 2.7	13 ± 2.6	0.66
Gender (% female)	69.2	67.1	0.80
Puberty stage (% pubertal)	92.3	93.4	0.82
Weight (kg)	74.1 ± 18.6	72.4 ± 19.2	0.61
Height (cm)	155.9 ± 12.8	155.1 ± 11.6	0.72
BMI (kg/m^2)	29.9 ± 4.4	29.5 ± 5	0.60
BMI SDS (kg/m^2)	2.03 ± 0.36	1.98 ± 0.35	0.44
Waist circumference (cm)	97.8 ± 11.6	94.5 ± 11.6	0.12
Hip circumference (cm)	105.6 ± 13.6	105.1 ± 11.9	0.83
Waist/Hip circumference ratio	0.93 ± 0.08	0.90 ± 0.06	**0.01**
SBP (mmHg)	116.6 ± 11.9	115.3 ± 13	0.58
DBP (mmHg)	73.1 ± 10.6	75.3 ± 11.6	0.29
GlcT0^i (mg/dL)	85.1 ± 7.5	86.0 ± 8.3	0.56
GlcT30^i (mg/dL)	136.3 ± 20.3	134.5 ± 21.9	0.64
GlcT60^i (mg/dL)	136 ± 29.8	132.6 ± 28.3	0.52
GlcT90^i (mg/dL)	126.2 ± 26.3	125.2 ± 33.1	0.85
GlcT120^i (mg/dL)	120.3 ± 22.1	122.1 ± 23.3	0.67
InsT0^i (µU/mL)	27.8 ± 12.8	23.4 ± 11.4	**0.045**
InsT30^i (µU/mL)	142.6 ± 77.5	117.8 ± 62.1	**0.049**
InsT60^i (µU/mL)	148.7 ± 91.6	113.3 ± 73.3	**0.017**
InsT90^i (µU/mL)	134.8 ± 91.3	117.8 ± 73.2	0.25
InsT120^i (µU/mL)	119.5 ± 89	105.7 ± 69.3	0.33
HOMA-IR	6.06 ± 3.1	5.04 ± 2.6	0.083
AST (IU/L)	27.5 ± 10.4	29.7 ± 21.2	0.49
ALT (IU/L)	31.7 ± 21.5	31 ± 28.1	0.87
Total cholesterol (mg/dL)	164.4 ± 28.3	158.3 ± 31.8	0.27
HDL-c (mg/dL)	45.2 ± 8.7	48.9 ± 10.1	**0.028**
LDL-c (mg/dL)	91.2 ± 21.3	83.8 ± 30.8	0.14
Triglycerides (mg/dL)	140.1 ± 66.5	124.7 ± 105.3	0.35
Urinary albumin excretion in a 24-hour urine collection	10.1 ± 9	10.2 ± 11.7	0.98
Protein/Creatinine ratio in a first-morning urine sample	0.62 ± 0.64	0.52 ± 0.61	0.41
Number of criteria related to MetS (26)	2.01 ± 0.9	1.60 ± 0.7	**0.009**[a]

ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: body mass index, DBP: diastolic blood pressure, GlcT0^i: fasting glucose, GlcT30^i: GlcT60^i, GlcT90^i, GlcT120^i: post-challenge glucose, HDL-c: high density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment of insulin resistance, InsT0^i: fasting insulin, InsT30^i: InsT60^i, InsT90^i, InsT120^i: post-challenge insulin, LDL-c: low density lipoprotein cholesterol, SBP: systolic blood pressure, SDS: standard deviation score, [a]Mann-Whitney U test

Table 2. Anthropometric, clinical and metabolic variables of the study population according to their uric acid levels

	Serum uric acid concentration		p
Variables	≥75th percentile (n = 52) n (%)	< 75th percentile (n = 76) n (%)	
Male	16 (30.8)	25 (32.9)	0.8
Female	36 (69.2)	51 (67.1)	
Extent of obesity			
Overweight	11 (21.2)	16 (21.1)	
Moderate	25 (48.1)	44 (57.9)	0.4
Severe	16 (30.8)	16 (21.1)	
Puberty			
Prepubertal	4 (7.7)	5 (6.6)	0.8
Pubertal	48 (92.3)	71 (93.4)	
Waist circumference			
Increased (≥90 p)	52 (100)	76 (100)	-
Normal (< 90 p)	-	-	
SBP			
Increased (≥95 p)	12 (23.1)	14 (18.4)	0.52
Normal	40 (76.9)	62 (81.6)	
DBP			
Increased (≥95 p)	7 (13.5)	12 (15.7)	0.84
Normal	45 (86.5)	64 (84.3)	
Glycemia			
Altered	13 (25)	21 (27.6)	0.74
Normal	39 (75)	55 (724)	
Triglycerides			
Altered (≥150 mg/dL)	18 (34.6)	13 (17.1)	**0.023**
Normal	34 (65.4)	63 (82.9)	
HDL-c			
Altered (< 40 mg/dL)	19 (36.5)	11 (17.8)	**0.004**
Normal	33 (63.5)	65 (85.5)	
Atherogenic risk 1			
Total cholesterol/HDL-c			
Present (> 4)	20 (38.5)	16 (21.1)	**0.03**
Absent	32 (61.5)	60 (78.9)	
Atherogenic risk 2			
TG/HDL-c			
Present (> 2.2)	20 (38.5)	33 (43.4)	0.044
Absent	32 (61.5)	43 (56.6)	
IR			
Present	40 (76.9)	41 (53.9)	**0.008**
Absent	12 (23.1)	35 (46.1)	
Metabolic syndrome			
Present	15 (28.8)	9 (11.8)	**0.015**
Absent	37 (42.3)	67 (88.2)	

DBP: diastolic blood pressure, HDL-c: high density lipoprotein cholesterol, IR: insulin resistance, SBP: systolic blood pressure, TG: triglycerides

Table 3. Correlations between serum uric acid levels and risk factors for metabolic syndrome, cardiovascular risk and renal injury

	Uric acid level (n = 128)	
Variables	r	p
BMI (kg/m^2)	0.13	0.14
BMI SDS (kg/m^2)	0.05	0.57
BMI percentile	0.019	0.83
Waist circumference (cm)	0.32	**< 0.0001**
Waist/Hip circumference ratio	0.20	**0.017**
SBP (mmHg)	0.07	0.42
DBP (mmHg)	-0.04	0.61
GlcT0^i (mg/dL)	-0.07	0.37
GlcT30^i (mg/dL)	0.09	0.27
GlcT60^i (mg/dL)	0.21	**0.013**
GlcT90^i (mg/dL)	0.11	0.21
GlcT120^i (mg/dL)	0.10	0.25
InsT0^i (µU/mL)	0.28	**0.001**
InsT30^i (µU/mL)	0.30	**0.001**
InsT60^i (µU/mL)	0.27	**0.002**
InsT90^i (µU/mL)	0.19	**0.03**
InsT120^i (µU/mL)	0.18	**0.03**
HOMA-IR	0.29	**0.001**
AST (IU/L)	-0.002	0.98
ALT (IU/L)	0.14	0.11
Total cholesterol (mg/dL)	0.06	0.45
HDL-c (mg/dL)	-0.26	**0.002**
LDL-c (mg/dL)	0.03	0.72
Triglycerides (mg/dL)	0.12	0.18
Total cholesterol/HDL-c ratio	0.27	**0.002**
TG/HDL-c ratio	0.24	**0.008**
The number of criteria related to metabolic syndrome	0.30	**< 0.0001**
Urinary albumin excretion in a 24-hour urine collection	-0.06	0.46
Protein/creatinine ratio in a first-morning urine sample	0.03	0.71

ALT: alanine aminotransferase, AST: aspartat aminotransferase, BMI: body mass index, DBP: diastolic blood pressure, GlcT0^i: fasting glucose, GlcT30^i, GlcT60^i, GlcT90^i, GlcT120^i: post-challenge glucose, HDL-c: high density lipoprotein cholesterol, HOMA-IR: homeostatic model assessmensst of insulin resistance, InsT0^i: fasting insulin, InsT30^i, InsT60^i, InsT90^i, InsT120^i: post-challenge insulin, LDL-c: low density lipoprotein cholesterol, SBP: systolic blood pressure, SDS: standard deviation score, TG: triglycerides

Discussion

Physiological UA concentrations have antioxidant and endothelial protective effects in the extracellular environment. However, increased SUAC has been reported

to play a pro-oxidant role and might promote several harmful effects (25). A relationship between increased SUAC and obesity-related comorbidities such as MetS, IR, cardiovascular risk factors and kidney diseases has been reported in OB adults and children (26,27). However, the results of these studies are still controversial.

Epidemiological studies on large populations have shown that the prevalence of MetS shows a gradual increase with increased SUAC (28). Despite the apparent role of SUA in contributing to MetS related metabolic impairment, studies in OW/OB children are rare. In the study of Ford et al. (7) which included 1370 adolescents aged between 12-17 years, patients meeting all the MetS criteria were found to have the highest SUAC. In the STYJOBS/EDECTA cohort study of 299 OW/OB Japanese children aged between 8-18 years, SUA was shown as the best predictor of unhealthy obesity. Patients in the highest quartile of the SUAC were found to be heavier, with worse lipid and insulin metabolism. The authors suggested that hyperuricemia should be considered as a cardiometabolic risk factor in early childhood (29). Our study confirms that the presence of MetS and the criteria related to MetS are significantly associated with elevated SUAC in OB/OW children. A growing number of studies suggest that UA should be added to the list of criteria used to diagnose MetS (30,31). Thus SUA requires more attention in the evaluation of the metabolic risk profile of OB children and adolescents. The pattern of fat distribution, rather than BMI, is important for metabolic and cardiovascular diseases (32). Our results showed that increased SUAC was significantly associated with greater WHR and correlated with higher WC. The strong association found by us and others with WC confirms the strong link between UA and visceral adiposity (7,24). The association of UA with regional distribution of abdominal adipose tissue in children is poorly understood. Increased dietary fructose consumption leads to hepatic lipogenesis, thus contributing to increased visceral fat accumulation and ultimately worsening of IR (33). In addition, dietary fructose activates the fructokinase metabolic system and upregulates *de novo* purine nucleotide synthesis in hepatocytes, thereby causing an increase SUA production and hyperuricemia (34). High SUAC-associated dyslipidemia has been shown to be a result of low serum HDL-c levels, not increased LDL or VLDL levels (15). This study confirmed associations of elevated SUA with lower HDL-c and hypertriglyceridemia. Recent evidence suggests that UA induces vascular inflammation and artery damage, leading to increased risk of atherosclerosis. Findings of the present study confirmed an association between SUA and increased atherogenic risk calculated with the ratio of TG to HDL-c and total cholesterol to HDL-c.

Recent prospective studies demonstrate that hyperuricemia is a predictor of IR (5). It was observed that, for every increase of 1 mg/dL in SUAC, there would be a 91 % increase in risk of IR. However, the pathophysiological mechanism of the association between hyperuricemia and hyperinsulinemia/IR is not yet clearly established. A double correlation has been proposed; in general, IR and hyperinsulinemia are thought to increase SUAC by reducing renal excretion and increasing production through the hexosemonophosphate shunt (1). Another possible link between hyperuricemia and IR could be hyperuricemia-mediated endothelial dysfunction which may lead to lower insulin uptake by reduced blood flow in peripheral tissues and may worsen the IR (35). Consistent with these pathogenic findings, hyperuricemic patients in our study had significantly higher insulin levels at 0, 30 and 60 minutes. SUAC showed a significantly positive correlation with insulin levels both at fasting and at all estimations following oral glucose loading. We also found that SUA is significantly associated with the HOMA-IR. Cardoso et al. (9) showed the association between MetS and SUAC by IR and they reported that while glycemia was not different, HOMA-IR significantly varied among quartiles of SUAC. In our study, we found a significant correlation between UA and glucose levels only at post challenge 60 minutes. Similarly Ricotti et al. (36) showed that hyperuricemic patients were at increased risk of having a 1-hour post-OGTT glycemia which was also associated with increased metabolic risk.

The association of higher SUAC with higher BP has been reported in adults and children in a number of studies (26,37). The lack of an association between SUA and BP in our sample may be related to the fact that duration of exposure to increased SUAC and related inflammation and oxidative stress was not evaluated in our study. In adults, in addition to microalbuminuria, hyperuricemia is a well-established risk factor for chronic kidney disease (26). However, data concerning the relationship between hyperuricemia and renal injury in OB children are still lacking. We did not find a significant association in our study group. Long-term prospective studies are needed on this subject.

Study Limitations

Our study has some limitations. We used percentages of the UA according to age and sex but SUAC may be affected by pubertal stage. We did not encounter any UA reference values which took into account sex and pubertal stages in the literature. Comprehensive studies are needed on this issue. The major limitation of our study is the relatively small size of the sample.

Conclusion

In conclusion, we believe that SUAC is a good alternative to assess cardiometabolic risk, even at a young age. Chronic hyperuricemia appears to be involved in the pathogenesis of metabolic impairment leading to MetS and subsequent comorbidities. The prevention of SUA elevation at an early age by SUA lowering agents may have a potential protective effect on hyperglycemia, hyperinsulinemia, IR, dislipidemia and hypertension (38). It is feasable to include assessment of UA in routine tests in primary care since its estimation is widely available, very cheap and reliable. We therefore suggest that measurement of SUAC should be included in the assessment protocols of OB/OW children and adolescents. We would also like to add that there is a need for prospective clinical studies to evaluate the clinical significance and to assess the cost effectiveness of measuring routinely SUAC in childhood obesity.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Manisa Celal Bayar University (20.478.486).

Informed Consent: It was not taken because it was a retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Deniz Özalp Kızılay, Betül Ersoy, Concept: Deniz Özalp Kızılay, Semra Şen, Design: Deniz Özalp Kızılay, Semra Şen, Data Collection or Processing: Deniz Özalp Kızılay, Semra Şen, Analysis or Interpretation: Deniz Özalp Kızılay, Literature Search: Deniz Özalp Kızılay, Writing: Deniz Özalp Kızılay.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2018.0098

J Clin Res Pediatr Endocrinol 2019;11(3):270-277

No Associations Between Serum Lipid Levels or HOMA-IR and Asthma in Children and Adolescents: A NHANES Analysis

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What is already known on this topic?

Being overweight in childhood is associated with an increased risk for development of allergic disease. A link has been shown between elevated lipid levels and the development of asthma/wheezing in children and adults. Hyperinsulinemia may be associated with the development of asthma.

What this study adds?

Multivariate analyses found no associations between reduced high-density lipoprotein cholesterol, elevated low-density lipoprotein cholesterol, total cholesterol and triglycerides and the presence of asthma in children or adolescents. Multivariate analyses found no associations between elevated fasting plasma glucose and the presence of asthma in children or adolescents. Multivariate analyses found no associations between homeostatic model assessment-insulin resistance and the presence of asthma in children or adolescents.

Abstract

Objective: Studies have reported inconsistent results on the associations between lipids and insulin resistance (IR) and asthma. The purpose of this study was to examine the associations between abnormal serum lipid levels and homeostatic model assessment-IR (HOMA-IR) and the presence of current asthma in children and adolescents.

Methods: The United States National Health and Nutrition Examination Survey database from 1999 to 2012 was randomly searched for children (aged 3-11 years) and adolescents (aged 12-19 years) with and without asthma and with complete demographic and clinical data of interest. Logistic regression analyses were performed to examine associations between abnormal serum lipids, glucose and HOMA-IR and the current presence of asthma.

Results: The data of 11,662 children (3 to 11 years of age) and 12,179 adolescents (12 to 19 years of age) were included in the analysis. The study group included 3,703 participants with asthma and 20,138 participants without asthma. The prevalence of self-reported current asthma was higher among participants aged between 3-11 years (52.9%) than among those aged between 12-19 years (50.7%). Multivariate analyses, after adjusting for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking, tobacco exposure, C-reactive protein level and body mass index Z-score, revealed no associations between elevated fasting plasma glucose, reduced high-density lipoprotein cholesterol, elevated low-density lipoprotein cholesterol, total cholesterol, triglycerides and HOMA-IR and the presence of current asthma in children or adolescents.

Conclusion: In this cross-sectional study, no association was found between abnormal serum lipids or HOMA-IR and the presence of current asthma in children or adolescents.

Keywords: Asthma, cholesterol, insulin resistance, lipid, lipoprotein, NHANES, obesity, wheezing

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Conflict of interest: None declared
Received: 24.04.2018
Accepted: 08.02.2019

Introduction

Asthma is primarily a disease of childhood and its increasing prevalence, beginning in the 1980s, has been referred to as an asthma epidemic (1). It has been estimated that the prevalence of asthma in 1980 was 3.6%, increased to 7.5% in 1995 and further increased to 9.3% in 2010. Since 2010, the overall prevalence of childhood asthma has remained unchanged or has decreased slightly (2).

While a number of risk factors are associated with the development of childhood asthma, the condition has been linked especially to obesity and metabolic syndrome (3,4,5). Being overweight in childhood has also been associated with an increased risk of the development of allergic disease (6). In addition, increasing attention has been given to the association between hypercholesterolemia and obesity, as well as that between hypercholesterolemia and obesity with airway hyper-responsiveness, suggesting a potential role of cholesterol and lipid homeostasis in lung physiology and asthma (7,8,9,10,11,12,13,14). Rastogi et al (15) have also suggested that hyperglycemia and hyperinsulinemia may result in airway hyper-responsiveness.

Results of a number of studies have also linked elevated lipid levels with the development of asthma/wheezing in children and adults (1,2,3,4,5,16). However, the results are inconsistent with those of other studies that show no association (12,13,17), or even a negative association, between elevated lipids and asthma/wheezing (11). The reported associations between asthma and lipid levels and insulin resistance (IR) appear to be independent of body mass index (BMI) (3). As such, dyslipidemia and hyperinsulinemia, precursors to cardiovascular disease and diabetes, may also be associated with the development of asthma and confound its epidemiologic link to obesity (3,5,18). The results of most studies, however, have been limited by a cross-sectional study design and a wide range of subjects studied.

Thus, the purpose of the current study was to use a national population-based database to examine the associations between lipid levels and IR and the presence of current asthma in children and adolescents.

Methods

Data Source

The United States National Health and Nutrition Examination Survey (NHANES) is an ongoing cross-sectional health survey that represents the non-institutionalized population of the United States. The program uses a complex, multistage design to collect and analyze data representative of different geographic regions. The NHANES data are collected through a combination of interviews and physical examinations of participants by highly trained personnel. The survey is administered by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). Further information about the NHANES program is available at the NHANES website: https://www.cdc.gov/Nchs/Nhanes/about_nhanes.htm

Detailed information about NHANES data collection methods is available at https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L13_C.htm and https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/L13AM_C.htm

The survey protocol and data collection methods for this present study were approved by the NHANES Institutional Review Board (IRB), and the NCHS Research Ethics Review Board (ERB) (Protocol#98-12, Protocol#2005-06, and Protocol #2011-17). All of the NHANES data were de-identified and analysis of the data by independent researchers does not require IRB approval or subject informed consent.

Study Population

Data from seven cycles of the NHANES, conducted during the period 1999-2012 were used. The data of children (aged 3-11 years) and adolescents (aged 12-19 years) with complete demographic and laboratory data, as well as that of other variables of interest, were included in the analysis. Exclusion criteria were: 1) diagnosis of diabetes mellitus (defined as a self-report of having been told by a doctor or health professional that the subject had diabetes or sugar diabetes, or currently taking diabetic pills or insulin); 2) pregnancy; 3) being underweight, defined as a BMI $< 5^{th}$ percentile for age and sex (19,20).

Dependent Variables (Y)

The primary outcome of the analysis was the presence of current asthma or wheezing (21). Current asthma was defined as those who reported ever being told that they had asthma and who had an asthma attack in the past year (https://wwwn.cdc.gov/Nchs/Nhanes/2007-2008/MCQ_E.htm). Wheezing was defined as wheezing or whistling in the chest in the course of the past year (https://wwwn.cdc.gov/Nchs/Nhanes/2003-2004/RDQ_C.htm).

Independent Variables (X)

The NHANES dataset provided laboratory results of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and fasting plasma glucose (FPG) levels. Of these, TG, LDL cholesterol and FPG were measured only in subsamples (adolescents), while TC and HDL cholesterol were measured

in all participants. Homeostatic model assessment-IR (HOMA-IR) was calculated using the equation: fasting glucose (mg/dL) × fasting insulin (pmol/L) /405 /6 (22,23,24) only in subsamples. The cutoff values for abnormal lipid and FPG levels, and HOMA-IR were: ≥170 mg/dL for elevated TC; ≤45 mg/dL for low HDL cholesterol; ≥110 mg/dL for elevated LDL cholesterol; ≥75 and ≥90 mg/dL for elevated TG for participants ≤9 years old and > 10 years old, respectively; ≥100 mg/dL for abnormal FPG; ≥3.0 for abnormal HOMA-IR, as reported by the expert panel of the United States National Heart, Lung, and Blood Institute (25) and used in prior investigations (15,26).

Covariates (Potential Confounders)

Demographic data examined as potential confounders included age, sex, family income-to-poverty ratio, prenatal maternal smoking, birth weight (low birth weight or not), and C-reactive protein (CRP) level. Age- and sex-specific BMI percentiles and BMI Z-scores were determined according to the 2000 CDC growth charts using a CDC SAS program www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm (6).

Tobacco exposure was defined by a "yes" response to the questions: "Have you ever tried cigarette smoking, even 1 or 2 puffs?" or "Does anyone who lives here smoke cigarettes, cigars, or pipes anywhere inside this home?"

To estimate physical activity before the year 2007, we summed the product of weekly time spent in each activity reported by the participant multiplied by the metabolic equivalent of task (MET) value for that activity yielding a MET-h index. One MET is the energy expenditure of 1 kcal/kg body weight per hour. For cycles after 2007, the physical activity questionnaire was changed. We estimated weekly MET-h for moderate and vigorous activities from questions asking participants about their participation in moderate and vigorous activities, the number of days per week engaged in these activities, and the number of minutes engaged in these activities on a typical day (27).

Statistical Analysis

Differences in categorical variables between participants with and without asthma were determined using the Rao-Scott chi-square test and differences of continuous variables between groups were examined using the Complex Samples General Linear Model. Demographic data and baseline characteristics are expressed as mean ± standard error for continuous variables, and unweighted counts (weighted %) for categorical variables. Univariate logistic regression analyses were performed to determine the association between serum lipids, glucose, HOMA-IR and current asthma. Extended-model approaches were used for covariate adjustment: Model 1 = gender, race, poverty income ratio, low birth weight (children only), prenatal maternal smoking (children only), tobacco exposure, and physical activity (adolescents only); Model 2 = Model 1 + CRP; Model 3 = Model 2 + BMI-Z-score. Participants with missing data of any covariates were not included in the regression analyses. All analyses included NHANES Medical Examination Center (MEC) sample weight or fasting subsample weight, stratum and primary sampling units per recommendations from the NCHS, to address oversampling, non-response, non-coverage and to provide nationally representative estimates. All statistical assessments were 2-sided and evaluated at the 0.05 level of significance. Statistical analyses were performed using the statistical software package SPSS complex sample module version 22.0 (IBM Corp, Armonk, NY, USA)

Results

A total of 26,158 participants aged between 3 and 19 years were identified in the NHANES 1999-2012 cycle. Participants with diabetes (n = 87), who were pregnant (n = 115), or who had a BMI Z-score less than the 5th percentile (n = 2,115) were excluded from the analysis, leaving 23,841 participants as the final sample.

This final eligible population included 20,138 participants without asthma and 3,703 participants with asthma, as shown in Table 1. The majority of participants were male (50.3% *vs* 55.1%, respectively), white (58.3% *vs* 58.6%, respectively), with a median income-to-poverty ratio (76.1% *vs* 75.2%, respectively), of normal birth weight (89.4% *vs* 87.3%, respectively), and with no tobacco exposure (69.7% *vs* 65.5%, respectively). The prevalence of current asthma was greater among participants aged between 3-11 years (52.9%) than among those aged between 12-19 years (50.7%). Using NHANES MEC sample weights, the analytic sample size (n = 23,841) was equivalent to a population-based sample size of 65,644,773 participants (55,246,119 without asthma and 10,398,654 with asthma). Significant differences were found in sex, race, low birth weight, prenatal maternal smoking, tobacco exposure, CRP level, and BMI Z-score between groups ($p < 0.05$).

As shown in Table 2, TC, HDL and non-HDL lipids were not associated with current asthma among children aged 3-11. In all multivariate analyses, no association was found between serum lipids and asthma after adjustment for demographic characteristics and smoking (Model 1). Addition of CRP level (Model 2), and of BMI Z-score to the analysis (Model 3) did not change the outcome (Table 3).

Univariate logistic regression showed that lower HDL [odds ratio (OR) = 1.229, 95% confidence interval (CI): 1.063 to 1.421], elevated TG (OR = 1.246, 95% CI: 1.013 to 1.533) and abnormal HOMA-IR (OR = 1.370, 95% CI: 1.077 to 1.742) were significantly associated with higher risk of asthma in adolescents. However, after adjusting for sex, race, poverty income ratio, tobacco exposure, and physical activity, the association between asthma and HDL (OR = 1.189, 95% CI: 0.992 to 1.424), TG (OR = 1.161, 95% CI: 0.908 to 1.484), and HOMA-IR (OR = 1.243, 95% CI: 0.950 to 1.628) became non-significant (Table 4). Again, no significant associations between serum lipid, glucose, HOMA-IR and asthma were found (Model 2 and Model 3).

Discussion

This study was based on the NHANES database to examine the relationships between lipids and IR with the presence of current asthma in children and adolescents. Although some associations were found in univariate analysis, after controlling for confounders, multivariate analysis found no associations between lipid levels or IR and asthma in children or adolescents. Consistent with the findings of the present cross-sectional study, two case-control studies had reported no associations of asthma with lipids and IR in adults (12,13).

The potential link between obesity, diabetes and asthma has been referred to as "metabolic asthma" (3). One hypothesis

Table 1. Demographic and basic characteristics of participants aged 3 to 19 years with and without asthma from NHANES 1999-2012 (unweighted n = 23,841; weighted n = 65,644,773[a])

	Without asthma (n = 20,138)	With asthma (n = 3,703)	p value
Sex			
Male	10048 (50.3)	2033 (55.1)	< 0.0001*
Female	10090 (49.7)	1670 (44.9)	
Race			
Mexican American	6412 (14.1)	764 (9.0)	< 0.0001*
Other Hispanic	1364 (6.5)	302 (7.1)	
White	5425 (58.3)	1051 (58.6)	
Black	5836 (14.0)	1347 (18.3)	
Other	1301 (7.1)	239 (7.1)	
Income-to-poverty ratio†			
Not poor	12263 (76.1)	2267 (75.2)	0.452
Poor	6301 (23.9)	1189 (24.8)	
Low birth weight†			
No	13380 (89.4)	2326 (87.3)	0.014*
Yes	1776 (10.6)	409 (12.7)	
Prenatal maternal smoking†			
No	12955 (83.6)	2222 (80.6)	0.003*
Yes	2015 (16.4)	478 (19.4)	
Tobacco exposure†			
No	13668 (69.7)	2338 (65.5)	0.001*
Yes	6345 (30.3)	1354 (34.5)	
Age group			
Children	9868 (52.9)	1794 (50.7)	0.061
Adolescents	10270 (47.1)	1909 (49.3)	
CRP†	0.16 ± 0.005	0.19 ± 0.014	0.004*
BMI Z-score	0.53 ± 0.013	0.74 ± 0.025	< 0.0001*
Physical activity MET score†	3574.7 ± 109.8	3701.6 ± 199.1	0.607

Data are reported as mean ± standard error, or number (weighted %).

[a]MEC sample weighting.

†There were missing data in the specific variables.

BMI: body mass index, CRP: C-reactive protein, MET: metabolic equivalent of task

is that the dysfunction of metabolic pathways that are present in obesity and diabetes exerts a direct and negative influence on the immune system, thereby affecting both adaptive and innate immunity and subsequently increasing the risk of asthma (3). Furthermore, an *in vitro* study implied that dysregulation of cholesterol transport in human airway

Table 2. Univariate logistic regression analysis of associations between current asthma and serum lipids, glucose, and homeostatic model assessment-insulin resistance

	Children, 3-11 years (n = 11,662)			Adolescents, 12-19 years (n = 12,179)		
	Proportion (%)	Prevalence of asthma (%)	Crude OR (95% CI)	Proportion (%)	Prevalence of asthma (%)	Crude OR (95%CI)
TC†						
< 170 mg/dL	61.7	14.6	Reference	66.9	16.9	Reference
≥170 mg/dL	38.3	16.4	1.153 (0.960, 1.384)	33.1	16.5	0.970 (0.827, 1.138)
HDL†						
> 45 mg/dL	71.4	14.8	Reference	63.8	15.7	Reference
≤45 mg/dL	28.6	16.4	1.128 (0.946, 1.315)	36.2	18.6	**1.229 (1.063, 1.421)**
Non-HDL†						
< 120 mg/dL	66.7	14.5	Reference	68.1	16.5	Reference
≥120 mg/dL	33.3	16.8	1.188 (0.966, 1.461)	31.9	17.3	1.052 (0.885, 1.251)
TG*						
< 90 mg/dL				60.7	16.6	Reference
≥90 mg/dL				39.3	17.1	**1.246 (1.013, 1.533)**
LDL*						
< 110 mg/dL				78.9	16.9	Reference
≥110 mg/dL				21.1	19.6	1.200 (0.933, 1.544)
FPG*						
< 100 mg/dL				84.2	17.5	Reference
≥100 mg/dL				15.8	17.0	0.967 (0.726, 1.287)
HOMA-IR*						
≤3				66.4	15.9	Reference
> 3				33.6	20.5	**1.370 (1.077, 1.742)**

†MEC sample weighting. *Fasting subsample weighting (there were around 27% of total participants with weighting data). Numbers in bold indicated statistical significance ($p < 0.05$).

