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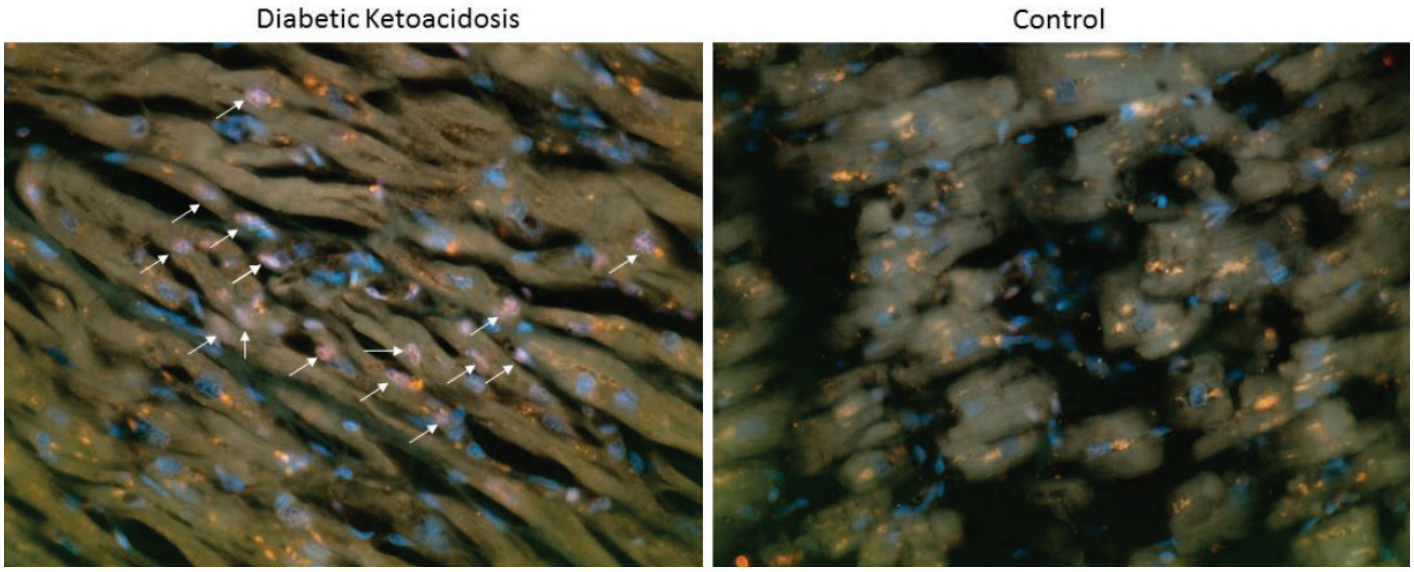

RAGE was prominently expressed in the diabetic ketoacidosis myocardium versus the gender and age matched control myocardium.

Soluble Receptor for Glycation End-products Concentration Increases Following the Treatment of Severe Diabetic Ketoacidosis

Hoffman WH et al.

Page: 160-167

Official Journal of
Turkish Pediatric Endocrinology
and Diabetes Society

galenos yayınevi

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

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

- Korneal bulutlanma
- Kronik sinüzit
- Kardiyovasküler hastalık
- Eklemlerde kontraktürler
- Umbilikal/inguinal herni
- Karpal tünel sendromu
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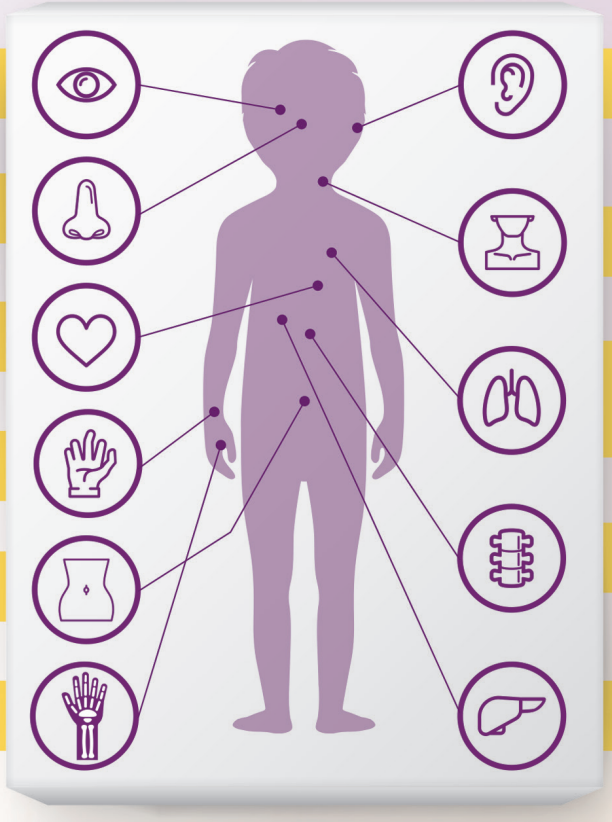

ALDURAZYME®, Mukopolisakkaridoz I (MPS I; a-L-iduronidaz eksikliği) tanısı konmuş hastalarda, hastalığın norolojik olmayan bulgularını tedavi etmek amacıyla uzun süreli enzim replasman tedavisinde endikedir.[8]

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

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

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

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What is already known on this topic?

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

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

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

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

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GENOTROPİN GOQUİCK® 16 IU (5,3 mg/ml) - 36 IU (12 mg/ml) enjeksiyonluk çözelti için toz ve çözücü içeren kullanıma hazır kalem **Formül:** Rekombinant DNA teknolojisiyle Escherichia Coli hücrelerinde üretilmiş 16 IU (5,3 mg/ml) - 36 IU (12 mg/ml) somatropin içerir. **Endikasyonları:** Büyüme hormonunun yetersiz salgılanmasına bağlı çocuklardaki büyüme bozukluklarında; gonadal disgenezi (Turner Sendromu) ile birlikte bulunan büyüme bozukluklarında; kronik böbrek yetersizliği olan prepubertal çocuklardaki büyüme bozukluklarında; SGA tedavisinde – doğum ağırlığı ve/veya uzunluğu -2 SD olan ve 4 yaşı ve sonrasında gerekli büyümeyi yakalayamamış (son 1 yılda yıllık boy kazanımı SDS<0) çocuklarda veya gestasyonel yaşına göre küçük doğmuş olan (SGA) kısa çocuklardaki büyüme bozukluklarında (uzunluk SDS<-2.5 ve ebeveyne uyarlanmış uzunluk SDS<-1) – ; hipotalamus-hipofizer hastalığı saptanan hipofizer cerrahi girişim geçirmiş, kraniyal radyoterapi görmüş veya çocuklukta başlamış büyüme hormonu yetmezliği olan erişkinler ile hipofizde adenomu olan hastalarda büyüme hormonu eksikliği varsa veya büyüme hormonu yetersizliğini düşündüren bulguların bulunması durumunda biyokimyasal tanı testleri ile büyüme hormonu eksikliği kesin olarak saptanan yetişkinlerde, özetle: konjenital veya idiopatik hipofiz hastalıkları, hipotalamus hipofiz tümörleri ve tedavileri sonunda, kraniofarenjioma tedavisinden sonra, cerrahi girişim hasarlarında, Sheehan sendromu ve vasküler sebeple gelişen iskemik sebepli büyüme hormonu yetersizlikleri, radyasyon, travma, kronik otoimmun, bakteriyel veya viral enflamasyonlar ile hemokromatozis ve amiloidoziste görülen hipofizer yetmezliklerde, septo-optik displazide meydana gelebilen aşikâr büyüme hormonu eksikliğinin replasmanı için büyüme hormonu replasman tedavisi endikasyonu vardır. **Pozoloji:** Çocuklardaki büyüme hormonu salgılanma yetersizliğine bağlı büyüme bozukluğunda: Genellikle 0,025 – 0,035 mg/kg veya 0,7 –1,0 mg/m² önerilmektedir. Turner Sendromuna bağlı büyüme bozukluğu: 0,045–0,050 mg/kg veya 1,4 mg/m² önerilir. Kronik böbrek yetmezliğine bağlı büyüme bozukluğu: 0,045–0,050 mg/kg (1,4 mg/m²) önerilir. Büyüme hızı çok düşükse daha yüksek dozlar gerekebilir. Gestasyonel yaşa göre küçük doğmuş (SGA) olan kısa boylu çocukların büyüme bozukluklarında: Final uzunluğa erişinceye kadar genellikle vücut ağırlığına göre günlük 0,035 mg/kg (1,0 mg/m²) önerilmektedir. Yetişkinlerdeki büyüme hormonu eksikliği: Çocukluk çağı BHY sonrasında büyüme hormonu tedavisine devam eden hastalarda önerilen yeniden başlangıç dozu 0,2-0,5 mg/gün' dür. Yetişkin başlangıçlı BHY olan hastalarda tedavi düşük doz (0,15-0,3 mg/gün) ile başlamalıdır. **Uygulama şekli:** Dozlama ve uygulama sıklığı bireyselleştirilmelidir. Enjeksiyonlar subkütan enjeksiyon şeklinde ve lipoatrofi gelişmesini önleyebilmek için her seferinde yeri değiştirilerek uygulanır. **Kontrendikasyonlar:** Etkin madde veya yardımcı maddelerden herhangi birine karşı aşırı duyarlılık durumunda kullanılmamalıdır. Somatropin, tümör aktivitesini gösteren herhangi bir bulgunun bulunması durumunda kullanılmamalıdır. Büyüme hormonu tedavisine başlanmadan önce intrakraniyal tümörler inaktif olmalı ve antitümör tedavi tamamlanmış olmalıdır. Tümör büyümesine ilişkin kanıt olması halinde tedavi sonlandırılmalıdır. GENOTROPİN GOQUİCK® epifizleri kapanmış çocuklarda büyümenin uyarılması için kullanılmamalıdır. Açık kalp ameliyatı, abdominal cerrahi, kazaya bağlı multipl travma, akut solunum yetmezliği veya benzeri durumları izleyen komplikasyonların bulunduğu akut kritik hastalığı olan hastalara GENOTROPİN GOQUİCK® uygulanmamalıdır. **Özel kullanım uyarıları ve önlemleri:** Hastalığın tanısı ve GENOTROPİN GOQUİCK® tedavisi, terapötik kullanım endikasyonunda; hastaların tanı ve tedavisinde yeterli nitelikte ve tecrübeli doktorlar tarafından başlatılmalı ve takip edilmelidir. Maksimum önerilen günlük doz aşılmamalıdır. Miyozit çok nadir bir advers olaydır ve koruyucu madde metakrezol ile ilişkili olabilir. Somatropin insülin hassasiyetini azaltabilir. Diabetes mellitus olan hastalarda somatropin tedavisine başlandıktan sonra insülin dozunun ayarlanması gerekebilir. Büyüme hormonu, T4'ün T3'e tiroit dışı dönüşümünü artırabilir ve bu durum serum T4'ünün azalmasına ve serum T3'ünün artmasına yol açabilir. Tiroit fonksiyonu tüm hastalarda takip edilmelidir. Malign bir hastalığın tedavisine sekonder büyüme hormonu yetersizliğinde malignitenin relaps belirtilerine dikkat edilmesi önerilmektedir. Çocukluk döneminde kanser sonrası sağ kalımlarda, somatropin ile tedavi edilen hastalarda ilk neoplazma sonrası ikinci bir neoplazma gelişiminde risk artışı bildirilmiştir. Büyüme hormonu yetersizliği dahil, endokrin bozukluğu olan hastalarda kalça ekleminde epifiz kayması genel popülasyondan daha sık görülebilir. Şiddetli veya tekrarlayan baş ağrısı, görme sorunları, bulantı ve/veya kusma gelişmesi halinde papilla ödemi için fundoskopi yapılması önerilmektedir. Büyüme hormonu eksikliği olan az sayıda hastada lösemi bildirilmiştir ve bu hastalardan bazıları somatropin ile tedavi edilmiştir. Somatropin içeren ürünlerin hepsinde olduğu gibi, hastaların düşük bir yüzdesinde GENOTROPİN GOQUİCK®'e karşı antikorlar gelişebilir. Seyrek görülmekle birlikte; somatropin ile tedavi edilen hastalarda; özellikle karın ağrısı gelişen çocuklarda pankreatit dikkate alınmalıdır. SGA olarak doğan kısa boylu çocuklarda tedaviye başlamadan önce büyüme bozukluğuna neden olacak diğer tıbbi nedenler veya tedaviler ekarte edilmelidir. SGA çocuklarda tedaviye başlamadan önce ve daha sonra yılda bir kez, açlık insülin ve kan glukozu düzeyleri ölçülmelidir. SGA çocuklarda tedaviye başlamadan önce ve daha sonra yılda iki kez, IGF-I değerleri ölçülmelidir. Kronik böbrek yetersizliğinde, tedavi başlangıcından önce böbrek fonksiyonu normalin %50 altında olmalıdır. Böbrek transplantasyonunda tedaviye devam edilmemelidir. **İlaç Etkileşimleri:** Glukokortikoidlerle eş zamanlı tedavi somatropin içeren ürünlerin büyümeyi tetikleyici etkilerini engelleyebilir. Büyüme hormonu eksikliği olan yetişkinlerde yapılan bir etkileşim çalışmasında somatropin uygulamasının sitokrom P450 izoenzimleriyle metabolize olduğu bilinen bileşiklerin klirensini artırdığı belirtilmektedir. **Gebelik kategorisi:** Gebelik kategorisi C'dir. Kontrasepsiyon kullanmayan çocuk doğurma potansiyeline sahip kadınlarda somatropin içeren ürünler önerilmemektedir. Emziren kadınlarda somatropin içeren ürünlerle ilgili klinik çalışmalar yapılmamıştır. Somatropinin anne sütüne geçip geçmediği bilinmemektedir, ancak yeni doğanlarda intakt proteinin gastrointestinal kanaldan emilme olasılığı oldukça düşüktür. Bu yüzden emziren kadınlara somatropin içeren ürünler verilirken dikkatli olunmalıdır. **Araç ve makine kullanımı üzerindeki etkiler:** GENOTROPİN GOQUİCK®'in araç ve makine kullanımı üzerinde etkisi bulunmamaktadır. **İstenmeyen etkiler:** Enjeksiyon bölgesi reaksiyonları, artralji, periferik ödem, parestezi, karpal tünel sendromu, miyalji, kas-iskelet sertliği çok yaygın ve yaygın görünen istenmeyen etkilerdir. **Doz aşımı ve tedavisi:** Akut doz aşımı başlangıçta hipoglisemi ve takiben hiperglisemiye neden olabilir. Uzun süreli doz aşımı fazla miktardaki insan büyüme hormonunun bilinen etkilerine benzer belirti ve bulgulara neden olabilir. **Saklama koşulları:** Sulandırılmadan önce: Buzdolabında (2°C - 8°C'de) veya 25°C'nin altında maksimum 1 ay boyunca saklayınız. İki kompartımanlı kartuşu/önceden doldurulmuş kalemi ışıktan korumak için dış kutusunda saklayınız. Sulandırıldıktan sonra: Buzdolabında (2°C - 8°C'de) saklayınız. Dondurmayınız. İki kompartımanlı kartuşu/önceden doldurulmuş kalemi ışıktan korumak için dış kutusunda saklayınız. **Raf Ömrü:** Sulandırılmadan önce: 25°C'nin altında oda sıcaklığında 1 ay, (2°C – 8°C)'de buzdolabında 36 ay. Sulandırıldıktan sonra: (2°C – 8°C) 'de buzdolabında 28 gün. Ürün, ışıktan ve donmaktan korunarak saklanmalıdır. **Ticari Takdim Şekli ve Ambalaj Muhtevası:** 16 IU, 36 IU GoQuick enjeksiyonluk çözelti için toz ve çözücü içeren 1 adet kullanıma hazır kalem. Reçete ile satılır. **Satış Fiyatı:** Genotropin GoQuick® 16 IU 288,96 TL (19.02.2020), Genotropin GoQuick® 36 IU 652,48 TL (19.02.2020). 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New Features for Child Metrics: Further Growth References and Blood Pressure Calculations

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Abstract

Many new features have recently been incorporated to ÇEDD Çözüm/Child Metrics, an online and freely accessible scientific toolset. Various auxological assessments can now be made with data of children with genetic diseases (Prader Willi syndrome, Noonan syndrome, Turner syndrome, Down syndrome, and Achondroplasia) and preterm and term newborns. More detailed reports for height, weight, and body mass index data of a given child are now available. Last but not least, office and 24-hour ambulatory blood pressure values can be analyzed according to normative data.

Keywords: Application, mobile, calculator, short stature, growth chart, hypertension, guideline, AAP

Introduction

There exist various calculators for pediatricians generated by, but not limited to, the World Health Organization (WHO) (an offline tool for anthropometric calculations), UpToDate (online calculators for many specialties which requires a subscription), and individual developers including online and offline tools developed using Excel or Java Software) (1,2,3,4). In order to meet the specific needs of pediatric endocrinologists, we had launched an online and freely accessible scientific toolset containing a wide array of formulae under the official auspices of the Turkish Pediatric Endocrinology and Diabetes Society in 2017: ÇEDD Çözüm/Child Metrics (www.ceddcozum.com, www.childmetrics.org). In addition, the mobile application of Child Metrics can be downloaded from the App Store and Google Play. Currently, 550-600 daily users across various medical centers in Turkey work with the tool.

A description of the system was previously published in this journal (5). Briefly, standard deviation (SD) scores and percentile values can be calculated for weight, height, body mass index (BMI), and head circumference, using reference data from the Centers for Disease Control (CDC), Neyzi et al., and the WHO, as well as upper/lower segment ratio, waist circumference, sitting height/height ratio, IGF1 and IGFBP3 concentrations, growth velocity, bone mineral density, and thyroid and ovarian volumes. SD scores for a given measurement (x) are mainly calculated using LMS data with the following formulae: L≠0, SD score = [(x/M)**L−1]/LS or L = 0, SD score = ln(x/M)/S (6). Interpolation by weighted mean is used to obtain L, M, and S values at finer intervals and that are not provided in the relevant references (7). When no LMS data are present for a variable, SD scores for a given measurement (x) are obtained by the following formula: SD score = (x−mean)/SD. Percentile values corresponding to calculated SD scores are obtained from a standard normal distribution table. In addition, various types of calculations

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for body surface area, target height, predicted adult height, growth hormone dose, tubular function tests, insulin resistance indexes, human chorionic gonadotropin test, and converting units of measurements are available (5).

With this review, we intended to present the newly added features and overview their scientific basis.

Growth

1. Genetic Diseases/Syndromes

There are numerous benefits of specific growth charts for children with genetic diseases. Most importantly it is possible to assess the natural growth process for any genetic condition included. In addition, assessment and monitoring growth of affected children compared to peers with the same condition can be done. Inadequate growth according to syndrome specific curves would necessitate assessment for an associated comorbidity. On the other hand, some reference data might be biased, possibly due to relatively low numbers of cases included and variation in disease severity (8).

For Child Metrics, we selected the syndromes which are the most-relevant for pediatric endocrinologists. Key characteristics of the incorporated reference data are summarized in Table 1. Data are obtained from the published articles unless otherwise stated in the following sections. In the relevant section of Child Metrics, the measurements of the subjects are analyzed according to reference data of both healthy children and syndrome specific growth reference at the same time. When applicable, the results are plotted on specific electronic growth charts as well.

1.1. Prader Willi Syndrome

In 2000, Hauffa et al (9) reported mixed cross-sectional and longitudinal data from German patients with genetically proven Prader Willi syndrome. Consequently, 123 data on height and 118 on weight and BMI were included in the analyses. They found no influence of genotypes or gender on SD scores of height, weight, and BMI.

Recently, Butler et al (10) published growth curves for both growth hormone-naive and -treated children with Prader Willi syndrome. In Child Metrics, we used the reference data belonging to white children who did not receive growth hormone. The majority of the measurements were obtained cross-sectionally. They noted that the height curves were found to be similar to previous German and USA graphs (9,10). The LMS data were obtained from Dr. Butler *via* personal communication.