FPG: fasting plasma glucose, HDL: high-density lipoprotein, HOMA-IR: homeostatic model assessment-insulin resistance, LDL: low-density lipoprotein, TC: total cholesterol, TG: triglycerides

Table 3. Multivariate logistic regression of the association between serum lipids and asthma in children (n = 11,662)

	Model 1[a]	Model 2[b]	Model 3[c]
	[a]OR (95% CI)	[a]OR (95% CI)	[a]OR (95% CI)
TC			
≥170 mg/dL *vs* < 170 mg/dL	1.145 (0.938, 1.396)	1.130 (0.884, 1.445)	1.109 (0.871, 1.412)
HDL			
≤45 mg/dL *vs* > 45 mg/dL	1.196 (0.988, 1.447)	1.112 (0.882, 1.402)	1.051 (0.821, 1.347)
Non-HDL			
≥120 mg/dL *vs* < 120 mg/dL	1.224 (0.986, 1.520)	1.149 (0.873, 1.513)	1.100 (0.839, 1.443)

[a]Adjusted for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking and tobacco exposure.

[b]Adjusted for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking, tobacco exposure and CRP.

[c]Adjusted for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking, tobacco exposure, CRP and BMI Z-score.

MEC sample weighting.

[a]OR: adjusted odds ratio, CRP: C-reactive protein, BMI: body mass index, CI: confidence interval, TC: total cholesterol, HDL: high-density lipoprotein

Table 4. Multivariate logistic regression of the association between serum lipids, glucose and homeostatic model assessment-insulin resistance and asthma in adolescents (n = 12,179)

	Model 1[a]	Model 2[b]	Model 3[c]
	[a]OR (95% CI)	[a]OR (95% CI)	[a]OR (95% CI)
TC†			
≥170 mg/dL *vs* <170 mg/dL	0.957 (0.801, 1.144)	1.048 (0.864, 1.271)	1.002 (0.830, 1.210)
HDL†			
≤45 mg/dL *vs* >45 mg/dL	1.189 (0.992,1.424)	1.179 (0.981, 1.417)	1.065 (0.874, 1.298)
Non-HDL†			
≥120 mg/dL *vs* <120 mg/dL	1.018 (0.828, 1.252)	1.097 (0.889, 1.355)	1.009 (0.812, 1.253)
TG*			
≥90 mg/dL *vs* <90 mg/dL	1.161 (0.908, 1.484)	1.055 (0.818, 1.362)	0.933 (0.710, 1.226)
LDL*			
≥110 mg/dL *vs* <120 mg/dL	1.152 (0.860, 1.542)	1.130 (0.828, 1.540)	1.049 (0.765, 1.438)
FPG*			
≥100 mg/dL *vs* <100 mg/dL	0.976 (0.683, 1.393)	1.023 (0.711, 1.473)	0.964 (0.676,1.374)
HOMA-IR*			
>3 *vs* ≤3	1.243 (0.950,1.628)	1.140 (0.861, 1.508)	0.891 (0.662, 1.198)

[a]Adjusted for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking and tobacco exposure.

[b]Adjusted for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking, tobacco exposure and CRP.

[c]Adjusted for sex, race, income-to-poverty ratio, low birth weight, prenatal maternal smoking, tobacco exposure, CRP and BMI Z-score.

†MEC sample weighting.

*Fasting subsample weighting. (there were around 27% of total participants with weighting data).

[a]OR: adjusted odds ratio, FPG: fasting plasma glucose, HDL: high-density lipoprotein, HOMA-IR: homeostatic model assessment-insulin resistance, LDL: low-density lipoprotein, TC: total cholesterol, TG: triglycerides, CI: confidence interval

smooth muscle cells may be important in the pathogenesis of asthma (14).

Yiallouros et al (16,18) have performed a series of studies examining the relationship between lipids and asthma in children and adolescents. In a cohort of 3,982 children from Cyprus, the authors found that low HDL cholesterol in childhood (11-12 years of age) was associated with the development of asthma in adolescence (age 15-17 years) (18). Utilizing a case-control design, these same authors found that adolescent asthma was associated with low serum HDL cholesterol levels independent of HDL levels in childhood (16). Furthermore, in a cohort of children from Cyprus, Yiallouros et al (28) found that two single nucleotide polymorphisms (SNPs) in different genetic loci were associated with both wheezing and HDL cholesterol levels, while the association between these two SNPs and asthma remains to be investigated.

Unlike most studies that did not distinguish lipid particles of different sizes, Scichilone et al (29) examined the associations between asthma and LDL subclasses in adults in a case-control study, and they found that asthma was associated with smaller LDL particles with a proinflammatory property. In addition, Barochia et al (30), in a case-control study, found that serum levels of large HDL_{NMR} particles are positively correlated with forced expiratory volume in 1 second (FEV_1) in adult patients with atopic asthma.

Two cross-sectional studies examined associations between obesity and lipids, respectively, with asthma based on the NHANES database (11,21). Visness et al (21) evaluated the association between obesity and atopic and non-atopic asthma in children and adolescents (aged 2-19) using the NHANES database (1999-2006). They found that obesity was significantly associated with current asthma among children and adolescents (OR = 1.68), and that the association was stronger in non-atopic (OR = 2.6) than atopic (OR = 1.34) children and adolescents (21). Moreover, Fessler et al (11) examined 7005 participants ≥6 years of age who participated in the NHANES 2005 to 2006 survey, and found that serum TC and non-HDL cholesterol were negatively associated with asthma. However, the authors noted that the association was chiefly due to the strong relationship with lipid metabolism previously found in Mexican American individuals (11).

The discussion of this topic would not be complete without drawing attention to studies that *did not* find an association between lipids and asthma. Recently, Fang

et al (17) compared the lipid profiles of obese asthmatic children with those of non-obese asthmatic children. The results showed that none of the asthmatic children had hypercholesterolemia and hypertriglyceridemia and that there were no differences in apo-A1 and apo-B between any of the BMI groups, nor were there differences in LDL levels (17). In a longitudinal study that followed children from birth to eight years of age, Murray et al (6) reported that although being overweight was associated with increased risk of allergic disease and wheezing, the strength of the association varied with sex, age and atopic phenotype.

Study Limitations

There are a number of limitations to this study that potentially may have affected the results. Like most studies examining this topic, this was a cross-sectional study and therefore temporal relations and causation cannot be determined. Body composition changes during growth and hormonal expression after puberty may influence the association between adiposity and asthma, particularly among girls. The study population is restricted to a non-institutionalized population in the NHANES database, which would likely cause under-representation of severe asthma patients who were hospitalized. Data on puberty were not available in the NHANES database. Due to the lack of certain other data, we were not able to control for other potential confounding factors such as poor asthma control, lung function and diet. Inaccurate reporting or recall bias may have occurred, because NHANES surveys are based on individual or parent interviews and questionnaires. On the other hand, data from NHANES are comprehensive and nationally representative, drawing from a large and diverse sample of participants of the population of the United States. Therefore, the findings are likely to reflect the overall United States population.

Conclusion

The results of this population-based cross-sectional study did not show an association between lipids or IR and the presence of childhood asthma. Further studies are necessary to fully understand the associations between lipids and IR and asthma.

Ethics

Ethics Committee Approval: The survey protocol and data collection methods for this present study were approved by the NHANES Institutional Review Board, and the NCHS Research Ethics Review Board (Protocol #98-12, Protocol #2005-06, and Protocol #2011-17).

Informed Consent: All of the NHANES data were de-identified and analysis of the data by independent researchers does not require IRB approval or subject informed consent.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Min Lu, Rong Qiao, Design: Min Lu, Rong Qiao, Data Collection or Processing: Xiaoyan Dong, Haoxiang Gu, Beirong Wu, Analysis or Interpretation: Min Lu, Beirong Wu, Rong Qiao, Ying Din, Literature Search: Rong Qiao, Haoxiang Gu, Writing: Min Lu, Beirong Wu, Rong Qiao, Haoxiang Gu, Ying Din, Xiaoyan Dong.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2019.0026

J Clin Res Pediatr Endocrinol 2019;11(3):278-286

The Development and Validation of a Turkish Insulin Treatment Self-management Scale Child Form (Ages 8-18) and Parent Form

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What is already known on this topic?

Personal management of insulin treatment is crucial for the success of diabetes treatment. There is currently no scale which measures insulin treatment self-management in Turkey. The absence of this kind of scale is a risk factor that may negatively affect the success of insulin therapy.

What this study adds?

Children are a very vulnerable group in terms of insulin treatment. Measuring insulin treatment self-management with a valid and reliable tool is a guide to health professionals like diabetes nurses, physician in assessing insulin treatment.

Abstract

Objective: The aim of the study was to develop an Insulin Treatment Self-management Scale; both Child Form and Parent Form for children ages 8-18 with type 1 diabetes.
Methods: Children with type 1 diabetes and their parents participated in the study. Development of a methodologically designed scale was conducted to investigate insulin treatment self-management of children with type 1 diabetes.
Results: A total of 331 children and their parents were recruited. Children and parents completed the data collection tools by themselves. The final scale had two subscales; one was related to cognitive and behavioural expressions regarding insulin treatment (self-efficacy) and the other to emotional aspects of self-maagement of insulin treatment (emotional impacts). The scale was shown to be valid and reliable.
Conclusion: This study was a valid and reliable scale for measuring insulin treatment self-management in children with type 1 diabetes. Thus can be used to assess insulin treatment self-management in children with type 1 diabetes and their parents as well as a tool for effective nursing care.
Keywords: Insulin treatment, self-management, scale development, type 1 diabetes

Introduction

Type 1 diabetes is a chronic metabolic disease caused by an autoimmune reaction to pancreatic beta cells which excrete insulin. It is characterized by absolute insulin deficiency. Type 1 diabetes usually begins during childhood or adolescence (1), mostly between the ages of 7 and 15 years. Type 1 diabetes constitutes 5-10% of all diabetic cases (2). Recently, type 1 diabetes incidence has shown a gradual increase. Worldwide, it is estimated that approximately 1,106,500 children between the ages of 0-19 (3) and 96,000 children under the age of 15 (4) live with type 1 diabetes, and that type 1 diabetes develops in 132,600 children every year (3).

The main aim of type 1 diabetes treatment is to ensure the stability of plasma insulin levels (5,6). Currently, there is no

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Conflict of interest: None declared
Received: 14.02.2019
Accepted: 20.03.2019

universally accepted insulin treatment for type 1 diabetes. Insulin treatment needs to be arranged for each child in an individualised way to provide optimal metabolic control while minimising interference with their psychosocial development (6,7).

Effective diabetes management depends on the harmony of several factors, such as insulin treatment, eating habits, exercise and personal control. Personal management of insulin treatment is crucial for its success. Patients with type 1 diabetes should have certain skills and attitudes, such as being aware of insulin types and treatment options; correct injection techniques; and the importance of giving the right dose at the right time. They should have sufficient information on insulin injection areas, absorption rates, factors affecting insulin absorption and insulin prevention conditions; understanding and overcoming the complications of insulin treatment; and arranging insulin doses according to food intake (8,9).

Teaching insulin management, which is an essential part of diabetes management, to children with type 1 diabetes and their caregivers is a fundamental part of a diabetes treatment plan. This also helps children and their parents to avoid diabetes-related complications such as hypoglycaemia or hyperglycaemia or, if such complications occur, to know how to treat them properly (8,10,11). Providing education and support to the child and parents is crucial for effective management of type 1 diabetes (11).

There do not yet exist, as far as we know, in the literature any tools to measure insulin treatment self-management levels of children with type 1 diabetes. Similarly, no tools are available for parents to evaluate their children's insulin management levels. Thus, the necessity to evaluate self-management skills regarding insulin treatment has emerged for both children and their parents. The present study was conducted in order to develop the Insulin Treatment Self-management Scale: Child Form and Parent Form for children of ages 8-18 with type 1 diabetes.

Methods

Participants

It has been suggested that, when developing a new scale or questionaire, the sample size should be 5-10 times greater than the total number of items in the scale (12,13,14). Concordantly, because the scale developed for this study included 50 items, the planned sample size was 250-500 participants. The study was thus conducted on 331 children with type 1 diabetes and their parents, as volunteer participants. The inclusion criteria for the children participants were: being followed-up on an outpatient basis; being between 8-18 years of age; having been diagnosed for a minimum of one year; using insulin; not having any other illnesses apart from diabetes; and not being hospitalised during the data collection phase. The inclusion criteria for their parents was not being under psychiatric treatment.

Procedure

Formation of an Item Pool: The item pool was primarily formed during the development of the Insulin Treatment Self-management Scale: Child Form and Parent Form. For both forms, 44 items were generated by the researchers in accordance with the literature (1,2,3,4,5,7,8,11). Items on the parent form were designed for them to evaluate their children. For instance, the item "I apply my injection as it was taught" on the child form was modified to "My child applies his/her injection as it was taught" for the parent form. Each of the items was prepared using a 5-point Likert-type scale ranging from 1 to 5, where 1 denotes "strongly disagree" and 5 "strongly agree". Scales were filled by scoring them one by one.

Content Validity: One of the logical methods to test the content validity of a study is to obtain expert opinions (15). The opinions of 14 experts were requested to assess the comprehensibility of the scale. This expert team consisted of clinicians and academic nurses focusing on diabetes. Furthermore, the content validity index (CVI) was utilised in order to prove both cultural and language equivalence and content validity in numeric values as well as a broad assessment of expert opinions (13). Experts assessed each of the items according to the Davis method (1992) (16), scoring them between 1 and 4, where 1 = not appropriate, 2 = the item should be reviewed, 3 = appropriate, but little changes needed and 4 = definitely appropriate. Following the assessment of scores by each of the experts, the items that received a 1 or 2 assessment were removed from the scale and redesigned. The CVI score is defined as 0.80 when 80% of the items score between 3 and 4. Having a score of 0.80 or above suggests appropriate content validity for the study (13). For this questionnaire, none of the items received a score of 1 or 2. Minor changes were made to the items that received a score of 3 in line with the experts' opinions. In addition, six more items recommended by experts were added to the scale, and their content validity was again tested as described.

Face Validity: Regarding scale development studies, the literature suggests that the outline of the scale should be tested with a similar sample group (17,18). Following the language and content validity, 15 children and their parents

were given the pre-application form by researchers in order to ensure necessary arrangements like complicated sentences or grammer mistakes in data collection tools to assess the face validity of the scale. Finally, the implementation phase was begun using the 50-item form.

Data Analysis

The data were analysed by Number Cruncher Statistical System 2007 (Kaysville, Utah, USA). Expert views were evaluated by CVI. The construct validity was assessed by a factor analysis. The reliability analysis of the scale was analysed as follows. Internal consistency was assessed using Cronbach's alpha coefficient and item total correlation, parallel form reliability was checked by Spearman's correlation analysis and split-half reliability was calculated. Socio-demographic data was analysed by descriptive statistical analysis [mean, standard deviation (SD) and percentage].

Data Collection

The study was carried out in İzmir and İstanbul, Turkey, in hospitals with pediatric diabetes centres, between June 2016 and December 2017. These hospitals were selected because they have high populations of paediatric diabetes. Children and parents filled out the data collection tools by themselves. Duration of data collection was recorded as minutes by researchers for each of participants separately.

Ethical Considerations

Ethical permission was obtained from the Medical Faculty Clinical Researches Ethic Committee of Marmara University (IRB no: 15.07.2016 09.2016.432). In addition, written permission was received from the participating hospitals. Participants were informed about the study, and written consent was obtained from them.

Results

Patients with Type 1 Diabetes

A total of 171 (51.7%) of the participating children were girls, and 160 (48.3%) were boys, making a total of 331 patients. For the whole group the mean ± SD chronological age was 14.25 ± 2.84 (range: 7-18) and mean ± SD age at diagnosis was 6.08 ± 4.00 (range: 1-17) years. The mean ± SD HbA1c value of the subjects was 8.92 ± 2.14. Of the parents who participated, 81.0% (n = 268) were mothers, 17.5% (n = 58) were fathers and 1.5% (n = 5) were other guardians. Data collection tools were filled out by participants in minimum 15 minutes and maximum 20 minutes.

Content Validity

The adjustments were proved for expert views on Insulin Treatment Self-management Scale Child Form and Parent Form according to Kendall's W adjustment analysis realised to ensure content validity (Kendall's $W^a_{Child\ \ Form} = 0.109$, $df = 41$, $p = 0.170$; Kendall's $W^a_{Parent\ Form} = 0.009$, $df = 43$, $p = 0.420$). The CVI, analysed via the opinions of experts according to the Davis method (1992) (16), was 0.93 for the child form and 0.94 for the parent form. Fifteen children and their parents were given the pre-application form by researchers and Cronbach's alpha coefficient for child form was 0.87 and parent form was 0.88.

Construct Validity

An exploratory factor analysis (EFA) was conducted in order to identify the structure of the scale. In addition, the Kaiser-Meyer-Olkin (KMO) test and Bartlett's test of sphericity were applied in order to determine the appropriateness of the data to the factor analysis. The KMO value was 0.889 for the child form and 0.901 for the parent form. The sphericity was statistically significant for both of the forms (child form: $\chi^2 = 4417.66$, $p < 0.001$; parent form: $\chi^2 = 4511.27$, $p < 0.001$).

For the EFA, the varimax vertical rotation technique was applied. As a result of the analysis, 10 items with an item load below 0.30 and nine items with loads from multiple factors were removed from the child and parent forms. The variant analysis showed that both of the forms had a two-factor structure. The two-factor structure explained 40.79% of the total variance for the child form and 40.82% for the parent form. For the child form, the first factor explained 26.78% of the variance, and the second one explained 14.00%. For the parent form, the first factor explained 28.73% of the variance, and the second one explained 12.09%. Scree plots present the factorial structure of the scale (Figures 1A, 1B).

Factor 1: Items 1-10, 13-17, 21, 22, 24 and 27-31 were gathered under this factor. These items include cognitive and behavioural expressions regarding insulin treatment. Thus, the factor was named self-efficacy.

Factor 2: Items 11, 12, 18-20, 23, 25 and 26 were gathered under this factor. These items include negative emotional expressions. Thus, the factor was named emotional impacts.

The item loads ranged from 0.42 to 0.83 for the child form and from 0.40 to 0.80 for the parent form. The findings obtained from the EFA are presented in Table 1.

Reliability

Item total correlation, inner consistency reliability, split-half test and parallel test techniques were utilised in order to test the reliability of the scale. The item total correlation for the Insulin Treatment Self-management Scale ranged from 0.21 to 0.58 for the child form and from 0.25 to 0.64 for the parent form (Table 1). In order to determine the inner consistency reliability of the scale, Cronbach's alpha was used. To determine two halves reliability, Spearman-Brown and Guttman split-half coefficients were calculated. These values are presented in Table 2. The correlation between the child and parent forms was examined for parallel test reliability, and the results are presented in Table 3.

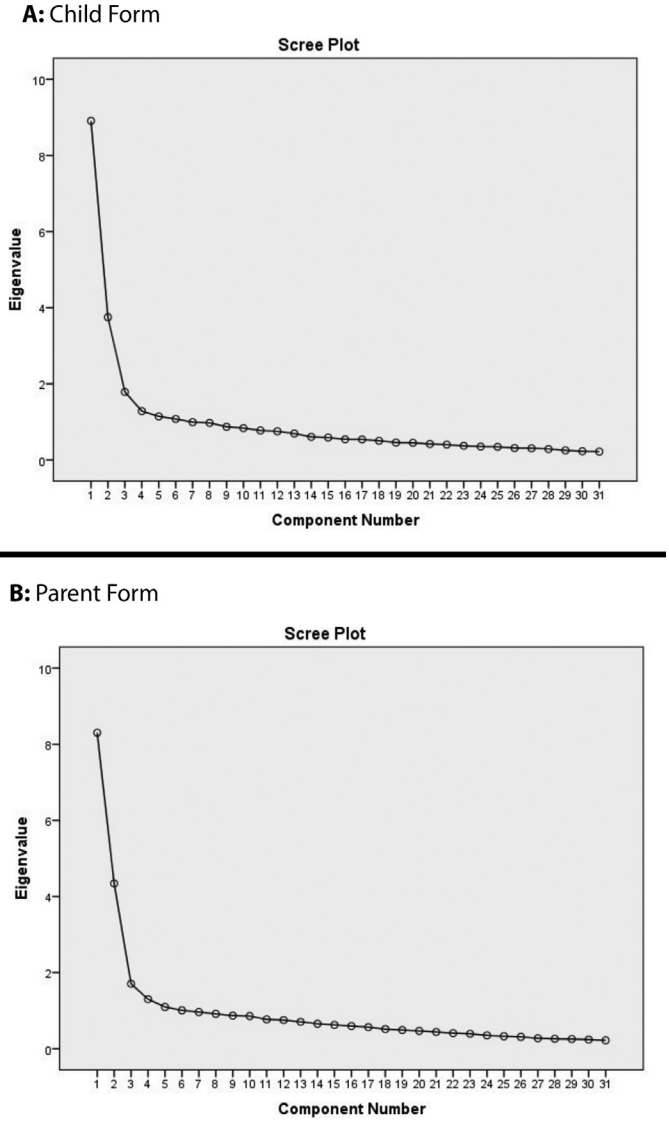

Figure 1. A, B). The scree plots present the factorial structure of the scale

Scoring the Insulin Self-management Scale

The 5-point Likert-type scale includes 31 items and two sub-groups. The first factor consists of 23 items. The minimum score for this factor was 23 and the maximum was 115. Higher scores imply a higher level of self-efficacy. The second factor consists of eight items with a minimum score of eight and a maximum score of 40. The items for this factor have a reverse scoring. After reverse scoring, higher scores indicate the respondent's positive feelings towards insulin management.

The overall score of the scale ranged from 31 (minimum) to 155 (maximum). Higher scores indicated a higher level of self-management regarding insulin treatment.

Discussion

Development of the scale began by searching for similar studies in the literature. However, no specific scales for measuring the self-management skills of children with type 1 diabetes were found in either our country or in others. This scale is important for identifying insulin treatment self-management skills as well as nursing care and self-management needs and to attain a desired level by the diabetes nurses. It is also important for developing individualised education programmes.

This methodologically designed scale was created to identify insulin treatment self-management for children with type 1 diabetes. A newly-developed scale should meet two important criteria: validity and reliability. Validity refers to how well a scientific test or a scale actually measures what it sets out to or how well it reflects the reality it claims to represent. Thus, if a scale correctly measures what it sets out to without interfering with other factors, then that scale can be accepted as valid (16). A valid scale should be reliable. Reliability is defined as the consistency between participants' responses to the scale's items (15). Content and construct validity were utilised in our study to test the reliability.

Content Validity

Content validity is the indicator of how sufficiently the items qualitatively and quantitatively measure the intended behaviour (15,19). According to the results of Kendall's W adjustment analysis, no significant differences were detected between the experts' opinions of the scale. Such a result shows that the items were understood similarly by the experts. Thus, the scale to measure insulin self-management skills was comprehensible.

Construct Validity

Construct validity refers to the degree to which a test measures a discrete concept in terms of desired behaviours.

Table 1. Characteristics of subscales of the Insulin Treatment Self-management Scale: Child Form and Parent Form (n = 331)

Child Form	Parent Form	Child Form		Parent Form	
		Factor load	Item to total correlations	Factor load	Item to total correlations
Factor 1: Self-efficacy					
1. I apply my insulin injection at the recommended time	My child applies his/her insulin injection at the recommended time	0.64	0.53	0.66	0.52
2. I apply my insulin injection as I was taught	My child applies his/her insulin injection as he/she was taught	0.70	0.51	0.69	0.51
3. Diabetes education is important for insulin treatment	Diabetes education is important for my child's insulin treatment	0.58	0.35	0.60	0.44
4. Keeping insulin under suitable conditions is important	Keeping insulin under suitable conditions is important for my child	0.52	0.33	0.59	0.43
5. Insulin treatment keeps blood glucose at normal levels	My child knows that insulin treatment keeps blood glucose at normal levels	0.64	0.40	0.70	0.54
6. I preserve insulin by storing it in the fridge	My child preserves insulin by storing it in the fridge	0.42	0.27	0.70	0.53
7. I can adjust my insulin dose according to my blood-glucose result	My child can adjust the insulin dose according to his/her blood-glucose result	0.66	0.44	0.66	0.44
8. I increase or reduce my insulin dose when I do sports	My child increases or reduces the insulin dose when he/she does sports	0.42	0.42	0.41	0.32
9. Being able to adjust the insulin dose according to my blood-glucose result is important	Being able to adjust the insulin dose according to blood-glucose results is important for my child	0.62	0.36	0.79	0.64
10. I am aware of what could possibly happen if I apply my insulin dose incorrectly	My child is aware of what can happen if he/she applies the insulin dose incorrectly	0.55	0.41	0.73	0.57
13. I know the problems that result from insulin injection (hypoglycaemia, swelling of injection areas, etc.)	My child knows the problems that result from insulin injection (hypoglycaemia, swelling of injection areas, etc.)	0.69	0.54	0.60	0.52
14. I know what I have to do in order to prevent hypoglycaemia from occurring as a result of insulin injection	My child knows what she/he has to do in order to prevent hypoglycaemia from occurring as a result of insulin injection	0.72	0.48	0.80	0.63
15. I know what I have to do to prevent lipohypertrophy/lipoatrophy (swelling/sinking in the injection area)	My child knows what she/he has to do to prevent lipohypertrophy/lipoatrophy (swelling/sinking in the injection area)	0.51	0.46	0.68	0.60
16. I know how much additional insulin I need to use in a state of severe hyperglycaemia	My child knows how much additional insulin he/she has to use in a state of severe hyperglycaemia	0.59	0.35	0.54	0.31
17. I know how much additional insulin I need to use when ketone is seen in my urine	My child knows how much additional insulin he/she has to use when ketone is seen in his/her urine	0.49	0.36	0.42	0.30
21. It is important to apply the insulin injection in a different area every time	It is important for my child to apply the insulin injection in a different area every time	0.70	0.50	0.60	0.44
22. I use my insulin injection needle tips only once	My child uses his/her insulin injection needle tips only once	0.57	0.45	0.54	0.43
24. Insulin injection areas must be controlled regularly	My child knows that insulin injection areas must be controlled regularly	0.58	0.49	0.58	0.49
27. I know that insulin absorption differs in different areas (arm, leg, hips and around the belly)	My child knows that insulin absorption differs in different areas (arm, leg, hips and around the belly)	0.59	0.42	0.61	0.50
28. I pay special attention to applying insulin around the belly since it is absorbed faster there	My child pays special attention to applying insulin around the belly since it is absorbed faster there	0.54	0.43	0.40	0.28

Table 1. Characteristics of subscales of the Insulin Treatment Self-management Scale: Child Form and Parent Form (n = 331) (Continued)

Child Form	Parent Form	Child Form		Parent Form	
		Factor load	Item to total correlations	Factor load	Item to total correlations
Factor 1: Self-efficacy					
29. I know the onset, peak and duration of action differ depending on the type of insulin (short-acting insulin, fast-acting insulin, mid-acting insulin, long-acting insulin and ready-made insulin mixtures)	My child knows the onset, peak and duration of action differ depending on the type of insulin (short-acting insulin, fast-acting insulin, mid-acting insulin, long-acting insulin and ready-made insulin mixtures)	0.48	0.41	0.43	0.40
30. I know it is necessary to keep glucagon handy at home in case of severe hypoglycaemia	My child knows it is necessary to keep glucagon handy at home in case of severe hypoglycaemia.	0.76	0.58	0.64	0.56
31. I know it is necessary to keep glucagon handy at school in case of severe hypoglycaemia	My child knows it is necessary to keep glucagon handy at school in case of severe hypoglycaemia	0.59	0.52	0.41	0.40
Factor 2: Emotional Impacts					
11. I want to be alone while injecting insulin	My child wants to be alone while injecting insulin	0.43	0.21	0.48	0.25
12. Injecting insulin causes pain	To my child, injecting insulin causes pain	0.67	0.31	0.70	0.44
18. Insulin causes weight gain	My child believes that insulin causes weight gain	0.70	0.33	0.63	0.43
19. Insulin injection interferes with my fun activities	Insulin injection interferes with his/her fun activities	0.83	0.36	0.78	0.41
20. I am unhappy that I must use insulin.	My child is unhappy that he/she must use insulin	0.56	0.29	0.64	0.27
23. Insulin injection ruins the shape of my body	My child thinks that insulin injection ruins the shape of his/her body	0.79	0.36	0.71	0.47
25. Insulin injection makes the fulfilment of my responsibilities both at home and at school more difficult	Insulin injection makes the fulfilment of his/her responsibilities both at home and at school more difficult	0.81	0.30	0.75	0.35
26. It is difficult for me to inject insulin at the right time or place every day	It is difficult for my child to inject insulin at the right time or place every day	0.71	0.30	0.67	0.27

Table 2. Cronbach's alpha and Split-Half Test Reliability Results for the Insulin Treatment Self-Management Scale: Child Form and Parent Form

Subscales	Items	Child Form			Parent Form		
		Cronbach's alpha coefficient	Split-half test reliability		Cronbach's alpha coefficient	Split-half test reliability	
			Spearman-Brown coefficient	Guttman Split-half coefficient		Spearman-Brown coefficient	Guttman Split-half coefficient
Factor 1	23	0.91	0.83	0.83	0.91	0.84	0.84
Factor 2	8	0.85	0.85	0.85	0.83	0.81	0.81
Total Scale	31	0.86	0.70	0.70	0.88	0.75	0.75

One of the techniques to test construct validity is factor analysis (15). The EFA is a technique to determine the number of sub-groups of items in a scale as well as the relation between them (17,20). The EFA was used to test the construct validity of the scale. However, prior to the EFA, the KMO test and Bartlett's test of sphericity were utilised in order to determine whether the number of samples was sufficient and if there was a desired level of relation between the variables. The KMO test is an index comparing observed correlation coefficients with partial correlation coefficients. The KMO values range between 0 and 1, and a value >0.80 is expected for a successful factor analysis. In Bartlett's test of sphericity, having a $p<0.05$ indicates an appropriate level of relation among

Table 3. Intercorrelation (Parallel Form reliability) between the Parent and Child Forms for the Insulin Treatment Self-management Scale (n = 331)

		Parent Form					
		Factor 1		Factor 2		Scale total score	
		r	p	r	p	r	p
Child Form	Factor 1	0.51	**0.000**	-0.60	0.275	0.30	**0.000**
	Factor 2	0.17	**0.001**	0.76	**0.000**	0.65	**0.000**
	Scale total score	0.50	**0.000**	0.54	**0.000**	0.71	**0.000**

r: Spearman's correlation coefficient, $p < 0.01$

variables for a factor analysis (21). It has been reported that a KMO value > 0.50 is enough to realize a factor analysis (13,15). In the present study, the KMO value for the Insulin Treatment Self-management Scale was 0.88 for the child form and 0.90 for the parent form, indicating its suitability for factor analysis. Furthermore, for Bartlett's test of sphericity, the p value was significant for both the child ($p < 0.001$) and parent ($p < 0.001$) forms, indicating that the correlation matrix for the items in the scale is suitable for the factor analysis.

The eigenvalue of items in the factor analysis should be at least 1.00, and the item factor load value should be at least 0.30 with a difference of at least 0.20 between items to have enough factor load between two different factors (20). The result of the factor analysis was 2.00. The scree plots present the factorial structure of the scale (Figures 1A, 1B). According to the scree plot, the distance between two points is accepted one factor and following the second factor the distance between two points was both little and similar (20) so that the scale was accepted as possessing two-factors. It is not recommended to have a factor load below 0.30 (21). Regarding the factor load, 0.71 and above is accepted as perfect, 0.63 is very good, 0.55 is good, 0.45 is acceptable and 0.32 is weak (22). In our study, the factor loads were high (Table 1), which confirmed the structure of the scale was appropriate.

Reliability

Reliability is the extent to which a scale measures what it sets out to measure (15,18,23,24). Reliability emphasises consistency (a factor affecting the validity) that does not change with time. Although a valid test is always reliable, a reliable test is not always valid (15,18).

The reliability of the Insulin Treatment Self-management Scale was tested through internal consistency, the split-half test and item total score correlation techniques. Internal consistency refers to the extent to which characteristics and mean behaviours are similar to each other (15). One of the most common methods to test reliability is Cronbach's alpha. A Cronbach's alpha coefficient of $0.00 < \alpha < 0.40$ shows that the scale is not reliable, $0.40 < \alpha < 0.60$ indicates low reliability, $0.60 < \alpha < 0.80$ indicates reliability and $0.80 < \alpha < 1.00$ shows high reliability (25,26). For our scale, the Cronbach's alpha was 0.86 for the child form and 0.88 for the parent form, indicating high reliability.

Item total correlation explains the relation between the scores obtained from each of the test items and the total score. A higher item total score correlation indicates a higher level of internal consistency and similar sampling behaviours. It has been suggested that items with a score of 0.30 and greater differentiate the participants quite well and should be kept, 0.20-0.30 might be removed and below 0.20 should be removed from the scale (15). For Items 6, 11 and 20 on the child form and 11, 20, 26 and 28 on the parent form, the total score correlations were between 0.20 and 0.30. However, the factor loads for these items were between 0.40 and 0.64, so they were retained in the scale.

One of the most common ways to test the reliability of a scale is to use the split-half test technique. Split-half test reliability refers to the correlation coefficient calculated for the overall scale in that test items' being separated into two halves and by using the correlations of these two halves with Spearman-Brown formulas and Guttman split-half formulas (15). Having a reliability coefficient of 0.70 or higher indicates a reliable measurement for the scale (17,20). In our study, the Spearman-Brown split-half test correlation was 0.70 for the child form and 0.75 for the parent form. The Guttman split-half coefficient was 0.70 for the child form and 0.75 for the parent form (Table 2). These reliability coefficients show that both forms have reliable measurements.

In order to ensure the reliability of the parallel forms, the correlation between the child form and parent form was examined. The correlation coefficients used were the Pearson correlation coefficient and Spearman's correlation coefficient (27). Both the Pearson correlation coefficient and Spearman's correlation coefficient (r) measure the strength of the linear association between

variables. The value of a correlation ranges between -1 and + 1. Negative values indicate a negative linear relation and positive values indicate a positive linear relation. Both the Pearson correlation coefficient and Spearman's correlation coefficient are interpreted as follows: 0.00 = no correlation, 0.01-0.29 = lower-level correlation, 0.30-0.70 = mid-level correlation, 0.71-0.99 = high-level correlation and 1.00 = perfect correlation (28). For this measurement, a high-level positive correlation was found between the two scales ($r = 0.71$, $p < 0.001$) (Table 3).

Study Limitations

In this study, we were unable to determine the test-retest reliability due to time limitations.

Conclusion

There is strong evidence that the psychometric characteristics of the scale are valid and reliable. In this study, a valid and reliable scale was developed in order to measure insulin treatment self-management of children with type 1 diabetes and their parents In addition, since there is no similar scale in the literature, it could be used in future studies on this issue.

Acknowledgement

We would like to thank all of the children and their parents for participating in the study.

Ethics

Ethics Committee Approval: Ethical permission was obtained from the Medical Faculty Clinical Researches Ethic Committee of Marmara University (IRB no: 15.07.2016 09.2016.432).

Informed Consent: Participants were informed about the study, and written consent was obtained from them.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Çağrı Çövener Özçelik, Design: Çağrı Çövener Özçelik, Eda Aktaş, Gülten Karahan Okuroğlu, Data Collection or Processing: Çağrı Çövener Özçelik, Nesrin Şen Celasin, Şükriye Şahin, Analysis or Interpretation: Çağrı Çövener Özçelik, Gülten Karahan Okuroğlu, Literature Search: Çağrı Çövener Özçelik, Eda Aktaş, Nesrin Şen Celasin, Writing: Çağrı Çövener Özçelik, Eda Aktaş, Nesrin Şen Celasin.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2018.0293

J Clin Res Pediatr Endocrinol 2019;11(3):287-292

Antimüllerian Hormone Levels of Infants with Premature Thelarche

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What is already known on this topic?

Antimüllerian hormone (AMH) levels during mini puberty are higher than those of the prepubertal period. AMH inhibits both initial follicle recruitment and follicle-stimulating hormone (FSH)-dependent follicle growth. Therefore the rising levels of AMH during mini-puberty may be an ovarian response to prevent FSH-induced follicle growth. It has been proposed that premature thelarche (PT) in infants results from transient, partial activation of the hypothalamic-pituitary-ovarian axis with excessive secretion of FSH.

What this study adds?

This is the first study to investigate AMH concentrations in infants with PT. It was found that AMH concentrations in infants with PT were lower when compared to healthy controls and a negative correlation between AMH and FSH was identified. It was concluded that a decreased level of AMH may cause PT in infants.

Abstract

Objective: Antimüllerian hormone (AMH) concentrations in mini puberty are higher than those reported for the prepubertal period. In this study we investigated AMH concentrations in infants with premature thelarche (PT). A healthy control group was used for comparison.

Methods: Forty five female infants with PT, aged between one and three years and a control group consisting of 37 healthy girls in the same age range were included in the study. Bone age, pelvic ultrasonography, and concentrations of luteinizing hormone, follicle-stimulating hormone (FSH), estradiol and AMH of the patient group were evaluated. Only serum AMH concentration of the control group was evaluated.

Results: Median (range) serum AMH concentrations in the subjects were 1.66 ng/mL (11.85 pmol/L) [0.15-6.32 ng/mL (1.07-45.12 pmol/L)] and were significantly lower ($p = 0.025$) than for the control group; 1.96 ng/mL (13.99 pmol/L) [0.60-8.49 ng/mL (4.28-60.64 pmol/L)]. AMH and FSH were negatively correlated ($r = -0.360$, $p = 0.015$) in infants with PT. There was no correlation between AMH and uterine size, uterine volume, endometrial thickness, fundocervical ratio, ovarian size or volume, follicle size and follicle number.

Conclusion: This is the first study that investigates AMH concentrations in infants with PT. The low AMH levels in these infants and the negative correlation between AMH and FSH suggests that AMH may play a role in suppressing pubertal findings during infancy and that decreased AMH may cause PT in infancy.

Keywords: AMH, premature thelarche, infancy, mini-puberty

Introduction

Mini-puberty of infancy refers to the transient activation of the hypothalamic-pituitary-gonadal (HPG) axis during the first few months of life. The follicle-stimulating hormone (FSH) concentration in girls decreases at delivery and increases again with activation of the HPG axis. Activation of the HPG axis reaches a peak at six to eight weeks after delivery. In this period, the mini-puberty, the levels of sex steroids are similar to early-middle pubertal levels but their peripheral effects are not evident. Increased FSH in girls continues until the age of between two and four years, although estradiol is elevated from the second to the fourth month after delivery (1).

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Conflict of interest: None declared
Received: 13.12.2018
Accepted: 08.03.2019

Premature thelarche (PT) refers to the precocious appearance of breast development in girls with no other signs of sexual maturation. It is mostly encountered during the first two years of life. PT has been postulated to result from transient, partial activation of the HPG axis with excessive secretion of FSH. The physiologic baseline event in PT is the increase in FSH level (2).

In females, antimüllerian hormone (AMH) is produced by the granulosa cells of primary, preantral and early antral follicles (3). AMH has at least two functions during follicular development. First, AMH plays an inhibitory role during initial recruitment, when resting primordial follicles are initiated to grow, and second, it may modify preantral and small antral follicle growth by decreasing the FSH responsiveness of the follicle. The second effect is important during cyclic recruitment, when some large preantral and small antral follicles are recruited to grow on to the preovulatory follicle stage (4,5,6).

AMH levels increase during infancy, but remain stable from childhood to early adulthood (7,8). In a recent study, it was reported that, after mini-puberty, AMH concentrations decreased by 30% during the first two years of life (9). It has also been reported that AMH concentrations in patients with central precocious puberty (CPP) were lower than those in the PT group and that there was a negative correlation between AMH and basal gonadotropin levels (10). These findings support the view that AMH may play a role in suppressing puberty. High concentrations of AMH in the mini-puberty period, when the peripheral effects of hormones are not usually observed despite the presence of hormonal values similar to those found during the pubertal period, supports the view that AMH may have a suppressive effect.

The aim of this study was to investigate AMH levels in infants with PT who are presumed to have a prolonged mini-puberty due to inadequate and/or late suppression of pubertal activation. We hypothesized that, the AMH mediated ovarian response which may prevent FSH-induced follicle growth is deficient in infants with PT.

Methods

Forty five consecutive girls, aged between one and three years, who had been admitted to our hospital between July 2015 and September 2016 and who had PT, defined as breast development with no other signs of sexual maturation or bone age (BA) advancement, were included in the study. All parents received oral and written information before signing a consent form. The study was approved by the Local Ethical Committee (Zekai Tahir Burak Training and Research Hospital, no: 44/2015). Exclusion criteria were: central and peripheral precocious puberty; thyroid disorder; intake of any medication; acute or chronic disease; and small for gestational age.

Pubertal staging was performed according to the method of Marshall and Tanner (11) by the same pediatric endocrinologist in all subjects. If breast stages differed between the two breasts, the more advanced stage was assumed to represent breast development stage. All patients presented with breast budding as the only sign of puberty. Standard deviation (SD) scores (SDS) for height, weight and relative weight were calculated using national reference data (12). BA was evaluated using the Greulich and Pyle method by the same endocrinologist (13).

In all subjects, blood sampling was performed at 8:00 am, via an intravenous cannula inserted into an antecubital vein. The blood samples were drawn into standard vacuum tubes. For the AMH assay, samples were centrifuged (3000 × g for 10 min) within 30 minutes of drawing and serum was analysed immediately. AMH was measured by an enzyme immunoassay method (Anshlab AMH/MIS ELISA kit, Webster, Texas, USA). Luteinizing hormone (LH), FSH and estradiol were measured using a chemiluminescence method (Advia Centaur XP, Siemens AG, Munich, Germany).

All patients were prospectively examined by pelvic ultrasonography (US) performed by the same experienced pediatric radiologist, who was blinded to their clinical and laboratory findings. US was performed using a Logiq 6 US scanner (General Electric Co. Milwaukee, WI, USA) and a 7.5-MHz linear-array small parts transducer. Patients and controls were scanned several times until images with a full bladder could be captured. Foley catheterization was not performed in any of the participants. The three dimensions of the uterus, endometrial thickness, and the three dimensions of each ovary were measured. The fundocervical ratio was assessed as >1 or ≤ 1 as a simple and fast expression of uterine maturation. Endometrial echogenicity was checked with the uterus scanned in the sagittal plane. Ovarian volume, as cubic centimeters, was calculated using the ellipsoid formula (longitudinal dimension × AP dimension × transverse dimension × 0.52).

As the control group, 37 healthy, age-matched Tanner stage 1 infants were included in the study. SDS for height, weight and relative weight were calculated and AMH concentrations were measured in the control group.

Statistical Analysis

The results of tests were expressed as the number of observations (n), mean ± SD, median and range or median

and interquartile range as appropriate. The results of the homogeneity (Levene's test) and normality tests (Shapiro-Wilk) were used to decide which statistical methods to apply in the comparison of the study groups. According to these test results, parametric test assumptions were not suitable for variables, so the comparisons of two independent groups were performed by using Mann-Whitney U test. A statistical significance level of $p < 0.05$ was considered significant. Pearson's correlation coefficient test was used for data with parametric test preconditions to determine the relationship between two continuous variables. In parametric test for variables that do not meet the pre-conditions, the Spearman correlation coefficient was used. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) software, version 17 (IBM Inc., Chicago, IL, USA).

Results

The anthropometric data of the patient and the control groups are given in Table 1. The mean ± SD chronologic age (CA) of the patients was 1.76 ± 0.54 (median 1.70; range 1-3) years. The mean ± SD age of onset of breast development was 8.19 ± 6.76 (median 8; range 0.8-22) months. The mean ± SD duration of breast budding was 12.39 ± 10.29 (median 10; range 1-34) months and the patient's breast stages ranged from stage 2 to stage 3. The mean ± SD CA and BA difference was 0.1 ± 0.34 years (median 0.15; range -0.6 to + 0.7). In the follow-up period (median 9 months; range 6-18 months), no pubertal progression was found in any patient. Serum AMH concentrations of PT subjects [median 1.66 ng/mL (11.85 pmol/L); range 0.15-6.32 ng/mL (1.07-45.12 pmol/L)] were significantly lower ($p = 0.025$) than those of the control group [median 1.96 ng/mL (13.99 pmol/L); range 0.60-8.49 ng/mL (4.28-60.64 pmol/L)] (Table 1). When the patients were grouped by breast stage, there was no significant difference between their AMH levels ($p = 0.585$). AMH was not correlated with CA and BA. There was no relationship between AMH and CA-BA difference.

Laboratory and pelvic US findings of the infants with PT are given in Table 2. AMH and FSH were negatively correlated ($r = -0.360$; $p = 0.015$) in infants with PT (Figure 1). The correlation between FSH and AMH levels was significant after controlling for the effect of age ($r = -0.366$; $p = 0.014$). No correlation was found between AMH and LH and only four (7.3%) patients had a baseline LH concentration above the measurement limit, as expected. No correlation was found between AMH and estradiol concentration because estradiol concentrations were above the measurement limit in only 16 (35.5%) patients. No correlation was found between AMH and LH concentrations. Uterine length was greater than 34 mm in only two patients and uterine volume was above 2 mL in five patients. Endometrial echo

Table 1. Anthropometric properties of infants with premature thelarche and the control group

	Premature thelarche (n = 45)			Controls (n = 37)			
	Median	IQR	25-75p	Median	IQR	25-75p	p
Chronological age (years)	1.70	0.75	1.35 - 2.1	1.90	1.05	1.35 - 2.5	0.127
Height (cm)	83.20	7.6	80.40 - 88.05	84.60	10.5	79.25 - 89.75	0.621
Height SDS	0.16	1.34	-0.56 - + 0.79	-0.40	1.18	-1.13 - + 0.05	0.012
Weight (kg)	11.50	1.92	10.65 - 12.58	11.10	3.05	9.90 - 12.95	0.518
Weight SDS	0.21	1.47	-0.46 - + 1.02	-0.34	1.49	-1.19 - + 0.30	0.021
Relative weight (%)	103	15.5	96 - 111.5	99	12.5	94 - 106.5	0.08
AMH (ng/mL)	1.66	1.65	1.03 - 2.67	1.96	2.21	1.54 - 3.76	0.025

Mann-Whitney U test

AMH: antimüllerian hormone, IQR: interquartile range, SDS: standard deviation scores

Table 2. Laboratory and ultrasound findings of infants with premature thelarche

	Median	IQR	25-75p
Follicle-stimulating hormone (IU/L)	3.39	2.86	2.54 - 5.37
Luteinizing hormone (IU/L)	0.07	0.001	0.07 - 0.07
Estradiol (pg/mL)	11.80	4.98	11.8 - 15.56
Uterine length (mm)	27.0	6.5	22.5 - 29.5
Uterine volume (mL)	1.17	0.61	0.79 - 1.54
Total ovarian volume (mL)	1.40	1.21	0.66 - 1.87

IQR: interquartile range

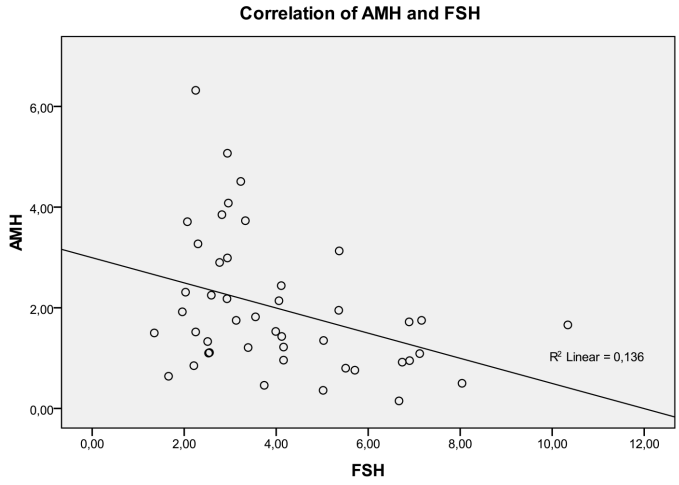

Figure 1. Correlation between antimüllerian hormone and follicle-stimulating hormone levels

AMH: antimüllerian hormone, FSH: follicle-stimulating hormone

was detected in four patients. There were six patients with a fundocervical ratio above one. There was no correlation between AMH levels and uterine size, uterine volume, endometrial thickness, fundocervical ratio, ovarian size or volume, follicle size and follicle number.

Discussion

This is the first study investigating AMH concentrations in infants with PT. In this study, serum AMH levels in infants with PT were significantly lower than those of healthy girls and a negative correlation between FSH and AMH was detected. Evidence in the literature suggests that AMH concentrations increase gradually from three years before pubertal onset to the beginning of puberty and then decrease by approximately 30% during the following two years (9,14,15). In a recent study, it was reported that the AMH levels of patients with CPP were lower than AMH concentrations in PT patients, aged between 4.5-8 years and that a negative correlation existed between AMH and basal and stimulated gonadotropin levels (10). AMH plays an inhibitory role during initial recruitment, when resting primordial follicles are initiated to grow and at the same time AMH decreases the sensitivity of primordial follicles to FSH and inhibits granulosa cell aromatase, which results in a decreased chance of the follicle moving towards cyclic recruitment and estrogen biosynthesis (4,5,6). The decrease in AMH concentration during the activation of the hypothalamus-pituitary-ovarian axis causes cyclic follicle recruitment. Partial activation of the hypothalamus-pituitary-ovarian axis, during so-called mini puberty, in the first months of life in girls has been demonstrated. An increase in AMH concentrations from birth to three months of age has also been reported (7). As AMH inhibits both initial follicle recruitment (primordial to primary follicles) and FSH-dependent follicular growth (preantral and antral follicles), these authors suggested that rising levels of AMH during mini puberty may be an ovarian response to prevent FSH-induced follicular growth at a time of life when further differentiation of follicles would be inappropriate. Elevated AMH concentrations might prevent the progress of puberty.

PT is a condition seen during the period of mini-puberty and it has been postulated to result from prolonged mini-puberty due to inadequate and/or late suppression of pubertal activation (1,2). In this present study, detection of somewhat lower AMH concentrations in infants with PT and a negative correlation between FSH and AMH are findings which support the hypothesis that an AMH-related ovarian response which inhibits FSH-induced follicular growth is deficient in infants with PT (power of test = 45%). However, the role of AMH in the pathogenesis of PT is not well clarified. It is known that many factors have complex interactions during the mini-puberty period. The findings of this present study support our proposition that AMH may play a role in this complex interaction.

Results reported from five other studies investigating AMH in early pubertal development are controversial (Table 3) (10,16,17,18,19). In one study, the number of subjects was very limited and no comparison was made with healthy controls (16). In a second study, the groups consisted of girls of ages 4-8 years and the AMH concentrations in patients with CPP were found to be lower than those of the PT group; results consistent with the results of this present study (10). In another study, compared with slowly progressive CPP, girls with more rapidly progressive CPP were reported to have lower AMH concentrations (18). This result also supports the proposition that pubertal progression is associated with decreased AMH concentrations. In another study, however, no difference in AMH concentrations was reported between CPP patients and the control group. However, in this study the patients were older, with more advanced pubertal stages (17). Thus, it was not possible to determine if the decrease in AMH reported at the onset of puberty was due to advanced pubertal stage. In another recent study, serum AMH concentrations in girls with PT were found to be higher than those of prepubertal girls. However, in this study the age of the control group was significantly different to that of the PT group, making a comparison inappropriate (19).

Pelvic ultrasound might be useful for diagnosis of precocious puberty. However no significant differences in uterine and ovarian ultrasound measurements were detected between

Table 3. Reported antimüllerian hormone levels in early pubertal development

	Hagen et al (16)	Sahin et al (10)	Nam et al (17)	Savas-Erdeve et al (19)	Chen et al (18)	Present study
	Median (min-max)	Median (min-max)	Mean ± SD	Mean ± SD	Median (min-max)	Median (min-max)
Controls						
n	-	25	55	22	20	49
Age (years)		8.1 (2.7-10)	9.4 ± 0.5[b]	6.52 ± 1.10[a]	6.7 (5.4-7.9)	1.6 ± 0.8 (0.4-3)
FSH		NA	2.30 ± 1.30[b]	2.21 ± 1.70	0.85 (< 0.1-2.13)	NA
LH		NA	0.30 ± 0.40	0.09 ± 0.78	< 0.07 (< 0.07-0.09)	NA
AMH (ng/mL)		1.81 (0.07-8.53)[a,b]	5.40 ± 3.70	2.10 ± 0.85[a]	2.14 (0.79-4.14)	2.46 (0.60-8.49)[b]
PT						
n	-	37	-	24	65	55
Age (years)		7.6 (4.7-8)		7.16 ± 0.62[b]	6.75 (5.2-8.0)	1.6 ± 0.7 (0.3-3)
FSH (mU/mL)		1.4 (0.23-3.88)[a]		1.84 ± 1.45	2.37 (< 0.1-5.4)	4.32 ± 2.26
LH (mU/mL)		0.05 (0-0.58)[a]		0.10 ± 0.64	< 0.07 (< 0.07-0.26)	0.10 ± 0.16
AMH (ng/mL)		2.39 (0.77-8.88)[a]		3.70 ± 3.00[b]	NA	1.66 (0.15-7.28)[a]
CPP					**Progressive CPP**	**Slowly progressive CPP**
n	15	37	98	21	55	28	-
Age (years)	7.7 (7.2-8.6)	8.0 (4.5-8)	8.4 ± 0.5[a]	7.45 ± 0.87[b]	6.5 (5.0-8.0)	6.75 (5.25-8.0)	
FSH (mU/mL)	NA	2.46 (1.05-9.31)[b]	3.50 ± 2.50[a]	2.61 ± 1.45	2.63 (0.74-7.35)	2.42 (0.79-5.46)	
LH (mU/mL)	NA	0.11 (0.04-4.79)[b]	0.40 ± 0.60	0.28 ± 0.33	0.33 (0.11-1.92)	0.25 (0.12-0.41)	
AMH (ng/mL)	2.84 (0.28-4.20)	1.55 (0.48-5.52)[b]	5.90 ± 3.60	2.70 ± 1.61[a,b]	2.82 (1.04-6.16)[a]	5.39 (1.94-11.15)[b]	

PT: premature thelarche, CPP: central precocious puberty, NA: non assessment, min: minimum, max: maximum, SD: standard deviation, FSH: follicle-stimulating hormone, LH: luteinizing hormone, AMH: antimüllerian hormone

[a,b]The difference between the group means with different letters significant ($p < 0.05$)

children with PT and controls (2). In a recent study, AMH was reported to be proportional to the number of small (2-3 mm) and medium (4-6 mm) follicles. Thus, in early puberty (Tanner breast stage 1-3), the number of AMH-producing follicles (2-6 mm) correlated positively with pubertal stages, whereas AMH levels were unaffected (20). In our study, ultrasound findings being prepubertal in the great majority of our patients, as expected, we were not able to determine a correlation between AMH and pelvic ultrasound findings.

Study Limitations

The study and control groups were composed of infants. Thus, due to ethical reasons, only AMH concentrations were measured in the control group. Although a negative correlation between AMH and FSH was found in the PT group, the relationship between FSH and AMH in the controls would have allowed comparison with the PT group if FSH concentrations had also been available from the control group.

Conclusion

In conclusion, AMH may play a role in suppressing pubertal findings during infancy. This effect of AMH might be due to the decrease in the sensitivity of primordial follicles to FSH and inhibition of granulosa cell aromatase which results in a decreased chance for the follicle to move toward cyclic recruitment and estrogen biosynthesis. Decreased AMH may cause PT in infants. In this present study, AMH concentrations in infants with PT were significantly lower than those found in healthy controls of the same age. A negative correlation was also found between AMH and FSH. Although our findings support this hypothesis, the opposite hypothesis, namely, that an excessive activation of the ovary results in lower AMH production cannot be completely excluded and the influence of other factors involved in mini-puberty cannot be ruled out. The cause of somewhat lower AMH concentrations and the role of AMH in the etiopathogenesis of PT should be clarified by further studies evaluating AMH levels in mini-puberty and related disorders in infancy.

Ethics

Ethics Committee Approval: The study was approved by the Local Ethical Committee (Zekai Tahir Burak Training and Research Hospital, no: 44/2015).

Informed Consent: All parents received oral and written information before signing a consent form.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Nursel Muratoğlu Şahin, Elvan Bayramoğlu, Hatice Nursun Özcan, Erdal Kurnaz, Melikşah Keskin, Şenay Savaş-Erdeve, Semra Çetinkaya, Zehra Aycan, Concept: Nursel Muratoğlu Şahin, Design: Nursel Muratoğlu Şahin, Data Collection or Processing: Nursel Muratoğlu Şahin, Elvan Bayramoğlu, Hatice Nursun Özcan, Erdal Kurnaz, Melikşah Keskin, Şenay Savaş-Erdeve, Semra Çetinkaya, Zehra Aycan, Analysis or Interpretation: Nursel Muratoğlu Şahin, Semra Çetinkaya, Zehra Aycan, Literature Search: Nursel Muratoğlu Şahin, Writing: Nursel Muratoğlu Şahin.

Financial Disclosure: The authors declared that this study received no financial support.

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ORIGINAL ARTICLE

DOI: 10.4274/jcrpe.galenos.2019.2018.0236

J Clin Res Pediatr Endocrinol 2019;11(3):293-300

Intrauterine Twin Discordancy and Partial Postnatal Catch-up Growth in a Girl with a Pathogenic *IGF1R* Mutation

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What is already known on this topic?

IGF1R mutations cause prenatal and postnatal decrease in linear growth. This mutation (p.Glu1050Lys) has been tested *in vitro* in fibroblasts which showed a decrease in phosphorylation of STAT5, a protein that, when activated, acts as a transcription factor in the nucleus.