1.2. Noonan Syndrome

Ranke et al (11) published their mixed longitudinal and cross-sectional data in 1988 before the genetic diagnosis of Noonan syndrome was available. The data were collected retrospectively from the patient files of two medical centers with a long-standing interest in Noonan syndrome.

Table 1. The characteristics of reference data for genetic diseases incorporated into Child Metrics

	Prader Willi syndrome		Noonan syndrome		Turner syndrome	Down syndrome	Achondroplasia
First author, publication year	Hauffa, 2000	Butler, 2015	Ranke, 1988	Malaquias, 2012	Ranke, 1988	Zemel, 2015	Hoover-Fong, 2017
Reference	9	10	11	12	14	15	16
Country	Germany	USA	West Germany	Brazil	West Germany	USA	USA
Number of cases	n = 100	n = 120	n = 144	n = 119	n = 150	n = 637	n = 293
Age range (years)	1-21	3-18	1-20	0-20	2-20	0-20	0-16
Data	H/L, W, BMI	H, W, BMI, HC	H/L	H/L, BMI	H	H/L, W, BMI, WFL, HC	H
Method	Mean ± SD for H LMS for W and BMI	LMS	Mean ± SD	LMS	Mean ± SD	LMS	Mean ± SD
Growth-promoting treatment	No	No	No	No	No	No	No
Molecular diagnosis	Yes	Yes	N/A at that time, all had normal karyotype	Yes	Yes	N/A	N/A

H: height, H/L: height or length, W: weight, BMI: body mass index, WFL: weight-for-length, HC: head circumference, SD: standard deviation, N/A: not available

In 2012, Malaquias et al (12) reported reference data and growth curves for patients with pathogenic mutations in RAS/MAPK-related genes. The study included 137 patients (Noonan syndrome, n = 119; Noonan syndrome with multiple lentigines, n = 4, Noonan-like syndrome with loose anagen hair, n = 4, and *CBL*-mutation associated syndrome n = 10). Height and weight data were collected in a mixed longitudinal and cross-sectional method resulting in 536 observations. In each age group, approximately two-thirds of measurements were performed in children harboring *PTPN11* mutations. Among all genotypes, patients with *SHOC2* mutations were the shortest compared to subjects with other genotypes. The LMS data were obtained from Dr. Malaquias *via* personal communication.

1.3. Turner Syndrome

The highest number of publications regarding condition-specific growth curves is in relation to Turner syndrome compared to other genetic diseases (13). We incorporated the widely accepted data of Ranke et al (14) published in 1983 and 1988. Among the included patients (n = 150), 60 % had 45,X karyotype. Reference data were generated in a mixed longitudinal and cross-sectional method.

1.4. Down Syndrome

In their CDC-funded study published in Pediatrics in 2015, Zemel et al (15) reported growth charts for children with Down syndrome in the USA, mostly from the Philadelphia area. The majority of them were non-Hispanic white (73 %). Researchers took a total of 1520 measurements from 637 individuals. Nearly two-thirds of subjects underwent measurement more than once and the average number of visits per subject was three (range, 1-9).

1.5. Achondroplasia

Hoover-Fong et al (16) from the USA reported growth data at one-month intervals from 293 children with achondroplasia collected by a single observer between 1967-2004. Average numbers of height measurements per subject were 3.3 (range, 1-9) and 5.4 (1-22) among children below 3 years of age and between 2-16 years of age, respectively.

2. Detailed Reports

In addition to reporting centile and SD scores of height, weight, and BMI data, the following calculations are now made where available:

- Adult height in centimeter corresponding to current SD score of the given case.

- Height values in centimeters corresponding to -2, 0, and 2 SD scores and weight values in kilogram corresponding to the 3rd, 50th, 85th, and 95th BMI centiles (equivalent to SD scores of -1.88, 0, 1.04, and 1.65, respectively) of given gender and age using the following formula: $e^{((\ln((x)*L*S)+1)/L)+\ln(M))}$, where x is the desired SD score.

- BMI centile for height age of the given case, instead of calendar age (17).

- (If obese) Ratio of the BMI value of the given case to the 95th centile of given gender and age (18).

Newborns

We incorporated Turkish and USA reference data to assess length, weight, and head circumference of preterm and term newborns. Both data sets were based on the LMS method.

In 2012, Kurtoğlu et al (19) published their cross-sectional data collected retrospectively from the medical records of infants (n = 4750, 52.5 % male, 60.6 % term) born at 28-42 weeks of gestational age during one year in 11 hospitals in Kayseri, a Central Anatolian city in Turkey. Infants whose mothers had chronic diseases, who were smokers or who had undergone multiple deliveries had been excluded, together with all infants who had fetal health problems, congenital malformations, and those with missing auxological data. Due to the low number of cases, some age groups were combined into the groupings 28-29, 30-31, 32-33 and 41-42 gestational weeks. The remaining data were given as per week of gestation.

In 2013, Fenton and Kim (20) published their data, a combination of six large population-based surveys with different exclusion criteria. They were performed between 1991-2007 including 3,986,456 infants (34,639 births < 30 weeks) from Germany, United States, Italy, Australia, Scotland, and Canada. The individual datasets were found to have good agreement with each other. The final LMS data were obtained from Dr. Fenton *via* personal communication. This dataset provides two different calculations according to gestational age input: (i) completed week (for weight: starting from 22 to 49 weeks, for length and head circumference 23-49 weeks), (ii) completed week + day (for weight: 22 weeks + 4 days to 50 weeks, for length and head circumference 23 weeks + 4 days to 50 weeks) (20).

3. Blood Pressure (BP)

BP values normally increase with age as the body grows; thus, comparing BP levels in mmHg among children are misleading. Instead, SD scores of office and ambulatory BP measurement (ABPM) values should be used.

3.1. Office Measurements

In the 2017 Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents, endorsed by American Academy of Pediatrics, detailed normative BP tables based on auscultatory measurements obtained from approximately 50,000 normal-weight children and adolescents (those with a BMI < 85th percentile between 1-17 years of age) are provided (21). Rosner et al (22) had published the methodology (quantile regression) used and a part of this normative data previously. For Child Metrics, equations and relevant regression coefficients were obtained from Dr. Rosner *via* personal communication. First, reference systolic and diastolic BP values corresponding to each of the 1st through the 99th centiles are generated for the given child using age, gender, and height/length data. Among these 99 reference values, the centile of BP that is closest to the child's observed BP is reported. For example, a systolic BP of 95 mmHg corresponds to 52nd centile for an 8-year-old girl with a height of 123 cm (Figure 1). The centile value is then converted to the corresponding SD score. The system also reports five BP values, decimals of which are omitted, corresponding to clinically relevant reference centiles, which indicate hypertension stages or target treatment thresholds: 50th, 75th, 90th, 95th centile, and 95th centile + 12 mmHg (21). The relevant BP values are 94 mmHg, 100 mmHg, 107 mmHg, 111 mmHg, and 123 mmHg, respectively, for the above-mentioned example.

3.2. Ambulatory Blood Pressure Measurements

Before application of most ABPM devices, BP levels corresponding to 95th centile values according to gender and height should be entered. These data are most frequently obtained from the publications of Wühl et al (23) in 2002 and Flynn et al (24) in 2014. The data included in the latter article are reproduced based on the references reported by Wühl et al (23), which are generated with the LMS method. The articles provide sample reference BP data only for every 5 cm starting from 120 cm of height up to 175 cm for girls and 185 cm for boys; however, Child Metrics can provide relevant BP data for finer intervals based on the dataset presented by Wühl et al (23). As a result, individualized BP limits can be accurately established and while assessing the results of ABPM more proper BP loads may be calculated. In addition, SD scores of mean values of 24-hour, day, and night systolic, diastolic, and mean arterial pressure and centile values based on the same dataset can also be derived (23).

1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
71.75297	74.86064	76.66156	78.25584	79.47868	80.45668	81.12397	81.73286	82.29531	82.94691
11%	12%	13%	14%	15%	16%	17%	18%	19%	20%
83.50347	83.99772	84.56093	84.89357	85.19153	85.591	85.96851	86.28016	86.52923	86.86385
21%	22%	23%	24%	25%	26%	27%	28%	29%	30%
87.21521	87.42311	87.67691	87.89638	88.11847	88.51659	88.81239	89.04817	89.4107	89.63064
31%	32%	33%	34%	35%	36%	37%	38%	39%	40%
89.75469	90.03538	90.21972	90.55341	90.74061	90.98968	91.25694	91.48897	91.76162	91.98838
41%	42%	43%	44%	45%	46%	47%	48%	49%	50%
92.28186	92.53902	92.78613	93.0832	93.38083	93.67087	93.83705	94.11279	94.34935	94.49245
51%	52%	53%	54%	55%	56%	57%	58%	59%	60%
94.77854	94.99403	95.20445	95.38161	95.56668	95.80081	95.98722	96.21596	96.40707	96.68254
61%	62%	63%	64%	65%	66%	67%	68%	69%	70%
96.88304	97.11062	97.30416	97.63383	97.84923	98.13857	98.45074	98.60639	98.8837	99.1503
71%	72%	73%	74%	75%	76%	77%	78%	79%	80%
99.47953	99.80236	100.0076	100.3997	100.6352	101.0518	101.4893	101.8736	102.3324	102.7792
81%	82%	83%	84%	85%	86%	87%	88%	89%	90%
103.1847	103.6683	104.1513	104.6462	105.1414	105.5773	106.1356	106.5553	106.9724	107.701
91%	92%	93%	94%	95%	96%	97%	98%	99%	
108.2981	108.975	109.567	110.0854	111.2749	112.7681	114.4791	116.4757	119.5459	

Figure 1. Reference centiles and corresponding systolic blood pressure values (mmHg) for an 8-year-old girl with a height of 123 cm generated by the system using relevant formulae and regression coefficients

4. Future Agenda

We are working on solutions for analyzing multiple data (e.g. auxological data of 200 subjects) at once, creating growth curves including various data belonging to more than one visit, and increase the spectrum of IGF1 calculations by adding other types of kits available on the market.

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REVIEW

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A Proposal for the Interpretation of Serum IGF-I Concentration as Part of Laboratory Screening in Children with Growth Failure

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Abstract

The serum insulin-like growth factor-I (IGF-I) concentration is commonly used as a screening tool for growth hormone deficiency (GHD), but there is no consensus on the cut-off limit of IGF-I standard deviation score (SDS) to perform GH stimulation tests for confirmation or exclusion of GHD. We argue that the cut-off limit is dependent on the clinical pre-test likelihood of GHD and propose a diagnostic strategy in which the cut-off limit varies between zero to -2 SDS.

Keywords: Short stature, growth disorders, IGF-I, IGFBP-3

Introduction

In guidelines on the diagnostic approach to children referred to a paediatrician or paediatric endocrinologist for short stature and/or growth faltering (from now on referred to as "growth failure", abbreviated as GF), including the consensus paper on Idiopathic short stature (ISS) (1) and the recent Dutch guideline (2), it is advised to perform laboratory screening for potential subclinical pathological causes, including serum insulin-like growth factor-I (IGF-I) [with or without serum IGF-binding protein-3 (IGFBP-3)], in order to screen for growth hormone deficiency (GHD). During the pre-final phase of the Dutch guideline, when authorization was sought from representatives of the Paediatric Association of the Netherlands and other specialist societies, the working group was asked to add specific instructions for the general paediatrician at which cut-off point of IGF-I a GH stimulation test should be performed to confirm or exclude GHD. Since a literature search did not provide a clear answer to this question, we performed a stepwise analysis of the decision procedure, taking established clinical epidemiological techniques into consideration. This led to several subsequent versions of an addendum to the Dutch Guideline on Triage and Diagnosis of Growth disorders in children (2), which were reviewed by members of the Section of Paediatric Endocrinology and the Growth Hormone Advisory Group of the Paediatric Association of the Netherlands and discussed at several meetings. In the present commentary we present an English adaptation of the addendum to the official guideline that will be published in Dutch on the internet.

Definition, Subcategories and Varying Levels of Uncertainty of GHD

Impaired secretion of GH in children is causally related to impaired growth, anthropometric characteristics (e.g., normal proportions, relatively large head) and changes of body composition (e.g., sarcopenia, excess of body fat and low bone mineral density) as well as functional abnormalities (e.g., hypoglycaemia) (3,4). However, for practical and financial reasons it is troublesome to measure the GH secretion over 24 hours in individual patients, so that as a proxy indicator of spontaneous GH secretion the GH response to a stimulus is commonly used. The various problems of such stimulation tests are well known (5) and also low serum levels of GH-dependent proteins (IGF-I and IGFBP-3) cannot be considered gold standards.

GHD can be subcategorized into acquired and non-acquired (congenital) forms (4). The acquired form is usually caused by space-occupying processes in the brain, such as tumours (e.g., craniopharyngioma), but can also be the consequence

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of brain trauma, infections, irradiation, histiocytosis or vascular anomalies. The congenital form can be subdivided into three subgroups (6). The first is an (almost) certain form where the etiology is known, for example if GHD is associated with cerebral or facial malformations, anatomic pituitary or hypothalamic abnormalities detected with magnetic resonance imaging (MRI), other pituitary hormone deficiencies, or established genetic causes of GHD, such as mutations of *GH1*, *GHRHR* or *GHSR* in isolated GHD (IGHD) and of multiple transcription factors in multiple pituitary hormone deficiency. In line with Ranke et al (6) this etiologic subgroup is abbreviated as cGHD. The second and most frequent subgroup is idiopathic IGHD (IIGHD). The third subgroup is growth hormone neurosecretory dysfunction (NSD), characterised by clinical features (including a "typical" growth curve) and low serum IGF-I which lead to suspicion of GHD, in combination with a normal GH peak during a stimulation test. In the original papers it was shown that such children had a low spontaneous GH secretion over 24 hours in contrast to a normal GH peak in response to a GH stimulation test (7,8,9). Due to the practical and financial hurdles of performing 24-hour GH profiles, this diagnosis has also been assumed without proof of a decreased spontaneous GH secretion (6).

The diagnostic process of acquired GHD and cGHD is straightforward, although sometimes with considerable diagnostic delay, but this does not apply to IIGHD and NSD. Retesting of the hypothalamic-pituitary-GH/IGF-I axis in puberty and after reaching adult height of patients with IIGHD leads in most cases to normal results regarding the GH peak in a GH stimulation test (10,11,12). This phenomenon is usually interpreted as an initial false-positive result of GH testing, although a transient form of GHD cannot be excluded. The diagnosis of NSD has been controversial from the beginning, although an adequate growth response to GH treatment of children diagnosed as NSD has been documented (7,8,9).

Diagnostic Approach with Respect to GHD in Children Referred for Growth Failure

There is no doubt that the diagnostic process for children referred for GF should be aimed at detecting and treating all children with acquired GHD or cGHD as soon as possible. In the remaining children (IIGHD and NSD), which constitute the majority of cases (6), the clinician has to face the challenge to distinguish as best as possible IIGHD or NSD from other causes of GF or short stature of unknown origin (ISS). In children it is particularly difficult to distinguish IIGHD from the non-familial form of ISS with maturational delay (1,3,4). Many prepubertal short children who later present with delayed puberty, have a prepubertal growth pattern characterised by a low height velocity [decreasing height standard deviation score (SDS)] (13,14). However, the diagnosis of "Constitutional Delay of Growth and Puberty" can only be diagnosed with certainty if the onset of puberty (Tanner genital or breast stage 2) is indeed delayed (boys > 14 years, girls > 13 years).

For an estimate of the probability of GHD in a child with GF (in comparison to another or unknown cause), the clinician needs to take the following steps:

1. Be aware of the general prevalence of GHD in children referred for GF in the specific clinic;

2. Estimate the *individual pre-test likelihood of GHD* in the patient, based on weighing the diagnostic clues from the medical history (including family history), physical examination, growth curve, laboratory screening and skeletal maturation;

3. Perform *laboratory screening* using serum IGF-I and (in young children) IGFBP-3, and interpret the result taking into consideration age, sex, pubertal status and body mass index;

4. Calculate the *post-test likelihood of GHD* based on serum IGF-I (with or without IGFBP-3) and individual pre-test likelihood;

5. If the post-test likelihood is considered sufficiently high, perform GH-provocation tests (GH-stimulation tests);

6. If the results of the GH stimulation test are compatible with GHD, perform an *MRI of the hypothalamus-pituitary area* (and in specific cases genetic testing can also be considered);

7. If the GH peak is normal in the setting of a severely decreased serum IGF-I, consider performing an IGF-I generation test (to differentiate between rare syndromes with either normal or decreased GH sensitivity) and/or genetic tests, preferably after consultation with a paediatric endocrinologist and/or clinical geneticist knowledgeable in genetic causes of GF.

In this Critical Review we shall discuss the first four steps. For the remaining three steps we refer to previous documents, references 15,16,17,18.

Prevalence of GHD and Its Subcategories in Children Referred for Growth Failure

The prevalence of GHD in the general population of children is uncertain. Estimates vary from 1:3,400 to 1:30,000 (19,20,21,22,23). If one estimates a prevalence of 1:5000,

and assumes that almost all cases present with a height below -2 SDS (2.3rd percentile), the prevalence of GHD in short children in the population would be approximately 1%. The reported prevalence of GHD in children referred for GF to a paediatric (endocrine) clinic also varies. In Dutch studies the prevalence was between 0.5-3% (24,25,26) and in other studies prevalences between 0 and 23% were reported (27,28,29,30,31,32,33,34). For this analysis we estimated the general prevalence of GHD in children referred for GF at 2%, in line with a recent publication (35), but this percentage may differ between a general and academic paediatric clinic. Furthermore, the variation in diagnostic approaches, for example whether sex hormone priming is used when a GH stimulation test is performed in the pre- to early-pubertal age range (36), can also be expected to have a significant impact on the percentage of GHD diagnosed in referred children.

We have not found data on the proportion of acquired and congenital GHD, but based on our clinical experience, acquired GHD is rare. Within the group of congenital GHD an indication of the relative proportions can be obtained from the report on 50 years' experience in Tübingen: out of 636 patients, 122 had cGHD (19.2%), 455 IIGHD (71.7%) and 58 (9.1%) NSD (6).

Estimate of the Individual Pre-test Likelihood of GHD in a Patient Referred for Growth Failure

A full clinical assessment of the child will lead to an estimate of the individual clinical pre-test likelihood of GHD, based on an inventory of all relevant clinical features. These comprise diagnostic clues from the medical history, physical examination, growth curve, general laboratory screening and skeletal age. Although objective data are lacking, we believe that the relative weight of these diagnostic clues is different. Table 1 shows the diagnostic clues and our personal opinion on their relative importance. Future prospective studies are needed to collect observational data

Table 1. Positive and negative clinical clues for growth hormone deficiency

	Positive clues	Negative clues
History	- Fatigue - Decrease of muscle power - Decrease of concentration - Symptoms of other pituitary deficiencies* - Positive family history for GHD* - Consanguinity - Breech delivery - Prolonged postnatal hypoglycaemia* - Prolonged neonatal jaundice* - Neurologic symptoms* - History of meningitis, cranial irradiation or brain injury*	Symptoms compatible with alternative explanation of growth failure
Physical examination	- Lobulated abdominal fat - Frontal bossing - High-pitched voice - Cryptorchidism - Neurologic signs* - Vision abnormalities* - Optic hypoplasia or other abnormalities at fundoscopy* - micropenis*	- Dysmorphisms* - Dysproportion* - Underweight - Any signs compatible with alternative explanation of growth failure
Growth	- Growth deceleration* - Absence of growth acceleration despite progression of puberty*	No or little growth faltering*
Laboratory screening	- Central hypothyroidism or deficiency of other pituitary axes* - Central diabetes insipidus*	Laboratory findings suggestive for an alternative explanation of growth failure (one of the secondary growth disorders or Turner syndrome)*
Radiograph of hand and wrist	Bone age delay*	Bone age close to chronological age or advanced*

*Diagnostic clues with relatively high weight in the opinion of the authors.

GHD: growth hormone deficiency

on the relative weight of the various diagnostic clues. Based on the balance of positive and negative diagnostic clues, the clinician can estimate the clinical pre-test likelihood of GHD in an individual patient. Thereafter, the result of serum IGF-I (and IGFBP-3) can be interpreted against the background of the pre-test likelihood of GHD.

The estimate of the pre-test likelihood of GHD is obviously a continuum and subjective, since objective data are lacking for the proportional weighting of diagnostic clues. For practical purposes, we propose five categories of pre-test likelihood:

1. High pre-test likelihood (≥≈50%), if there are several positive clues and no negative clues.

2. Moderate pre-test likelihood (≈20%), if there are some positive clues for GHD, and no negative clues.

3. Rather low pre-test likelihood (≈10%), if there are few positive clues for GHD and no negative clues.

4. Low pre-test likelihood (≈5%), if there are few positive clues and at least one negative clue for GHD.

5. Very low pre-test likelihood (≤≈2%), if there are no positive clues and/or ≥1 negative clues for GHD; this is roughly equal to (or less than) the estimated prevalence in children referred for GF.

In case of a high pre-test likelihood of GHD the clinician will directly perform an MRI of the pituitary/hypothalamic area, particularly in neonates or if there is clinical suspicion of acquired GHD. In neonates with clinical and biochemical features of hypothalamic/pituitary deficiency an abnormal brain MRI in combination with a random serum GH concentration <7 µg/L in the first week of life has been proposed as sufficient to make the diagnosis of GHD (37). A low serum IGF-I supports the diagnosis, but its specificity at that age is low because the lower limit of the reference range is then below the sensitivity of the assay. A low IGFBP-3 (<-2.0 SDS) is also supportive, and in general its specificity appears to be higher than that of IGF-I (38,39,40).

In addition, when an acquired form of GHD is suspected, the clinician will promptly perform a brain MRI, as well as further diagnostic investigations into hypothalamic/pituitary function. Such investigations will usually be repeated following neurosurgical or oncological treatment. During follow-up of neuro-oncological patients with an increased risk of developing GHD, a recent international guideline advised to perform GH stimulation tests when there is clinical suspicion of GHD, irrespective of the result of serum IGF-I, because in such patients a low serum IGF-I appears to have a low sensitivity (41). It has been suggested that if multiple pituitary axes are affected, a GH stimulation test can be omitted because of the high pre-test likelihood of GHD (41). In patients with possible IIGHD, a brain MRI is only performed after GHD has been confirmed by GH stimulation tests.

Laboratory Screening Using Serum IGF-I and Interpreting the Result Adjusting for Age, Sex, Pubertal Status and Body Mass Index

The serum concentration of IGF-I is not only influenced by GH secretion, but also by biological factors, such as nutritional status, age, sex, and pubertal stage, as well as analytical factors. This implies that if the serum IGF-I concentration is measured in the context of laboratory screening as an indicator of GHD in children with GF, the result should first be adjusted for confounding factors.

In most countries different commercial IGF-I assays are used with a considerable inter-assay variation. In our country, most of the inter-assay variation of 5-20% is compensated for because of a national harmonization program. For each assay there is also some intra-assay variation [e.g., a coefficient of variation of 6-8% in the assay used in Tübingen (6) and a total coefficient of variation 3.4-8.7% in the iSYS assay (42)], so that repetition of an IGF-I determination should be considered, particularly if there is a discrepancy between the clinical features and the laboratory results. Given the inter-assay variation, it is advisable to use the same assay during follow-up of an individual patient.

For a proper interpretation of the serum IGF-I concentration, the first step is to express IGF-I as SDS for age and sex. Obviously, for this purpose adequate reference data from a large population of healthy children are needed. Given the differences between assays, reference data should be gathered for each assay separately.

It is generally assumed that in prepubertal children in the age range where puberty usually has not yet started IGF-I SDS can be calculated for age and sex. However, the maximum age for this approach is arbitrary. One could argue that the 10th percentile of the age at start of puberty in the population would be a rational choice (9 years in girls and 10 years in boys in our country), but other investigators suggested a cut-off of 8 and 9 years, respectively (35).

Regarding adjustment for pubertal status in older children, the situation is more complex, and a further adjustment step has to be performed. For this purpose, there are essentially two possible approaches. For a few assays reference data have been reported on IGF-I per pubertal stage [for example (42,43,44)], but the assays used in the two oldest reports were calibrated to old standards. IGF-I percentiles according

to pubertal (Tanner) stage for the IDS iSYS assay are shown in Table 2 (42).

If an assay is used for which such reference data are unavailable, one can calculate SDS for skeletal age (35), because a (relatively) delayed puberty is usually associated with a (relatively) delayed skeletal age. However, its predicted power is reportedly slightly lower than that of the SDS for pubertal stage (35).

Since the expression of IGF-I as an SDS is more informative than a percentile position, we calculated mean, SD and cut-off limits for various SDS for children with different Tanner stages based on the raw data that were used for the construction of reference data for the IDS iSYS assay (Table 3) (42).

A further complication when assessing serum IGF-I occurs if BMI SDS is low or high (45). In a child with a low BMI

Table 2. Percentiles of insulin-like growth factor-I (ng/mL) according to sexual maturation, based on 854 samples (age 0-20 years), as measured with iSYS (42)*

		IGF-I, ng/mL, percentiles				
Tanner	**Age**	**2.5%**	**25%**	**50%**	**75%**	**97.5%**
Males						
I	6.1-12.9	81.3	132.5	160.0	187.9	255.3
II	8.1-14.8	106.2	212.4	276.9	331.8	432.3
III	10.9-16.0	244.9	341.2	407.2	449.0	511.4
IV	12.4-17.1	222.6	364.5	439.0	492.4	577.7
V	13.5-20.0	227.4	308.6	355.7	412.3	517.8
Females						
I	5.8-12.1	85.9	152.6	187.7	235.3	323.0
II	9.3-14.1	117.5	190.0	247.3	323.2	451.3
III	9.3-15.1	258.3	335.5	382.8	430.8	528.5
IV	11.8-16.6	224.2	339.8	378.3	437.5	585.8
V	12.5-19.9	188.2	277.4	339.1	394.9	511.6

*For results expressed in nmol/L, multiply by 0.131.

IGF-I: insulin-like growth factor-I

Table 3. Cut-off points at -2, -1, 0 +1 and +2 SDS of serum insulin-like growth factor-I (ng/mL) according to sexual maturation as measured with iSYS (Bidlingmaier, personal communication 2019)*

		IGF-I (ng/mL) SD and SDS positions based on Box-Cox transformation					
Tanner stage	**Age range**	**SD^**	**-2.0 SDS**	**-1.0 SDS**	**0.0 SDS**	**+1.0 SDS**	**+2.0 SDS**
Boys							
I	6.1-12.9	44.0	82.0	117.7	161.5	205.4	257.1
II	8.1-14.8	83.2	110.5	192.3	275.6	358.9	443.2
III	10.9-16.0	69.7	230.9	329.2	396.5	464.2	517.8
IV	12.4-17.1	90.1	211.1	340.8	426.7	513.6	582.2
V	13.5-20.0	74.7	226.9	288.1	362.5	437.0	525.5
Girls							
I	5.8-12.1	60.0	88.5	134.0	193.64	253.3	327.1
II	9.3-14.1	90.3	101.6#	167.1	256.59	346.7	460.8
III	9.3-15.1	69.0	246.5	313.6	382.67	451.7	522.3
IV	11.8-16.6	86.1	235.2	304.8	390.25	475.9	577.9
V	12.5-19.9	82.1	184.7	256.6	338.74	420.9	512.3

IGF-I: insulin-like growth factor-I, SD: standard deviation, SDS: standard deviation score.

*For results expressed in nmol/L, multiply by 0.131

^Here, the untransformed SD is shown. For calculations of the various SDS cut-off points the Box-Cox transformation was used.

#This value is lower than the P2.5 obtained by the Harrell-Davis transformation (117.5 ng/mL) used in Bidlingmaier et al (42). For clinical practice, one could consider an IGF-I < 117.5 in a girl with Tanner breast stage II as roughly equivalent to < -2.0 SDS

SDS, another sign of undernutrition, or a recent disease associated with decreased appetite, IGF-I is relatively low. This would imply that in such children a low serum IGF-I has a relatively low predictive value. If feasible, one may wish to first improve the nutritional condition before repeating further IGF-I measurements. In a child or adult with a high BMI SDS, GH secretion is usually low in contrast to a serum IGF-I in the upper half of the reference range. In overweight children, one could therefore expect that the optimal cut-off point of IGF-I for the decision to perform a GH stimulation test may be higher than in children with a normal BMI SDS. Due to the lack of observational data, a formal adjustment of the IGF-I result for BMI is not possible, so that the clinician can only use his/her subjective clinical judgement.

When a low serum IGF-I is found in a child with a low clinical pre-test likelihood we suggest to repeat serum IGF-I, preferably in combination with a determination of serum IGFBP-3, before one decides to perform a GH stimulation test. If IGFBP-3 SDS is remarkably lower than IGF-I SDS, one should consider the possibility of a homozygous mutation of *IGFALS* (46).

Calculation of the Post-test Likelihood of GHD Based on Serum IGF-I (with or without IGFBP-3) and Individual Pre-test Likelihood of GHD

In a previous study by our group (47), various parameters for quantifying the diagnostic value of serum IGF-I and IGFBP-3 and other markers were investigated in a Dutch cohort of children from four years of age with GHD or ISS. The optimal cut-off point for IGF-I for the diagnosis of GHD was -0.83 SDS [with an area under the curve (AUC) of 0.80 in the ROC analysis], but specificity was low at that point (47%). The optimal cut-off point for IGFBP-3 was -0.47 SDS (AUC 0.69) with a specificity of 22%. In the same publication data were shown on the frequencies of a serum IGF-I or IGFBP-3 SDS below <-2, <-1 and <0 in children with either GHD or ISS, as well as the positive likelihood ratio (LR+) and negative likelihood ratio (LR-) (Table 4).

For IGF-I, the sensitivity (65%) and specificity (78%) of a cut-off at -2 SDS in the Dutch study (47) were similar to those reported in other studies, as summarized in a meta-analysis (39), where average sensitivity and specificity of IGF-I were 0.66 and 0.69, with positive and negative LRs similar to those in the Dutch study. Also for IGFBP-3 similar findings were reported in the Dutch study (sensitivity 53%, specificity 81%) as in the later meta-analysis (50% and 79%). It is noteworthy that a low IGFBP-3 is less sensitive but more specific than a low IGF-I.

In the literature there is no consensus whether IGFBP-3 should be added to an IGF-I measurement in the screening phase (48). In light of the higher sensitivity of IGF-I (38,39), and in order to reduce expenses, in the recent Dutch guideline we decided to limit IGFBP-3 determinations in this phase to children below three years of age, because in that age range the 3rd percentile of the reference range of IGF-I is close to the detection limit of most assays (40).

We also advised to add an IGFBP-3 determination when an IGF-I measurement is repeated, for example in children with a low IGF-I but a low pre-test likelihood of GHD. In such children, a low IGFBP-3 (because of its high specificity) considerably increases the likelihood of GHD (or a homozygous *IGFALS* defect). Also if a GH stimulation test is performed, we advise to repeat IGF-I and IGFBP-3 determinations at baseline.

A so far unexplored issue is which cut-off for serum IGF-I should be used for the decision to perform a GH stimulation test. Most papers suggest that IGF-I should be below -2 SDS, but it is obvious that this would imply that approximately 35% of children with GHD would not be detected. In one paper a cut-off of -1 SDS was suggested (49). However, according to general clinical epidemiological principles, the diagnostic value of a test depends on the clinical pre-test likelihood, which implies that different cut-off limits should be used for different ranges of pre-test likelihood.

Table 4. Frequency of children with growth hormone deficiency or idiopathic short stature with a serum insulin-like growth factor-I (IGF-I) or IGF-binding protein-3 <-2, <-1 and <0 standard deviation score, and positive and negative likelihood ratios [likelihood ratio (LR)+ en LR-]

IGF-I SDS	<-2	<-1	<0	**IGFBP-3 SDS**	<-2	<-1	<0
GHD	65%	86%	96%	**GHD**	53%	78%	96%
ISS	22%	50%	88%	**ISS**	19%	56%	88%
LR+	3.0	1.7	1.1	**LR+**	2.8	1.4	1.1
LR-	0.44	0.29	0.33	**LR-**	0.58	0.51	0.33

IGF-I: insulin-like growth factor-I, GHD: growth hormone deficiency, ISS: idiopathic short stature, LR: likelihood ratio, IGFBP-3: IGF-binding protein-3, SDS: standard deviation score

Ref. 47

We therefore calculated the post-test likelihood depending on pre-test likelihood (in five arbitrary categories) and IGF-I result (<-2, <-1 and <0 SDS) (Table 5). Based on this post-test likelihood, the clinician can make the decision if a GH stimulation test would be indicated to confirm or reject the diagnosis of GHD. We appreciate that the decision about which likelihood is sufficiently high to warrant GH stimulation tests is subjective. For the sake of argument we assumed that a post-test likelihood of >10% would be sufficient to decide to perform GH stimulation tests. The consequences of this analysis for different categories are described in the following paragraph. We emphasize that the IGF-I SDS that is used for decision-making should have been adjusted as well as possible for confounding factors such as puberty and nutritional status, although we acknowledge that in contrast to the adjustment for pubertal stage, there are no numerical data that enable the recalculatation of IGF-I SDS adjusted for nutritional status.

For each of the five categories of clinical pre-test likelihood a rational choice of cut-off for serum IGF-I can be made, as explained below. A schematic representation of this advice is shown in Table 6.

High (≥50%) clinical pre-test likelihood: In such children a GH stimulation test is indicated regardless of the result of IGF-I. If IGF-I is <0 SDS all post-test likelihoods are high (>52-70%) and even in the rare cases with an IGF-I above 0 SDS [4% in the Dutch study (47)] the post-test likelihood will remain far above 10%. In fact, in children treated for brain tumours, for example using irradiation, or obese children, the IGF-I result is not a determing factor for performing a GH stimulation test (41).

Moderate (20%) clinical pre-test likelihood: At a cut-off of 0 SDS the post-test likelihood is approximately equal to the pre-test likelihood, and at a cut-off of -1 SDS it is 30%. We suggest that in such cases a serum IGF-I

Table 5. Post-test likelihood of growth hormone deficiency according to clinical pre-testlikelihood and serum insulin-like growth factor-I*

Category of pre-test likelihood	IGF-I SDS cut-off	LR+	Pre-test likelihood	Pre-test odds	Post-test odds	Post-test likelihood
High (≥50%)	<0	1.1	50%	1	1.1	**52%**
	<-1	1.7		1	1.7	**63%**
	<-2	3.0		1	3.0	**70%**
Moderate (20%)	<0	1.1	20%	0.25	0.275	**21.5%**
	<-1	1.7		0.25	0.425	**30%**
	<-2	3.0		0.25	0.75	**43%**
Rather low (10%)	<0	1.1	10%	0.11	0.12	**11%**
	<-1	1.7		0.11	0.19	**16%**
	<-2	3.0		0.11	0.33	**25%**
Low (5%)	<0	1.1	5%	0.05	0.055	5%
	<-1	1.7		0.05	0.085	8%
	<-2	3.0		0.05	0.158	**14%**
Very low (≤2%)	<0	1.1	2%	0.02	0.022	2%
	<-1	1.7		0.02	0.034	3.3%
	<-2	3.0		0.02	0.06	5.7%

IGF-I: insulin-like growth factor-I, SDS: standard deviation score, LR: likelihood ratio.

*The following formulas were used: Pre-test odds = pre-test likelihood/(1 minus pre-test likelihood. Post-test odds = Positive likelihood (LR+) x pre-test odds. Post-test likelihood = post-test odds/(post-test odds + 1). Percentages >10% are in bold print

Table 6. Suggested insulin-like growth factor-I cut-off limits in the screening phase to guide the decision to perform growth hormone stimulation tests

Clinical pre-test likelihood of GHD	IGF-I SDS cut-off for GH stimulation tests
High (≥50%)	No
Moderate (20%)	<0 SDS
Rather low (10%)	<-1 SDS (or eventually <0 SDS)
Low (5%) or very low (≤2%)	<-2 SDS; consider alternative diagnosis

GH: growth hormone, GHD: GH deficiency, IGF-I: insulin-like growth factor-I, SDS: standard deviation score

<0 SDS can be used for the decision to perform a GH stimulation test.

Rather low (10%) clinical pre-test likelihood: At a cut-off point of -1.0 SDS the post-test likelihood increases from 10 to 16%, which is in our opinion sufficient to perform a GH stimulation test. When IGF-I is between -1 and 0 SDS the post-test likelihood is not different from the pre-test likelihood and we assume that the clinician's subjective assessment will influence the decision to perform further testing.

Low (5%) or very low (≤2%) clinical pre-test likelihood: At a low pre-test likelihood the post-test likelihood is only above 10% at a cut-off point of -2.0 SDS, while at a very low likelihood the post-test likelihood remains below 10% at all values of the IGF-I results. We suggest that at (very) low pre-test likelihood a rational first step would be to repeat an IGF-I determination, in combination with serum IGFBP-3. If this IGF-I result is in line with the first one and is supported by a low or low-normal IGFBP-3 SDS, further investigations are warranted to diagnose either GHD or rare syndromes characterized by low IGF-I and IGFBP-3. Such syndromes can be divided into two sugroups: those with normal sensitivity to GH [NSD, bio-inactive GH (Kowarski syndrome) or *GHSR* mutation] and those with low or absent sensitivity to GH (GH resistance, such as in children with mutations of *GHR, IGFALS, STAT5B, STAT3* and *IGF1*)(50). In order to differentiate between the two subgroups one can consider performing an IGF-generation test, in spite of its imperfect diagnostic value (51). In a short child with an IGF-I <-2 SDS and GH peak >10 ng/mL one can also consider consulting with a paediatric endocrinologist and/or clinical geneticist to discuss performing a growth-specific whole exome based gene panel (50,52).

Obviously, one should realize that the estimate of the pre-test likelihood of GHD is subjective, and probably will vary between clinicians. The same applies to the cut-off point of post-test likelihood which is considered to be sufficiently high for performing a GH stimulation test.

Interpretation of a Serum IGF-I >0 SDS in a Short Child

A serum IGF-I >0 SDS can be seen in children with ISS, although mean IGF-I is approximately -1 SDS. If such IGF-I concentrations are found in a short child with low or low-normal birth size or low or low-normal head circumference, this is suggestive of a mutation or deletion of *IGF1R* [for a clinical score, see (53)]. Also, in children with Silver-Russell syndrome (caused by various (epi)genetic disorders, including IGF2 and other gene mutations), a *PAPPA2* mutation, Bloom syndrome and *IGF1* mutations, IGF-I can be increased or in the upper half of the reference range (54).

Conclusion

In the child referred for short stature and/or growth faltering determination of serum IGF-I is a useful component of laboratory screening. The result should be expressed as SDS for age and sex and adjusted to physiologic (particularly pubertal stage and nutritional status) and analytic factors, and interpreted according to the clinical pre-test likelihood of GHD. The post-test likelihood can guide the clinician's decision to perform GH stimulation tests.