What this study adds?

The effect of this mutation on intrauterine growth was tested for the first time in discordant twins. The affected girl's weight decreased by 36% and her length by 12%. This case highlights that intrauterine twin discordancy can occur in some patients carrying IGF1R mutations.

Abstract

Objective: Insulin like growth factors-1 (IGF-1) is essential for normal *in utero* and postnatal human growth. It mediates its effects through the IGF-1 receptor (IGF1R), a widely expressed cell surface tyrosine kinase receptor. The aim of the study was to analyze pre- and post-natal growth, clinical features and laboratory findings in a small for gestational age (SGA) girl in whom discordant postnatal growth persisted and her appropriate for gestational age (AGA) brother.

Methods: A girl born with a low weight and length [-2.3 and -2.4 standard deviation (SD) score (SDS), respectively] but borderline low head circumference (-1.6 SD) presented with a height of -1.7 SDS, in contrast to a normal height twin brother (0.0 SDS). IGF-1 resistance was suspected because of elevated serum IGF-1 levels.

Results: Sequencing revealed the presence of a previously described pathogenic heterozygous mutation (p.Glu1050Lys) in the SGA girl which was not present in the parents nor in the AGA twin brother.

Conclusion: The pathogenic *IGF1R* mutation in this girl led to intrauterine growth retardation followed by partial postnatal catch-up growth. Height in mid-childhood was in the lower half of the reference range, but still 1.7 SD shorter than her twin brother.

Keywords: Insulin-like growth factor type-1, insulin-like growth factor type-1 receptor, small for gestational age, postnatal growth, intrauterine discordancy

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Conflict of interest: None declared

Received: 26.11.2018

Accepted: 08.03.2019

Introduction

Insulin like growth factors (IGFs) are essential for intrauterine and postnatal growth and development (1). The mitogenic effects of IGF-1 are mediated through the IGF-1 receptor (IGF1R), a cell surface tyrosine kinase receptor encoded by IGF1R (15q26.3) (2). Synthesized as a single polypeptide precursor, the IGF1R undergoes proteolytic cleavage into α- and β-chains and forms a tetramer ($\alpha_2\beta_2$), with the extracellular α_2-subunits involved in ligand binding and the β_2-subunits carrying intrinsic tyrosine kinase activities (2). Ligand association leads to IGF1R autophosphorylation and activation of multiple downstream signaling pathways (3). This signaling results in fetal somatic growth, whereas postnatal somatic growth is achieved through the synergistic interaction of growth hormone (GH) and IGFs, among other factors (4).

The role of IGFs and their receptors in growth and development was first studied in animal models in which the invalidation of the *Igf1* and *Igf1r* genes in mice causes pre- and post-natal growth retardation (5). Later, genetic studies in short children showed that absent or decreased expression of IGF-1 leads to severe pre- and post-natal growth failure, and microcephaly (6,7,8), while heterozygous (or compound heterozygous hypomorphic) mutations or deletions of *IGF1R* lead to a variable degree of pre- and post-natal growth failure and microcephaly (9,10,11).

Intrauterine growth retardation (IUGR) is not a rare condition and can lead to a small body size for gestational age (SGA) (12). It can be caused by maternal, placental or fetal factors. Approximately 90% of children born SGA show catch-up growth in the first years of life (13,14). In these children no further diagnostic tests are carried out. In children born SGA with persistent short stature multiple genetic causes have been detected (15).

We report a twin girl born SGA with partial catch-up growth, but still 1.7 standard deviation (SD) shorter than her appropriate for gestational age (AGA) born twin brother. Her serum IGF-1 level was unexpectedly elevated, due to a previously described pathogenic mutation in *IGF1R* (c.3148G > A, p.Glu1050Lys).

Methods

Subjects

Informed consent was obtained from the family to participate and provide samples (DNA, whole blood), in compliance with the Institutional Ethics Committee at San Borja-Arriarán's Hospital (Santiago, Chile).

Sample Procurement

Genomic DNA was isolated from peripheral blood from the patient, her sibling and from both parents. The samples were sent to the Laboratory for Diagnostic Genome Analysis, Department of Clinical Genetics at the Leiden University Medical Center (LUMC) for routine genetic testing of *IGF1R*. Targeted Sanger sequencing of the complete coding region exon 1-21 including intron/exon boundaries (NM_000875.3) was performed as previously reported (10,16). Multiplex ligation-dependent probe amplification (MLPA) assay (MRC Holland kit P217-B2) containing probes for *IGF1R* exon 1-21 was performed for the detection of deletions or duplications (16).

Statistical Analysis

Comparisons between groups were not performed in this study.

Results

Clinical Presentation of the Index Patient

The Chilean female index patient was part of a bichorial biamniotic twin, born after a pregnancy interrupted due to premature membrane rupture and metrorrhagia. The patient showed *in utero* growth discordancy at week 21 and was born SGA at 33 weeks of gestational age, with a birth weight of 1.48 kg [-2.4 SD score (SDS)] (17), a birth length of 39 cm (-2.4 SDS) and a head circumference of 29.5 cm (-1.6 SDS) (Figure 1A). During her first days of life, she was hospitalized for gastric distress. Several episodes of gastro-oesophageal reflux with and without cyanosis were reported after hospitalization.

The parents were not consanguineous. Paternal and maternal heights were 176.9 cm (-0.1 SDS) and 157.9 cm (-1.0 SDS), respectively, with a target height of -0.45 SDS (18). The father reported normally timed puberty and the mother's pubertal development was slightly delayed (menarche 14 years). Paternal grandfather and -mother had a height of 170 cm (-0.9 SDS) and 165 cm (0.4 SDS), and maternal grandparental heights were 162 cm (-2.1 SDS) and 157 cm (-1.0 SDS), respectively (Figure 2).

The patient was referred to the pediatric endocrine unit for evaluation of short stature at age 1.25 years, because of postnatal growth discordancy with her twin brother (Table 1). Height to arm span ratio was abnormal (≥1.0), weight 6.87 kg (-2.8 SDS for age), weight for height -2.2 SDS (19), and head circumference 44.8 cm (-1.3 SDS). Physical examination revealed normal body proportions and a small midface, mild frontal bossing, a thin upper lip,

and mild hypertelorism. Bone age was delayed by three months. A normal female karyotype (46 XX) was found. Serum IGF-1 concentration was high (194 ng/mL; reference range (RR) < 131 ng/mL) and IGFBP-3 levels in the upper normal range (3.1 mg/L; RR = 1.1-3.6 mg/L). Independent walking was achieved at 1.25 years. Her appetite was poor and selective.

Over the subsequent eight years she visited the clinic several times (Table 1). Psychomotor development was normal. Height remained below -2 SDS up to three years of age and then increased (Figure 1B). Bone age at 3.75 years was delayed but identical to chronological age by 6.33 years. At age 8.92 years she was prepubertal and a small diffuse goiter was noted, confirmed by the finding of a small thyroid cyst at ultrasound. Thyroid function was normal during follow-up. Over the years, her circulating IGF-1 levels and IGFBP-3 concentrations remained high (Table 1).

The Patient's Twin Brother

The male twin brother of the index patient was born at 33 weeks of gestational age with a weight of 2.0 kg and length of 44 cm. Growth data are shown in Table 1. At 1.75 years of age, his height was 83 cm and weight was 13.3 kg (Figure 1C). Thereafter his height SDS increased to close to the reference mean (Table 1) and was slightly above conditional target height SDS, and remained stable afterwards (Figure 1D). He has no associated morbidities nor dysmorphic features (Figure 3).

Genetic Studies

Since the clinical and biochemical characteristics of the index patient were consistent with IGF-1 resistance which could be caused by a deletion or an inactivating mutation in the gene encoding IGF1R, targeted sequencing and MLPA was performed for *IGF1R* on genomic DNA from whole blood from the index patient. Sequence analysis showed a heterozygous nucleotide substitution at position 3148 (c.3148G > A), changing glutamic acid to lysine at position 1050 of the mature IGF1R protein (p.Glu1050Lys). This heterozygous mutation was not encountered in the twin brother nor in either parent. It was confirmed by PP16 analysis that the index patient was the daughter of this couple.

Discussion

In this study, we report a patient who presented with pre- and post-natal growth retardation resulting from a *de novo* heterozygous *IGF1R* mutation in exon 16 (c.3148G > A, p.Glu1050Lys). Substitution of this highly conserved amino acid residue, located in the intracellular tyrosine kinase domain, is associated with a change in charge of the amino acid and *in silico* analysis predicts inactivation of the IGF1R leading to a partial resistance to IGF-1. This mutation was not identified in the patient´s twin AGA born normal-statured brother nor in other family members.

Fetal growth and development are influenced by maternal, placental and fetal factors (1). A variety of maternal and utero-placental factors may constrain the growth of the

Table 1. Clinical and biochemical characteristics of the index patient and her twin brother

	Index patient							Sibling			
Age (years)	Height cm (SDS)	Weight kg (SDS)	BMI (SDS)	HC cm (SDS)	Bone age	IGF-1 ng/mL (RR)	IGFBP-3 mg/L (RR)	Height cm (SDS)	Weight kg (SDS)	BMI (SDS)	HC cm (SDS)
Birth data	39 (-2.5)	1.48 (-2.4)		29.5 (-1.6)	NA	NA	NA	44 (0.0)	2.0 (-0.6)		NA
1.25	69.4 (-3.0)	6.87 (-2.8)	14.3 (-1.3)	44.8 (-1.3)	1 year	194 (< 131)	3.1 (1.1-3.6)	NA	10.7 (0.2)	NA	48 (0.9)
3.08	86.2 (-2.1)	10 (-3.5)	13.5 (-2.3)	NA	NA	269 (< 289)	4.3 (< 4.3)	NA	NA	NA	NA
3.75	NA	NA	NA	NA	3 year	NA	NA	NA	NA	NA	NA
4.25	95.5 (-1.6)	13.1 (-1.9)	14.4 (-0.8)	NA	NA	330 (< 289)	4.5 (< 4.3)	104.8 (0.2)	20.9 (1.6)	19.0 (2.4)	NA
4.75	NA	NA	NA	NA	4 year	NA	NA	NA	NA	NA	NA
6.33	109 (-1.6)	17 (-1.7)	14.3 (-0.7)	49 (-1.7)	6.5 year	417 (< 286)	NA	118.3 (0.2)	22 (0.1)	15.7 (0.2)	53.5 (1.0)
8.92	122.0 (-1.8)	21 (-2.1)	14.1 (-1.3)	NA	NA	NA	NA	132.2 (-0.1)	37.7 (1.4)	21.6 (1.7)	NA

BMI: body mass index, HC: head circumference, NA: not available, RR: reference range, SDS: standard deviation score, IGF-1: insulin like growth factors-1

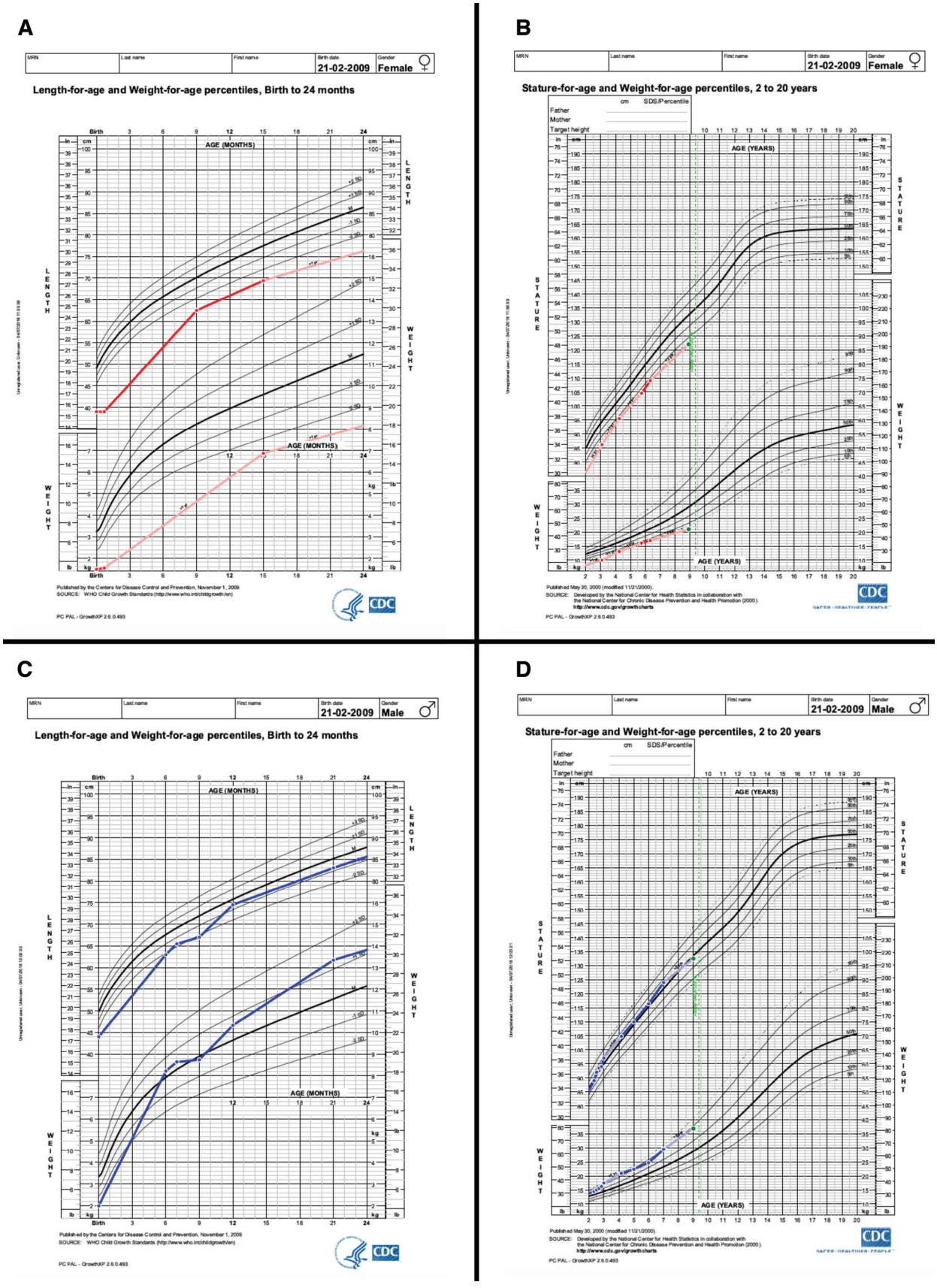

Figure 1. Growth chart of the patient (A) and her twin brother (B). Growth charts of the patient carrying the mutation (C) and (D) growth charts of the normal statured brother

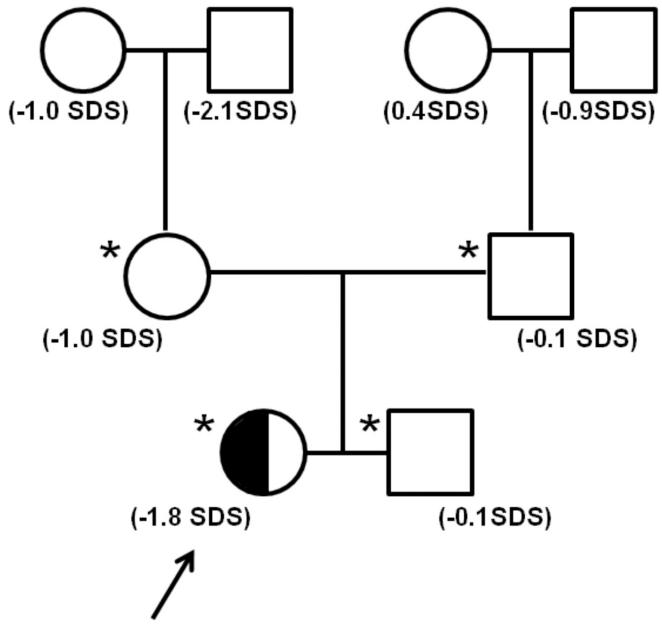

Figure 2. Pedigree of the index patient with the *IGFIR* mutation. Height standard deviation score is indicated in brackets and persons who were checked for the *IGFIR* mutations are indicated (*)

SDS: standard deviation score

Figure 3. Picture of the twins taken in July 2014

fetus. In this interesting experiment of nature the role of maternal and placental factors are well controlled and separated from the role of fetal factors. A series of elegant investigations in mice, complemented by case studies in humans, have convincingly demonstrated the critical role of the IGF system in pre- and post-natal growth (5). Targeted disruption of the gene encoding Igf-2 in mice resulted in a 40 percent reduction in fetal growth with normal postnatal growth, demonstrating the important role of IGF-2 in intrauterine growth. Disruption of the gene for Igf-1 led to a similar decrease in birth weight but also led to persistent postnatal growth failure. Furthermore, deletion of the gene encoding Igf1r, which mediates the growth-promoting actions of both Igfs, resulted in birth weights that were only 45 percent of normal and these mice generally died within hours after birth from respiratory insufficiency due to muscular hypoplasia (5). The relevance of these findings for human growth was supported by reports on humans. Homozygous mutations of *IGF-1* were found in a few patients presenting with severe pre- and post-natal growth failure, microcephaly and deafness (6,7). Several reports have been published of patients with IGF-1 resistance due to molecular defects in the *IGF1R* who present with a variable degree of pre-and post-natal growth retardation (9).

Short stature is a common problem confronting pediatric endocrinologists. After exclusion of systemic or skeletal diseases or overt hormonal deficiencies, clinicians are often unable to provide a definitive diagnosis for the etiology of an individual patient's short stature. An important clue for the cause of short stature is to register whether prenatal growth was normal or reduced. We suspected a mutation within the IGF-1 signaling cascade because of the persistent short stature in our patient and the high IGF-1 levels. Our hypothesis led us to the detection of a *de novo* heterozygous mutation of *IGF1R* in exon 16, resulting in the replacement of a Glu residue at position 1050 by a Lys residue. So far, mutations in *IGF1R* were almost always reported to result in IUGR, and postnatal catch-up growth had not been documented. Aberrant *IGF1R* expression is described to lead to *IGF1R* haploinsufficiency (20,21), disturbed processing of the proreceptor (22,23), decreased ligand binding (24), abrogated IGF1R tyrosine kinase activity and reduced receptor autophosphorylation (10,25,26).

In line with the previously reported adult patient (with a birth weight and length of -2.1 and -0.3 SDS, respectively, and a height SDS of -3.3 at presentation, and an adult head circumference SDS of -3.0), the mutation led to a clinically significant prenatal and postnatal growth failure, though

postnatal growth of our patient is less affected compared to almost all cases with *IGF1R* haploinsufficiency described to date. This mutation was also associated with microcephaly, but it did not affect intellectual development. Our patient was reported to have feeding problems during the first year of life and poor appetite, which previously has been associated with the same and other *IGF1R* mutations (10). This mutation was not present in her twin brother and parents, who all have normal stature. Our results provide strong evidence that this variant is likely to be the underlying cause of the IUGR and mild postnatal short stature observed in this patient.

Most of the *IGF1R* mutations have been described in children born SGA. The first human IGF1R defects were described by Abuzzahab et al (9) in 2003 and only a few compound heterozygous cases have been described thereafter (9,27). Most of the described cases are heterozygous carriers of *IGF1R* mutations (10,20,21,22,23,25,26,28,29,30,31,32,33, 34). To date only two single patients carrying a homozygous mutation have been described (35,36). The phenotype is variable, presumably depending on the impact of the mutation on the function of the IGF1R. The most common feature described in the reported patients included IUGR (11,37), postnatal growth failure and microcephaly (11,37,38).

Study Limitations

The affected Glu residue at position 1050, is located in the strongly conserved serine-threonine/tyrosine-protein kinase catalytic domain. A study limitation was the absence of functional studies, as fibroblasts from skin biopsies were not available. However, functional analysis of fibroblasts from a previously described patient with the same mutation showed a marked reduction of autophosphorylation of the IGF1R and of activation of PKB/Akt upon a challenge with IGF-1. Furthermore, [^{3}H]thymidine incorporation in that patient's cells after a challenge with a dose range of IGF-1 in comparison with a panel of fibroblast cultures of eight non-growth-retarded individuals (controls) showed a 50% reduction (10).

Conclusion

In conclusion, we describe a discordant pair of twins in whom the effect of this *IGF1R* mutation in the context of a similar intrauterine environment is unmasked. This clinical observation shows that while it is assumed that most patients carrying IGF1R mutations remain short postnatally, partial catch-up growth can occur, possibly related to increased GH and IGF-1 secretion.

Ethics

Ethics Committee Approval: It is in compliance with the Institutional Ethics Committee at San Borja-Arriarán's Hospital (Santiago, Chile).

Informed Consent: It was obtained from the family to participate and provide samples (DNA, whole blood).

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Veronica Mericq, Concept: Veronica Mericq, Jan M. Wit, Design: Veronica Mericq, Jan M. Wit, Data Collection or Processing: Monique Losekoot, Veronica Mericq, Analysis or Interpretation: Paula Ocaranza, Monique Losekoot, Literature Search: Paula Ocaranza, Veronica Mericq, Marie J. E. Walenkamp, Christiaan De Bruin, Writing: Paula Ocaranza, Veronica Mericq, Jan M. Wit.

Financial Disclosure: This work did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

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CASE REPORT

DOI: 10.4274/jcrpe.galenos.2018.2018.0211

J Clin Res Pediatr Endocrinol 2019;11(3):301-305

A Case of Cleidocranial Dysplasia with a Novel Mutation and Growth Velocity Gain with Growth Hormone Treatment

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What is already known on this topic?

Classical cleidocranial dysplasia (CCD) is characterised by hypoplasia or aplasia of clavicles, failure of cranial suture closure and dental anomalies. Short stature is a frequent feature of the syndrome. Nearly two hundred mutations associated with CCD have been reported.

What this study adds?

We present a likely novel mutation for CCD. Although data about growth hormone (GH) therapy for CCD with severe short stature is very limited, we observed a gain in growth velocity with GH treatment.

Abstract

Cleidocranial dysplasia (CCD) is a rare congenital autosomal dominant skeletal disorder that is characterized by hypoplasia or aplasia of clavicles, failure of cranial suture closure, dental anomalies, short stature and other changes in skeletal patterning and growth. The gene responsible for pathogenesis has been mapped to the short arm of chromosome 6p21, core binding factor alpha-1 *(CBFA1)* or runt related transcription factor-2 *(RUNX2)*. Here we describe a CCD patient with a novel mutation in the *RUNX2* gene. A five-and-a-half year old girl presented with severe short stature, dysmorphic facial appearance (hypertelorism, prominent forehead, high palate, midfacial hypoplasia), macrocephaly, large anterior fontanelle, increased anteroposterior chest diameter. Her shoulders were close to each other and her bilateral clavicles appeared short on physical examination. Bilateral hypoplastic clavicles, coxa valga, hypoplasia of iliac bones, wide symphysis pubis and phalangeal dysplastic features were detected on her skeletal X-ray examination. She was diagnosed as having CCD. Molecular analysis detected a novel heterozygous mutation 'NM_001024630.3p.T155P(c.463A > C)' in the *RUNX2* gene. At age seven years and two months old, because of her severe short stature, growth hormone (GH) treatment was started and she responded well to GH therapy with no adverse effects. In conclusion, hypoplasia or aplasia of the clavicles, failure of cranial suture closure, dental anomalies and short stature should bring CCD to mind. We present a novel mutation in the *RUNX2* gene for CCD. We obtained growth velocity gain with GH treatment in our patient.

Keywords: Cleidocranial dysplasia, *RUNX2*, severe short stature

Introduction

Cleidocranial dysplasia (CCD) (CCD; OMIM:119600) is a skeletal dysplasia characterized by hypoplasia or aplasia of the clavicles permitting abnormal facility in apposing the shoulders, by persistently open skull sutures with bulging calvaria and by dental anomalies including delayed exfoliation of primary teeth, delayed eruption of permanent teeth and multiple impacted supernumerary teeth. Short stature, generalized bone dysplasia, vertebral malformations, a depressed nasal bridge and a wide pubic symphysis can also be seen (1). The estimated prevalence of CCD is one per million births, which is most likely underdiagnosis, and there is no sex predilection (2).

CCD is caused by heterozygous loss-of-function mutation in the runt related transcription factor-2 *(RUNX2)* gene, encoding the transcription factor core binding factor

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Conflict of interest: None declared
Received: 31.08.2018
Accepted: 22.11.2018

alpha-1 *(CBFA1)* on chromosome 6p21 (1,3). The human *RUNX2 (CBFA1)* gene consists of eight exons and it controls differentiation of precursor cells into osteoblasts and is essential for membraneous and endochondral bone formation (3,4). It is a master regulatory gene for skeletal development and morphogenesis. The majority of *RUNX2* mutations in classic CCD patients are missense or nonsense mutations. Frame shift and exon skipping mutations (4), insertions and deletions have also been described (3). The disease is commonly autosomal dominantly inherited but can be sporadic.

Here we present a CCD patient with significant short stature with typical characteristics of CCD and a novel *RUNX2* mutation. She had growth hormone (GH) therapy with growth velocity gain.

Case Report

A five-and-a-half year old girl was admitted to our hospital due to her short stature and dysmorphic features. Her anthropometric measurements and standard deviation (SD) scores (SDS), according to Turkish standards (5), were as follows: height was 94.3 cm (-3.69 SD); weight was 13.7 kg (-2.45 SD); body mass index (BMI) was 15.4 (-0.05 SD); head circumference was 52 cm (0.77 SD); upper/lower segment ratio of 1.25 (> +2 SD); and mid parental target height was 161.15 cm (-0.31 SD). The parents had no history of constitutional delay of puberty and growth. The patient had a dysmorphic face with hypertelorism, a prominent forehead, high palate, midfacial hypoplasia and down-slanting palpebral fissures. In addition she had macrocephaly, large anterior fontanelle, increased anteroposterior chest diameter, laxity in her distal joints and pes planus. Her shoulders were close to one another and her clavicles appeared too short (Figure 1). Exfoliation of her primary teeth was delayed. She had normal developmental milestones and intelligence, except for a mild speech delay. Her neurological examination was normal.

Bone age was 3-3.5 years according to the method of Greulich and Pyle. Skeletal X-rays showed bilateral hypoplastic clavicles, a wide and open anterior fontanelle, coxa valga, hypoplasia of iliac bones and a wide symphysis pubis (see Figures 2, 3). Her hand X-ray examination revealed cone shaped epiphyses, a pseudo-epiphysis of the second metacarpal, tapering of distal phalanges, severe dysplasia of the middle phalanx in the fifth finger and a wide phalangeal epiphysis. These findings were compatible with the diagnosis of CCD. She had no scoliosis.

In laboratory studies her blood count, biochemical tests, thyroid function tests and urine examination results

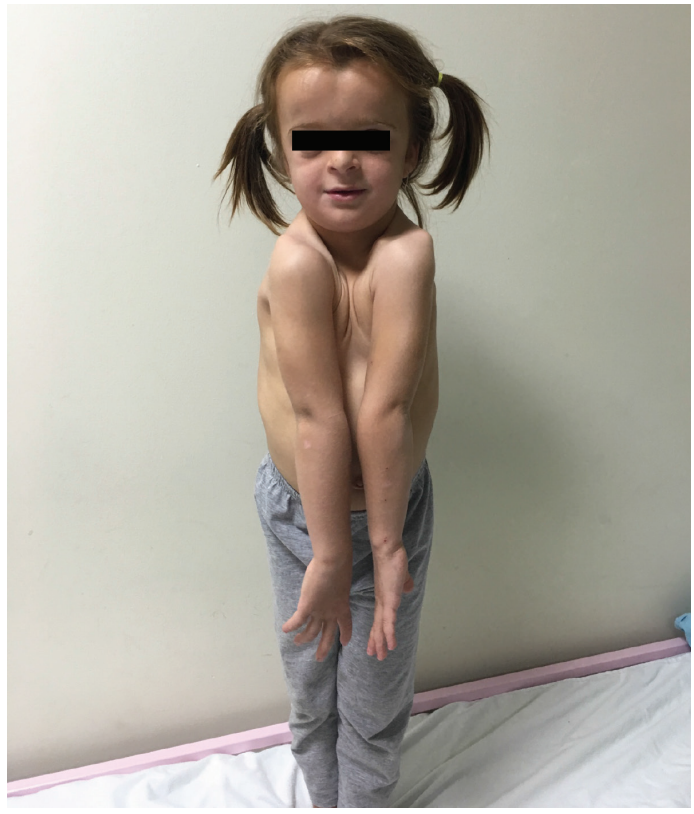

Figure 1. Hypoplasia of the clavicles permitting abnormal facility in apposing the shoulders

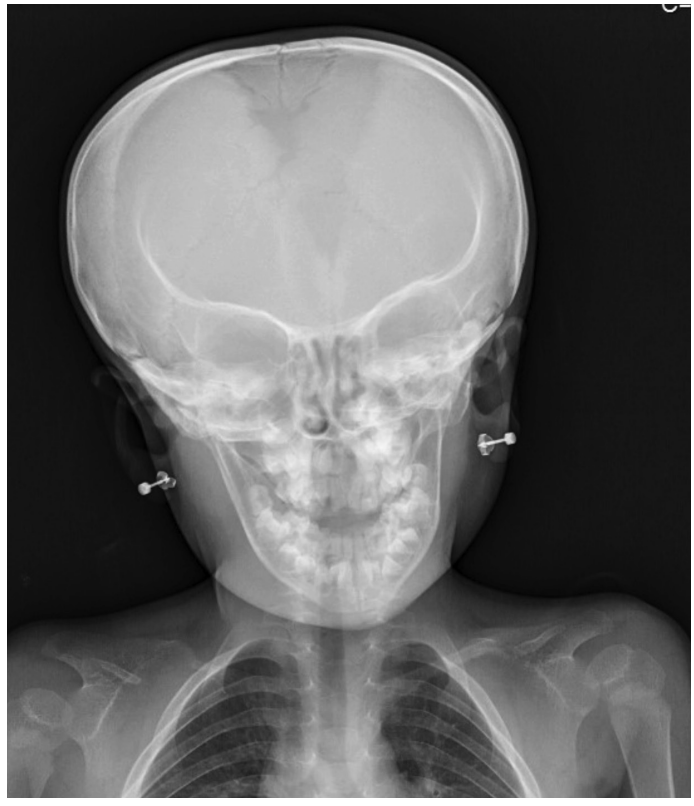

Figure 2. Skull X-ray of patient shows wide and open anterior fontanelle

were normal. Tissue transglutaminase antibodies were negative. Insulin like growth factor-1 (IGF1) and IGF binding protein-3 (IGFBP3) concentrations were 74 ng/mL (-1.15 SD) and 2860 ng/mL (-0.12 SD) respectively. Her peak GH concentration following L-DOPA stimulation was 13.4 ng/mL (non-deficient). Karyotype was 46,XX.

After genetic consultation, next generation sequencing (NGS) detected a novel heterozygous mutation 'NM_001024630.3p.T155P(c.463A > C)' in the *RUNX2* gene (Figure 4). *RUNX2* gene sequence analysis was performed by using MiSeq NGS platform, an FDA approved diagnostic system (Illumina Inc., San Diego, CA, USA). Genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All coding exons of the *RUNX2* gene and their flanking splice site junctions were amplified using polymerase chain reaction (PCR) primers, designed with PRIMER©-Primer Designer v.2.0 (Scientific and Educational Software programme) software. PCRs were validated by using agarose gel electrophoresis. After PCR amplification, the libraries were prepared with the Nextera XT kit (Illumina Inc., San Diego, CA, USA), according to the manufacturer's instructions. Next-gene sequencing was carried on MiSeq

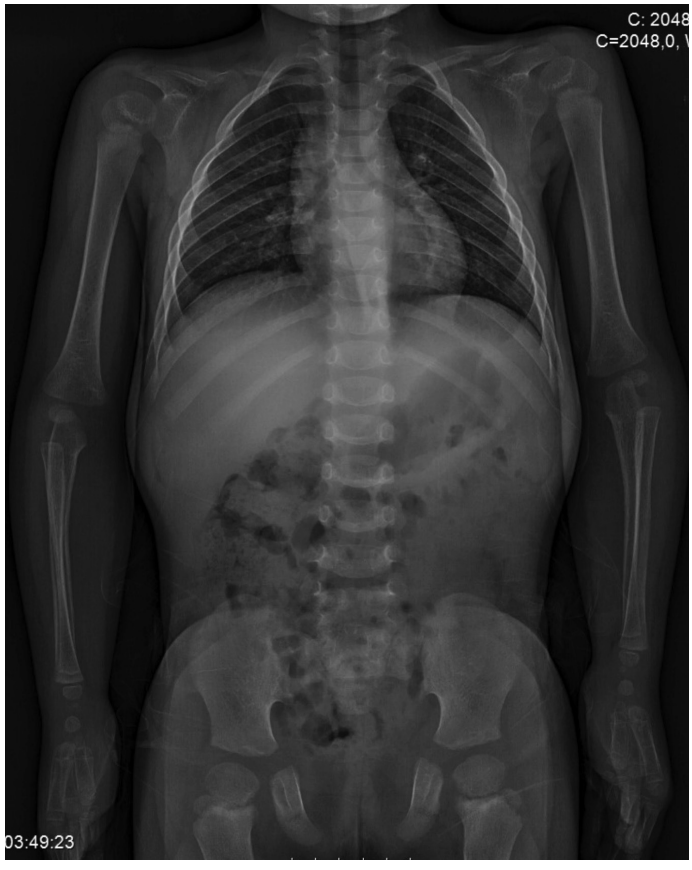

Figure 3. X-ray of her trunk shows bilateral hypoplastic clavicles, hypoplasia of iliac bones, wide symphysis pubis

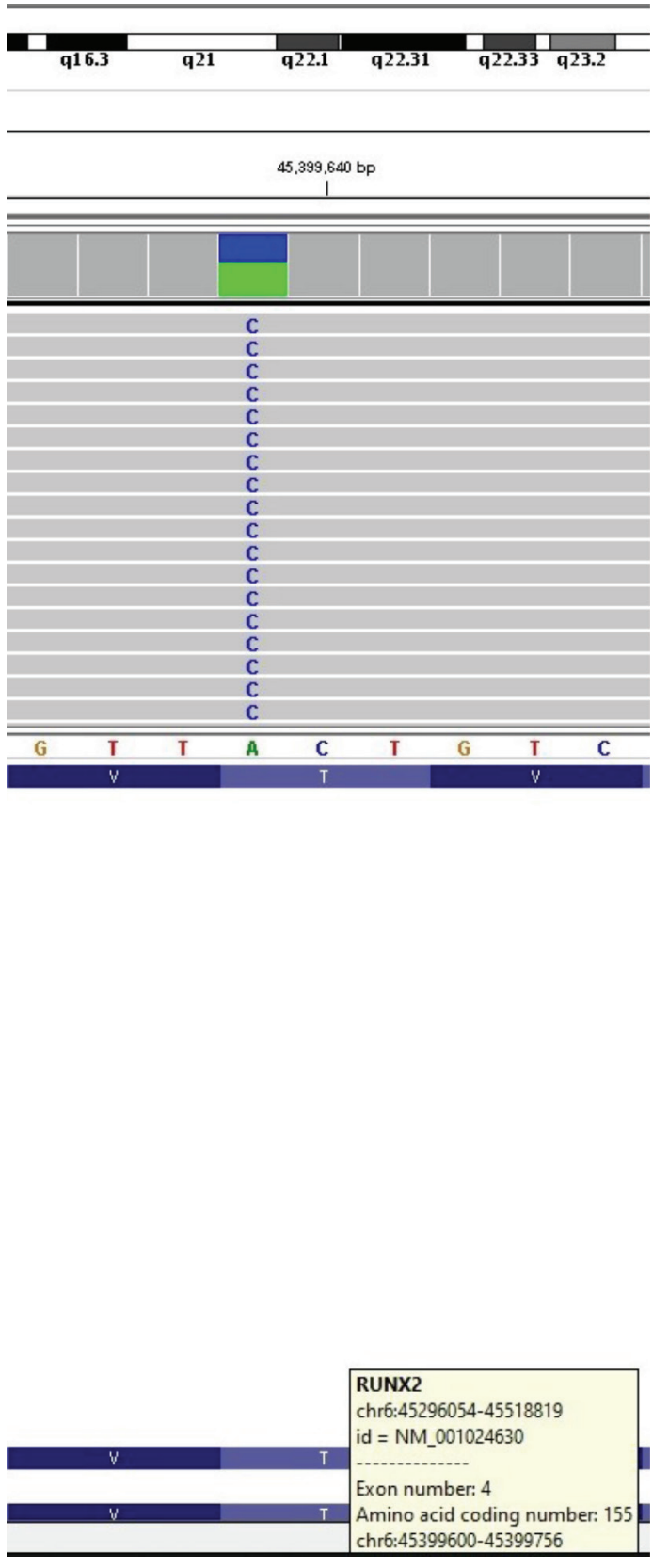

Figure 4. MISEQ sequence image of the mutation in *RUNX2* gene

(Illumina Inc., San Diego, CA, USA). Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc., San Diego, CA, USA). Visualisation of the data was performed with IGV 2.3 (Broad Institute, Cambridge, MA, USA) software.

This mutation has not been reported previously and it is highly likely to be pathogenic according to the PolyPhen-2 (score = 1.00, sensitivity: 0.00, specificity: 1.00) (http://genetics.bwh.harvard.edu/pph2), SIFT (score = 0.0001 converted rank score = 0.912), Provean (score = -5.46 -5.53 converted rankscore = 0.86) and Mutation Taster (score = 0.99) software analysis. Her mother's genotype was normal for this mutation. It was not possible to perform the father's genetic analysis.

At age seven years and two months old, the patient's anthropometric characteristics were: height 104.1 cm; height SDS -3.8 SD; body weight 17.1 kg (-2.3 SD); and upper/lower segment ratio 1.28. Her bone age was estimated as five years.

An IGF generation test was performed with 0.1 mg/kg/day GH for four days because of her severe short stature. The test revealed a 200% increase in IGF1. Subcutaneous GH treatment was started at a dose of 30 mcg/kg/day. After one year of treatment (at age 8 years and three months) her growth velocity was found to have increased to 8.2 cm/year from 5.28 cm/year before treatment. Height SDS had increased to -3.15 SD. She was still prepubertal and her bone age was 6.5-7 years. Her IGF1 concentration was 123 ng/mL (-0.03 SD) and IGFBP3 concentration was 5460 ng/mL (1.08 SD) after GH treatment. She was followed-up every three months and no adverse side effects were observed which could be associated with GH treatment. She was also followed by an orthopedist for pes planus and a pediatric dentist for delayed exfoliation of primary teeth. She continues to receive GH therapy. After 21 months of GH therapy, at age 9 years, she was prepubertal and her anthropometric measures were as follows: height 119.2 cm (-2.28 SD); weight 22.6 kg (-1.48); BMI 15.9 (-0.26 SD); upper/lower segment ratio 1.16 (> +2 SD) (+2 SD = 1.08); and arm span 115 cm. Her body disproportion had not worsened.

A written informed consent was obtained from the patient's family regarding the scientific publication of the patient's photographs and her medical data.

Discussion

CCD is generally diagnosed clinically and the diagnosis is supported by radiography. Genetic analysis reveals a *RUNX2* mutation in almost 70% of patients. Our patient was diagnosed due to her classical phenotypic and radiological features.

Short stature can be a feature of CCD due to generalized bone dysplasia. Reports of gender differences and severity of short stature are controversial (2,6,7). Short stature is usually mildly disproportional but it also can be proportional (2,7). Studies which include younger CCD patients indicate that birth lengths are normal, but heights decrease to around -2 SD at ages of 4-8 years (6).

Jensen (6) investigated somatic development in 17 Danish CCD patients, aged 5-46 years. Stature was documented in six males and eight females and compared with Danish reference data. The report noted growth retardation, especially in females. Heights of CCD males were clustered between the 5th and 50th percentiles, but heights in all CCD females were below the 5th percentile (between -1.81 SD and -3.56 SD). Due to the small number of patients, having no data about parental heights and noting that four females belonged to the same family, the authors concluded that the observed severity of short stature in females may have occurred by chance. It was also noted that the females had smaller head circumference values than the boys (-0.77 SD versus +0.27 SD, respectively).

Our female patient had a significant short stature and her height SDS was significantly lower than her mid-parental height SDS. Her head circumference was relatively macrocephalic (head circumference +0.77 SD).

In the study of Cooper et al (2), 21 female and 21 male CCD patients aged >18 years were evaluated for height. The authors observed that their patients had shorter statures than their healthy relatives. The mean height percentiles of girls and boys were 10th percentile (38% were <5th percentile) and <5th percentile (62% were <5th percentile), respectively. Unlike Jensen's (6) report, short stature in this study was more prominent in males and severe short stature was not observed among the cases. Most prevalent skeletal deformities of the cases were genu valga and pes planus.

Dinçsoy Bir et al (7) reported 15 CCD cases in 11 independent families. Short stature was observed in three males (height SDS were -4.2, -2.9 and -2.24) and a female (height SDS was -2.55) and all were proportional. Three of these short patients had low IGF1 levels (<-2 SD). The female patient showed partial GH deficiency on GH stimulation tests and had normal hypophyseal magnetic resonance imaging. She had not received GH therapy at the time of the report. The male patient with a height SDS of -4.2 did not have low IGF1 and no GH deficiency was found. He did not respond to one

year of GH therapy at the age of 15 years, but his bone age was not reported in the study.

Short stature of different frequency and severity, reported in CCD patients, can be explained with the variety in the effects of the known mutations. There are studies investigating genotype-phenotype correlations for *RUNX2* mutations (4,8). Yoshida et al (8), studied genotype-phenotype correlations in 17 Japanese CCD patients and they reported that *RUNX2* mutations which affect the Runt domain (responsible for binding to DNA) are correlated with short stature and its severity. They showed that patients had normal stature when they had mutations with an intact Runt domain. The mutation detected in our patient ['NM_001024630.3p.T155P(c.463A > C)'] was a missense mutation leading to a change in 155th amino acid in Exon 4 and located within the Runt domain. It was likely to be pathogenic in the *in silico* analysis. This situation can explain our patient's severe short stature.

Yoshida et al (8), found that short stature and the number of supernumerary teeth were correlated significantly. Different studies have shown mutations which affect the Runt domain of the *RUNX2* gene also cause the classical CCD phenotype and are associated with severe dental anomalies (4). Genotype-phenotype correlation studies also showed that mutations of the *RUNX2* gene could lead to various phenotypic features even in the same family (4).

Data on GH treatment for CCD patients are very limited. In the study of Dinçsoy Bir et al (7), one patient with CCD who was treated with GH for one year did not benefit from this treatment but the patient was 15 years old and his bone age data were missing. Our patient's height increased by 8.2 cm/year (prepubertal), an increase of 3 cm/year more than in the year prior to GH treatment. This corresponded to an increase in height SDS of 0.65 SD/year. The initiation of GH therapy at an early age could have been the reason for the better outcome observed in this patient. However, in terms of efficacy and safety of GH therapy, there is a need for randomized controlled trials involving more patients.

It has been suggested that there can be increased bone fragility in CCD patients. Cooper et al (2) reported two patients with multiple bone fractures, but they found similar fracture and osteoporosis rates in 90 CCD patients and in the control group. Dinçsoy Bir et al (7) reported that more than 50% of their patients had osteoporosis and they also reported no relationship between osteoporosis in their patients and vitamin D deficiency. Our patient had no bone fractures and the radiology of her vertebrae showed no fractures or scoliosis.

In conclusion, we report a patient who presented with severe short stature, failure of cranial suture closure and hypoplasia of clavicles, who was diagnosed as having CCD. A novel mutation in the *RUNX2* gene for CCD was detected. We obtained a growth velocity gain with GH treatment for severe short stature, with no side effects. Randomized controlled trials are necessary however, for evaluating the effectiveness and safety of GH therapy for this population.

Ethics

Informed Consent: A written informed consent was obtained from the patient's family regarding the scientific publication of the patient's photographs, medical informations and imagines.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Emine Çamtosun, Ayşehan Akıncı, Concept: Emine Çamtosun, Ayşehan Akıncı, Design: Emine Çamtosun, Ayşehan Akıncı, Data Collection or Processing: Emine Çamtosun, Emine Demiral, İbrahim Tekedereli, Ahmet Sığırcı, Analysis or Interpretation: Emine Çamtosun, Emine Demiral, İbrahim Tekedereli, Ahmet Sığırcı, Literature Search: Emine Çamtosun, Writing: Emine Çamtosun, İbrahim Tekedereli.

Financial Disclosure: The authors declared that this study received no financial support.

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CASE REPORT

DOI: 10.4274/jcrpe.galenos.2018.2018.0217

J Clin Res Pediatr Endocrinol 2019;11(3):306-310

A Case of the Perinatal Form Hypophosphatasia Caused by a Novel Large Duplication of the *ALPL* Gene and Report of One Year Follow-up with Enzyme Replacement Therapy

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What is already known on this topic?

Hypophosphatasia (HPP) is caused by mutations in the gene encoding tissue-non-specific isoenzyme of alkaline phosphatase. Missense mutations are the most common type of mutations described for this gene, while duplications are rarely described. It has been shown that enzyme replacement therapy (ERT) mineralizes the skeleton and improves respiratory function and survival in the life-threatening perinatal form of HPP.

What this study adds?

We report a novel, large, homozygous duplication encompassing exons 2 to 6 of the *ALPL* gene. Early diagnosis and rapid intervention with ERT is life-saving in the severe form of HPP.

Abstract

Hypophosphatasia (HPP) is a rare disease caused by mutations in the *ALPL* gene encoding tissue-non-specific isoenzyme of alkaline phosphatase (TNSALP). Duplications of the *ALPL* gene account for fewer than 1 % of the mutations causing HPP. It has been shown that asfotase alfa enzyme replacement treatment (ERT) mineralizes the skeleton and improves respiratory function and survival in severe forms of HPP. Our patient was a newborn infant evaluated for respiratory failure and generalized hypotonia after birth. Diagnosis of HPP was based on low-serum ALP activity, high concentrations of substrates of the TNSALP and radiologic findings. On day 21 after birth, ERT using asfotase alfa (2 mg/kg three times per week, subcutaneous injection) was started. His respiratory support was gradually reduced and skeletal mineralization improved during treatment. We were able to discharge the patient when he was seven months old. No mutation was detected in the *ALPL* gene by all exon sequencing, and additional analysis was done by quantitative polymerase chain reaction (qPCR). As a result, a novel homozygote duplication encompassing exons 2 to 6 was detected. Early diagnosis and rapid intervention with ERT is life-saving in the severe form of HPP. qPCR can detect duplications if a mutation cannot be detected by sequence analysis in these patients.

Keywords: Hypophosphatasia, perinatal form, *ALPL* gene, duplication, enzyme replacement therapy

Introduction

Hypophosphatasia (HPP) is a rare disease caused by mutations in the gene encoding tissue-non-specific isoenzyme of alkaline phosphatase (TNSALP) (1). It is estimated that the incidence of severe forms of the disease is approximately 1 in 300,000 in Europe and approximately 1 in 435,517 in Turkey (2,3). Patients have been classified traditionally as having perinatal, infantile, childhood, or adult HPP based on symptom severity and presentation age. Currently, specific bone-targeted recombinant enzyme replacement therapy (ERT) (asfotase alfa; STRENSIQ®,

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Conflict of interest: None declared
Received: 05.09.2018
Accepted: 22.11.2018

Alexion Pharmaceuticals, Inc. NASDAQ: ALXN, Boston, Massachusetts, U.S.) is available for HPP patients, and it is suggested for patients with pediatric-onset HPP (1,4).

ALPs are membrane-bound ectoenzymes that hydrolyze monophosphate esters. Human ALP is classified into four types: TNSALP, intestinal, placental-like ALP (PLAP) and germ cell. The expression of TNSALP is widespread, especially in the liver, bone, kidney, neuronal cells and neutrophils. It is expressed on the cell membrane of hypertrophic chondrocytes, osteoblasts and odontoblasts and is also concentrated on the membranes of budding matrix vesicles in these cells. TNSALP is essential for tissue biomineralization (5,6,7).

TNSALP is encoded by an ALP-liver *(ALPL)* gene on chromosome 1p36.12. To date, over 300 different mutations in *ALPL* gene have been identified. Missense mutations are the most common type of mutation. Duplications in this gene have been reported very rarely. Herein, we report a novel duplication in the *ALPL* gene in a patient with the perinatal form of HPP, as well as the patient's clinical characteristics and a brief report of the results of 12-months follow-up with ERT.

Case Report

The patient was evaluated at birth for respiratory failure and generalized hypotonia. He was born from second cousin consanguineous parents at full-term weighing 3,440 g. The birth length was 50 cm and the head circumference was 35 cm. Diagnosis of HPP was based on low-serum ALP activity, high levels of substrates of TNSALP (see Table 1) and radiologic findings (see Figure 1). The parents were of Turkish origin and healthy. At the time of the assessment, when father's age was 37 years and mother's age was 32 years, neither parent had clinical symptoms of HPP.

No mutation was detected in the *ALPL* gene by full gene sequencing, and thus, we decided to do an additional analysis by quantitative polymerase chain reaction (qPCR). Blood samples were collected from the patient and parents. DNA isolation was performed by a salt precipitation method. The qPCR analysis was performed by LightCycler 480 Software (Roche, Basel, Switzerland), and a relative quantification analysis was performed that compared the target DNA sequence (that of the patient) with a reference DNA sequence (used for normalization of the ratio). Primers were designed for the coding exons 2 to 12 of the gene of interest; 0.5 µL primer forward, 0.5 µL primer reverse, 10 µL SYBR Green I Master (Roche, Basel, Switzerland) and 2 µL DNA were used for reaction mix in a total volume of 20 µL. As a result, a novel homozygous duplication encompassing exons 2 to 6 was detected (Figure 2). This mutation was classified as likely pathogenic (class 2) according to the American College of Medical Genetics (ACMG) and Centogene's guidelines. Genetic analysis of the parents demonstrated that both were carriers of the same mutation (Figure 3).

Asfotase alfa was kindly provided by Alexion Pharmaceuticals as part of the compassionate use program. On day 21 after birth, ERT using asfotase alfa (2 mg/kg three times per week, subcutaneous injection) was started.

After birth, the patient was intubated and ventilated by Synchronized Intermittent Mandatory Ventilation mode. The inspiratory requirement was gradually reduced during treatment. Due to an ongoing requirement for mechanical ventilation, tracheostomy was performed at the age of six months. The patient was discharged from the hospital at seven months of age. At 12 months he needed ventilation via tracheostomy only during sleep, equivalent to eight hours a day.

Improved mineralization was observed during the treatment (Figure 1). There was no significant hypercalcemia before the treatment and hypocalcemia was not observed during the same period. At age one year, the patient was able to sit up with support, with full head control, but he was not yet able to stand. No objective test, such as the Bayley Scales of Infant and Toddler Development, was performed during the first 12 months to evaluate neurocognitive functions. However we noted some signs of neuromotor development, for example placing a spoon in a cup. We could not evaluate speech function due to the tracheostomy. Seizures were not observed before or during treatment, and there was no sign of craniosynostosis at age 12 months. At this time his weight

Table 1. Biochemical findings in the patient and his parents

	Patient	Mother	Father
ALP (IU/L)	8 (100-380)	52.00 (25-94)	27.0 (33-107)
Pyridoxal 5'-phosphate (PLP) (µg/L)	14104.0 (5-30)	7.8 (5-50)	20.2 (5-50)
PEA (µmoL/g creatinine)	2767.5 (15-341)	41.6 (0-48)	21.8 (0-48)
PPi	NA	NA	NA

ALP: alkaline phosphatase, PLP: pyridoxal phosphate, PEA: phosphoethanolamine, PPi: inorganic pyrophosphate

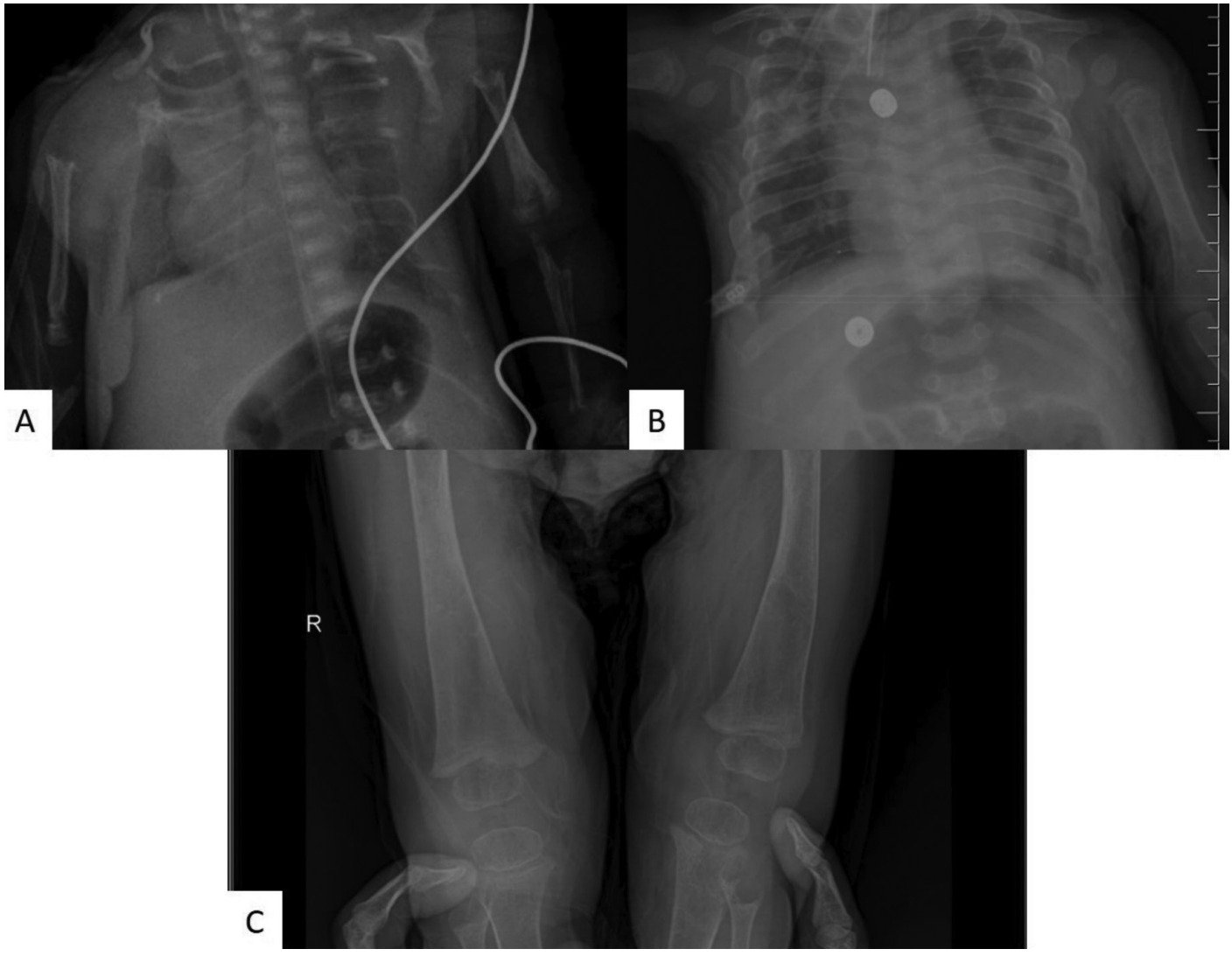

Figure 1. X-ray of the patient; (A) before treatment, (B and C) at 12 months of treatment. Note the general improvement of mineralization and of rachitic changes with asfotase alfa enzyme replacement therapy

Target/ ALPL Exon Ratio	
ex02	1,79
ex03	1,85
ex04	1,91
ex05	2,24
ex06	1,78
ex07	1,06
ex08	1,02
ex09	1,05
ex10	1,05
ex11	0,88
ex12	1,04

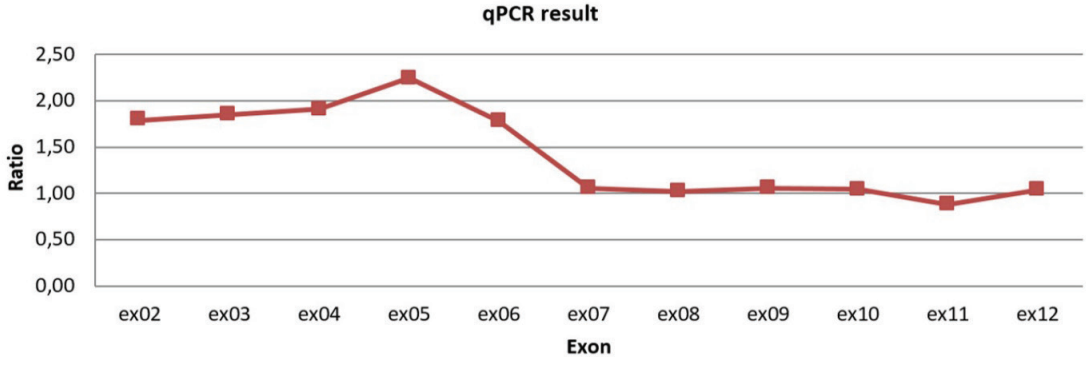

Figure 2. Quantitative polymerase chain reaction (qPCR) assay by using 11 gene-specific amplicons encompassing the coding exons 2 to 12 of the *ALPL* gene. Normalized qPCR ratios are WT (0.70-1.35) and homozygous duplication (4n) (1.75 -2.35)

qPCR: quantitative polymerase chain reaction

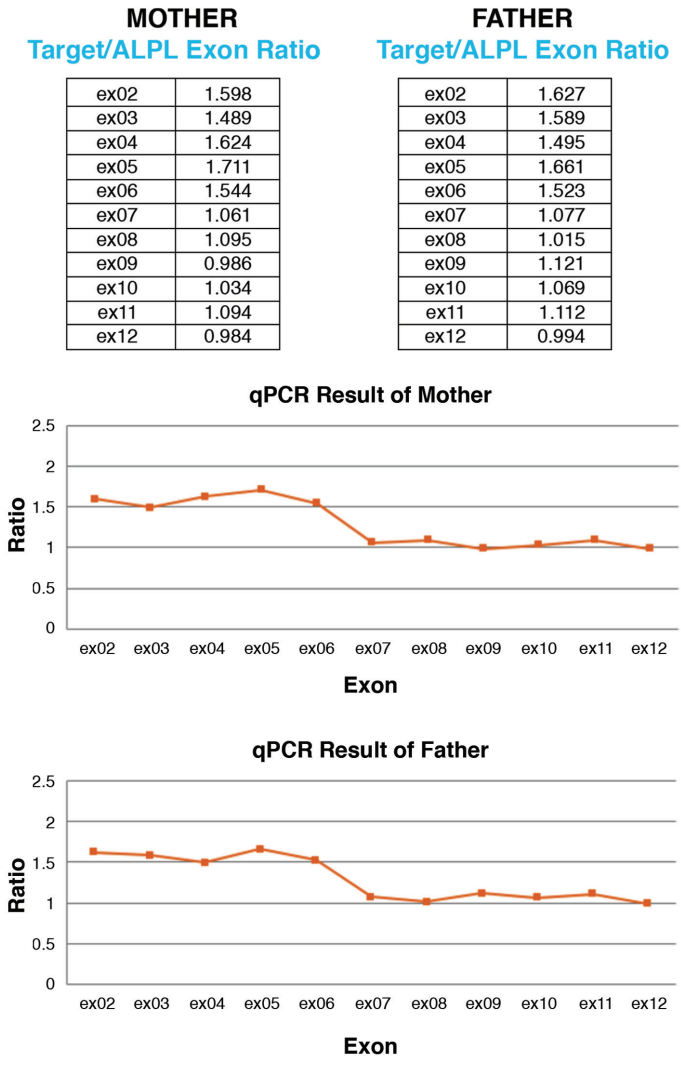

Figure 3. Mother and father heterozygous carriers of the same mutation

qPCR: quantitative polymerase chain reaction

was 8.0 kg [standard deviation score (SDS) -1.87], his height was 75.0 cm (SDS -0.59), and his head circumference was 46.0 cm (SDS -0.75). He continued to eat normally. Kidney function and renal ultrasound findings were normal. No side effects were observed during the first 12 months of treatment. The patient is currently still on treatment with asfotase alfa, and we hope to share long-term follow-up results in the future.