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COMMENTARY

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A Proposal to Develop New References for Serum IGF-I Levels in Children

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Commentary to Wit et al. 2019 "A proposal for the interpretation of the serum IGF-I concentration as part of laboratory screening in children with growth failure" J Clin Res Pediatr Endocrinol. 2019 Dec 17. doi: 10.4274/jcrpe.galenos.2019.2019.0176

Wit and collaborators (1) have again approached the issue of the value of IGF-I serum levels as a tool for diagnosing growth hormone deficiency (GHD). They have thoughtfully reviewed the existing literature and deliberated that the cut-off in terms of standard deviation (SD) score (SDS) should be interpreted based on the pre-test likelihood of GHD. I recommend reading this interesting article. In short, the authors concluded: In the case the pre-test likelihood for GHD - based on anamnestic information, physical findings, anthropometric measurements and laboratory results not specifically related to GHD - is high (>50%) further testing (including measurements of GH secretion) is recommended even if IGF-I levels are in the normal range. However, if the pre-test likelihood is low (<10%) only very low IGF-I levels (<-2 SDS) qualify these children for a further diagnostic work-up.

The diagnosis of GHD in childhood is in fact complex and is subject to an ongoing, partly controversial discussion (2). A number of - often rather less well-defined terms - have been used to subclassify GHD, including congenital, acquired, idiopathic, with or without (isolated) other pituitary hormone deficits, severe, less severe, total, and partial GHD (3). In addition, the clinical picture, as well as the diagnostic tools and criteria applied, are dependent on the age of the patient. The key issue remains the methodologically difficult quantification of GH secretion, which means defining the individual GH secretion as being insufficient (deficient) compared to normal. Components of the IGF system [IGF-I, IGF-binding protein-3 (IGFBP-3), and others] are qualitatively dependent on GH secretion. Their serum levels (IGF-1, IGFBP-3) have been proven to be positively correlated with the spontaneously secreted amount of GH in children and adolescents (4). Thus the attempt to use their blood levels (and their standardized derivatives) as a potential diagnostic indicator of GHD (5,6,7) is rational.

Modern classification system which are placing IGF-I into the center of a classification even distinguish between disorders with primary IGF-deficiency (such as the inability to primarily produce IGF-1) from those with secondary IGF-deficiency (such as GH deficiency) (3,8). Serum levels of IGF-I (or IGFBP-3) show little circadian variance (9) and their measurement is well standardized and generally accessible (10), factors which recommend their use as a diagnostic step before specific GH testing.

The idea proposed by Wit et al (1) to establish a pre-test likelihood of GHD makes sense and is probably intuitively used by every physician diagnosing short children. To transform this into an empirically based scoring system is certainly difficult, but potentially doable. However, in my view, much of the problem of using IGF-I (IGFBP-3) as diagnostic tools is caused by the available references. Since the measurement of Somatomedin C = IGF-I had become available by means of radioimmunoassay (11) several authors have published references for age and sex based on children, adolescents or adults from large cohorts using various immunoassay techniques (6,12,13,14,15,16). In general, these references show the following qualitative characteristics for IGF-I: 1. During the childhood years serum levels show a steady increase from very low levels

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at birth onwards with no quantitative differences between the sexes. 2. During puberty there is a steep increment with a peak at about mid-puberty with levels in females exceeding those in males. 3. Thereafter there is a gradual decline - females remaining higher than males - reaching very low levels during senescence. The levels in all age groups are not distributed by a Gaussian characteristic but are skewed positively (to the right of the mean). The latter phenomenon is observed to a lesser degree for IGFBP-3 as compared to IGF-I. In order to express the relative magnitude of the difference of a patient's value from the reference mean we are used to calculating the SDS for age: SDS = (patient's value - mean of patient's age-related reference) divided by the SD of mean of patient's age-related reference. If parameters are skewed specific mathematical transformations are needed to calculate means and SDs for a given age as the basis for such a calculation. In order to approximate towards a normal distribution of the references several methods have been applied such as logarithmic transformation or square root transformation (6,13,15,16).

The author always found it remarkable that that quantitative spread of the normal range for serum IGF-I (and other IGF parameters) for a given age is relatively high compared to other biological parameters. For example, in a 7 year old child, the normal range of serum IGF-I levels is from about 60 to about 250 µg/L, while for standing height it is only from about 114 to 134 centimeters. Authors who have reported references of IGF parameters have shown that IGF-I levels in serum of children are not only dependent on age and sex but also on pubertal stage and body mass index (BMI). Therefore it appears to be very likely that the enormously wide range (for the usual denominators age and sex) of normal of IGF-I levels is indeed caused by the variability of other effectors, which are not accounted for. The quantitative impact of pubertal development as expressed in terms of the rather crude Tanner stages has been investigated rather extensively (12,16). However, for the diagnosis of idiopathic GHD this appears to be of little practical relevance since the children in question have a delayed development and normal references for children in pubertal age but without puberty would be needed.

Alberti and collaborators (16) calculated that in short children a change of one SDS of height - or of BMI - corresponds to +/- 0.2 SDS in IGF-I for age and sex. Thus, if a short and obese boy (e.g. BMI = +2.5 SDS], has an IGF-I level for age of – 0.5 SDS, this figure must be interpreted as being about 0.5 SDS too low for the condition of overweight. In addition, some authors have observed that height and weight also play an independent role for IGF-I levels. If such relevant co-variants (pubertal stage, body composition, and others) should play a major role for IGF serum levels they should also be measured when establishing references of normally tall children. The co-variants should also be truly quantified (e.g. levels of sex hormones rather than just rough pubertal staging; exact non-invasively measured masses of muscle, fat, bone, and tissue size rather than calculating BMI alone; - and using automatically determined bone age in addition to chronological age; etc.).

If the quantitative effect of these factors on the reference values were known the expected mean reference value (and its SD) for the individual patient in question could be calculated with the help of a multivariate regression algorithm. A somewhat similar approach has been used for references of serum Leptin levels by Blum et al (17). Such individually calculated (predicted) reference figures (mean, SD) could then be used to calculate an individual (adjusted for the individual co-variants) SD-score. The approach of developing such "conditional" references should at least be attempted for children during the (prepubertal) childhood age (about 4-11 years), at an age range when isolated, idiopathic GHD, which has the lowest pre-test likelihood, is diagnosed most frequently in childhood. The information collected for establishing the reference of Bereket et al (15) or Alberti et al (16) could probably be used for such an analysis. Such a novel approach may be an additional step to further substantiate the diagnostic value of measuring IGF-parameters in short children and reach a higher likelihood of GHD before further extensive testing. Multidimensional reference region combining the levels of various IGF parameters as used in adults (18) may perhaps also augment their diagnostic value in short children.

Ethics

Peer-review: Internally peer-reviewed.

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Evaluation of Thyroid Function Tests in Children with Chronic Liver Diseases

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What is already known on this topic?

Thyroid hormone metabolism may be impaired in adult chronic liver diseases and subclinical hypothyroidism (SH) (≈3.5%) or euthyroid sick syndrome (7-30%) may occur. There are very few studies in the pediatric age group investigating this issue.

What this study adds?

The proportion of pediatric/adolescent chronic liver disease patients with SH or euthyroid sick syndrome was around 10%. It should be kept in mind that children with chronic liver disease may have SH.

Abstract

Objective: Studies examining changes in thyroid function in the course of chronic liver disease have mostly been conducted in adults. The aim of this study was to investigate thyroid dysfunction in children with chronic liver diseases.
Methods: Between 2005 and 2018, patients aged up to 18 years of age, diagnosed with chronic liver disease and had thyroid function test results available were included. Anthropometric characteristics, liver and thyroid function results were collected and analyzed.
Results: The study included 107 (53 female; 49.5%) patients aged between one month and 18 years-old. Of the 107 patients, 96 (89.7%) had normal thyroid function results, seven (6.5%) had subclinical hypothyroidism (SH) and four (3.7%) had euthyroid sick syndrome. Of the patients with SH, one (14.2%) had glycogen storage diasease, one (14.2%) had biliary atresia, one (14.2%) had undiagnosed cholestatic liver disease, one (14.2%) had Alagille syndrome, one (14.2%) had idiopatic hepatitis, one (14.2%) had progressive familial intra-hepatic cholestasis and one (14.2%) had congenital hepatic fibrosis. Spearman correlation analysis showed a negative correlation between free tri-iodothyronine and direct bilirubin ($r = -0.329$, $p = 0.027$).
Conclusion: In conclusion, euthyroid sick syndrome or SH may affect up to 10% of children with chronic liver diseases. It is suggested that thyroid function should be evaluated in cases of pediatric chronic liver disease at diagnosis and during follow-up. Moreover, this study is the first to show a negative correlation between free T3 levels and direct bilirubin, suggesting a possible association between liver disease severity and thyroid function.
Keywords: Pediatric or childhood chronic liver diseases, thyroid function test, euthyroid sick syndrome, subclinical hypothyroidism.

Introduction

Thyroid hormone synthesis occurs in the thyroid gland and is mainly controlled by thyroid stimulating hormone (TSH) secreted by the anterior pituitary gland. The hormone that is mostly synthesized from the thyroid gland is tetraiodothyronine (T4), while the active form at the level of the cell is triiodothyronine (T3) (1). Iodothyronine selenodeiodinase enzyme complex, which regulates thyroid hormone metabolism, consists of three types of enzymes: type 1, type 2 and type 3 deiodinase (2). Deiodineases are responsible for the conversion of T4 to T3 (active form), T4 to inactive reverse T3 (rT3) and the conversion of rT3 and T3 to diiodothyronine (T2) (2). T4, the main product of the

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thyroid gland, must be converted to T3 by type 1 or type 2 deiodinase enzymes in order to affect cell metabolism (1,2).

Studies have shown that chronic liver diseases can be associated with conditions indicating impaired thyroid hormone metabolism, including euthyroid sick syndrome and subclinical hypothyroidism (SH) (2,3,4,5,6). Euthyroid sick syndrome is a condition classically characterized by low serum T3, low or normal free T4 (fT4) and normal or low TSH (7). In a prospective study of 118 patients with cirrhosis, thyroid glandular volume increased by 17% compared to controls and moreover, low total or free T3 (fT3) and high rT3, suggestive of euthyroid sick syndrome, were demonstrated in thyroid hormone profiles (8). It has been suggested that this occurs as a result of a decrease in the activity of type 1 deiodinase and an increase in the activity of type 3 deiodinase (2). In a study of adult cases with acute and chronic liver diseases, the rate of thyroid dysfunction was found to be 16% and 7% of them were reported to have euthyroid sick syndrome (9). Caregaro et al (10) reported that the frequency of euthyroid sick syndrome to be 30.6% in a recent study of cirrhotic adult patients. SH is defined as a serum TSH level above the reference range with normal serum fT4 and fT3 levels (11). In the pediatric population, SH prevalence is reported to be slightly lower than 2%, although epidemiological studies concerning childhood and adolescence are scanty (12). In a study conducted with adult patients with acute and chronic liver diseases, SH was found in 3.5% of patients with thyroid dysfunction (9).

In the literature, there are only a few studies investigating the relationship between liver disease and thyroid dysfunction in the pediatric age group (13,14,15). In a study of children with glycogen storage disease (GSD), it was reported that fT4 levels were significantly lower in patients with GSD type 1a and type 1b and free T3 was reported to be significantly higher in patients with GSD type 1b than in the control group (13). In a study of children with cirrhosis it was reported that decreased levels of thyroid hormones correlated with the severity of disease, so that in more advanced cirrhosis, patients with decreased T4 concentrations were in imminent need of liver transplant although those with normal T4 concentrations, despite having severe cirrhosis, were able to delay liver transplant for longer (14). In another study of children with liver cirrhosis it was reported that fT3 concentrations were lower in patients than in controls (15).

It has been suggested that there is a relationship between hypothyroidism and autoimmune liver diseases, such as chronic active hepatitis and primary biliary cirrhosis (3). Thyroid dysfunction has been reported in 5% to 20% of patients with primary biliary cirrhosis and chronic active hepatitis, a frequency which is higher than reported for general population. There is insufficient data showing the relationship between childhood chronic liver disease and thyroid dysfunction. Therefore, the aim of this study was to identify thyroid dysfunction during the course of childhood chronic liver diseases and to contribute to the literature in order to better inform clinicians managing this group of patients.

Methods

Between January 2005 and June 2018, pediatric and adolescent patients who were diagnosed with chronic liver disease by the pediatric gastroenterology and hepatology and nutrition departments of our hospital were included in this study. Chronic liver involves a process of progressive and irreversible damage in the liver, due to some acquired or congenital conditions, and subsequent regeneration of liver parenchyma leading to fibrosis and cirrhosis. Patients with acute hepatic disease, drug use that might impair thyroid function (such as dopamine or glucocorticoid) and known thyroid disease were excluded. The current study was approved by the Dokuz Eylül University Local Ethics Committee (protocol no: 2017/09-10) and was performed in accordance with the Helsinki Declaration.

Anthropometric data including age, gender, body weight, height, body mass index (BMI) and laboratory data including liver function test results for alanine aminotrasferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, albumin, total protein, clotting studies which may be influenced by liver function (protrombin time, international normalized ratio) and thyroid function tests (TSH, fT4, fT3) were obtained from patient files. The BMI of each case was calculated by dividing weight (in kilograms) by the height (in squared meters). For calculation of percentiles and standard deviation scores (SDSs) of all anthropometrics according to Turkish child growth reference data, child metrics online calculator program (http://www.childmetrics.com) was used (16).

SH is defined as a serum TSH concentration above the reference range with normal serum fT4 and fT3 levels (11). Euthyroid sick syndrome was defined as low fT3, normal/low fT4 and normal/low TSH (17). TSH, fT3, and fT4 were analyzed by the 'ECLIA' chemiluminescence method on a Roche Elecsys E170 (Roche Diagnostics, Indianapolis, IN, USA). TSH, fT4 and fT3 concentrations of the patients included in the study were evaluated by reference to the age range of this kit (18).

Statistical Analysis

Statistical analyses of the data were performed with SPSS, version 24.0 (IBM Corp., Armonk, New York, USA). The distribution of data was evaluated with the Kolmogorov-Smirnov test. For numerical comparisons, the independent sample t-test or Mann-Whitney U tests were used for parametric and non-parametric distribution of the measured parameters, as appropriate. Categorical data were expressed as frequency (%), while numerical data were expressed as median (25-75th percentile) or mean ± standard deviation. In all statistical tests, p values <0.05 were considered significant. Spearman's rho correlation was used to identify the associations between variables.

Results

A total of 107 patients with chronic liver disease were included in the study. Of all cases, 53 (49.5%) were female and 54 (50.5%) were male and the median age was 1.25 (interquartile range: 0.28-10.3) years. The median BMI was 15.9 (14.2-17.4) kg/m^2 and the mean BMI SDS was -0.80 ± 1.79 (Table 1). The distribution of cases with chronic liver disease is shown in Table 2. Twenty-two patients (19.8%) had chronic liver disease due to congenital metabolic diseases, 14 (13.0%) had chronic viral hepatitis and 34 (31.8%) had cholestatic liver disease.

Of the 107 patients, 96 (89.7%) had normal thyroid function test, seven (6.5%) had SH and four (3.7%) had euthyroid sick syndrome. Of the seven patients with SH, one (14.2%) had GSD, one (14.2%) had biliary atresia, one (14.2%) had undiagnosed cholestatic liver disease, one (14.2%) had Alagille syndrome, one (14.2%) had idiopatic hepatitis, one (14.2%) had progressive familial intrahepatic cholestasis and one (14.2%) had congenital hepatic fibrosis. The distribution of patients with euthyroid sick syndrome was as follows: one patient (25%) had congenital hepatic fibrosis, two patients (50%) had undiagnosed cholestatic liver disease, and one patient (25%) had cryptogenic cirrhosis (Table 3). Correlation coefficients between thyroid function tests and liver function tests are summarized in Table 4. There was a negative correlation between fT3 and direct bilirubin ($r=-0.329$, $p=0.027$) and between fT4 and total albumin ($r=-0.273$, $p=0.005$). The weight, height and BMI SDS values of patients with abnormal thyroid function tests were significantly lower than those of patients with normal throid function test ($p<0.01$, $p=0.043$ and $p=0.014$, respectively) (Table 5).

Table 1. Age, sex distribution and anthropometric characteristics of patients

Parameters	Values
Age at diagnosis (year)	1.25 (0.28-10.3)
Gender [female (%) / male (%)]	53 (49.5%) / 54 (50.5%)
Weight (kg)	10.1 (4.9-32.0)
Weight SDS	-0.94 ± 1.44
Height (cm)	76.5 (55.8-140.0)
Height SDS	-0.76 ± 1.41
BMI (kg/m^2)	15.9 (14.2-17.4)
BMI SDS	-0.80 ± 1.79

BMI: body mass index, SDS: standard deviation score.

Data are given as mean ± standard deviation or median (25th-75th percentile)

Discussion

In the present study, thyroid function tests were found to be normal in 89.7% of pediatric/adolescent chronic liver disease patients. However, SH was identified in 6.5% and euthyroid sick syndrome in a further 3.7% of these cases with chronic liver diseases. In a study of adult patients conducted by Kharb et al (9) it was reported that 18.7% (14 of 75 cases) with acute and chronic liver disease had thyroid dysfunction and, additionally, 8% (6 of 75 cases) of these patients were reported to have euthyroid sick syndrome. In one study of patients with cirrhosis, the frequence of euthyroid sick syndrome was reported to be 30.6% (10). Experimental studies have shown that the

Table 2. Classification of patients according to their diagnosis

Diagnosis	Number (n)	Percent (%)
Chronic viral hepatitis*	14	13.0
Autoimmune hepatitis	5	4.7
Wilson disease	11	10.2
Chronic liver disease due to congenital metabolic diseases**	22	19.8
Idiopathic chronic hepatitis	8	7.5
Cirrhosis***	5	4.5
Cholestatic liver disease	34	31.5
Cystic fibrosis	3	2.8
Idiopathic portal hypertension	3	2.8
Hydatid cyst	2	1.9
Total	107	100

Chronic viral hepatitis*: 13 cases with hepatitis B ve one with hepatitis C

Chronic liver disease due to congenital metabolic diseases**: Tyrosinemia, glycogen storage diseases, Zelweger's disease, galactosemia, hemochromatosis

Chirrosis***: Three cases with cryptogenic cirrhosis, one case with congenital hepatic fibrosis and one case with liver fibrosis secondary to chemoteropy

synthesis and release of T4 and T3 are adversely affected by elevated proinflammatory cytokine concentrations (17). In addition, an increase in interleukin 1 beta expression during acute inflammation has been shown to reduce TSH receptor expression (17). The conversion of T4 to T3 was shown to decrease and rT3 concentrations increased, due to decreased deiodinase type 1 activity. Deiodinase type 1 catalyzes both the transformation of serum T4 to active T3 by deiodination of the outer ring and the degradation of T4 to inactive rT3 by deiodination

Table 3. Age, free T3, free T4 and thyroid stimulating hormone levels of patients with thyroid dysfunction*

Patient no	Age	Diagnosis	Thyroid dysfunction	TSH	Free T4	Free T3
9	2 months	Biliary atresia	SH	22.80	1.13	1.67
19	17.0 years	PFIC	SH	4.55	1.10	1.99
35	1 year	Cholestatic liver disease	SH	7.70	1.04	3.95
48	13 years	Congenital hepatic fibrosis	SH	5.03	1.11	3.12
82	1 year	Idiopathic hepatitis	SH	6.40	1.10	3.65
89	1.5 years	Glycogen storage disease	SH	11.87	0.93	3.56
96	2 months	Alagille syndrome	SH	18.80	0.96	3.21
17	10 years	Hepatic fibrosis secondary to chemoteropy	ESS	0.19	0.79	1.78
20	1.5 months	Cholestatic liver disease	ESS	0.17	0.94	1.17
50	8 years	Cryptogenic cirrhosis	ESS	0.23	0.85	1.20
69	1.5 months	Cholestatic liver disease	ESS	0.09	1.13	0.90

SH: subclinical hypothyroidism, ESS: euthyroid sick syndrome, PFIC: progressive familial intrahepatic cholestasis, TSH: thyroid stimulating hormone.