Written informed consent was obtained from the parents of the patients.

Discussion

To our knowledge large duplications on the *ALPL* gene have not been reported to date, but minor duplications are not rare (8,9). Herein, we report a novel large homozygote duplication encompassing exons 2 to 6 of the *ALPL* gene. Missense mutations are the most commonly reported type of mutation in this gene (9). This case highlights that, in a patient clinically diagnosed with HPP, duplication or deletion analysis should be performed if a mutation cannot be detected by sequencing.

Recently, it has been shown that ERT with asfotase alfa mineralizes the skeleton and improves respiratory function and survival in the life-threatening perinatal form of HPP (9,10). For patients with a perinatal form of HPP who receive ERT, many of whom had previously died in infancy, survival is the main goal, but not the only goal. Other goals of the treatment are improvement of respiratory status, skeletal mineralization, improvement of growth and physical development, promotion of normal developmental milestones, treatment of craniosynostosis, seizure control and reduced hospitalization requirement (11). We were able to discharge our patient when he was seven months old. His respiratory support was gradually reduced and skeletal mineralization improved during treatment. Asfotase alfa treatment has a good safety profile for children. Common adverse reactions are hypersensitivity reactions, localized lipodystrophy, ectopic calcification of the eye and nephrocalcinosis. Severe hypocalcemia has also been reported (11,12). We monitored our patient according to the current guidelines, and no adverse reactions were observed during the first 12 months (11).

In conclusion, this report describes a child diagnosed with the perinatal form of HPP with a novel large duplication in the *ALPL* gene. Although we were unable to perform cDNA studies/mRNA, this large duplication is very likely to be pathogenic based on ACMG guidelines. Early diagnosis and rapid intervention with ERT is life-saving in the severe form HPP. In patients with HPP duplications can be detected by qPCR, if a mutation cannot be detected by sequence analysis.

Ethics

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Bülent Hacıhamdioğlu, Gülşen Acar, Gamze Özgürhan, Serdar Cömert, Concept: Bülent Hacıhamdioğlu, Design: Bülent Hacıhamdioğlu, Data Collection or Processing: Bülent Hacıhamdioğlu, Analysis or Interpretation: Bülent Hacıhamdioğlu, Catarina Pereira, Emre

Tepeli, Literature Search: Bülent Hacıhamdioğlu, Gamze Özgürhan, Gülşen Acar, Writing: Bülent Hacıhamdioğlu, Catarina Pereira.

Financial Disclosure: Dr. BH received payment from Alexion Pharmaceuticals Company for being part of the HPP advisory board. Other authors have no financial relationships relevant to this article to disclose.

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CASE REPORT

DOI: 10.4274/jcrpe.galenos.2018.2018.0204

J Clin Res Pediatr Endocrinol 2019;11(3):311-318

Magnesium and Anti-phosphate Treatment with Bisphosphonates for Generalised Arterial Calcification of Infancy: A Case Report

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What is already known on this topic?

Generalized arterial calcification of infancy (GACI) is a rare disease that is associated with a high mortality rate owing to the development of severe hypertension and cardiovascular complications. GACI may begin *in utero* during the third trimester and 50% of children with GACI present with well-developed large arterial calcifications within the first week of life. Although there is no definitive treatment, it is claimed that patients treated with bisphosphonates have better survival rates. In contrast, children not treated with bisphosphonates were also reported to have spontaneous regression of large arterial calcifications. Furthermore, magnesium treatment has been reported to be beneficial in some experimental animal models.

What this study adds?

To date, only a few experimental treatment modes, other than bisphosphonates, have been proposed for GACI patients. This is a report of a patient who did not respond to bisphosphonates alone, but who subsequently improved clinically after treatment with magnesium and anti-phosphate therapy (calcium carbonate) along with continued bisphosphonates therapy.

Abstract

Generalized arterial calcification of infancy (GACI) is a rare autosomal-recessive disorder, characterized by calcification of the internal elastic lamina, fibrotic myointimal proliferation of muscular arteries and resultant arterial stenosis. Treatment with bisphosphonates has been proposed as a means of reducing arterial calcifications in GACI patients, although there is no formalized treatment approach. The case reported here was a patient with severe GACI diagnosed at three months of age who had no response to bisphosphonate treatment, but clinically improved after the initiation of magnesium and anti-phosphate (using calcium carbonate) treatments. In patients unresponsive to bisphosphonate, magnesium and anti-phosphate treatment may be attempted.
Keywords: Generalized arterial calcification, infant, treatment, magnesium, etidronate

Introduction

Generalized arterial calcification of infancy (GACI) is a rare autosomal-recessive disorder, characterized by calcification of the internal elastic lamina, fibrotic myointimal proliferation of muscular arteries and resultant arterial stenosis (1,2). An extravascular feature is that foci of periarticular calcification occur in many of the affected subjects. Depending on the severity and the local distribution of the calcific stenoses, affected patients can present with neonatal heart failure, arterial hypertension and death within the first six months of life (3,4).

GACI is linked to mutations in the ectonucleotide pyrophosphatase/phosphodiesterase 1 *(ENPP1)* gene, which encodes for ectonucleotide pyrophosphatase/

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Conflict of interest: None declared
Received: 08.08.2018
Accepted: 06.12.2018

phosphodiesterase 1 *(ENPP1)*. This enzyme facilitates hydrolysis of adenosine triphosphate to adenosine 5'-phosphate and inorganic pyrophosphate (PPi). PPi is a potent inhibitor of hydroxyapatite crystal deposition, while inorganic phosphate (Pi) serves as a pro-mineralization factor. Thus an appropriate ratio of PPi/Pi is required to prevent spontaneous calcium phosphate precipitation. In patients with GACI, deficiency of the ENPP1 enzyme leads to reduced PPi/Pi and ectopic mineralization (5,6,7). In addition, *ENNP1* gene mutations have been identified in some patients with pseudoxanthoma elasticum (PXE), another hereditary ectopic mineralization disorder. Most cases with PXE also harbor mutations in the *ABCC6* gene (8). Recent studies have demonstrated a considerable genotypic and phenotypic overlap between PXE and GACI (9).

There is no effective and formalized treatment approach for patients affected by GACI (6). After the original report by Meradji et al (10), first-generation bisphosphonates, which are synthetic analogues of PPi, have been widely used in an attempt to treat GACI patients. First-generation bisphosphanates have a stronger effect in inhibiting formation and further growth of hydroxyapatite crystals compared to newer generation bisphosphonates (5,11). However, a potential complication of bisphosphonates is severe skeletal toxicity associated with prolonged use in patients with GACI (6). In addition to the skeletal toxicity, bisphosphonate treated children were also reported to experience persistent calcifications, which is an unwanted side effect of the treatment. Furthermore, some children not treated with bisphosphonates were reported to have spontaneous regression of large arterial calcifications (12,13,14). The lack of consistency and limited data concerning the efficacy of these compounds has created difficulties in reaching a consensus on the safety and efficacy of bisphosphonate treatment for GACI.

Li et al (15) investigated the dual effects of bisphosphonates on ectopic skin and vascular soft tissue mineralization versus bone microarchitecture in a mouse model of GACI. Their results suggested that bisphosphonate treatment may be beneficial for preventing ectopic soft tissue mineralization while correcting decreased bone mineralization. Effects of etidronate and alendronate on ectopic calcifications in the *ENPP1^{asj}* mice were assessed at three different concentrations; doses the same as or five or 12 times greater than those used for treatment of osteoporosis. It was found that five times and 12 times greater doses of etidronate provided some benefit for reducing calcifications of the kidney, heart, descending thoracic aorta, or the eye.

Albright et al (16) used the identical animal model to evaluate the efficacy of ENPP1 enzyme replacement therapy in GACI. In this study, the breeding pairs were placed on the 'acceleration diet' to mimic the *in utero* calcification induced by ENPP1 deficiency and death was used as a preclinical endpoint. The results using ENPP1-enzyme replacement in this more severe preclinical study was a complete suppression of all ectopic calcification, as well as elimination of mortality. This study suggested that the efficacy of bisphosphonates was quite limited compared with what can be achieved with other more rational therapeutic interventions.

In a recent clinical trial of patients with PXE, the possibility of supplementing the diet with magnesium as a way of preventing mineralization was investigated (17). Kingman et al (8) showed the effects of dietary magnesium supplementation on ectopic mineralization in the vascular tissues in mice, a model for GACI, which shares genotypic and phenotypic overlap with PXE. Furthermore Rutsch et al (4) reported that application of a phosphate poor diet or a phosphate binding agent would be of interest with respect to early intervention in GACI. However, because this was a retrospective, small sized study with 55 subjects, it was difficult to draw any definite conclusion that patients with GACI may benefit from anti-phosphate treatment consisting of calcium carbonate supplementation.

In this case report, we report a case of a 3-month-old boy diagnosed with severe GACI who was unresponsive to bisphosphonate therapy but recovered after magnesium and calcium carbonate treatment in conjunction with continued bisphosphonate therapy.

Case Report

A three month old male infant with para-articular calcification was referred to the paediatric endocrinology department of our hospital. The patient's history revealed referral to the neonatology clinic at age 17 days because of arthritis in the right hip which had been noted in the first week of life. The infant was the second child of a 39-year old healthy mother and a 37-year old healthy father who were first degree cousins. He also had a three year-old healthy brother. The patient had been delivered by caesarean section at the gestational age of 38 weeks. Birthweight was 3680 g.

Septic arthritis was suspected, but acute phase reactants and cultures were negative. Histopathologic investigation of a biopsy specimen obtained from the right hip joint revealed severe calcification in the arterial walls with no evidence of inflammation.

At presentation, the patient's weight was 4900 g [-1.72 standard deviation (SD) score (SDS)] and his length was 58

cm (-1.22 SDS). He had prominent ears. Systemic physical examination was normal except for a swollen, painful and restricted right hip joint. Arterial blood pressure was measured at 121/84 mmHg, which was high (> 95th percentile) for a three month old boy. Echocardiography showed a normal left ventricle wall and coronary artery thickness. Audiologic and ophthalmologic assessments were normal. Routine biochemical tests were normal while plasma renin activity and aldosterone levels were above normal reference ranges (Table 1). Non-contrast abdominal computed tomography (CT) was performed. Diffuse narrowing of the abdominal aorta, bilateral renal arteries and iliac arteries was observed (Figure 1A). Soft tissue calcifications were observed in the paratracheal region at the laryngeal level and around the hyoid bone (Figure 1B). There were linear hyperdensities, consistent with calcification, in the mesenteric artery and its branches (Figure 1C). Periarticular calcifications in the right shoulder and right hip were observed (Figure 1D). Baseline radiographic images revealed arterial calcifications in the brachial and radial arteries on the left side and intra- and peri-articular calcifications in the left elbow and wrist joints (Figure 1E). There was no evidence of calcification in the cerebral arterial vessels on cranial CT. Due to the severe arterial calcification noted in the histopathologic investigation, a diagnosis of GACI was considered and *ENPP1* gene analysis was performed. A previously identified homozygote (c.2677G > T p.E893*) (p.Glu893*) mutation was detected in the *ENPP1* gene. The genetic analyses of the parents was not performed since the mutation was a previously reported one; however they have received genetic counselling.

Intravenous disodium pamidronate was administered as three doses on days 0, 7 and 10. On the fifth day of pamidronate treatment, oral etidronate was initiated at a dose of 10 mg/kg/day which was increased to 20 mg/kg/day after three days. After six months of etidronate treatment, calcifications on direct radiographs and CT persisted (Figure 2A, 2B, 2C) as well as intermittent swelling and restriction of joints. This suggested an inadequate response to biphosphonate treatment. Calcium carbonate treatment at a dose of 250 mg twice a day and magnesium oxide treatment 150 mg twice a day were started with a simultaneous reduction in Etidronate to a dose of 10 mg/kg/day. While calcium, phosphorus and other laboratory parameters were normal at baseline (Table 1), serum phosphorus concentration decreased following the anti-phosphate treatment, as expected. After the initiation of calcium carbonate and magnesium treatment, restriction and swelling of the joints gradually improved. No adverse effects were experienced in the follow-up period. A marked decrease of calcifications was seen in the radiographs which were taken during the sixth month of treatment. Calcium carbonate and magnesium treatments were continued while etidronate was further reduced to a dose of 5 mg/kg/day.

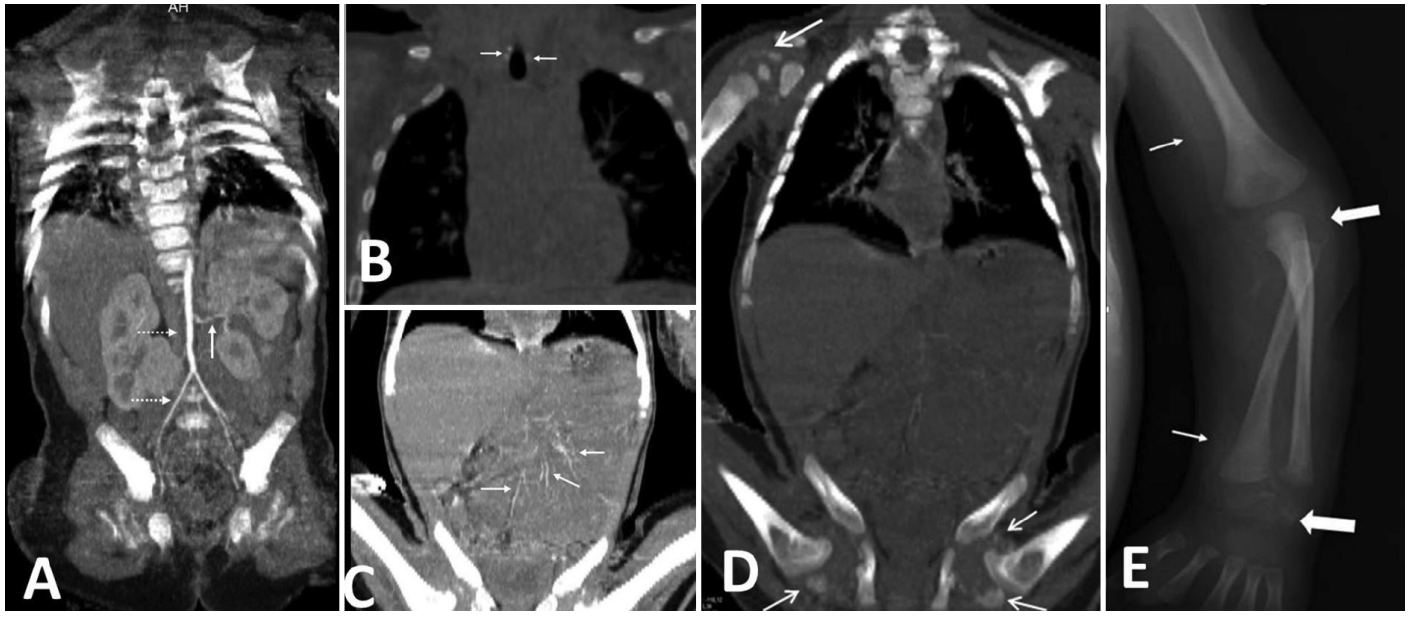

Figure 1. At age three months, coronal non-contrast computed tomography of abdomen and chest, A) Diffuse narrowing is seen in bilateral renal arteries, abdominal aorta and bilateral iliac arteries, B) At the level of the larynx, soft tissue calcifications are observed in the paratracheal region and around the hyoid bone, C) There are linear hyperdensities consistent with calcification in the mesenteric artery and branches, D) Periarticular calcification in the right shoulder joint and in the right hip joint, E) At age three months, on baseline radiograph; the left wrist shows arterial calcification of the brachial and radial arteries (thin arrow) and intra- and peri-articular calcifications in left elbow and wrist joints (thick arrow)

CT and CT angiography were performed at the end of the first year of calcium carbonate and magnesium treatments. The calcifications previously observed in the abdominal and mesenteric arteries had disappeared, there was no longer any narrowing of renal arteries evident and there was a significant reduction in calcifications in hip and shoulder joints (Figure 3A, 3B, 3C, 3D). In addition there was a significant clinical improvement in joint functions and motor development. At the most recent examination of the patient, at the age of 23 months, his weight was 10 kg (-1.93 SD), height was 85 cm (-0.7 SD), arterial blood pressure measurements were normal, joint movements were comfortable and neuromotor development was improving. The etidronate treatment was stopped and magnesium

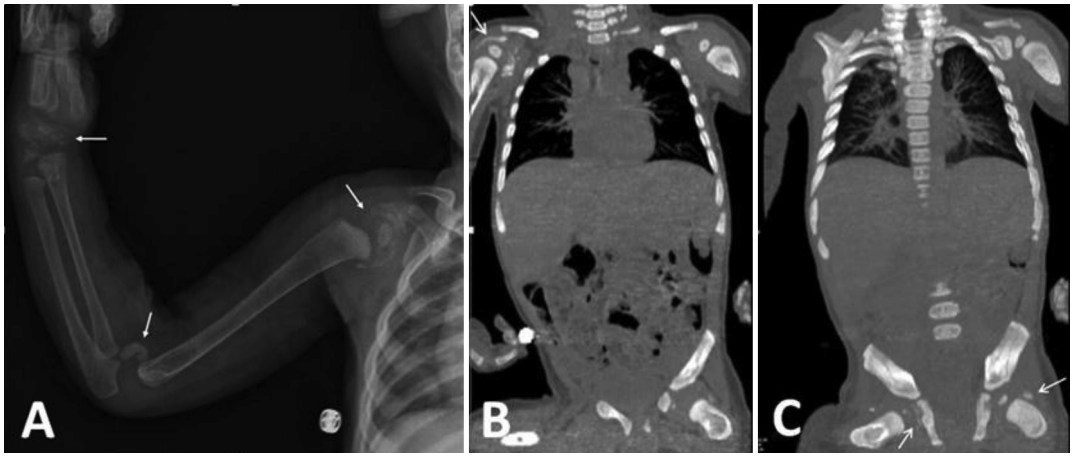

Figure 2. At age nine months, radiograph before magnesium and anti-phosphate treatment, A) Radiograph shows progression of periarticular calcification in the right shoulder, elbow and wrist joint, B) Coronal non-contrast computed tomography of abdomen demonstrates periarticular calcification in the right shoulder joint, and C) shows periarticular calcification in right hip joint

Table 1. Laboratory and auxological findings of the patient under treatment

Age (months)	3 months (diagnosis)	6 months	9 months	12 months	18 months	23 months
Weight (SDS)	-1.72	-2	-1.8	-1.66	-1.75	-1.93
Height (SDS)	-	-0.95	-0.66	-0.53	-0.45	-0.7
Etidronate dose (mg/kg/day)	20	20	10	5	5	-
Mg dose (mg/day)	-	-	300	300	300	300
Anti-phosphate dose (mg/day)	-	-	375	375	-	-
Ca (mg/dL) (NR: 9-11.5)	11.4	10.5	10.4	9.6	9.4	9.6
P (mg/dL) (NR: 4-6.5)	5.5	5.9	4.5	3.2	3	3.2
Mg (mg/dL) (NR: 1.7-2.3)	1.9	1.8	2.2	2.3	2.6	2.5
ALP (U/L) (NR: < 455)	226	185	261	255	231	156
PTH (pg/mL) (NR: 11-67)	49	18.1	25.3	51.6	50	51
25(OH)D_3 (ng/mL) (NR: > 20)	38.9	31.1	33.9	-	20.8	17
Renin (ng/mL/h) (NR: 0.3-1.9)	18.5	15.8	3.6	-	2.05	-
Aldosterone (pg/mL) (NR: 10-160)	1243	489	133	207	21.6	-

SDS: standard deviation score, Mg: magnesium, Ca: calcium, P: phosphate, ALP: alkaline phosphatase, PTH: parathyroid hormone, 25(OH)D_3: 25-hydroxy vitamin D_3, NR: normal range

treatment was continued. The course of treatment is shown in Figure 4.

Informed consent was obtained from the parents of the patient for publication of this case.

Discussion

Our patient presented with arthritis at the age of three months. He was diagnosed as GACI and treated with etidronate, magnesium and anti-phosphate. Approximately 50% of children with GACI present within the first week of life with large arterial calcifications which are reported to develop as early as the third trimester of pregnancy. The course of these children may be less favorable than children who present later (4). Although our patient was diagnosed with GACI at three months old, clinical findings consistent with GACI were reported to have been present during the first week of life.

Respiratory distress is one of the presenting features in more than 50% of cases, followed by feeding intolerance, poor weight gain, tachypnea, tachycardia and cyanosis (4,18,19). The disease usually results in death in infancy due to progressive ischemic heart failure associated with coronary calcification. Survivors of GACI frequently present with periarticular calcifications rather than coronary calcification (4).

Treatment options in GACI are limited to the use of bisphosphonates, such as etidronate and pamidronate (18). Bisphosphonates are synthetic analogs of inorganic pyrophosphate, which block the conversion of calcium phosphate to hydroxyapatite and thus may reduce

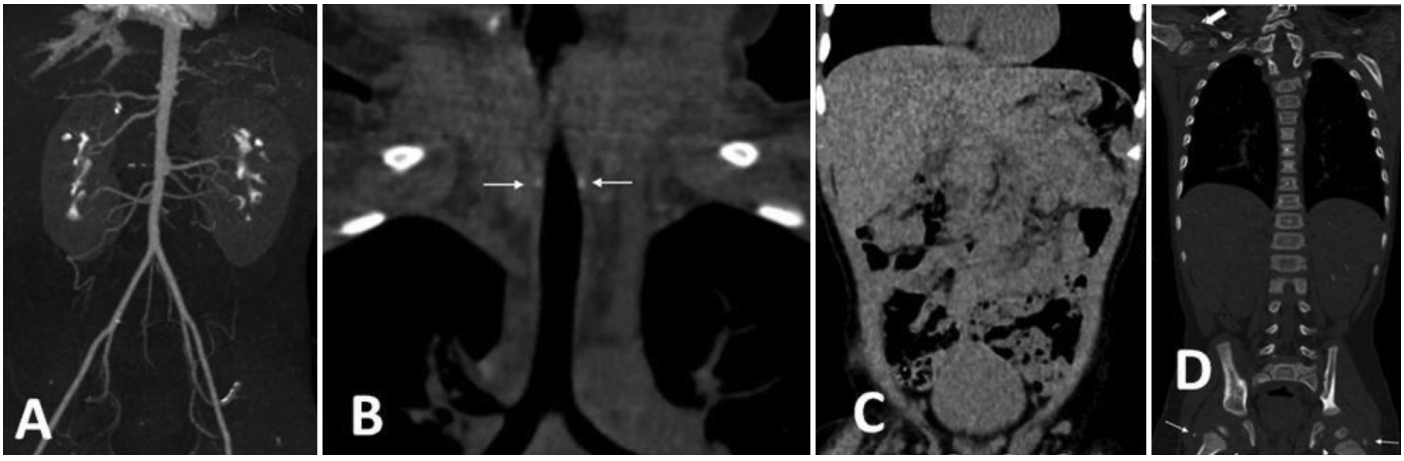

Figure 3. Computed tomography (CT) angiography images and non-contrast CT of abdomen and chest after treatment with magnesium demonstrates: A) Normal appearance of abdominal aorta, bilateral internal and external iliac arteries, femoral artery, renal and mesenteric arteries, B) At the level of the larynx, soft tissue calcifications are reduced in the paratracheal region and around the hyoid bone. C) Mesenteric artery wall calcifications are not observed. D) Periarticular calcification in the right shoulder joint and in the right hip joint are reduced

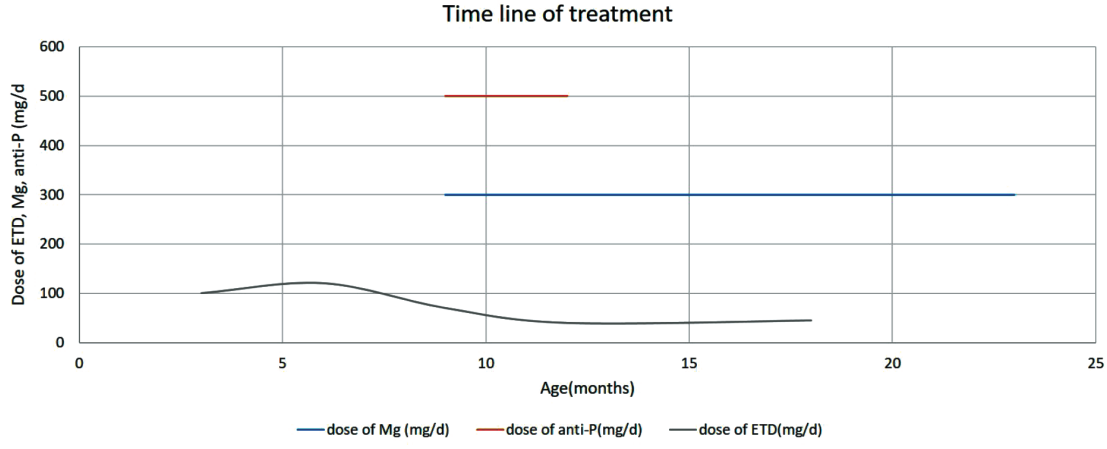

Figure 4. Time line chart of treatments received by the patient

Mg: magnesium, ETD: etidronate, anti-P: anti-phosphate

ectopic calcification (19). Etidronate, as a first-generation bisphosphonate, has been used most frequently at a dose of 5-35 mg/kg/per day orally (5). Etidronate has a stronger effect in inhibiting mineralization compared to the newer aminobisphosphonates and shows no adverse effect on growth (19). However, high-dose etidronate injections have been shown to induce vitamin D-resistant rickets in rats (20). Other nitrogen-containing bisphosphonates, which have been used in earlier case series reports of GACI, include intravenous pamidronate and oral risedronate (5,21). *In vitro* studies have shown that bisphosphonates accumulate within vessel walls suggesting that these drugs may have a direct effect on calcification (6). As the starting treatment, we administered three intravenous doses of pamidronate infusion, in accordance with previous reports (6). Subsequently, oral etidronate was added to the treatment.

It is difficult to evaluate whether recovery occurs spontaneously or with the effect of bisphosphonates. Long-term survival has been reported in GACI patients with no specific therapy, thus the possibility of spontaneous resolution of calcification should be considered (12,14). In a retrospective study it was reported that 17 of 55 patients affected by GACI were treated with bisphosphonates, namely etidronate, pamidronate, clodronate or risedronate. Survival rate of these treated patients was found to be 65%, while 69% of patients who were not treated with bisphosphonates had died in infancy (4). These authors have also claimed that children treated with bisphosphonates have a survival advantage, but this claim was based on observations in a retrospective study with a small sample size rather than a blinded clinical trial. Indeed, the survival advantage suggested by these authors was not statistically significant. More favorable outcomes in some children could be related to disease severity. Patients with less severe disease may survive long enough to be transported to a medical center, evaluated and treated with bisphosphonates. The persistence of calcifications, especially in periarticular regions leading to severe restriction and contractures, indicated a need for exploration of alternative therapeutic options. Magnesium treatment was reported to be effective in *ENPP1* knockout mice. In a recent study, Kingman et al (8) showed that elevated dietary magnesium during pregnancy and postnatal life prevents ectopic mineralization in *ENPP1*^{asj} mice, a model for GACI. Based on this experimental report, oral magnesium oxide treatment at a dose of 150 mg twice a day was commenced in our patient.