*Normal TSH levels by age group: 0.72-11 µIU/mL from 6 days to 3 months; 0.73-8.35 µIU/mL for 4-12 months; 0.70-5.93 µIU/mL for 1-6 years; 0.60-4.84 µIU/mL for 7-11 years; 0.51-4.30 µIU/mL for 12-20 years.

Normal free T4 levels by age group: 0.89-2.20 ng/dL from 6 days to 3 months; 0.92-1.99 ng/dL for 4-12 months; 0.96-1.77 ng/dL for 1-6 years; 0.97-1.67 ng/dL for 7-11 years; 0.98-1.63 ng/dL for 12-20 years.

Normal free T3 levels by age group: 1.95-6.04 pg/mL from 6 days to 3 months; 2.15-5.83 pg/mL for 4-12 months; 2.41-5.50 pg/mL for 1-6 years; 2.53-5.22 pg/mL for 7-11 years; 2.56-5.01 pg/mL for 12-20 years.

Table 4. Correlation coefficients between thyroid function tests and liver function tests

		TSH	Free T3	Free T4
AST	r*	-0.093	0.012	-0.053
	p	0.340	0.936	0.586
ALT	r*	-0.081	0.083	-0.078
	p	0.406	0.590	0.423
GGT	r*	0.115	0.088	0.016
	p	0.239	0.565	0.869
ALP	r*	-0.023	-0.46	0.105
	p	0.811	0.765	0.280
Total bilirubin	r*	-0.017	-0.159	0.040
	p	0.858	0.297	0.684
Direct bilirubin	r*	-0.102	-0.329	0.051
	p	0.298	**0.027**	0.601
Indirect bilirubin	r*	0.070	0.043	0.012
	p	0.476	0.779	0.902
Total protein	r*	-0.098	0.188	-0.273
	p	0.319	0.221	**0.005**

ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma glutamyl transferase, ALP: alkaline phosphatase, TSH: thyroid stimulating hormone.

*Spearman's correlation analysis; Serum free T3, free T4, and TSH as the dependent variable

Table 5. Comparison of body weight height and body mass index standard deviation score of patients with normal and impaired thyroid function tests

	Normal thyroid function test (n = 96)	Impaired thyroid function test (n = 11)	p
Age (years)	1.50 (0.3-11)	1.2 (0.1-10)	0.640[a]
Weight SDS	-0.77 ± 1.36	-2.36 ± 1.44	**< 0.001[b]**
Height SDS	-0.67 ± 1.40	-1.56 ± 1.38	**0.043[b]**
BMI SDS	-0.44 [(-1.72)-(0.23)	-2.70 [(-4.90)-(-0.60)]	**0.014[a]**

SDS: standard deviation score, BMI: body mass index.

[a]Mann-Whitney U test, [b]Student's t-test. Data are given as mean ± standard deviation or median (25^{th}-75^{th} percentile)

of the outer ring. Type 1 deiodinase is the main enzyme involved in the production of active T3 and as such, reduction in this enzyme activity would contribute to the clinical picture regarding thyroid dysfunction (17). As a result, during acute or chronic inflammation, some cytokines may reduce TSH expression, potentially leading to SH in order to achieve sufficient levels of active thyroid hormone for normal metabolism, and reduce deiodinase type 1 enzyme activity, leading to the biochemical picture of euthyroid sick syndrome (low/normal fT4 and fT3 and elevated rT3).

Clinical management of thyroid dysfunction should be decided according to the presence of possible clinical features of hypothyroidism, the degree of TSH elevation, and changes over time in TSH and free T4 concentrations. In the current study, SH was detected in seven patients. Melis et al (13) investigated pediatric patients with GSD and reported that fT4 concentrations were significantly lower in GSD type 1a and type 1b patients and, moreover, fT3 levels were significantly higher in patients with GSD type 1b than a control group. In the same study, although four of seven patients with GSD type 1b had some degree of hypothyroidism (clinical or subclinical), this was not reported in the patients with GSD type 1a. The present study included 15 cases of GSD and one of them (6.6%) with GSD type 1a, had SH. The prevalence of clinical and SH has been reported to be higher in adult GSD type 1b cases compared to other types (19). It is noteworthy that the rate of detecting thyroid dysfunction in cases with GSD was higher than the other patient groups, which is in accordance with the literature. However, due to the small number of GSD cases in our study, further studies including large case series are needed to confirm if this putative association between is pediatric GSD and thyroid dysfunction is genuine.

Congenital liver disease and hypothyroidism may be manifestations of an underlying genetic defect. For example, *JAG1* gene defect causes Alagille syndrome type 1 and was also tought to be associated with hypothyroidism. In a study that was conducted in 21 Alagille and 100 congenital hypothyroidism patients by de Filippis et al (20), some variants in the *JAG1* gene were found in cases with congenital thyroid defects and unexplained mild hypothyroidism was present in a 28.6% of the Alagille syndrome patients (20). In our study, one case with Alagille syndrome had SH. However, this study was designed as a cross-sectional study and long-term thyroid function test monitoring was not performed. The findings of the de Fillipis et al (20) study suggest that monitoring of thyroid function in patients with Alagille syndrome is warranted.

One (14.2%) of seven patients with SH had biliary atresia. The relationship between biliary atresia and thyroid abnormalities is not yet clear. In a study investigating the genetic etiology of 35 cases with biliary atresia by using single nucleotide polymorphism (SNP) genotyping arrays, heterozygous 2q37.3 deletions was detected in two of these patients, one of whom had congenital hypothyroidism (21). Further studies have shown that 2q37 region contains 18 genes and two of these genes *(ATG4B, ING5)* are highly expressed in thyroid gland except T/B cells (21). Further studies regarding these genetic regions may help us in the elucidation of thyroid hormone abnormalities in patients with biliary atresia.

It was reported that there is a relationship between primary sclerosing cholangitis and autoimmune thyroiditis (22). However, there were no cases of primary sclerosing cholangitis among the patients with cholestasis with SH in our study. Additionally, in the present study, the correlation analysis between thyroid function tests and liver function tests revealed a negative correlation between fT3 and direct bilirubin ($r = -0.329$, $p = 0.027$). It has been reported that serum fT3 concentrations are associated with the severity of liver function, based on liver function test results. However, there was no data on the relationship between direct bilirubin and fT3 (23). Furthermore, in the present study, weight SDS, height SDS and BMI SDS values of patients with impaired thyroid function tests were found to be significantly lower

than those with normal thyroid function test, despite all having chronic liver disease. This could be due to nutritional deterioration, which may be more pronounced in children with thyroid hormone dysfunction, which, in turn, may be related to disease severity or duration. There are a lack of studies regarding this issue and further studies are needed to better define this relationship and also the association between thyroid dysfunction and cholestatic liver diseases.

Study Limitations

There are some major limitations of our study. These are: i) low number of cases; ii) evaluation based on one thyroid function test measurement and lack of long-term follow-up of thyroid function tests; and iii) ignoring the impact of other drugs on thyroid functions. Moreover, in this retrospective study, since no further investigations such as thyroid antibody testing or thyroid imaging, was performed in patients with thyroid function test abnormalities, no definitive diagnoses could be made in this respect.

Conclusion

In conclusion, euthyroid sick syndrome or SH may be present in around 10% of children and adolescents with chronic liver diseases. Therefore, thyroid function testing is suggested in these cases at diagnosis and during follow-up. In addition, different types of thyroid dysfunction, according to the types of liver disease, may be seen. Further studies with large case series are needed to better understand the effect of chronic liver disease on thyroid function.

Ethics

Ethics Committee Approval: The study were approved by the Dokuz Eylül University of Local Ethics Committee (protocol no: 2017/09-10, date: 27.04.2017).

Informed Consent: Study was designed retrospectively, informed consent doesn't need.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş. Şebnem Ön, Korcan Demir, Ayhan Abacı, Concept: Ece Böber, Design: Ece Böber, Data Collection or Processing: Ş. Şebnem Ön, Sinem Kahveci Çelik, Yeşim Öztürk, Analysis or Interpretation: Ş. Şebnem Ön, Ece Böber, Literature Search: Sezer Acar, Ş. Şebnem Ön, Writing: Korcan Demir, Ayhan Abacı.

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Nationwide Turkish Cohort Study of Hypophosphatemic Rickets

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What is already known on this topic?

Hypophosphatemic rickets (HR) is a rare renal phosphate wasting disorder commonly with X-linked inheritance. There is no nationwide data on HR with initial and follow-up findings.

What this study adds?

The age of diagnosis was similar in good and bad responders to conventional therapy. Good responders had better height standard deviation score on admission. Higher treatment doses led to nephrocalcinosis without any change in serum levels of phosphorus. Awareness of the importance of early diagnosis and treatment complications should be improved.

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Abstract

Objective: Hypophosphatemic rickets (HR) is a rare renal phosphate-wasting disorder, which is usually X-linked and is commonly caused by PHEX mutations. The treatment and follow-up of HR is challenging due to imperfect treatment options.
Methods: Here we present nationwide initial and follow-up data on HR.
Results: From 24 centers, 166 patients were included in the study. Genetic analysis (n = 75) showed *PHEX* mutation in 80 % of patients. The mean follow-up period was 6.7 ± 2.4 years. During the first 3-years of treatment (n = 91), mild increase in phosphate, decrease in alkaline phosphatase and elevation in parathyroid hormone (PTH) levels were detected. The height standard deviation scores were -2.38, -2.77, -2.72, -2.47 at initial, 1st, 2nd and 3rd year of treatment, respectively ($p > 0.05$). On follow-up 36 % of the patients showed complete or significant improvement in leg deformities and these patients had similar phosphate levels at presentation with better levels in 1st and 2nd years of treatment; even the treatment doses of phosphate were similar. Furthermore, 27 patients developed nephrocalcinosis (NC), the patients showed no difference in biochemical differences at presentation and follow-up, but 3rd year PTH was higher. However, higher treatment doses of phosphate and calcitriol were found in the NC group.
Conclusion: HR treatment and follow-up is challenging and our results showed higher treatment doses were associated with NC without any change in serum phosphate levels, suggesting that giving higher doses led to increased phosphaturia, probably through stimulation of fibroblast growth factor 23. However, higher calcitriol doses could improve bone deformities. Safer and more efficacious therapies are needed.
Keywords: Hypophosphatemic rickets, *PHEX*, treatment

Introduction

Hypophosphatemic rickets (HR) is a rare renal phosphate-wasting disorder caused by several genetic mutations in factors leading to increase in fibroblast growth factor 23 (FGF23) signalling or secretion, and in renal phosphate transporters (1). The most common inherited form of HR is X-linked HR (XLH; OMIM: #307800), where inactivating mutations of the Phosphate Regulating Endopeptidase Homolog, X-Linked (*PHEX*, OMIM: #300550) gene lead to local and systemic effects (2). The incidence of XLH is 1/20 000 live births, and it accounts for approximately 80 % of familial cases (3).

PHEX is predominantly expressed in osteoblasts and down-regulation of PHEX increases serum levels of the phosphatonin, FGF23. FGF23 has a central role in HR pathophysiology. Elevated levels of serum FGF23 increase urinary phosphate excretion by downregulating renal sodium-phosphate transporters, and decrease intestinal phosphate absorption by restricting active vitamin D synthesis (2). The other rare genetic disorders of excess FGF23 are autosomal dominant HR (caused by missense mutation in *FGF23*), autosomal recessive HR (type 1 caused by mutations in the gene encoding dentin matrix protein *(DMP1)*, type 2 caused by mutations in ectonucleotide pyrophosphate/phosphodiesterase 1 *(ENPP1)* and type 3 caused by mutation in the family with sequence similarity 20, member C *(FAM20C)* gene (4).

Clinical presentation of HR includes rickets, osteomalacia, short stature, leg deformities, dental abscesses, premature cranial synostosis, and muscle weakness in children, and also pseudofractures, osteoarthritis, and entesopathy in adults. The phenotype can vary widely, even in the same family and diagnosis can be delayed (5). In addition to the rarity and diagnostic difficulties, which has a significant impact on patient outcomes, treatment and follow-up of HR is very challenging.

Conventional treatment of HR includes a combination of oral phosphorus and active vitamin D. Unfortunately this therapy was unsuccessful in a significant proportion of patients in respect to healing of rickets and improvement in deformities, and can be associated with treatment related side effects (4,6).

Information about the clinical, laboratory, genetic and follow-up characteristics of HR patients is very scarce for our population and only a few small series have been reported (7,8).

The aim was to present nationwide data on HR with initial and follow-up data on the patients presenting to pediatric endocrinology clinics before the age of 18 years.

Methods

In this study, the data of 166 children and adolescents with HR who were being followed in 24 centers in Turkey were cross-sectionally analyzed . A nation-wide web-based CEDD-NET Data System (http://cedd.saglik-network.org/) was used for data collection between December 2016 and April 2018. A proforma, including clinical, genetic, laboratory and follow-up information about the patients was uploaded to the website and filled by the patient's doctors. Study approval was given by the Ankara University Ethics Committee (approval number: 06-229-16).

Patients aged between 0 to18 years were included in the study and patients with calciopenic rickets (related to

vitamin D deficiency or hypocalcemia, vitamin D dependent rickets, and the like) were excluded.

The following data on the patients' admission, clinical and laboratory characteristics were collected: birth weight, age at diagnosis, age of first symptoms, positive family history, the time of starting to walk, height, weight, height standard deviation (SD) score (SDS), limb deformities (genu varum, genu valgum, etc.) with intercondylar and intermalleolar distance, other skeletal deformities (cranial, thoracal) and craniosynostosis, dental abscess, serum calcium (Ca), phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25-hydroxyvitamin D (25-OHD3) levels, tubular phosphate reabsorption (TPR), urinary Ca/creatinine ratio, and radiological findings. The researchers were also asked to enter other clinical features that were not included on the questionnaire form to the system. ALP SD of patients were calculated according to reference data (9).

The questionnaire form also included the genetic test results of the patient. Information concerning genetic analysis of PHEX, and other genes causing HR were requested.

If there was a specific diagnosis causing hypophosphatemia, such as tubulopathy or McCune Albright syndrome, clinicians were asked to specify this.

The participating centers were also asked to enter onto the system if there were any other known pathological laboratory or clinical findings.

The researchers were also asked to enter onto the system the treatment doses of phosphate and calcitiriol, and any other treatments. The yearly heights and improvement of deformities of patients who were given conventional treatment were recorded. The compliance with treatment were evaluated by asking if there were any missed planned visits and/or failure to give recommended dose of therapy by parents.

Follow-up patients were grouped as good responder (complete or significant improvement of deformities either clinically and radiologically) or bad responder (no improvement or worsening of deformities).

The follow-up form also included complications [nephrocalcinosis (NC), hyperparathyroidism, hypertension, dental abscess, cranial synostosis, entesopathy etc.] which developed during the follow-up. Entering additional information not included in the questionnaire form was optional.

Statistical Analysis

Statistical analyses were performed by using SPSS for Windows, version 22.0, statistical software (IBM Inc., Chicago, Ill., USA). Frequencies and percentages represented the descriptive statistics for categorical variables, and mean ± SD values, median (minimum-maximum), when required, were used for continuous variables. Student's t-test, chi-square, Fisher exact tests, repeated measures of ANOVA for normally distributed continuous variables and Friedman ANOVA as nonparametric test were used. *Post hoc* multiple comparison test was also used.

Statistically significance was regarded as $p < 0.05$.

Results

From 24 centers, data on 166 patients of whom 98 (59%) were females, 68 males and 18 (10.8%) pubertal and 148 prepubertal, were included in the study. The mean age of diagnosis and was 5.1 ± 3.7 years. The mean age at beginning of symptoms and start of walking were 1.89 ± 1.96 years and 15.5 ± 3.82 months respectively (Table 1). Almost half of patients (n = 80, 48.2%) had a history of at least one affected family member.

Clinical and Laboratory Characteristics on Admission

The mean height SDS was -2.43 ± 1.35. The most frequent reported clinical findings were lower limb deformities including genu varum 80.1% (n = 133), genu valgum 7.8% (n = 13). Bone pain 16.8% (n = 28), widening of wrist 31% (n = 51), rachitic rosary/thoracal abnomalities 8.4% (n = 14) and frontal bossing 7.2% (n = 12) were also reported. Late walking, lordosis, and congenital hip dysplasia were among the reported findings. Seven (4.2%) asymptomatic patients were detected due to a positive family history. No craniosynostosis was reported in our patient cohort.

Table 1. Clinical and laboratory characteristics of all cases on admission

Parameter	Mean ± SD	Median (min.-max.)
Age of presentation	5.1 ± 3.7 years	3.6 (0.12-16)
Age of first symptoms (years)	1.89 ± 1.96	1.8 (0.5-9)
Height SDS	-2.43 ± 1.35	-2.6 (-5.52-1.18)
BMI	17.7 ± 2.62	17.6 (11.3-28)
Calcium (mg/dL)	9.5 ± 0.46	9.6 (8.2-10.9)
Phosphate (mg/dL)	2.51 ± 0.49	2.5 (1.1-5)
ALP (U/L)	738 ± 481	607 (143-3200)
ALP SD	1.08 ± 2.73	0.48 (-3.9-12.5)
Tubular phosphate reabsorption (%)	73.2 ± 16.2	79 (16-98)
PTH (pg/mL)	66.8 ± 45.37	57.1 (2.3-254)

SDS: standard deviation score, PTH: parathyroid hormone, BMI: body mass index, ALP: alkaline phosphatase, min.: minimum, max.: maximum

Laboratory features were consistent with HR with hypophosphatemia, normocalcemia, high ALP, normal/normal- high PTH, and low TPR (Table 1). Mean 25-OH Vitamin D levels were 35.97 ± 15.61 ng/mL (n = 139). Low 25-OH Vitamin D levels (< 20 ng/dL) were detected in 27 patients. High dose vitamin D ingestion was also reported in 16 cases due to misdiagnosis of nutritional rickets before the admission to a pediatric endocrinology clinic.

Etiology of HR

Seven patients in our cohort had specific diagnosis from additional clinical and laboratory findings including cystinosis in three, tyrosinemia in two, Dent diseases with *CLCN5* mutation in one and McCune Albright syndrome in one patient.

Genetic analysis had been performed in 75 of 159 (47.2 %) patients, and 65 of them showed a genetic mutation: *PHEX* mutation in 60 (80 %); *DMP1* mutation in three (4 %); *SLC34A3* mutation in two (2.6 %) and no mutation was detected in 10 patients who were screened for *PHEX* gene by sequencing (Figure 1).

Treatment and Follow-up

Almost all patients were treated with oral phosphate and vitamin D (calcitriol) supplements. Patients with *SLC34A3* mutation were treated solely with phosphate replacement. The mean follow-up period of the patients was 6.67 ± 2.3 years. The first three years treatment response was evaluated for 91 patients, who had all completed at least three full years follow-up. Although an increasing trend in serum phosphate and PTH levels were seen, no statistical significant differences from initial to 1st, 2nd and 3rd year of follow-up was detected (p = 0.563 and p = 0.796 for serum phosphate and PTH, respectively). A decrease in ALP and ALP SD was evident (Table 2). The height SDSs were -2.38, -2.77, -2.72, -2.47 at initial, 1st, 2nd and 3rd year of treatment, respectively (p = 0.570).

On follow-up, 36 % of 159 HR patients without specific etiology (such as cystinosis) showed complete or significant improvement in leg deformities. Improvement in leg deformities was evaluated clinically and radiologically by each center. The patients with leg deformity improvements were compared with those who did not and both had similar ages at the time of diagnosis (4.39 vs 5.3 years, p = 0.12). However, good responders had better height SDS (-2.07 vs -2.61, p = 0.039), worse TPR (70 % vs 77 %, p = 0.046), and worse ALP SDS at presentation (p = 0.03). When the following years were evaluated, both groups had similar phosphate levels at presentation with better levels in the 1st and 2nd years of treatment, and even the treatment doses of phosphate were similar. However, significantly higher calcitriol treatment doses in the 1st and 3rd years were found in the improved group (Table 3, Table 4).

Complications During Follow-up

When the complications of treatment were evaluated, 27 out of 159 patients (17 %) developed NC on follow-up. The patients who developed NC showed no difference in biochemical characteristics at presentation and follow-up, however, their PTH levels at the 3rd year were higher (145 vs. 78 pg/mL, p = 0.002), and they had higher treatment dose of both phosphate and calcitriol (p < 0.05) (Table 5, Table 6).

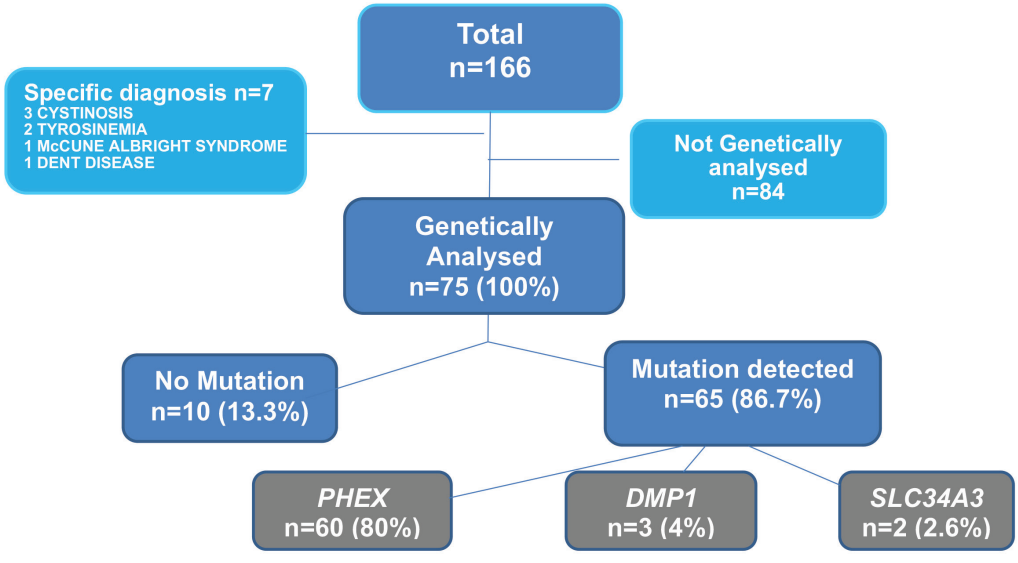

Figure 1. The results of genetic analysis of patients

Table 2. Laboratory evaluation of 91 cases during 3 years of follow-up

n = 91	On admission	1. year	2. year	3. year	p value
Calcium (mg/dL)	9.48 ± 0.51 9.5 (8.2-10.9)	9.45 ± 0.51 9.4 (7.4-10.3)	9.56 ± 0.49 9.6 (8.4-10.7)	9.58 ± 0.46 9.6 (8-10.9)	0.062
Phosphate (mg/dL)	2.58 ± 0.55 2.5 (1.1-5)	2.76 ± 0.62 2.7 (1.53-5.54)	2.83 ± 0.71 2.7 (1.6-5.4)	2.85 ± 0.73 2.83 (1.69-4.7)	0.563
ALP (U/L)	786 ± 522 624 (45-3200)	627 ± 449 510 (173-2957)	561 ± 319 450 (169-1650)	546 ± 327 458 (142-25349)	< 0.001
ALP SD	1.08 ± 2.73 0.80 (-3.9-12.5)	0.33 ± 2.47 -0.04 (-4.1-8.8)	0.12 ± 2 -0.4 (-3.3-5.1)	0.03 ± 2.09 -0.19 (-3.9-5.99)	< 0.001
PTH (pg/mL)	68.4 ± 48 60 (5-254)	85.9 ± 77 75.6 (4.6-537)	80.68 ± 65.9 66.5 (3.4-419)	93.15 ± 99 64.4 (5.2-574)	0.796

PTH: parathyroid hormone, ALP: alkaline phosphatase, SD: standard deviation

Table 3. Laboratory characteristics of good responder and bad responder groups for 3 years follow-up

	Phosphate level (mg/dL)			Calcium level (mg/dL)		
	Good responder	Bad responder	p values	Good responder	Bad responder	p values
At diagnosis	**2.53 ± 0.52**	**2.6 ± 0.58**	0.24	**9.52 ± 0.56**	**9.46 ± 0.47**	**0.58**
1. year	**2.95 ± 0.79**	**2.63 ± 0.46**	0.01	**9.4 ± 0.55**	**9.48 ± 0.48**	**0.52**
2. year	**3.02 ± 0.85**	**2.72 ± 0.58**	**0.044**	**9.54 ± 0.51**	**9.57 ± 0.47**	**0.78**
3. year	**2.94 ± 0.77**	**2.73 ± 0.61**	0.08	**9.63 ± 0.51**	**9.55 ± 0.43**	**0.45**

Table 3. Continued

	ALP SDS			PTH level		
	Good responder	Bad responder	p values	Good responder	Bad responder	p values
At diagnosis	**2.1 ± 3.81**	**0.95 ± 2.02**	**0.01**	**68.35 ± 41.5**	68.6 ± 44	0.47
1. year	**1.1 ± 3.06**	**-0.16 ± 1.86**	**0.009**	**91 ± 101**	82.35 ± 58.5	0.38
2. year	**0.80 ± 2.3**	**-0.3 ± 1.67**	**0.014**	**73.3 ± 50.2**	84.79 ± 73.3	0.18
3. year	**0.35 ± 2.46**	**-0.14 ± 1.85**	**0.28**	**109.6 ± 139**	83.66 ± 65.8	0.12

ALP: alkaline phosphatase, SDS: standard deviation score, PTH: parathyroid hormone

Additionally, osteotomy was required in 15 subjects (9.4%), while dental abscess occurred in 14 (8.4%) subjects, parathyroid hyperplasia developed in four subjects (2%), hypertension in one subject (< 1%), and depression in one subject (< 1%) among 159 cases.

Growth hormone (GH) therapy was given to 10 patients. Although duration and dose of treatment were variable, the patients treated with GH in addition to conventional therapy had similar height SDS before and after GH treatment (-3.47 and -3.3, respectively, with delta height SDS of 0.173).

Discussion

Characteristics at Presentation

This is the first description of a large national series of HR patient with clinical, laboratory, follow-up and etiological characteristics from Turkey. There was a delay of more than three years between the mean age of diagnosis (5.1 years) and the mean age of onset of symptoms (1.89 years). In a Norwegian series, the age of diagnosis was 2.1 years (10), also three years earlier than diagnosis in our cohort. Early diagnosis is very important for treatment response and healing especially. If treatment is started < 1 year of age, it has been reported that height SDS outcome is improved (10,11).

Among the clinical findings, short stature and lower limb deformities were the most striking features. In our cohort the mean height SDS was -2.41 at presentation. Disproportionate short stature is a definitive features of HR and is primarily related to reduced growth of long bones and the limb deformities (2,11). Wide variability of height among individuals with HR has been reported and adults with XLH have a significantly reduced final height of up to 20 cm (-1.9 height SDS) (12). Almost half of our cases (80/166) had at

Table 4. Doses of treatment and growth characteristics of good responder and bad responder groups for 3 years follow-up

	Phosphate dose (mg/kg)			Calcitriol dose (ng/kg)			Height SDS		
	Good responder	Bad responder	p values	Good responder	Bad responder	p values	Good responder	Bad responder	p values
At diagnosis	61.6 ± 42.7	60.8 ± 38.4	0.765	34.33 ± 24.6	26.9 ± 12.2	0.129	-2.07 ± 1.01	-2.61 ± 1.4	0.039
1. year	66.25 ± 38.14	62.1 ± 42.2	0.268	33.43 ± 19.1	24.9 ± 13.22	0.031	-2.07 ± 0.87	-2.67 ± 1.37	0.021
2. year	58.72 ± 28.11	56.5 ± 33.7	0.298	29.21 ± 14	26.3 ± 11.62	0.366	-2.07 ± 0.86	-2.71 ± 1.25	0.012
3. year	66.34 ± 31.8	54.69 ± 32.3	0.079	26.9 ± 10.61	22.06 ± 11.3	0.04	-1.94 ± 0.89	-2.8 ± 1.28	0.002

SDS: standard deviation score

Table 5. Laboratory characteristics of patients according to development of nephrocalcinosis

	Phosphate level (mg/dL)			Calcium level (mg/dL)		
	NC (+)	NC (-)	p	NC (+)	NC (-)	p
At diagnosis	**2.54 ± 0.76**	**2.59 ± 0.52**	0.209	**9.44 ± 0.49**	**9.5 ± 0.51**	0.25
1. year	**2.95 ± 0.82**	**2.71 ± 0.56**	0.114	**9.32 ± 0.65**	**9.48 ± 0.46**	0.11
2. year	**2.99 ± 0.97**	**2.81 ± 0.64**	0.24	**9.54 ± 9.46**	**9.56 ± 0.5**	0.42
3. year	**2.92 ± 0.85**	**2.82 ± 0.64**	0.36	**9.5 ± 0.41**	**9.6 ± 0.47**	0.21

Table 5. Continued

	ALP SDS			PTH level		
	NC (+)	NC (-)	p	NC (+)	NC (-)	p
At diagnosis	**1.88 ± 2.6**	**1.28 ± 2.9**	0.29	**50.5 ± 24.8**	**72.7 ± 50.8**	0.06
1. year	**0.44 ± 1.74**	**0.31 ± 2.6**	0.4	**114.7 ± 154**	**79.57 ± 48**	0.069
2. year	**0.13 ± 1.7**	**0.11 ± 2.08**	0.44	**85.6 ± 102**	**79.6 ± 56**	0.36
3. year	**0.31 ± 1.94**	**-0.02 ± 2.1**	0.37	**154.5 ± 171**	**78.28 ± 66**	0.002

NC: nephrocalcinosis, ALP: alkaline phosphatase, SDS: standard deviation score, PTH: parathyroid hormone

Table 6. Treatment characteristics of patients according to development of nephrocalcinosis

	Phosphate dose (mg/kg)			Calcitriol dose (ng/kg)		
	NC (+)	NC (-)	p values	NC (+)	NC (-)	p values
Begining of treatment	89.9 ± 46.4	55.95 ± 32.4	0.003	62.37 ± 26.7	27.92 ± 11.5	0.006
1. year	74.31 ± 37.1	60.61 ± 42.7	0.13	34.9 ± 12.4	26.4 ± 10.5	0.04
2. year	71.93 ± 38.3	54.69 ± 28.8	0.033	26.43 ± 11.8	27.17 ± 12.6	0.48
3. year	69.4 ± 29.7	56.76 ± 33.3	0.096	18.66 ± 10.4	25.16 ± 11.8	0.035

NC: nephrocalcinosis

least one individual in family with a pre-existing diagnosis of HR diagnosis but despite this positive family history, diagnosis was comparatively late in our series.

At diagnosis, one patient was diagnosed as McCune Albright syndrome, two patients with *PHEX* mutation, and two asymptomatic patients were screened because of affected siblings have normal TPR. In addition three patients had normal ALP levels, despite hypophosphatemia and low TPR on admission. Patients characteristics, mistake in sampling, or a variety of methodological problems may have led to the normal laboratory results in these patients. During follow-up all of these patients had a revised diagnosis of HR made.

Before diagnosis most of the cases were given therapeutic doses of vitamin D, on the assumption that they had calciopenic rickets. So the percentage of vitamin D deficiency (below 20 ng/dL) was correspondingly low at 19.4% (27/139) in our series at the time of diagnosis. Serum PTH levels were very helpful for diagnosis of HR. While calciopenic rickets is associated with increased PTH concentrations, our patients with HR had normal or upper normal levels of PTH.

Etiology of HR

Seven cases in this HR cohort had a specific diagnosis of tubulopathy (cystinosis, Dent disease, tyrosinemia) or

McCune Albright syndrome. Genetic analysis was performed in 75 of 159 cases. Results showed that the most frequent reason etiology of HR was XLH and 80% of patient had a *PHEX* mutation. Interestingly, this frequency was similar to other reported series in the literature in which disease-causing genetic variants were identified (3,7,8,13). We were expecting a higher frequency of autosomal recessive forms of HR in our population due to high consanguineous marriage rates. Thus our findings show that *PHEX* mutation is the most prominent form of HR regardless of the population and consanguinity rates. PHEX protein is a member of the neutral endopeptidase family of zinc metalloproteinases and predominantly expressed in osteocytes and osteoblasts (3,5). It ameliorates the inhibitory effect of matrix extracellular phosphoglycoprotein (MEPE) proteins on bone mineralization. PHEX forms a trimeric complex with DMP1 and $\alpha 5\beta 3$-integrin. The resulting complex restricts FGF23 expression. While MEPE derived "acidic serine and aspartate rich motif" (ASARM) peptides inhibit the trimeric complex. So inactivating *PHEX* mutations lead to an increase both in ASARM peptides, and serum FGF23 levels (3,14). All of these caused FGF23 related phosphaturia, hypophosphatemia, short stature, and bone deformities as in our cases.

Loss of function mutation in *PHEX* causes X-linked dominant HR. Males to female ratio has previously been reported as equal (5) although in our series it was 21/32 (0.65), male to female.

In addition we found autosomal recessive HR type 1 which was detected in three cases from one family, all of whom had a *DMP1* mutation. Clinical and laboratory finding of these cases have been reported previously (15) and were similar to those seen in patients with XLH. *DMP1* mutation results in increased FGF23 production and clinical, laboratory and radiological findings could be expected to resembling those in XLH (3).

Two cases had *SLC34A3* mutations that lead to hereditary HR with hypercalciuria. This disease is characterized by hypophosphatemia and rickets, due to renal phosphorus wasting (3,8). As serum 1.25(OH)2D is high and FGF23 and PTH are reduced in such cases, secondary hypercalcemia and medullary calcinosis together with urolithiasis may be expected (3,8).

In 10 cases in which *PHEX* mutation were negative, *FGF23* had also been analyzed in six although no variants were found. Other mutations leading HR and deletions and duplication which cannot be detected by Sanger sequencing should be considered in cases with negative *PHEX* mutations.

Treatment Results

The conventional treatment for HR includes active vitamin D analogues and phosphate supplementations. The recommended doses of calcitirol is 0.5-1.5 mcg/day or 20-30 ng/kg/day, and for phosphorus this is 20-60 mg/kg/day (16,17). In our series the doses of calcitriol and phosphorus were appropriate for recommendations but compliance of patients with treatment was a problem. This is almost always poor, due to the frequent daily dosing of drugs (4-6 times for phosphate and 1-2 times for calcitriol) and the bitter taste of the phosphate solutions. In addition the optimal dose of treatment can vary from patient to patient and higher doses will be needed during rapid growth phases and at initiation of treatment (15).

After the 3rd year of conventional treatment, patients showed no improvement in height SDS. This was dissapointing but may be partly related to the late diagnosis of our patients. It is known that 25-40% of the patients with HR who are closely followed-up and compliant with treatment still have growth retardation (4). Almost 2/3 of our HR cases did not show good response to conventional treatment. Although earlier diagnosis and immediate treatment can be important prognostic factor for height improvement, we could not find any statistical differences between the good responder and bad responder groups when we stratified for age of diagnosis. The most striking feature was better height SDS in good responders compared with bad responders at presentation. This finding may indicate that bad responder were more severely affected than good responder in respect to bone pathology. Additionally, similar serum Ca and PTH levels were found in the good responder and bad responder groups. Good responders had higher serum phosphate levels despite having similar doses of phosphate replacement and higher calcitriol doses were given during follow-up,

This study has shown that conventional treatment could not lead to an additional growth promoting effect in all HR patients, but this treatment seems to stop further deterioration of growth. The effects of conventional therapy on growth have been reported previously, usually in a small case series. In a study, 13 cases showed height increment from -1.58 to -1.25 after two years of conventional therapy. In untreated historical controls (n = 16) height SDS was reported as -2.02 ± 1.30 (18). In another study, 36 cases treated with vitamin D and phosphate replacement showed improvement in linear growth, as height SDS increased from -2.89 to -1.98 (19).

In general, bone deformities and abnormal growth of skeleton is not adequately treatable despite early and optimal doses of phosphorus and calcitriol and the lack of

success with therapy for HR has led to the search for other treatment options. GH treatment is one of the therapies that has been tried in HR patients. In our series, recombinant GH (rGH) therapy was given to 10 patients. However, these patients showed similar height increment to patients receiving only conventional treatment. rGH treatment in HR has usually been reported in limited pilot studies, although these have suggested a beneficial effect. While one randomized study showed significant improvement in height SDS in eight severely short XLH children treated with rGH for three years, follow-up showed that this treatment did not significantly increase adult height (20,21).

During follow-up, corrective osteotomy was required in 15 (9.4%) among the 159 subjects.

Development of progressive bone deformities in HR could lead to progressive gait disturbance, functional impairment, and severe arthritis. In such conditions surgical treatment may help improve patients quality of life (22). Each patient should be evaluated carefully for requirement for corrective surgery.

Complications During Follow-up

The compliance of treatment is a difficult issue in HR, since phosphate needs 4-6 daily doses and calcitriol needs 1-2 daily doses. During conventional treatment of HR, several complications may occur including NC, dental abscesses, entesopathy, and craniosynostosis. Among these, NC is more frequently reported (1,2,3,4,17).

In this national cohort 17% of all hereditary hypophosphatemia patients had NC, which is a relatively low rate compared to other series, with NC being reported in 22% to 100% of XLH patients who are under conventional therapy (1,2,3,4). However, these were small series and diagnosis was earlier than in the present study. We speculate that earlier therapy may lead to an increased frequency of NC.

NC usually develops after conventional treatment of HR, and is accepted as a treatment complication. It is known that higher doses of phosphate replacement caused both to NC and hyperparathyroidism. Higher doses of phosphate replacement may increase FGF23, and phosphaturia will be increased (13). Similarly, our cases with NC were treated with higher doses of phosphate than the cases without NC. In addition to higher dose of phosphate replacement, calcitriol may lead to hypercalcemia which may be additive in the likelihood of NC occurrence. In our cases higher doses of calcitriol during initial therapy seems to have had an additional effect on NC development. The reason for high dose therapy were not recorded in all cases. However, some physicians might have belived that response to therapy was insufficient and were attempting to maintain the serum phosphate levels within the reference values. In fact, the primary aim in conventional HR therapy should be to keep serum Ca, ALP, PTH, and urinary Ca excretion in normal limits rather thn the phosphorus concentration. It is salutory that although higher doses of phosphate and calcitriol were given, possibly predisposing some patients to NC, this NC group did not have better growth outcomes than the lower dose patients who did not develop NC.

While dental abscess was present in 14 cases, other complications were infrequently reported, with parathyroid hyperplasia in four (2%), hypertension in one (<1%), and no neurological complications including craniosynostosis. Awareness of the complications of HR should be improved among medical professionals.

The aim of conventional treatment is to compensate for renal phosphate wasting and to counter 1,25OHD deficiency (4), which is commonly related to excess levels of FGF23 in HR. Recently, some strategies that manipulate FGF23 signaling have been described. Burosumab, a monoclonal antibody directed at FGF23, is one (23). In children with HR, treatment with burosumab has been reported to improve renal tubular phosphate reabsorbsion, serum phosphate levels, linear growth, and severity of rickets (24) but burosumab is expensive and long-term outcomes are not yet available.

Conventional therapy is still the first line therapy for HR. When patients are correctly treated with conventional therapy, the incidence of NC should be lower, and good proportion of patients would have a good response.

Study Limitations

Ascertainment of all case data for the whole country was not complete and inevitably some findings were not reported. In addition, as the radiological findings were evaluated in each center, follow-up characteristics were mainly based on the healing of skeletal deformities, which was assessed subjectively by many different radiologists. Unfortunately genetic diagnosis was not made in all cases. Almost half of all cases were analysed and definitive PHEX mutation was found in 80% of genetically analysed cases but this amounted to only 36% of all cases.