The mechanism for the inhibition of ectopic mineralization by magnesium may involve direct interactions between magnesium and calcium ions in the mineralization process. Magnesium competes with calcium, reduces calcium-phosphate binding and forms magnesium phosphate complexes. These complexes, which are soluble, prevent mineral deposition (8).

Phosphate levels are high in healthy newborns, probably due to low glomerular filtration rate and retention of phosphate (22). These higher levels may lead to an increased risk of arterial calcification in young patients with ENPP1 deficiency during the first few months of life, which may decline with age (6). PPi and Pi seem to have mutually antagonistic roles in tissue mineralization. Significantly, a phosphate-poor diet induces hypophosphatemia with markedly decreased artery calcification and periarticular calcifications. Furthermore, several mutations in the *ENPP1* gene result in the phenotype of autosomal recessive hypophosphatemic rickets (ARHR2) without any arterial calcifications (5,23). Furthermore in some patients with generalised arterial calcification due to *ENPP1* mutations in infancy, hypophosphatemic rickets developed in the following years. Treating these patients with calcitriol and phosphorus led to the recurrence of calcifications. It has been reported that hypophosphatemia is a protective factor against vascular calcification (24). Rutsch et al (4) found that both hypophosphatemia and hyperphosphaturia are associated with GACI survival. Both the hypophosphatemia and hyperphosphaturia were linked to increased fibroblast growth factor 23 (FGF23) concentrations. FGF23 is a hormone which induces phosphate wasting in the urine. GACI patients with elevated FGF23 and low phosphate are expected to have reduced vascular calcifications. Therefore, phosphate wasting via increased FGF23 production may be an adaptive mechanism in GACI to accommodate the low plasma PPi by reducing plasma Pi in an attempt to preserve the Pi/PPi ratio. A consequence of the hyperphosphaturia is osteomalacia and rickets, seen in ARHR2. This may indicate an association between ARHR2 and GACI (25). Since arterial calcifications could be lethal in infancy, thesepreviously reported observations encouraged the hypothesis that creating a controlled hypophosphatemia could decrease mortality. A careful and closely monitored balance between bone demineralisation and arterial calcifications should be sought. In our patient, treatment with both magnesium and calcium carbonate was started with the aim of lowering phosphate levels and keeping the magnesium levels within the upper limit. As shown in Table 1, there was a significant clinical improvement after the initiation of concurrent magnesium and calcium carbonate treatment. Joint mobility improved and hypertension recovered. Marked reduction in calcifications was detected on direct radiographs and CT performed at

the after 12 months of this combined therapy. No adverse effects were observed during the treatment process.

The limitation of this study include a lack of adequate experience in treating patients with GACI and, since recovery of calcifications in GACI occur spontaneously in some of the survivors in the absence of any therapeutic intervention, it is difficult to conclude that the improvement was due to the treatment protocols. Due to inadequate knowledge of the natural history of GACI, we hesitated to cease the bisphosphonate treatment at the beginning of the magnesium and calcium carbonate treatment. Between the sixth and twelfth month of this additional therapy, the patient also received etidronate at gradually reduced doses, together with other treatments. Thus, in this case it is impossible to ascertain the relative benefits of the treatments given, including the effects of varying doses of etidronate, although the outcome at nearly two years of age appears clinically good.

In the current case, a sufficient clinical response was not obtained after six months of etidronate treatment. Bisphosphonate treatment had not made a significant impact on the regression of calcifications. Although there was insufficient information in the literature concerning magnesium and calcium carbonate treatment, there are animal data showing that these could be effective. A significant reduction in calcifications after the initiation of magnesium and calcium carbonate treatment was observed in our patient. Thus we believe that this treatment option should be considered in GACI patients, especially in those in whom bisphosphonates appear clinically ineffective. Further case reports and, ideally, carefully designed studies would help to resolve this matter in the future.

Ethics

Informed Consent: Informed consent was obtained from the parents of the patient for publication of this case.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Medical Practices: Fatma Dursun, Betül Sözeri, Concept: Fatma Dursun, Design: Fatma Dursun, Data collection or processing: Fatma Dursun, Serçin Güven, Tülay Atasoy Öztürk, Betül Sözeri, Analysis or interpretation: Fatma Dursun, Serçin Güven, Gülcan Seymen Karabulut, Heves Kırmızıbekmez, Sevinç Kalın,Tülay Atasoy Öztürk, Literature Search: Fatma Dursun, Serçin Güven, Tülay Atasoy Öztürk, Heves Kırmızıbekmez, Gülcan Seymen Karabulut, Writing: Fatma Dursun, Gülcan Seymen Karabulut, Betül Sözeri, Heves Kırmızıbekmez, Tülay Atasoy Öztürk.

Financial Disclosure: The authors declare that this study has received no financial support.

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CASE REPORT

DOI: 10.4274/jcrpe.galenos.2018.2018.0227

J Clin Res Pediatr Endocrinol 2019;11(3):319-326

A Patient with Berardinelli-Seip Syndrome, Novel *AGPAT2* Splicesite Mutation and Concomitant Development of Non-diabetic Polyneuropathy

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What is already known on this topic?

Within Berardinelli-Seip syndrome (congenital lipodystrophy disorders characterized by total absence of both metabolic and mechanical fat tissue) patients, only congenital generalized lipodystrophy type 1 (CGL1) retains mechanical fat and is exclusively associated with 1-acylglycerol-3-phosphate O-acyltransferase 2 *(AGPAT2)* gene mutations. Polyneuropathies and learning deficiencies are currently unknown in the context of classical CGL1 disease and *AGPAT2* lesions.

What this study adds?

The case history of a patient with classical CGL1 followed for 27 years is presented. The patient, in addition to mechanical fat retention, developed polyneuropathy and learning deficiencies. A new *AGPAT2* intronic deletion was detected in this patient. Our results describe a phenotype expansion for CGL1 and suggest that certain *AGPAT2* gene lesions cause neuropathy which blurs clinical presentation boundaries for CGL and other fat biology disorders.

Abstract

Primary polyneuropathy in the context of Seip-Berardinelli type 1 seipinopathy, or congenital generalized lipodystrophy type 1 (CGL1) has not been previously reported. We report the case history of a 27 year old female CGL1 patient presenting with an unusual additional development of non-diabetic peripheral neuropathy and learning disabilities in early adolescence. Whole exome sequencing (WES) of the patient genome identified a novel variant, homozygous for a 52 bp intronic deletion in the *AGPAT2* locus, coding for 1-acylglycerol-3-phosphate O-acyltransferase 2, which is uniquely associated with CGL1 seipinopathies, with no molecular evidence for dual diagnosis. Functional studies using RNA isolated from patient peripheral blood leucocytes showed abnormal RNA splicing resulting in the loss of 25 amino acids from the patient AGPAT2 protein coding sequence. Stability and transcription levels for the misspliced *AGPAT2* mRNA in our patient nonetheless remained normal. Any AGPAT2 protein produced in our patient is therefore likely to be dysfunctional. However, formal linkage of this deletion to the neuropathy observed remains to be shown. The classical clinical presentation of a patient with *AGPAT2*-associated lipodystrophy shows normal cognition and no development of polyneuropathy. Cognitive disabilities and polyneuropathy are features associated exclusively with clinical CGL type 2 arising from seipin *(BSCL2)* gene mutations. This case study suggests that in some genetic contexts, *AGPAT2* mutations can also produce phenotypes with primary polyneuropathy.

Keywords: Berardinelli-Seip syndrome, seipinopathy, congenital generalized lipodystrophy, polyneuropathy, *AGPAT2*, fat biology

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Conflict of interest: None declared
Received: 19.09.2018
Accepted: 17.12.2018

Introduction

Berardinelli-Seip syndrome, also known as congenital generalized lipodystrophy (CGL), occurs in approximately 1 in 10 million of the world population and can result from mutation in four genes, giving rise to four clinically similar but distinguishable subsyndromes affecting fat biology (1). CGL type 1 (OMIM#608594) is autosomal, recessive and uniquely associated with mutation in the *AGPAT2* gene encoding 1-acylglycerol-3-phosphate O-acyltransferase 2 (2). This enzyme is integral to phospholipid biosynthesis, triglygeride/fat formation and storage, adipocyte formation and fat metabolism pathways and has multiple molecular interaction partners (1).

Clinical symptoms associated with CGL1 described to date involve both metabolic malfunctions and physical malformations present in all forms of CGL. Complete lack of all metabolic body fat (adipose tissue that stores energy) from birth is the central clinical characteristic for all forms. CGL1 patients alone, however, retain mechanical fat (adipose tissue that provides protective padding for joints and points of impact, i.e. palms, soles of feet, joints, scalp facial bones). This fat distribution is specific and differentially diagnostic for CGL1 (1). Clinical neuropathy and cognitive deficits are associated with CGL2 and mutations in seipin *(BSCL2)*, and are rare but not unknown for CGL3 and CGL4 syndromes [associated with caveolin-1 *(CAV1)* and cavin (RNA polymerase 1 and transcript release factor: *PTRF*) gene mutations, respectively] (1,2). Primary neuropathy and cognitive deficit in the context of CGL1, in the absence of diabetic or other secondary disease complications, are previously unreported traits.

We present the natural history of a female CGL1 patient, continuously recorded from infancy to adulthood. We further demonstrate that this patient carries a previously unknown homozygous intronic deletion variant g.12562_12613del p.(Val197Glufs*32) in the *AGPAT2* gene. Our functional studies show that the deletion disrupts normal *AGPAT2* transcriptional processing and mRNA coding content consistent with a dysfunctional and therefore potentially pathogenic effect for this deletion.

Case Report

Table 1 lists the clinical symptoms from infancy (three months) to current age (27 years) in chronological order of emergence in this female patient.

Family history disclosed distant parental consanguinity; identity by descent is corroborated by extensive absence of heterozygosity (AOH) totalling 46 Mbp, in the patient genome, with an average AOH region size of 321 Kbp. The AOH block encompassing the patient *AGPAT2* gene is 1.1 Mbp (see Methods). Direct ancestors on both sides lived in the same village for many generations. Both parents are clinically asymptomatic for CGL1. One grandfather however, presented with lipodystrophy and diabetes mellitus (DM) (Column 4, Table 1).

The patient was born to a 34 year old multigravida mother by spontaneous vaginal delivery. The father was 39 years old. Parents reported an unremarkable prenatal history. Clinical evaluation of the patient at age three months revealed the presence of numerous dysmorphic and metabolic features associated with CGL (Table 1) including seven clinical features diagnostic for CGL (1-7, Table 1). Physical examination revealed generalized lipodystophy, large hands and feet, and enlarged tongue (8-10, Table 1), a low anterior hairline and low set ears (11-12, Table 1), hepatomegaly and an umbilical hernia (13, Table 1).

Imaging techniques revealed that the patient had cardiovascular system abnormalities including concentric hypertrophic cardiomyopathy, left ventricle enlargement and thickened intraventricular septum (5, Table 1). Heart muscle contractility was good. Abdominal ultrasound imaging showed a hyperechogenic liver (3, Table 1). Pathological evaluation of a liver biopsy specimen showed microvesicular steatosis and intertrabecular fibrosis (3, Table 1).

Metabolic abnormalities present from birth included elevated serum triglycerides (4.01 mmol/L; normal range 0.4-1.8 mmol/L) and low high-density lipoprotein-cholesterol (HDL-C) (0.58 mmol/L; normal range 0.9-2.0 mmol/L).

Oral glucose tolerance test (OGTT) was normal; both fasting glucose and 120 minute glucose were 4.7 mmol/L and glycated hemoglobin (HbA1c) was 4.82% (normal range 4.5-6.5%). Other abnormal laboratory studies included mildly elevated serum alanine aminotransferase (50.2 U/L; normal range 0-41 U/L) and high alkaline phosphatase (ALP) (351 U/L; normal range 20-150 U/L). All of these metabolic imbalance findings, with the exception of elevated ALP, were progressive conditions (1-6, Table 1).

At age seven years the patient displayed acanthosis nigricans on the nape of the neck, in the axillary and popliteal regions and had prominent musculature, due to general absence of metabolic fat tissue and abnormal fat deposition in muscles, (14-17, Table 1). At this age it was noted that despite absence of metabolic fat, mechanical fat tissue was maintained. Accelerated linear growth velocity was evident, concomitant with an accelerated skeletal maturation of two years. Serum growth hormone

Table 1. Developmental timeline for clinical emergence of Seip-Berardinelli syndrome and neuropathology features in our patient

	Features	HPO no.	Age at emergence	Familial features (grandfather)
1	Congenital generalized lipodystrophy x	HP:0009059	+ + + 3 m ●	+ + + ▲
2	Hypertriglyceridemia	HP:0002155	+ + + 3 m ●	Nd
3	Hepatomegaly x	HP:0002240	+ + 3 m ●	Nd
4	Hepatic steatosis x	HP:0001397	+ + 3 m ●	Nd
5	Concentric hypertrophic cardiomiopathy x	HP:0005157	+ + 3 m ●	Nd
6	Elevated hepatic transaminases (alanine aminotransferase)	HP:0002910	+ 3 m ●	Nd
7	Elevated alkaline phosphatase	HP:0003155	+ 3 m	Nd
8	Large hands x	HP:0001176	+ 3 m	Nd
9	Large feet x	HP:0001833	+ 3 m	Nd
10	Increased tongue size	HP:0000158	+ 3 m	Nd
11	Low anterior hairline	HP:0000294	+ 3 m	Nd
12	Low set ears	HP:0000369	+ 3 m	Nd
13	Umbilical hernia x	HP:0001537	+ 3 m	Nd
14	Acanthosis nigricans x	HP:0000956	+ + + 7 y ●	+ + + ▲
15	Accelerated skeletal maturation x	HP:0005616	+ 7 y	Nd
16	Accelerated linear growth x	HP:0000098	+ 7 y	Nd
17	Abnormality of the musculature x	HP:0003011	+ 7 y	Nd
18	Intellectual disability	HP:0001256	+ 14 y	Nd
19	Acroparesthesia (hands)	HP:0031006	+ 14 y	Nd
20	EMG: neuropathic changes	HP:0003445	+ 14 y	Nd
21	Decreased motor NCV	HP:0003431	+ 14 y (median nerve and peroneal nerves)	Nd
22	Decreased sensory NCV	HP:0003448	+ 14 y (median and sural nerves)	Nd
23	Amenorrhea x	HP:0000141	+ + + 16 y	Na
24	Polycystic ovarian syndrome x	HP:0000147	+ + + 16 y	Na
25	Hypoplasia of the ovary	HP:0008724	+ + + 16 y	Na
26	Hyperinsulinemia x	HP:0000842	+ + + 16 y	Nd
27	Noninsulin-dependent diabetes mellitus x	HP:0005978	+ + 16 y ●	+ + ▲
28	Increased circulating androgen level	HP:0030348	+ + 16 y	Nd
29	Labial hypertrophy	HP:0000065	+ + 16 y	Na
30	Clitoromegaly x	HP:0008665	+ + 16 y	Na
31	Weakness of the intrinsic hand muscles (asymmetric)	HP:0009005	+ 16 y	Nd
32	Hirsutism x	HP:0001007	+ 16 y	Nd

Seip-Berardinelli symptoms are shown on white background, primary neuropathologies are shaded grey; HPO no.: Human Phenotype Ontology database identification number for phenotypic abnormality, EMG: electromyogram, NCV: nerve conduction velocity.

+ + +: strong presentation, + +: medium presentation, +: mild presentation, Nd: no data, Na: not applicable, y: years, m: months, ●: progressive condition, ▲: age at emergence unknown, x: diagnostic for CGL1

concentration was low [0.83 ng/mL (result below 1 ng/mL excludes acromegaly)] and a magnetic resonance imaging (MRI) scan of the hypophysis was normal. Gigantism was not observed, despite the appearance of acromegaloid features.

At age 14, neurological symptoms (18-22, Table 1) began to emerge. Learning disability (IQ score 88) (18, Table 1) was first noted at this age. We also noted acroparaesthaesiae in both hands (19, Table 1). In addition we found changes in electromyogram (EMG) tracings, a mildly reduced neurogenic

pattern and decreased motor fibre nerve conduction velocity (NCV) in median, sural and peroneal nerves which was also present in the sensory fibres of the median and sural nerves (20-22, Table 1). MRI scans however showed no sign of median nerve compression. Normal serum calcium, phosphorus, magnesium and parathyroid hormone concentrations further excluded hypoparathyroidism.

At age 16, the patient presented with multiple endocrine abnormalities. At this time a number of clinical features emerged relating to hormonal disturbances (23-30, 32 Table 1). She presented with primary amenorrhoea with clinical and laboratory findings of hyperandrogenism including clitoral enlargement, elevated free testosterone 11.33 pg/mL (normal range 1.1-6.3 pg/mL) and elevated androstenedione 3.60 ng/mL (normal range 0.8-2.4 ng/mL) (28,30 Table 1). Sonographic evaluation showed atrophic ovaries with no ovarian follicles (28, Table 1). Hirsutism was also first noted at this age (32, Table 1). Hormonal function of the hypophysis was normal.

The patient also developed DM (27, Table 1) identified on the basis of an OGTT (0 min - 3.7 mmol/L; 120 min - 11.9 mmol/L) with a high homeostasis model assessment of insulin resistance value [10.39 (normal < 2.5)]. HbA1c was 4.6%.

Current status: At 27 years, our patient has graduated from college and is employed as a clerk in an office. Paraesthesiae of the hands has not worsened since its first appearance. Diabetes is well-controlled (HbA1c - 5.1%), but dyslipidemia persists despite aggressive therapy (serum triglycerides - 3.8 mmol/L; HDL-C - 0.29 mmol/L). Current medications include metformin (3 g/day), fenofibrate (267 mg/day), rosuvastatin (10 mg/day) and insulin (1.5 UI/kg/day).

Molecular Analyses

WES analysis revealed a homozygous 52 bp intronic deletion, g.12562_12613del p.(Val197Glufs*32), affecting the 5'splice site for exon 5 of the *AGPAT2* gene. Bioinformatic prediction software (MutationTaster) indicates the deletion (g.12562_12613del) is of unknown pathogenicity. This variant is absent from the ExAC and 1000 G databases. No rare variant alleles in other known disease associated genes were found which could potentially explain the lipodystrophy phenotype or the neuropathy observed in our patient (3).

Sanger sequencing confirmed the *AGPAT2* deletion variant and cosegregation with the disease trait according to Mendelian expectations (Figure 1A) and also showed the expected reference sequence around the deletion at the nucleotide level (Figure 1B). This shows the patient is homozygous for the g.12562_12613del p.(Val197Glufs*32) allele. Also, each clinically asymptomatic parent is heterozygous for the identical variant allele (Figure 1C, 1D).

Standard polymerase chain reaction (PCR) (PCR; see Figure 1E, upper image) on genomic template DNA from both patient and parents generated a PCR product shorter (red arrowhead) than the wildtype (grey arrowhead), consistent with a 52 bp genomic deletion. Standard PCR on cDNA templates (lower image) revealed a PCR product shorter by 75 bp for the patient *AGPAT2* mRNA (red arrow) relative to the wildtype control individual mRNA (grey arrowhead). Direct Sanger sequence of the cDNA PCR products showed complete deletion of exon 5 (75 bp), leaving exon 4 joined to exon 6 with a frameshifted coding sequence downstream of the join, creating a premature translation stop signal. The parents are each heterozygous for the deletion and generate both forms of mRNA, and therefore show both mutated and wildtype PCR products (Figure 1E, mother, father).

To assess mutant *AGPAT2* mRNA expression levels and/or stability we used real time-PCR (RT-PCR) to quantify mutant and WT *AGPAT2* mRNA. Expression levels of *AGPAT2* mRNA generated from patient (homozygous for deletion allele) and both parent (heterozygous for deletion allelle) mRNA samples are comparable to those of a healthy control individual (homozygous for wiltdtype allele) (Figure 1F). Stability and/or transcription levels therefore appear unaffected for the mutant mRNA. We concluded that *AGPAT2* mRNA expression levels and stability in our patient remain unaffected by this deletion.

Methods Used in the Genetic Analysis

Genomic DNA samples were isolated from blood leucocytes from each individual using automatic magnetic bead-based method (MagnaPure, Roche). Copy number variations were identified using array Comparative Genomic Hybridization (aCGH: CytoSure Constitutional v3 8x60K, Oxford Gene Technology) and bioinformatic analyses using XHMM (4) and HMZDelFinder (5) algorithms; single nucleotide variation was determined by WES analysis (6), and confirmed by Sanger sequencing. Chromosomal regions demonstrating AOH were detected by analyzing B-allele frequency data obtained from WES (6) by running BafCalculator accessible from https://github.com/BCM-Lupskilab/BafCalculator (7). *AGPAT2* expression was measured by quantitative RT-PCR (TaqMan Gene Expression Assay for *AGPAT2* gene, Life Technologies, Grand Island, NY, USA), on blood lymphocyte mRNA isolated using High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Grand Island, NY, USA). Level of *AGPAT2* expression was corrected to the mRNA level of the housekeeping genes GAPDH and TBN. Expression

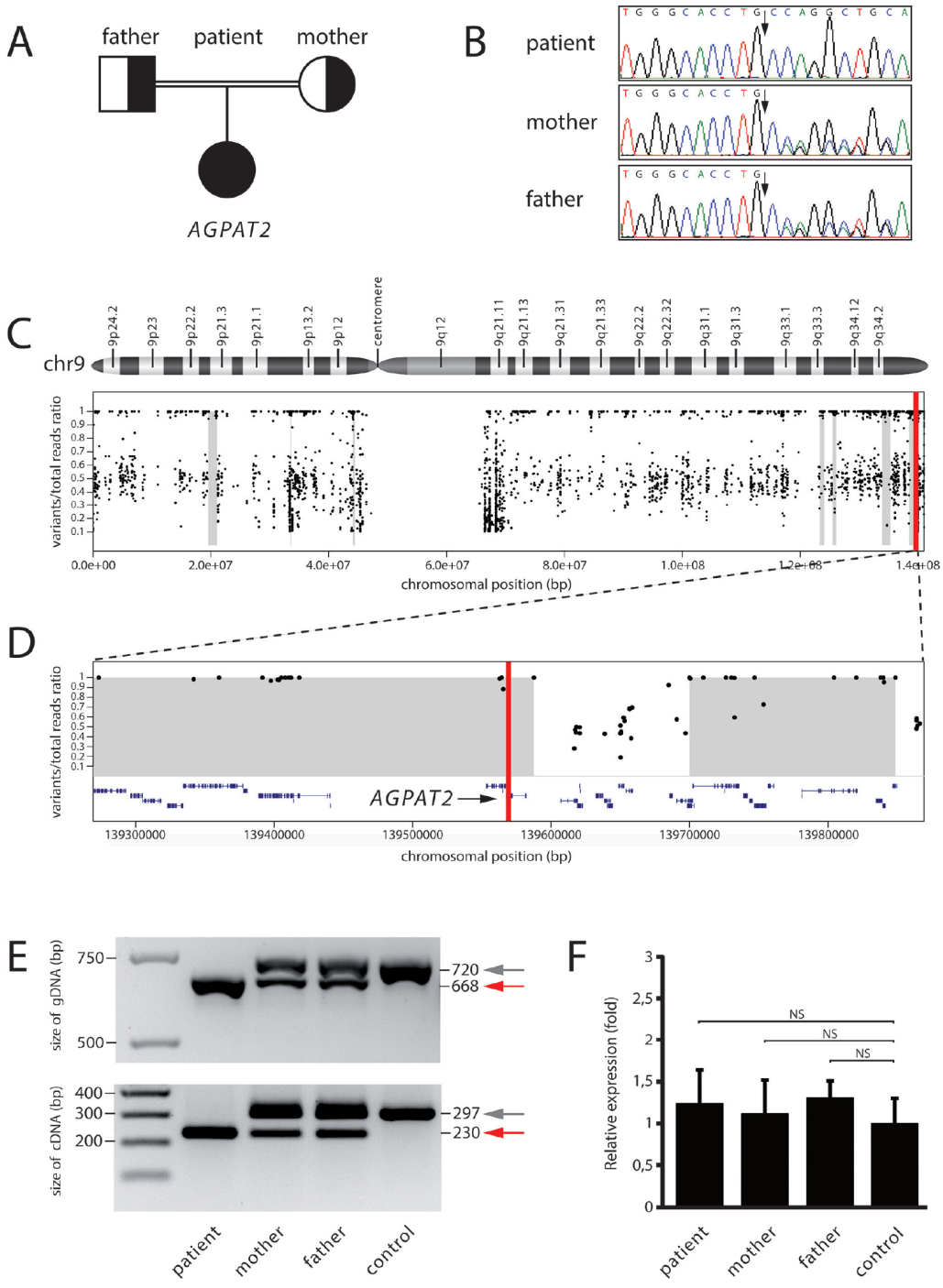

Figure 1. A) Patient pedigree for homozygous *AGPAT2* deletion mutation c.589-55_589-4del p.(Val197Glufs*32). B) Sanger sequence confirmation of biparental inheritance for the mutation. Black arrow shows point of deletion. C) Genomic context of *AGPAT2* mutation. Cartoon shows Chromosome 9 organization. Grey blocks denote regions with absence of heterozygosity (AOH). Scattered dots indicate single nucleotide variation (SNV) for proband along chromosome 9. Absence of SNV in centromere-adjacent areas reflects lack of reference sequence for these region. D) Detail for AOH region surrounding the *AGPAT2* mutation (vertical red line at around 13 958 000 base pair). E) PCR products for *AGPAT2* in patient (mutation homozygous), each parent (mutation heterozygous) and control individual (wildtype homozygous). Red arrowhead, reduced *AGPAT2* product size reflecting deletion mutation. Grey arrowhead, normal size wildtype *AGPAT2* product. Genomic gDNA template, upper image; complementary cDNA template lower image. F) Real time-polymerase chain reaction products for *AGPAT2* mRNA expression levels in patient, parents and control are comparable

NS: non-significant

data reflected the means of three independent experiments each performed in triplicate.

Primers and probes:

gDNA PCR

F: CTCACTGGCTTCCTGAGATGG; R: GGTCCATCCGTGTGAAGTCT

cDNA PCR

F: GGGAGAACCTCAAAGTGTGG; R: GGTCTTGGAGATGTGGAGGA

RT-PCR

TaqMan Gene Expression Assay, Thermofisher labelled probes cat. no. HS00944961.

The study was approved by the Bioethics Committee of the Institute of Mother and Child, Warsaw. Informed consent was obtained from the patient and her parents.

Discussion

The detailed, lifelong clinical case history revealed findings diagnostic for Berardinelli-Seip syndrome from infancy. Childhood mechanical fat distribution was diagnostic for CGL1. This patient also showed development of polyneuropathy and cognitive disability in early adolescence, symptoms not previously reported in CGL1 patients.

Intellectual disability is typical of CGL2 and rare in other forms of CGL. Primary neuropathy in the absence of DM complications leading to neural pathology, has been associated with CGL2 but has not been reported for CGL1. Polyneuropathy has been associated with a range of lipodystrophic disorders, but in CGL1 patients, the neuropathy reported to date arises from diabetic complications or other secondary conditions. In our patient, laboratory evidence of diabetes was only found two years after initial development of neuropathy. We suggest that the polyneuropathy observed was therefore unlikely to be a diabetic complication. EMG/NCV results (see 20-21, Table 1) suggest demyelinating neuropathy, similar to that caused by duplications in the *PMP22* gene, responsible for Charcot-Marie Tooth (CMT) type 1A syndrome. However, no *PMP22* duplication was detected, nor were any recessive CMT genes found to map within AOH intervals. The possibility that elevated patient triglyceride levels contribute to the clinical manifestation of peripheral neuropathy however, cannot be excluded.

All forms of CGL involve complete lack of metabolic fat (body fat) from birth and the majority show early development of severe hypertriglyceridemia, hepatic steatosis, hepatosplenomegaly, acanthosis nigricans and insulin resistance, generally leading to diabetes in early adolescence. Enlargement of liver tissue and slightly enlarged hands and feet are also typical. Myocardiopathies arise in approximately 25% of individuals. In the case described here the emergence at age 16 of multiple symptoms related to hormonal disturbances after puberty (eg. polycystic ovarian syndrome and hyperinsulinemia) is also typical for all Berardinelli-Seip syndromes including CGL1. Our patient presented with all these CGL-associated symptoms by early-mid adolescence, with concomitant emergence of neuropathological symptoms and learning disability in early adolescence (8). In addition, umbilical hernia, present in our patient at three months, was reported to be associated only with *BSCL2* mutations in one patient (9). The clinical picture therefore suggests CGL2, despite the normal mechanical fat distribution differentially diagnostic for CGL1 (1).

Our genomic investigation nonetheless confirms a previously unknown, single exon, homozygous 52bp deletion in *AGPAT2*; a gene uniquely associated with CGL1 seipinopathy. Given the inability to identify any other known disease genes that might explain the unusual phenotypic features (i.e. polyneuropathy, cognitive deficiency) associated with known *bona fide AGPAT2*-related CGL1 clinical manifestations in this patient, we suggest a potential phenotypic expansion. Whether this new mutation causes the neuropathology observed however, remains unresolved. Our molecular analyses show the intronic 5'splice site deletion eliminates 25 codons of protein coding sequence and generates a frameshift resulting in a premature translation stop codon. There is no evidence for mutant *AGPAT2* mRNA instability in the blood cell studies. Structure and function of any AGPAT2 protein in our patient however would likely be impaired.