Conclusion

Age of diagnosis in HR patients was late in our series, despite some having positive family history, and the most frequent etiology was *PHEX* mutation. HR treatment and follow-up is challenging and our results showed an association between

higher treatment doses and NC without any change in serum levels or growth outcomes, suggesting that higher doses lead to increased phoshaturia, probably through the stimulation FGF23. However, higher calcitriol doses appear to improve bone deformities.

Ethics

Ethics Committee Approval: The study were approved by the Ankara University Ethics Committee (approval number: 06-229-16).

Informed Consent: Retrospective data and ethical approval were given.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Zeynep Şıklar, Merih Berberoğlu, Serap Turan, Concept: Zeynep Şıklar, Merih Berberoğlu, Serap Turan, Design: Zeynep Şıklar, Merih Berberoğlu, Serap Turan, Data Collection or Processing: Zeynep Şıklar, Merih Berberoğlu, Serap Turan, Abdullah Bereket, Firdevs Baş, Tülay Güran, Azad Akberzade, Ayhan Abacı, Korcan Demir, Ece Böber, Mehmet Nuri Özbek, Cengiz Kara, Şükran Poyrazoğlu, Murat Aydın, Aslı Kardelen, Ömer Tarım, Erdal Eren, Nihal Hatipoğlu, Muammer Büyükinan, Nesibe Akyürek, Semra Çetinkaya, Elvan Bayramoğlu, Beray Selver Eklioğlu, Ahmet Uçaktürk, Saygın Abalı, Damla Gökşen, Yılmaz Kor, Edip Ünal, İhsan Esen, Ruken Yıldırım, Onur Akın, Atilla Çayır, Emine Dilek, Birgül Kırel, Ahmet Anık, Gönül Çatlı, Analysis or Interpretation: Zeynep Şıklar, Merih Berberoğlu, Serap Turan, Literature Search: Zeynep Şıklar, Merih Berberoğlu, Serap Turan, Writing: Zeynep Şıklar, Merih Berberoğlu, Serap Turan.

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Soluble Receptor for Glycation End-products Concentration Increases Following the Treatment of Severe Diabetic Ketoacidosis

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What is already known on this topic?

The advanced glycation end-products/receptor for glycation end-products (AGE-RAGE) axis is a significant factor in the pathogenesis of type 1 diabetes complications. It has been proposed that soluble RAGE may act in a protective role during diabetic ketoacidosis (DKA) episodes.

What this study adds?

This is the first study of its kind. A longitudinal study of DKA measuring the marker for AGE-RAGE, soluble RAGE (sRAGE) and examining the systemic pattern of this inflammatory pathway during DKA treatment. This inflammation was expressed very early in the heart tissue of a young person who died of DKA without treatment. This study again stresses the serious implications of even one episode of DKA.

Abstract

Objective: To determine the time relationships of soluble receptor for glycation end-products (sRAGE), [a decoy of the advanced glycation end-products (AGE)-RAGE axis] and D-lactate, (a metabolite of methylglyoxal) in the inflammatory response to diabetic ketoacidosis (DKA).

Methods: Sixteen children and adolescents with type 1 diabetes (T1D) had blood samples obtained, 6-12 hours into treatment, at three weeks and three months post start of treatment. sRAGE and D-lactate concentrations at three months were considered baseline. Expression of RAGE was investigated in the myocardium of a newly diagnosed and untreated young person with fatal T1D/DKA.

Results: sRAGE 6-12 hours after the start of treatment was 39% lower than the values at two weeks ($p = 0.0036$) and at three months ($p = 0.0023$) post treatment. D-lactate was higher during treatment than at three weeks ($p = 0.04$) and at three months ($p = 0.035$).

Conclusion: sRAGE concentration was decreased during treatment, compared to concentrations at two weeks and three months after treatment. The increased D-lactate during treatment was in keeping with the known increase in dicarbonyls at this time. The finding of RAGE expression in a young myocardium prior to DKA treatment suggested cardiovascular inflammation pre-treatment and at a young age.

Keywords: Diabetic ketoacidosis, D-lactate, myocarditis, soluble receptor for advanced glycation end-products

Introduction

Suboptimal metabolic control caused by the insulin deficiency of type 1 diabetes (T1D) involves varying degrees of metabolic and immunologic dysregulation, resulting in a milieu that mediates oxidative stress (1,2) and inflammation (3). With significant insulin deficits and poor control, this dysregulation leads to the medical crisis of diabetic ketoacidosis (DKA) and the increased potential of comorbidities. Prior to DKA there is a gradual/

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dysfunctional increase in an array of inflammatory cytokines, chemokines (4,5,6) and complement (7), followed by a systemic inflammatory response (SIR) shortly after the initiation of DKA treatment (4,8,9). The metabolic stress of hyperglycemia, hyperketonemia and increased reactive oxygen species also initiates the non-enzymatic glycosylation of glucose with free amino acids to form the toxic α-dicarbonyls (10,11). These precursors/intermediates lead to the formation of advanced glycation end products (AGEs), ligands for the receptor of AGE (RAGE) and for soluble RAGE (sRAGE) (12). RAGE is ubiquitous, and has a major role in the pathogenesis of diabetic cardiovascular comorbidities, even in newly diagnosed patients with diabetes (13,14). sRAGE is a proteolytic, cleaved, secretory isoform, a natural competitor of RAGE and is a protective "decoy" that abrogates the insults that otherwise occur as a result of AGE ligands transferring to, binding to and activating RAGE (13).

Despite impressive advances in understanding the pathogenesis of the AGE-RAGE axis in acute and chronic medical conditions, uncertainties remain in the pathogenesis of T1D comorbidities and in DKA (15), a relative frequent medical crisis in children and adolescents (16). The recent article by Rawshani et al (17) gives reason to reconsider the seriousness of poorly controlled T1D in terms of longevity in children, even though DKA is not referred to. The importance of DKA can be deduced because of its common occurrence when the age of onset is before 10 years, and with the resulting loss of approximately 15 life-years for both women and men. This unfortunate statistic does not fully consider quality of life, including achievement, a factor that is much more difficult to quantify.

This data prompted us to examine the systemic inflammatory marker sRAGE during and after DKA treatment when an increase of toxic and inflammatory factors, such as the dicarbonyls and inflammatory cytokines, are expressed at the same approximate times (4,5,8,10,11). D-lactate was used as the metabolic marker of flux or catabolism of methylglyoxal (MG) (18), the precursor for the AGE ligands hydroimidazolone-1 (MG-H1), the most abundant human AGE; and N(epsilon)-(carboxymethyl) lysine (19). The myocardial expression of RAGE was also investigated in an undiagnosed and untreated, fatal case of T1D/DKA (20) to give insight into: 1) the role of treatment in RAGE expression; and 2) the likely developmental sequence of chronic cardiovascular complications of RAGE that result from severe DKA.

Methods

Study Design and Patients

A prospective longitudinal study design was utilized to study a cohort of children and adolescents with T1D/DKA. The study received Expedited Approval by the institutional review board at East Carolina University (ECU) Brody School of Medicine, since blood samples were only obtained at the time of routine blood sampling for treatment of DKA and follow up visits. The study was conducted in accordance with the Declaration of Helsinki. A total of sixteen children and adolescents between the ages of 9.5 and 17 years, presenting with DKA (total CO_2 = / < 12 mmol/L) were invited to enroll in the study. Informed consent was signed by the legal guardian and assent was obtained from the patients of nine years and over, when not prohibited by severity of illness. In such cases, patient assent was obtained when clinical improvement permitted. Patients referred from outlying hospitals were stabilized prior to being transported to ECU after consultation with the accepting attending physician in the Pediatric Intensive Care Unit. Treatment was according to previously published guidelines (21) with each patient serving as their own control and the three-month follow-up time point (T3) served as the baseline. Transfer of children and adolescents for treatment of DKA was routine in this part of North Carolina at the time of the study.

Study Evaluation and Analysis

Pretreatment values were obtained for blood pressure (BP), heart rate, complete blood count (CBC), blood glucose (BG), electrolytes, urea nitrogen (BUN), and creatinine at the referring hospitals. The start of treatment was defined as the initiation of continuous intravenous insulin. In addition to the pretreatment BP, BPs were recorded hourly with an automated oscillometric device and appropriately sized BP cuff. BPs were also obtained hourly after initiation of treatment) (T1); at discharge; two weeks post discharge (T2); and at baseline which was three months post discharge (T3). BG measurements were obtained hourly, electrolytes, and BUN were measured every two to four hours. A CBC and differential was repeated at 24 hours. None of the patients were known to have hypertension, diabetic retinopathy, nephropathy or coronary artery disease. Exclusion criteria included a history or physical findings suggestive of an acute or chronic infection, emotional or physical disability or autoimmune conditions other than chronic lymphocytic thyroiditis.

Serum samples were analyzed undiluted using an enzyme-linked immunoabsorbent assay according to the manufacturer's instructions (Human RAGE ELISA, R&D

Systems, Minneapolis, MN., USA). The inter-assay coefficient of variation was 7.6%, while the intra-assay coefficient of variation was 3.5%. The analysis included the pool of both circulating esRAGE and sRAGE.

Serum D-lactate was measured by kinetic spectrophometric assay, using the D-lactate Colormetric Assay kit MAK058 (Sigma, St. Louis, MO., USA). D-lactate was employed as it is the end-product of MG catabolism by glyoxalase 1 and 2. In this method, D-lactate is specifically oxidized by bacterial D-lactate dehydrogenase (LDH). To increase the sensitivity of the assay we incubated samples at 37 °C and the reaction was followed kinetically to achieve maximal sensitivity and linearity. To eliminate interference by the reaction of serum L-LDH with L-lactate, serum was ultrafiltered. The < 10 kDA fraction was separated by ultrafiltration through 0.5 mL Amicon Ultra Centrifugal filters spun at 14,000 g for 30 minutes in a refrigerated centrifuge at 4 °C. The ultrafiltrate was used to measure D-lactate. The limit of detection was 1 µM and the reaction was linear up to 15 µM. The intra-assay CV at 2 µM and 10 µM was 5% and 3% respectively. To further ensure specificity, the reaction was performed with and without 1 mmol/l L-lactate (upper limit of reference range in serum) and identified < 5% interference ($p < 0.05$), in agreement with data in the literature.

Immunohistochemistry (IHC) for myocardial RAGE was studied in the left ventricles of the undiagnosed and untreated case of DKA and the control. Sections were deparaffinized in two changes of xylene and two changes of absolute ethanol for 10 minutes each. Antigen retrieval was performed in 10 mM buffer, pH 6.0 in a microwave for 30 minutes then rinsed in phosphate buffered saline (PBS). Sections were blocked in 5% donkey serum for 20 minutes, then PBS for two minutes. Rabbit anti-RAGE, diluted 1:1000 (GeneTex, Irvine, CA., USA), was applied for 40 minutes at room temperature in a humid chamber, then unbound antibody removed with three changes of PBS for two minutes each. The secondary antibody, donkey anti-rabbit-Cy3, diluted 1:1,500 was then applied for 40 minutes at room temperature in a humid chamber. Unbound antibody was removed with three further changes of PBS for two minutes each. The sections were then counterstained with Dapl and viewed with an epi fluorescent microscope. All images were documented using the same magnification (x200).

Statistical Analysis

Normality of the data was determined using the Shapiro-Wilk test. For the variables that did not show a normal distribution they are described with median and interquartile range (IQR). Tests for differences between [A (T1), B (T2) and C (T3) (baseline/3 months post-discharge)] in least-square means of RAGE and D-lactate scores were performed with a repeated measures ANOVA model, with the Tukey adjustment for multiple tests applied to the p values. A correlation analysis of Spearman was used to determine relationships between variables. Results were considered significant with $p < 0.05$. Statistical analyses were performed using the SPSS software statistical package for Mac, version 19.0 (SPSS, Chicago, IL, USA).

Results

The cohort was representative of the middle Eastern coast of North Carolina. The median age of the 16 patients was 13.6 (9.7-16.9) years. The mean duration of T1D for the 10 previously diagnosed patients was 5.7 (1-12) years. Six patients were newly diagnosed with T1D at the time of admission (duration one day). There were seven males and nine females; six Caucasians (C) and 10 African Americans (AA). Patients were within 2 standard deviation (SD) of their height for age and had weights within 1.5 SD of their ideal weight for height (data not shown). All patients had uneventful correction of DKA (neurocognitive testing was not performed). All patients had at least one positive islet cell autoantibody test (IAA, IA-2 and GAD65; data not shown).

Laboratory Findings in Patients

At T1 the following biochemistry results (median and IQR) were found: BG 26.6 (14.4-46.9) mmol/L which at discharge had fallen to 10 (5.3-12.2) mmol/L; sodium 135.8 (130-144) mmol/L; potassium 5.2 (3.9-6.7) mmol/L; chloride 100.3 (90-111) mmol/L; total CO_2 10.5 (9-11) mmol/L; and BUN 6.4 (4.3-15) mmol/L. None of these admission parameters, other than the increased BG concentration, had significant associations with the studied metabolic inflammatory markers (see below).

Median (IQR) sRAGE concentration (pg/mL) was significantly lower at T1 at 332.18 (257.85-506.85) compared with T3 546.20 (390.42-739.19) ($p = 0.0023$), representing a 39% difference (Table 1). There was a strong negative correlation between the decreased sRAGE concentration (T1) and increased BG concentration [$r = -0.59$; $p = < 0.0001$). sRAGE concentration at T3 was higher in:

1) Females vs males 237.3 (176.4-446.2) vs 156.5 (76.4-191.8) pg/mL, $p = 0.04$;

2) C vs AA at 867.9 (585.0-1,243.1) vs 459.7 (356.1-546.2) pg/mL, $p = 0.003$; and

3) For the patients with newly diagnosed T1D/DKA vs previously diagnosed patients with DKA at 721.6 (585-768.1) vs 549.7 (356.1-571.8) pg/mL, $p = 0.04$.

D-lactate concentrations significantly (p = 0.035) decreased from T1 (14.1 µmol/L) to T3 (3.7 µmol/L). There was also a negative correlation which approached significance between the increased D-lactate concentration and decreased sRAGE concentration at T1 (r = -0.32; p = 0.05). The D-lactate at T3 of newly diagnosed patients with T1D/DKA was significantly lower compared to patients who had an existing diagnosis of T1D but presented with DKA [2.3 (1.2-2.8) vs 8.6 (3.2-15.9) µmol/L; p = 0.04] (Table 1).

Tissue Expression of RAGE in Myocardium

The young woman with undiagnosed T1D/DKA was found dead in her apartment, approximately 24 hours after her death. She was approximately 22 years old with a height of 156 cms weight of 37.3 kg and a BMI of 15.3 (20). IHC of myocardial RAGE expression is shown in Figure 1. Compared to control tissue, there was marked albeit subjective increase in the staining for RAGE in the myocardial samples of the young woman with undiagnosed T1D/DKA.

Discussion

To the best of our knowledge this is the first longitudinal report of sRAGE concentrations during and after the correction of severe DKA. We report a 39% lower concentration of sRAGE during DKA treatment (T1; 6-12 hours into treatment) compared with three months after the episode (p = 0.0023). There was also a significant increase in sRAGE at T1 compared with T2, two weeks post treatment, (p = 0.0036). The trend to increase in sRAGE concentrations continued to the final study point at three months, however

Table 1. Comparison of median sRAGE and D-lactate concentrations between the different time points examined in the study

Time points	DKA, 3 weeks and 3 months			
	sRAGE (pg/mL)	p value	D-lactate (µmol/ L)	p value
DKA (T1)	332.18 (257.85-506.85)		14.1 (10.5-18.0)	
	T1 vs T2	0.0036		0.04
	T1 vs T3	0.0023		0.035
3 weeks (T2)	521.84 (411.65-726.55)		5.4 (4.5-9.3)	
	T2 vs T3	NS		NS
3 months (T3)	546.20 (390.42-739.19)		3.7 (2.5-12.8)	

Tests for differences between times in least-square means of marker scores performed with a repeated measures ANOVA model, with the Tukey adjustment for multiple tests applied to the p values.

DKA: diabetic ketoacidosis, sRAGE: soluble receptor for glycation end-products, AGE: advanced glycation end-products, NS: not significant

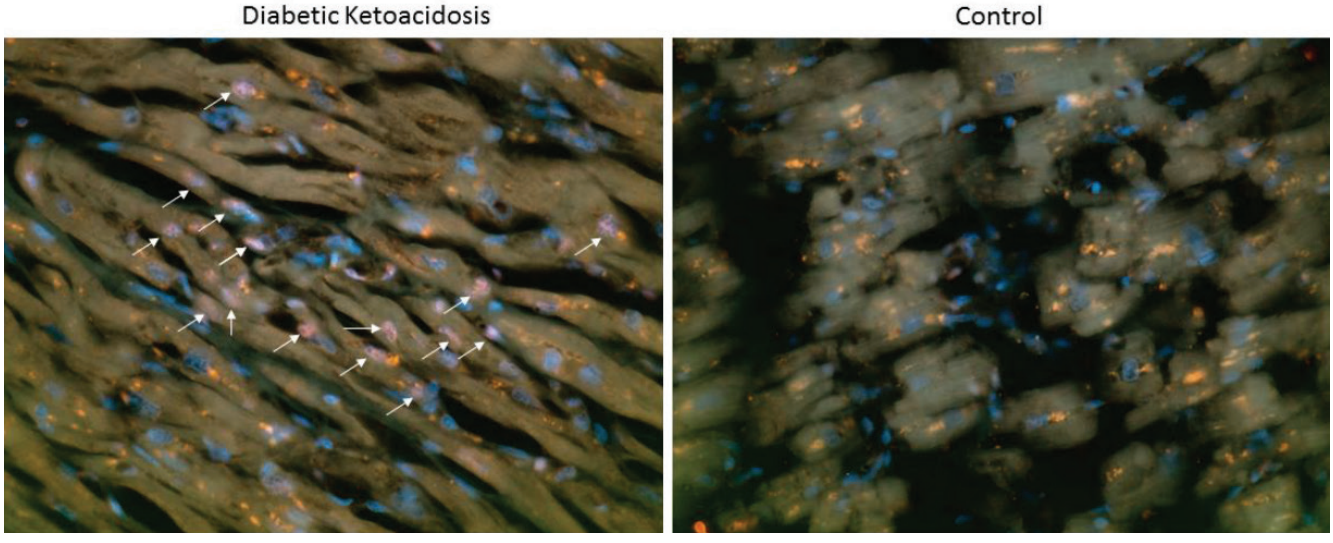

Figure 1. RAGE was prominently expressed in the diabetic ketoacidosis myocardium versus the gender and age matched control myocardium.

RAGE expression in the myocardium (left ventricle) of a young, Japanese woman, aged approximately 22 years, who died of undiagnosed (new onset) T1D/DKA prior to treatment. The arrows show positively stained RAGE cells. No stain was present in the age and gender matched control. Magnification x200

there was no significant difference between concentrations of sRAGE measured at T2 and T3 (p = NS). We hypothesize that the decrease in the sRAGE (decoy) concentrations at T1 occurred early in, or possibly prior to, DKA treatment as the result of the significantly increased concentrations of dicarbonyls (10,11), with accompanying AGE ligand formation followed by sRAGE sequestration. This sequence minimizes or prevents ligand binding, and activation of RAGE. Depending on the extent of the initial ligand binding both mediators of capillary perturbation -MG and MGH1- are candidates to be involved in the pathogenesis of pretreatment subclinical brain edema (22) and interstitial pulmonary edema (23) that occurs in severe DKA. In this regard an additional ligand of sRAGE, malondialdehyde, which is a highly reactive and a damaging β dicarbonyl, is also elevated during DKA (24), resulting in lipid peroxidation, breakdown of phospholipids, and increased vascular endothelial permeability (25,26). These pretreatment subclinical capillary perturbations are relatively common (27) and can progress during DKA treatment, but rarely to the extent of causing signs/symptoms (22,28). A decrease of early vascular perturbators at the time of treatment of severe DKA is in keeping with the hypotheses of Grossin et al (29) and Salonen et al (30), which is that sRAGE has a protective effect. With the decrease of sRAGE and its ligand sequestrations, the residual unsequestered ligand can then activate RAGE.