Precisely how this would affect physiological pathways involving *AGPAT2* is unknown. The AGPAT2 protein is located in the membrane of the endoplasmic reticulum and is primarily involved in triglyceride and phospholipid biosynthesis, with multiple interaction partners involved in lipid biosynthesis/degradation and related pathways. These include acyl chain remodelling of phosphatidylethanolamine (10), adipose droplet formation, lipid signalling and ER and mitochondrial membrane transport pathways (11,12). Many CMT neuropathy genes involve this transport biology, hinting at possible overlapping molecular bases for the polyneuropathy observed in this patient. Disruption of post-translational protein-protein interactions central to lipid homeostasis and of related pathway function, such as cholesterol metabolism, are highly probable and likely to have fundamental physiological effects. Other mutations leading to similar disequilibrium of lipid homeostasis, phospholipid degradation and remodelling in ER and

mitochondrial membranes for example, have been linked to neural degeneration and epileptic seizures in other species including flies and worms, with similar phenotypes for mutations in human gene counterparts (13). Disruption of phospholipid homeostasis has been reported to be associated with α-synuclein protein aggregation, implicated in the pathology of Parkinson's disease (11,14). Disrupted cholesterol metabolism has also been linked to protein aggregation leading to mitochondrial distribution defects and neurodegenerative disease (15,16).

It is notable that known *AGPAT2* regulatory circular RNA (circRNA) (circRNAs; non-coding post-transcriptional splicing products) expression levels are high in normal foetal tissues, including adrenal tissue which regulates circulating hormonal levels in the developing foetus (17,18). Recent elucidation of fundamental functions for circRNA in eukaryotic gene expression programs (19) has highlighted the potential for future investigations into defective RNA processing during foetal development as a possible contributor to genetic disorders.

Finally, we would add an epidemiological note. *AGPAT2* mutations predominate in American CGL cases of African descent. The vast majority of European seipinopathies arise from mutations in *BSCL2* (20). The newly identified *AGPAT2* deletion in our patient was located within a large block (approximately 1.1 Mb) of a chromosome sequence with both alleles identical at nucleotide sequence level (Figure 1C, 1D). Blocks of AOH arise when parentage is related, as was the case for this patient. The rare deletion in *AGPAT2* identified appears to have arisen in an individual from a small European village and accumulated in the relatively static local population over generations.

This case is the first report of primary polyneuropathy within the classical clinical CGL1 syndrome exhibiting differentially diagnostic mechanical fat retention, establishing a potential phenotypic expansion for CGL1 disease. We further identified a new, recessive intronic splice site deletion in the CGL1-associated *AGPAT2* locus, resulting in an apparently translatable truncated mRNA species with missense coding. Precisely how the splicing defect identified affects AGPAT2 protein physiology or noncoding transcriptional regulatory RNA functions remains undefined. This however, is the case for all *AGPAT2* mutations linked to a CGL1 phenotype and the mechanism of action has not been defined for any thus far (12).

Acknowledgements

James R. Lupski has stock ownership in 23andMe and is a paid consultant for Regeneron. James R. Lupski is a coinventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases and bacterial genomic fingerprinting. The Department of Molecular and Human Genetics at Baylor College of Medicine derives revenue from the chromosomal microarray analysis (CMA) and clinical exome sequencing offered at Baylor Genetics (MGL; http://www.bcm.edu/geneticlabs/).

Ethics

Informed Consent: Informed consent was obtained from the patient and her parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Joanna Oswiecimska, Katarzyna Ziora, Marta Marek, Anna Obuchowicz, Alicja Sikora, Wojciech Wiszniewski, Concept: Joanna Oswiecimska, Wojciech Wiszniewski, Pawel Gawlinski, Design: Wojciech Wiszniewski, Pawel Gawlinski, Data Collection or Processing: Joanna Oswiecimska, Mateusz Dawidziuk, Tomasz Gambin, Sylwia Rzonca, D. Lys Guilbride, Shalini N. Jhangiani, Anna Obuchowicz, Wojciech Wiszniewski, Pawel Gawlinski, Analysis or Interpretation: Joanna Oswiecimska, Tomasz Gambin, Sylwia Rzonca, D. Lys Guilbride, Anna Obuchowicz, James R. Lupski, Wojciech Wiszniewski, Pawel Gawlinski, Literature Search: Joanna Oswiecimska, D. Lys Guilbride, Pawel Gawlinski, Writing: Joanna Oswiecimska, Anna Obuchowicz, Tomasz Gambin, Sylwia Rzonca, D. Lys Guilbride, Pawel Gawlinski.

Financial Disclosure: National Science Centre, Poland 2015/19/B/NZ2/01824. Also funded in part by the US National Human Genome Research Institute (NHGRI)/National Heart Lung and Blood Institute (NHLBI) grant number UM1HG006542 to the Baylor-Hopkins Center for Mendelian Genomics (BHCMG).

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DOI: 10.4274/jcrpe.galenos.2019.2019.0103
J Clin Res Pediatr Endocrinol 2019;11(3):327-328

Vitamin D Deficiency and Insufficiency According to Current Criteria for Children: Vitamin D Status of Elementary School Children in Turkey

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Dear Editor,

We read the article of Hocaoğlu-Emre et al (1) entitled 'Vitamin D Deficiency and Insufficiency According to the Current Criteria for Children: Vitamin D Status of Elementary School Children in Turkey' in the Journal of Clinical Research in Pediatric Endocrinology with great interest. In this study, the researchers investigated serum vitamin D levels in 640 healthy children between the ages of 6 and 9 years. It was stated that serum vitamin D levels of the subjects were obtained from the hospital records. They explained further that vitamin D levels were checked in healthy children by an "annual check-up for vitamin D status" at the hospital. The authors conclude that close follow-up of vitamin D status especially in the winter and post-winter period is necessary and that vitamin D supplementation be given for a strong bone structure and healthy growth (1).

Vitamin D deficiency screening should aim to identify people with low vitamin D levels who theoretically could benefit from vitamin D supplementation. Only after this theoretical screening program, we would expect improvement in particular health outcomes e.g improved bone mineral density, reduced risk of falls etc. Furthermore in any screening programme, the intervention and subsequent treatment should be harmless (2). However, there is no firm evidence showing benefits of vitamin D deficiency screening for healthy children (3,4). Recent global consensus recommendations caution strongly against population-based screening for vitamin D deficiency in healthy children (3). According to this consensus, serum 25(OH)D measurement would be reasonable for patients with high risk of vitamin D deficiency, such as patients having rickets, chronic kidney disease, hepatic failure, malabsorption, hyperparathyroidism or granuloma-forming disorders (3). Similarly, the American Academy of Pediatrics advises screening only in patients who have disorders associated with low bone mass such as rickets and/or a history of recurrent, low-trauma fractures (4). In addition, there has been a significant increase in health costs related to vitamin D tests and prescriptions for children in primary care over the past decade (5).

In conclusion, current evidence is not sufficient to suggest that screening for vitamin D deficiency in a healthy population produces health benefits, is necessary, safe or cost-effective.

Ethics

Informed Consent: Consent form was filled out by all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: Ahmet Anık, Özgür Akbaba, Design: Ahmet Anık, Özgür Akbaba, Data Collection or Processing: Ahmet Anık, Analysis or Interpretation: Ahmet Anık, Özgür Akbaba, Literature Search: Ahmet Anık, Özgür Akbaba, Writing: Ahmet Anık, Özgür Akbaba.

Financial Disclosure: The authors declared that this study received no financial support.

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Conflict of interest: None declared
Received: 05.07.2019
Accepted: 13.07.2019

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Yetişkinlerde başlayan: İzole GH yetmezliğinde, 2 dinamik test ile 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. **Pozoloji ve Uygulama Şekli:** SAİZEN çoklu doz kullanımı için tasarlanmıştır. SAİZEN dozu, vücut yüzey alanı ya da vücut ağırlığı temel alınarak her hasta için bireysel olmalıdır. SAİZEN'in aşağıdaki dozlarda gece uyumadan önce uygulanması tavsiyeedilmektedir: *Çocuklarda ve ergenlerde:* Endojen büyüme hormonu salgısının yetersiz olduğu büyüme geriliği gösteren hastalarda: Subkutan uygulama ile günde 0.7-1.0 mg/m^2 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/m^2 vücut yüzey alanı ya da günde 0.045-0.050 mg/kg vücut ağırlığı. Turner sendromlu hastalarda non-androjenik anabolik steroidlerle birlikte tedavi büyümeye cevabı artırabilir. *Kronik böbrek yetmezliğinden ötürü büyüme geriliği gösteren prepubertal çocuklarda (CPF):* Subkutan uygulama ile günde 1.4 mg/m^2 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 ağırlığıdır. (ya da 1 mg/m^2/gün, eşittir 0.1 IU/kg/gün ya da 3 IU/m^2/gün) Uygun yetişkin boyu ulaşınca ya da epifizler kapanınca tedavi kesilmelidir. SGA ile doğan kısa çocuklardaki büyüme bozukluğunda, erişkin boya ulaşılıncaya kadar tedaviye devam edilmesi önerilmektedir. Eğer boy uzama hızı SDS'si +1'in altında ise, bir yıl sonunda tedavi sonlandırılmalıdır. Erişkin boya ulaşıldığında (boy uzama hızı <2 cm/yıl olarak tanımlanmıştır) ve teyit gerekiyorsa epifizyal büyüme plaklarının kapanmasına uygun olarak kemik yaşı < 14 (kızlardaki veya >16 (erkeklerde) olduğunda, tedavi sonlandırılmalıdır. Yetişkinlerde: büyüme hormonu eksikliğinde: Somatropin tedavisi başlangıcında, günlük subkutan enjeksiyon olarak 0.15-0.3 mg gibi düşük dozlar önerilir. Doz, İnsülin-benzeri Büyüme Faktörü 1 (IGF-1) değerleri ile kontrollu şekilde ayarlanmalıdır. Önerilen en fazla büyüme hormonu dozu nadiren 1.0 mg/gün değerini aşar. Genellikle en düşük etkili doz uygulanmalıdır. Yaşlılarda veya kilolu hastalarda, daha düşük dozlar gerekli olabilir. SAİZEN'i uygulamak için one.click oto-enjektör ya da cool.click iğnesiz oto-enjektör ya da easypod oto-enjektör ile sağlanan kullanım kılavuzunda ve hasta kullanma talimatında verilen talimatları takip ediniz. **Özel Popülasyonlara İlişkin Ek Bilgiler:** *Böbrek/Karaciğer yetmezliği:* Bu hasta grubunda pozolojı üzerine herhangi bir öneri yapılamamaktadır. *Pediyatrik popülasyon:* SAİZEN, büyüme hormonu eksikliği olan çocuklarda endikedir. *Geriyatrik popülasyon:* 60 yaş üstü hastalarda ve uzun süreli kullanımlarla ilgili sınırlı deneyim bulunmaktadır. **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 malın tümor bulgusu varlığında kontrendikedir. Tedaviye başlamadan önce, aktif intrakraniai 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 benzer durumlarla ilişkili akut hastalığı olan bireylerde somatropin kullanılmamalıdır. Kronik böbrek yetmezliği olan çocuklarda, böbrek nakli durumunda somatropin tedavisi kesilmelidir. **Özel kullanım uyarıları ve önlemleri:** Büyüme hormonu eksikliği olan hastaların teşhis ve tedavisinde deneyimli bir hekimin düzenli gözetimi altında tedavi takip edilmelidir. Önerilen günlük maksimum doz aşılmamalıdır. Büyüme hormonu ile tedavi alan remisyondaki intra ya da ekstrakraniai neoplazili hastalar hekim tarafından dikkatle ve düzenli aralıklarda kontrol edilmelidir. Intrakraniai tümöre bağlı sekonder büyüme hormonu eksikliği olan hastalar, altta yatan hastalığın herhangi bir ilerleme ya da yineleme durumu için sık sık muayene edilmelidir. Saizen tedavisi sırasında, özellikle pubertal olan ve/veya beraberinde tiroid tedavisi alan çocuklarda, kemik yaşı düzenli olarak takip edilmelidir. Bu şartlar altında, epifiz olgunlaşması hızlı gelişebilir. Epifizal füzyon meydana geldiğinde, büyüme hormonu eksikliği durumunun endokrin testleriyle yeniden konfirme edilmesi önerilir ve eğer varsa, yetişkinlerde büyüme hormonu eksikliği için endike olan dozlarda tedaviye devam edilebilir. Çocuklukta kanser atlatan ve sonrasında büyüme hormonu tedavisi alan hastalarda ikincil neoplasm (tümör) riskinin arttığı bildirilmiştir. İlk neoplazmları için kafa bölgesine radyoterapi uygulanarak tedavi edilen hastalarda, özellikle meningiyomlar olmak üzere intrakraniai tümörler, ikincil neoplazmlar içinde en yaygın olanlar. Saizen, 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. Ciddi obezite, üst solunum yolu tıkanıklığı öyküsü, uyku apnesi veya tanımlanmamış solunum yolu enfeksiyonu gibi risk faktörlerinden bir veya daha fazlasına sahip olan Prader-Willi sendromlu çocuklarda büyüme hormonu tedavisi sonrasında uyku apnesi ve ani ölüm rapor edilmiştir. Büyüme hormonu ile tedavi edilmiş veya edilmemiş büyüme hormonu eksikliği olan çocuklarda ksıtli sayda ölsemi vakaları rapor edilmiştir. Bununla beraber, predispoze faktörlerin yokluğunda büyüme hormonu kullananlarda ölsemi insidansının arttığına dair bir bulgu yoktur. Somatropin, insülin duyarlılığını azaltabileceğinden dolayı, hastalar glukoz intoleransı yönünden gözlenmelidir. Diabetes mellitus olan hastalarda, somatropin içeren tedaviye başlanması sonrasında insülin dozunu ayarlamak gerekebilir. Diabetes mellitusu ve glukoz intoleransı olan hastaların somatropin tedavisi süresince yakın takip edilmeleri gerekir. İlerlemeyen retinopati olgularında somatropin replasman tedavisi kesilmemelidir. Somatropin tedavisi, T4'den T3'e dönüşümü artırır ve başlangıç aşamasındaki hipotiroidizmin fark edilmesini önleyebilir. Bu yüzden somatropin ile tedaviye başladıktan sonra ve doz ayarı sonrası tüm hastalara tiroid fonksiyon testi yapılması tavsiye edilir. Hipopitüitarizm hastalarında somatropin tedavisine başlandığında, standart yerine koyma tedavisi yakından takip edilmelidir. Şiddetli ya da tekrarlayan başağrısı, görsel problemler, bulantı ve/veya kusma durumlarında, papiloödem için funduskopi önerilir. Papiloödem varsa, iyi huylu intrakraniai basınç artışı (psödotümor serebri) tanısı düşünülmeli ve uygunsa SAİZEN tedavisi kesilmelidir. Halen intrakraniai hipertansiyonlu hastalarda klinik karar vermede rehberlik edecek yeterli kanıt yoktur. Büyüme hormonu tedavisine tekrar başlanırsa, intrakraniai hipertansiyon semptomları için dikkatli gözlem gerekir ve intrakraniai hipertansiyon tekrarlarsa tedavi kesilmelidir. Nadir görülmesine rağmen, özellikle karın ağrısı gelişen çocuklarda olmak üzere somatropin ile tedavi edilen hastalarda pankreatit düşünülmelidir. Tüm somatropin içeren preparatlarda olduğu üzere, bazı hastalarda somatropin'e karşı antikorlar oluşabilir. Bu antikorların bağlanma kapasitesi düşük olup büyüme hızına etkileri yoktur. Tedaviye yanıt vermeyen hastalarda somatropin'e karşı antikor üretimi test edilmelidir. Büyüme hormonu eksikliği ve hipotiroidizm gibi endokrin bozukluğu olan hastalarda, femur başı epifizlerinde daha sık kayma meydana gelebilir. Büyüme hormonu ile tedavi edilen çocuklarda, femur başı epifizlerinde kayma ya altta yatan endokrin bozukluktan ya da tedavi ile meydana gelen artmış büyüme hızından ötürüdür. Büyüme sıçramaları, prepubertal büyüme sıçraması esnasında özellikle gerilim altında olan kalça eklemi gibi, eklemle ilgili problem riskini artırabilir. SAİZEN ile tedavi edilen hastalarda diz ağrısı ya da kalça şikayetleri ya da topallama gelişmesine karşı hekimler ve hasta yakınları dikkatli olmalıdır. Kronik böbrek yetmezliğinden ötürü büyüme geriliği olan hastalar, renal osteodistrofi ilerleme bulgusu için periyodik olarak kontrol edilmelidir. 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. Böbrek yetmezliği için konservatif tedavi ötedavi başlamadan önce 1 yıl süreyle asıdoz, hiperparatiroidizm ve beslenme kontrolunu içerir) saptanmalı ve büyüme hormonu tedavisi esnasında sürdürülmelidir. Tedavi böbrek nakli esnasında sonlandırılmalıdır. SGA ile doğan kısa çocuklarda, tedaviye başlamadan önce, büyüme bozukluğunu açıklayabilecek diğer medikal sebeplerin ve tedavilerin ekarte edilmesi gerekmektedir. SGA hastalarında, tedaviye başlamadan önce, açlık kan şekeri ve insülin ölçümlerinin yapılması ve sonrasında yılda bir kez tekrarlanması tavsiye edilmektedir. Diabetes Mellitus için artmış riske sahip hastalarda (örn. ailede diyabet öyküsü, şişmanlık, artmış vücut kitle indeksi, şiddetli insülin direnci, acanthosis nigricanş) oral glikoz toleransı testi (OGTT) yapılmalıdır. Diyabet varlığı kesin ise, büyüme hormonu uygulanmamalıdır. SGA hastalarında, tedaviye başlamadan önce, IGF-I seviyesi ölçümünün yapılması ve sonrasında yılda iki sefer tekrarlanması tavsiye edilmektedir. Tekrarlanan ölçümlerde IGF-I seviyeleri, yaş ve pubertal durumuna uygun referanslarla karşılaştırıldığında +2 SD aşarsa, doz ayarlaması için IGF-I / IGFBP-3 oranı değerlendirmeye alınabilir. SGA hastalarında, ergenlik başlangıcında tedaviye başlanması konusundaki deneyim sınırlıdır. Bu nedenle puberte dönemine yakın hastalarda tedaviye başlanması önerilmemektedir. Silver-Russell sendromuna sahip SGA hastalarındaki deneyimler sınırlıdır. SGA ile doğan kısa çocuklarda, eğer erişkin boyuna erişilmeden tedavi sonlandırılırsa, Somatropin tedavisi ile boy uzamasında elde edilen büyüme hızının bir kısmı kaybedilebilir. Yetişkinlerde büyüme hormonu replasman tedavisi esnasında sıvı retansiyonu beklenir. Devamlı ödem ya da şiddetli paraestezi durumunda karpal tünel sendromu gelişmesini önlemek için dozaji azaltılmalıdır. Akut kritik hastalık gelişen tüm hastalarda, büyüme hormonu ile tedavinin olası yararları; olabilecek potansiyel riskler düşünülerek değerlendirilmelidir. 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. SAİZEN her dozunda 1 mmol (23 mg)'dan daha az sodyum ihtiva eder, yani aslında "sodyum içermez". **Diğer Tıbbi Ürünler ile Etkileşimler ve Diğer Etkileşim Şekilleri:** Eşzamanlı olarak glukokortikoid kullanılması, somatropinin büyümeyi artırıcı etkisini engeller. ACTH eksikliği olan hastalarda büyüme hormonu tedavilerinin engellenmemesi için glukokortikoid tedavileri çok dikkatli ayarlanmalıdır. İlaveten, büyüme hormonu replasmanının başlatılması, inaktif kortizonun kortizole dönüşmesini sağlayan bir enzim olan 11β-hidroksisteroid dehidrojenaz, tip 1 (11β-HSD1)'in aktivitesini azaltarak bazı hastalarda ikincil adrenal yetmezliğin ortaya çıkmasına neden olabilir. Glukokortikoid replasman tedavisi alan hastalarda somatropinin başlatılması, kortizol eksikliğinin ortaya çıkmasına neden olabilir. Glukokortikoid dozunun ayarlanması gerekebilir. Oral östrojenler, serum IGF-1'in somatropin tedavisine cevabını azaltabileceğinden, oral östrojen replasmanı alan hastalarda daha yüksek somatropin dozları gerekebilir.Büyüme hormonu eksikliği olan erişkin hastalarda yapılan bir çalışma, büyüme hormonu uygulamasının sitokrom p450 enzim sistemi ile metabolize edilen ilaçların klirensini artırabileceğini göstermektedir. 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. Bu gözlemin klinik anlamı bilinmemektedir. **Gebelik ve Laktasyon:**Gebelikte kullanım kategorisi C'dir. Somatropin içeren ürünler kontrasepsiyon kullanmayan çocuk doğurma potansiyeline sahip kadınlarda kullanılmamalıdır. Gebelerde yapılmış klinik veri mevcut değildir. Hayvanlarda somatropin içeren ürünlerle ilgili yürütülen reprodüktif çalışmalarda embriyo veya fetüs için advers reaksiyonların arttığına dair bir bulgu bulunmamaktadır. Bununla birlikte somatropin içeren ilaçlar gebelikte ve kontrasepsiyon kullanmayan çocuk doğurma potansiyeli olan kadınlarda önerilmez. Somatropinin insan sütüyle atılıp atılmadığı bilinmemektedir. Somatropinin süt ile atılımı emziren kadınlarda araştırılmamıştır. Bu nedenle emziren kadınlarda somatropin uygulandığında dikkatli olunmalıdır. Emzirmenin durdurulup durdurulmayacağına ya da SAİZEN tedavisinin durdurulup durdurulmayacağına/tedaviden kaçınılıp kaçınılmayacağına ilişkin karar verilirken, emzirmenin çocuk açısından faydası ve SAİZEN tedavisinin emziren anne açısından faydası dikkate alınmalıdır. Üreme yeteneği / Fertilite: Klinik dışı toksisite çalışmaları, rekombinant insan büyüme hormonunun erkek ve kadın fertilitesi üzerinde advers etkileri olmadığını göstermiştir. Somatropin içeren ilaçların hastanın araç ya da makine kullanma kabiliyeti üzerine etkisi yoktur. **İstenmeyen Etkiler.** Hastaların %10'a kadarı, özellikle subkutan yol kullanıldığı zaman, enjeksiyon yerinde kızarıklık ve kaşıntı yaşayabilir. Yetişkinlerde büyüme hormonu replasman tedavisi esnasında sıvı retansiyonu beklenir. Ödem, eklemşişmesi, artralji, myalji, ve paresteziler sıvı retansiyonunun klinik manifestasyonları olabilir. Yine de bu semptomlar genellikle geçicidir ve doza bağlıdır. Çocukluk döneminde büyüme hormonu eksikliği tanısı konmuş yetişkin hastalarda ortaya çıkan istenmeyen etkiler, hastalığın yetişkin dönemde ortaya çıktığı hastalara göre daha azdır. Hastaların küçük bir yüzdesinde somatropin'e karşı antikorlar oluşabilir; bugüne kadar antikorların düşük bağlanma kapasitesine sahip olduğu görülmüş ve bu antikorlar ile, gen deleasyonu olan hastalar dışında, büyümede yavaşlama ilişkilendirilmemiştir. Büyüme hormonu gen kompleksinin delesyonuna bağlı boy kısalığı gibi nadir görülen durumlarda, büyüme hormonu ile tedavi büyümeyi gerileten antikorları indükleyebilir. Büyüme hormonu ile tedavi edilmiş veya edilmemiş büyüme hormonu eksik çocuklarda bazı ölsemi vakaları rapor edilmiştir. Bununla beraber, predispoze faktörlerin yokluğunda büyüme hormonu kullananlarda ölsemi insidansı arttığına dair bir bulgu yoktur. Pazarlama sonrası çalışmalarda büyüme hormonu tedavisi sırasında pankreatit bildirilmiştir. İstenmeyen etkiler, olduğu sıklığa göre aşağıdaki gibi sınıflandırılmıştır: Çok yaygın (≥1/10); yaygın (≥1/100 ila <1/10); yaygın olmayan (≥1/1.000 ila <1/100); seyrek (≥1/10.000 ila <1/1.000); çok seyrek (<1/10.000); bilinmiyor (eldeki verilerden hareketle tahmin edilemiyor). Bağışıklık sistemi hastalıkları: Bilinmiyor: Lokal veya genelleşmiş aşırı duyarlılık reaksiyonları. Endokrin hastalıkları: Çok seyrek: Hipotiroidizm. Metabolizma ve beslenme hastalıkları: Yaygın (Yetişkinlerde)/Yaygın olmayan (çocuklarda): Sıvı tutulması: periferal ödem, sertlik, artralji, myalji, parestezi, Bilinmiyor: İnsülin rezistansı hiperinsülinizm ve nadiren hiperglisemi ile sonuçlanabilir. Sinir sistemi hastalıkları: Yaygın: Baş ağrısı (izole), karpal tünel sendromu (yetişkinlerde). Yaygın olmayan: İdiopatik intrakranial hipertansiyon (iyi huylu intrakranial basınç artışı), karpal tünel sendromu (çocuklarda). Gastrointestinal hastalıklar: Bilinmiyor: Pankreatit Kas-iskelet bozuklukları, bağ doku ve kemik hastalıkları: Çok seyrek: Femur başı epifiz kayması, ya da femur başının avasküler nekrozları. Genel bozukluklar ve uygulama bölgesine ilişkin bozukluklar: Yaygın: Enjeksiyon yeri reaksiyonları, Lokalize lipoatrofi Yaygın olmayan: Jinekomasti **Doz Aşımı ve Tedavisi:** Önerilen dozları aşmak yan etkilere sebep olabilir. Doz aşımı hipoglisemi ve sonrasında hipergliserniye yol açabilir. Bundan başka, somatropin doz aşımı sıvı retansiyonuna sebep olabilir. **Saklamaya Yönelik Özel Tedbirler:** Raf ömrü 36 aydır. 25ºC altındaki oda sıcaklığında ve ambalajında saklayınız. Dondurmayınız. Rekonstitüye edildiğinde, kartuşda, 2ºC-8ºC arasında buzdolabında saklanmalı ve en geç 28 gün içinde kullanılmalıdır. Rekonstitüye edilmiş SAİZEN kartuşu içeren easypod ve one.click oto-enjektörler 2ºC-8ºC buzdolabında arasında saklanmalıdır. Cool.click iğnesiz oto-enjektör kullanıldığı zaman, sadece rekonstitüye edilmiş SAİZEN kartuşu 2ºC-8ºC arasında buzdolabında saklanmalıdır. Sulandırılmış enjeksiyonluk çözelti partikülsüz berrak olmalıdır. Çözelti partikül içeriyorsa, enjekte edilmemelidir. **Ruhsat Sahibi:** Merck İlaç Ecza ve Kimya Tic.A.Ş. Atatürk Mh. Ertuğrul Gazi Sk. Metropol İstanbul Sit. C2 Apt. 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TURSAI/0519/0011 22.05.2019

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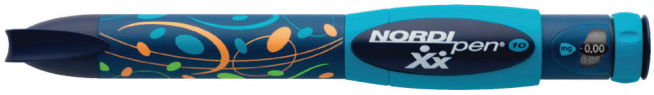

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Norditropin® SimpleXx® 5 mg/1.5 mL kalem

Norditropin® SimpleXx®
Bileşimi: 5 mg/1.5 mL kartuş ml'sinde 3.3 mg, 10 mg/1.5 mL kartuş ml'sinde 6.7 mg ve 15 mg/1.5 mL kartuş ml'sinde 10 mg somatropin (rekombinant büyüme hormonu) içerir. **Farmasötik Şekil:** Enjeksiyonluk çözelti içeren kartuş. **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 (su 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 kişilere 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; gebelik ve emzirme; kalp cerrahisi ve abdominal cerrahiyi takiben akut yaşamsal hastalık komplikasyonları, kazaya bağlı çoklu travma, akut solunum yetmezliği veya benzer durumlar; somatropine ya da bileşimindeki maddelerden herhangi birisine aşırı duyarlılık; kronik böbrek yetmezliği olan çocuklarda böbrek transplantasyonu; epifizleri kapanmış çocuklar. **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 eksikliği: 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. 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. Gebelik kategorisi: C. Gebelik döneminde somatropin tedavisinin güvenilirliği açısından yeterli kanıt bulunmamaktadır. Somatropinin anne sütüne geçme olasılığı göz ardı edilemez. **Yan Etkiler/Advers Etkiler:** Erişkinlerde periferik ödem, baş ağrısı, parestezi, artraljieklem 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. 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 28 gün saklayınız. Işıktan koruyunuz. Dondurmayınız. Ürün, alternatif olarak, 25°C'nin altında maksimum 21 gün 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® SimpleXx® 5mg; 07.01.2002-111/56, Norditropin® SimpleXx® 10mg; 25.12.2001-111/45, Norditropin® SimpleXx® 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: 09.10.2018. Norditropin®, SimpleXx® ve NordiPen® Novo Nordisk'in ticari markalarıdır. Daha geniş bilgi için firmamıza başvurunuz.

Referans: Norditropin® SimpleXx® Kısa Ürün Bilgisi.

TR19NSXX00002