D-lactate, the steroisomer of L-lactate, the other metabolic marker studied, was formerly viewed to be a metabolic by-product, but is now recognized as an active metabolite in signaling of pro-inflammatory circuits. In particular D-lactate controls T-cell migration (31) and also contributes to the anion gap in the metabolic acidosis of DKA. This marker of MG catabolism was increased at 6-12 hours (T1), and decreased two weeks following treatment (T2) (p = 0.04), with a further decrease at three months (T3) (p = 0.035). This systemic pattern of D-lactate is in keeping with an early decrease of sRAGE and later, subclinical perturbation of the myocardium (5). A serious effect of D-lactate is its limited ability to be an effective respiratory substrate in the rat heart and brain due to its altering of mitochondrial energy production (32). This raises the question: Does a lower D-lactate concentration act synergistically with other DKA perturbations resulting in subclinical cardiac insults?

The longitudinal study of pre-diabetic children by Salonen et al (30) reported a decrease of sRAGE prior to the seroconversion to positive pancreatic autoantibodies. Both this and the present DKA study document low sRAGE in relation to DKA insult. The decrease of sRAGE in the study of Salonen et al (30) was reported to occur approximately 30 days before the onset of DKA with no further sRAGE decrease. In contrast, our study identifies a period of sRAGE increase following DKA treatment. We believe these transitions could be influenced by a gradual change in the pH of the milieu. However, low sRAGE concentrations are reported to occur in various conditions during the acute clinical phase in addition to changes reported in DKA. These conditions include: 1) atrial fibrillation (33); 2) low sRAGE and high cardiac troponin in non-ST segment elevation myocardial infarction (34); and 3) the autoimmune condition of multiple sclerosis (35).

Our study was not intended to identify a cause and effect relationship between the two inflammatory pathways of the AGE-RAGE axis and the SIR of inflammatory cytokines, but rather, was intended to compare their systemic phenotypes during the treatment of DKA, a time of known myocardial perturbation (5,36) and post treatment. This difference between the two inflammatory pathways was evident during the 6-12 hour period of treatment (T1) with sRAGE being lower in comparison to the rapid increase in the majority of cytokines, possibly initiated by insulin treatment (4,5,8,9). While interactions between components of the two pathways are likely (37,38,39), we were unable to confirm interactions in this study. Of interest, we also noted a difference in sRAGE concentrations at three months post-DKA therapy by ethnicity and gender with C having significantly higher (p = 0.003) sRAGE concentrations compared with AA and this concentration was also significantly higher (p = 0.04) in females than males. However, the small sample size means that although these findings are interesting, they remain to be confirmed in larger cohorts.

Despite the dichotomy in systemic inflammatory patterns at approximately the same time during treatment, the transient systemic decrease of sRAGE and the rapid transient increase of inflammatory cytokines of the SIR (4) likely occurs shortly after (IV) insulin is initiated. In regard to this dichotomy of two systemic inflammatory patterns, including RAGE, it is of note that both inflammatory pathways have significant histologically proven expression in teenage brains following fatal DKA/BE (40,41,42). This pattern of brain expression is similar to the positive association between these two inflammatory systemic pathways reported in adults with T2D adults and may be unrelated to DKA (43).

The significant, myocardial IHC expression of the multiligand receptor RAGE in the young woman found “dead-in-bed” with new onset T1D/DKA (20) suggests early RAGE-mediated cellular activation and a positive feedback initiated by sRAGE-ligand and RAGE interaction. This expression is in keeping with a report of two young fatal T1D/DKA cases (deletion of phrase) both of whom had significant

myocardial expression of the inflammatory markers MCP-1 and IL-1 beta (44). These autopsy IHC studies, along with the synthesis of cardiac autoantibodies in uncomplicated severe DKA (36), support the hypothesis that subclinical myocarditis can be initiated by the inflammatory insults of the AGE-RAGE axis and by inflammatory cytokines in T1D/DKA, with eventual progression to diabetic cardiomyopathy in some T1D patients. Yang et al (45) reported that blocking of RAGE attenuates autoimmune myocarditis which is additional supportive evidence for this hypothesis.

Conclusion

While the limitation of this study is the size of the patient cohort, this study adds to the evidence that the AGE-RAGE axis is a contributor to the acute inflammatory insult during the medical crisis and treatment of DKA and acts as constant source of subclinical inflammation leading to chronic diabetic vascular complications, including those of the heart. Inflammation during DKA treatment involved a significant transient decrease of sRAGE, possibly prior to treatment, and a significant transient increase of D-lactate, both metabolic markers of AGE-RAGE activity. It is suggested that, following the dissipation of systemic sRAGE, RAGE expression would increase and D-lactate decrease. This pattern would supports the hypothesis of sRAGE being a protective "decoy" prior to cell perturbation, through sequestration of its ligand, RAGE (10,11,31). The finding of lower concentrations of sRAGE both for AAs compared to Cs, and for males compared to females at T3, and with the opposite relationship reported for inflammatory cytokines during severe uncomplicated DKA treatment (5) warrant further investigation with larger sample sizes. The significant myocardial RAGE expression of the young woman who died of undiagnosed and untreated DKA (20) adds to the previously reported increase in myocardial inflammatory cytokines in young patients who died during the treatment of severe DKA (44). Myocardial expression of RAGE suggests a further pathogenetic mechanism for the AGE-RAGE axis, unrelated to DKA treatment (20). Whether RAGE only becomes activated in the myocardium during the life-threatening crisis of severe DKA and whether early myocardial RAGE expression occurs in less severe forms of metabolic/immunologic DKA insults, in addition to the relationship with cardiac function, all require careful follow-up and further study.

Ethics

Ethics Committee Approval: The study received Expedited Approval by the institutional review board at East Carolina University (ECU) Brody School of Medicine.

Informed Consent: Informed consent was signed by the legal guardian and assent was obtained from the patients of nine years and over, when not prohibited by severity of illness.

Peer-review: Externally and internally peer-reviewed.

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The Effect of Pubertal Stage on the Concentrations of the Novel Adipomyokine, Irisin, in Male Adolescents

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What is already known on this topic?

Irisin is a recently discovered peptide and is defined as an adipomyokine. The amount of fat and muscle tissue and gender affect the release of irisin. The relationship between irisin levels and pubertal stages (2-5) after the onset of puberty has not been studied.

What this study adds?

This study adds to the limited evidence available concerning the relationship between pubertal stage and irisin concentrations and strengthens the view that irisin concentrations do not change in response to pubertal progression.

Abstract

Objective: Irisin is a recently discovered protein and is defined as an adipomyokine. The relation of irisin with carbohydrate metabolism and other hormone parameters have been investigated. However, studies evaluating the relationship between irisin and puberty are limited and inconclusive. The aim was to evaluate serum concentrations of irisin during different pubertal stages in male adolescents.

Methods: The study included normal weight pubertal male adolescents between the ages of $13^{6/12}$-$14^{11/12}$ who had entered puberty. Fasting serum irisin concentrations were evaluated, and bioelectrical impedance analysis was used to measure body fat ratio (BFR) and fat-free mass (FFM). BFR was also calculated by caliper measurement of subcutaneous fat at the triceps.

Results: Sixty-eight adolescents were enrolled. The number of adolescents in pubertal stage 2, 3, 4 and 5 were n = 17 (25%), n = 13 (19.1%), n = 21 (30.1%) and n = 17 (25%), respectively. The median values of the irisin are 8.80, 8.20, 9.15 and 7.24 ng/mL according to the 2-5 pubertal stages, respectively. The levels of circulating irisin did not differ according to the pubertal stage. Additionally, there was no significant relationship between irisin levels and body fat percentage or FFM.

Conclusion: Irisin levels do not differ after the onset of puberty or with progressing pubertal maturation. This study strengthens the evidence that there is no change in irisin concentration as puberty progresses. This may have important implications when using this adipomyokine in the future for diagnosis or treatment of obesity-related diseases.

Keywords: Irisin, male adolescent, puberal stage, body fat percentage, muscle mass

Introduction

Irisin is a myokine recently described by Boström et al (1) and is derived from the extracellular N-terminal domain of fibronectin type 3 domain-containing-5 (FNDC5), a myocyte transmembrane protein. The transfer of irisin from muscle to circulation after exercise is regulated by peroxisome proliferator-activated receptor-γ co-activator 1α. Irisin also contributes to the regulation of energy consumption and glucose metabolism by influencing the transformation of white fat tissue to brown fat tissue (2).

Some studies (3,4,5) have suggested that irisin is released from muscles in connection with exercise, and muscle mass is the determining factor for the levels of irisin in circulation. However, Pekkala et al (6) reported that irisin levels were not associated

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with either acute or chronic high intensity or low intensity exercise, and no association was reported between overweight or impaired glucose tolerance and irisin concentrations in the same study. Since high levels of irisin were found in people with obesity, it was also suggested that irisin was released from adipose tissue (7,8). This has led to irisin being considered as an adipomyokine, a hormone released from both muscle and adipose tissues and affects distant organs (9,10).

Several studies have investigated the relationship between irisin and body mass index (BMI), exercise, thyroid function tests, bone metabolism, regulation of blood glucose, and metabolic syndrome in adults (11,12,13,14). Similarly, the relation between irisin and BMI, exercise, weight loss, and metabolic and anthropometric measurements have also been investigated in pediatric age groups (15,16). All of these studies imply that irisin levels depend on the ratio of body fat and muscle mass. Muscle and body fat mass increases with growth during childhood and varies with gender during puberty. When the increase in an individual's body fat and muscle mass exceeds a critical, personal limit, the hypothalamic-pituitary-gonadal axis is stimulated, and puberty begins (17,18). During puberty, the amount and distribution of muscle and fat mass varies according to gender and pubertal stage. While the fat-free mass (FFM), most of which consists of muscle mass, and body fat are not different between girls and boys in the prepubertal period, at the end of puberty boys have 1.5 times more muscle mass than girls whereas girls have more body fat than boys (19). Since the level of irisin is reported to be associated with both fat and muscle mass (20), irisin levels may also vary along with the pubertal stages in adolescents.

To date, very few studies in children and adolescents have addressed the effects of puberty on irisin levels. In two studies it was concluded that the prepubertal/pubertal stage was not associated with irisin levels. However, in one study, it was reported that the level of irisin was higher in pubertal adolescents than in prepubertal children (21,22,23).

Considering the future utility of irisin in treatment, in relation to many factors such as metabolic or chronic disease and obesity, it may be important to know how concentrations of irisin change according to sex and pubertal stage. The objective of this study was to investigate whether irisin levels differ according to pubertal stages in male adolescents.

Methods

This cross-sectional analytical study was conducted with eligible participants from the adolescent outpatient clinic. The study was approved by the Research Ethics Committee at Hacettepe University (protocol number: GO 16/721-08, date of approval: 24.11.2016). Written informed consent was obtained from all participants and their parents. Eligible subjects were male adolescents, aged between $13^{6/12}$-$14^{11/12}$ years, of healthy weight, with no chronic illness, who had entered puberty and were attending well-child care visits. The study focused on male adolescents between $13^{6/12}$-$14^{11/12}$ years to control for the age variable and to ensure that all participants had entered puberty. Additionally, peak height velocity, minimum body fat ratio (BFR) and maximum FFM are all observed at the age of 14 in male adolescents (24). Those who had a psychiatric, or endocrine disease, were using chronic medication for any reason, were underweight (BMI equal to or under than the 5th percentile), overweight or obese (BMI equal to or higher than the 85th percentile), had exercised a day before the study, were elite athletes, had a special diet, or took food supplements were excluded from the study. In addition, patients with acute infection during the examination, and those with pathological findings related to lipid or glucose metabolism were excluded from the study. Maturation of sexual development was based on Tanner and Whitehouse (25) stages according to pubic hair stage and testis volume. Patients were examined by the same clinician for pubertal staging.

Body weight was measured to the nearest 0.1 kg using a body composition analyzer (Tanita SC-330). Height was measured using a fixed wall-scale to the nearest 1 mm. BMI (kg/m^2) was used to define healthy weight (5th to 85th percentile), according to age and sex-specific growth reference data (26). FFM (kg) and BFR were measured by the bioelectric impedance analysis (BIA-BFR) technique with Tanita SC-330 (Tanita Corp. Tokyo, Japan). BIA was performed without socks, shoes, and heavy clothing in the morning after eight hours of fasting.

Additionally, BFR was calculated by triceps skinfold thickness measurement. Skinfold thickness was measured with a Harpenden caliper at the tricep, at the middle point between the acromion process and olecranon process on the left arm (27). Subcutaneous adipose tissue was measured by gently pulling the skin and subcutaneous fatty tissue upwards while the patient was standing upright and arms drooping on both sides. The measurement was completed twice and repeated if the difference was more than 1 mm. All skinfold measurements were performed by the same specialist. The body fat percentage was calculated by Triceps Skinfold Thickness (Triceps-BFR) measurement, using the reference values for Turkish children and adolescents (28).

Irisin Measurement

Adolescents who met the inclusion criteria were invited to the clinic at 08.30-09.00 am after eight hours of fasting

to obtain blood samples for serum irisin measurements. Serum was separated and stored at -80 °C until the time of analysis, which was no more than three months in any case. Quantitative measurements of irisin were performed with human FNDC5 enzyme-linked immunosorbent assay (ELISA) kit (Catalog No: E-EL-H2254 Elabscience, Wuhan, China) with a sensitivity 0.10 ng/mL and detection range of 0.16-10 ng/mL and interassay coefficients of variation <6%. Irisin concentrations were expressed as ng/mL.

Statistical Analysis

Data from the study were analyzed using SPSS 23.0 for Windows, version 23.0 (IBM Inc., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviations, frequency distributions, and percentages. Chi-square test was used to analyze categorical variables. The normal distribution of variables was tested using visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov or Shapiro-Wilk Test). The variance equation was controlled by the Levene test. One-way analysis of variance was used when parametric test preconditions were met to determine whether there was a significant difference between the three groups and Bonferroni test was used for *post-hoc* tests for double comparisons. The Kruskal-Wallis-H test was used when data distribution was more than two non-parametric data. The relationship between variables was evaluated by Pearson correlation coefficient or the Spearman correlation coefficient as appropriate. Significance was assumed if $p < 0.05$.

Results

Sixty-eight adolescents were included in this study. The number of adolescents in Tanner stages 2, 3, 4 and 5 were n = 17 (25%), n = 13 (19.1%), n = 21 (30.1%) and n = 17 (25%), respectively. Table 1 shows mean body weight, mean height, mean BMI percentile (BMIp), irisin, BFR and FFM values by pubertal stage.

As might be expected a significant positive correlation was found between Triceps-BFR and BIA-BFR ($r = 0.444$ $p = 0.01$) and Triceps-BFR and FFM (kg) ($r = 0.446$; $p = 0.01$).

FFM did not differ between Tanner stages 2 and 3. The increase in mean FFM was significant between Tanner 2/3 and Tanner 4 and increased signidficantly again to Tanner 5. However, the change in mean irisin concentrations by pubertal stage was not statistically significant with no evident trend in concentrations (see Table 2).

Table 1. Body mass index percentile, irisin, body fat ratio (%) and fat-free mass values according to pubertal stages

	Pubertal stage			
	2 (n = 17)	3 (n = 13)	4 (n = 21)	5 (n = 17)
Body weight (kg) (mean ± SD)	45.6 ± 6.22	46 ± 8	49 ± 7	57 ± 6
Height (cm) (mean ± SD)	155 ± 4.35	155 ± 6	160 ± 5	170 ± 6
BMIp (mean ± SD)	40.71 ± 27.54	52.38 ± 24.81	53.57 ± 25.32	51.88 ± 25.31
Irisin (ng/mL) (mean ± SD)	10.50 ± 10.50	12.41 ± 11.48	14.05 ± 14.98	12.12 ± 12.95
BF (BIA) (%) (mean ± SD)	16.34 ± 4.76	16.69 ± 4.72	15.22 ± 3.56	16.49 ± 4.17
FFM (kg) (mean ± SD)	35.58 ± 3.30	37.67 ± 5.22	44.34 ± 7.40	46.91 ± 3.82
BF (Triceps) (%) (mean ± SD)	19.29 ± 4.54	20.92 ± 3.09	19.95 ± 3.26	18.94 ± 2.01

BMIp: body mass index percentile, BFR: body fat ratio, BIA: bioelectric impedance analysis, FFM: fat-free mass, SD: standard deviation

Table 2. Statistical analysis of variables according to pubertal stage

Pubertal stage	BMIp Median (25^{th}-75^{th} centile)	Irisin (ng/mL) Median (25^{th}-75^{th} centile)	BFR (BIA) (%) Mean ± SD	FFM (kg) Mean ± SD	BFR (Triceps) (%) Median (25^{th}-75^{th} centile)
2	39 (15.50-64)	8.80 (5.88-10.12)	16.34 ± 4.76	35.58 ± 3.30[a]	19 (14.50-24.00)
3	63 (27.50-73)	8.20 (6.32-15.91)	16.69 ± 4.72	37.67 ± 5.22[a]	20 (19.00-24.00)
4	62 (24.00-75.50)	9.15 (6.13-21.38)	15.22 ± 3.56	44.34 ± 7.40[b]	19 (18-23.50)
5	62 (24.00-75.50)	7.24 (4.50-14.63)	16.49 ± 4.17	46.91 ± 3.82[c]	19 (18.00-20.00)
p	0.467	0.917	0.796	< 0.001*	0.440

*Statistical differences between the pubertal stages with different letters are significant for FFM.

[a-b]$p < 0.001$, [a-c]$p < 0.001$, [b-c]$p < 0.001$

BMIp: body mass index percentile, BFR: body fat ratio, BIA: bioelectric impedance analysis, FFM: fat-free mass, SD: standard deviation

Correlation analysis of BMIp, BIA-BFR, FFM, and Triceps-BFR variables for each pubertal stage are given in Table 3. A significant correlation was found between BIA-BFR and Triceps-BFR and BMIp. There was a significant correlation between FFM and Triceps-BFR, BMIp and pubertal stage. Irisin was not found to be correlated with any of the parameters. The inter-correlations between the parameters investigated in this study are shown in Table 4.

Table 3. Correlation analysis of body mass index percentile, body fat ratio (BFR) (bioelectric impedance analysis) (%), fat-free mass (kg) and BFR (Triceps) (%) variables according to pubertal stages

Pubertal stage			BMIp	BFR (BIA) (%)	FFM (kg)	BFR (Triceps) (%)
2	Irisin (ng/mL)	r	-0.259	0.192	-0.233	-0.273
		p	0.316	0.530	0.443	0.288
	BMIp	r		0.476	0.732[a]	0.814[a]
		p		0.100	0.004	0.000
	BFR (BIA) (%)	r			0.212	0.112
		p			0.488	0.716
	FFM (kg)	r				0.574[b]
		p				0.040
3	Irisin (ng/mL)	r	0.300	0.233	-0.106	0.016
		p	0.319	0.545	0.786	0.959
	BMIp	r		0.814[a]	0.450	0.531
		p		0.008	0.224	0.062
	BFR (BIA) (%)	r			0.058	0.604
		p			0.883	0.085
	FFM (kg)	r				0.611
		p				0.081
4	Irisin (ng/mL)	r	0.056	-0.194	-0.059	-0.146
		p	0.810	0.472	0.827	0.529
	BMIp	r		0.528[b]	0.857[a]	0.773[a]
		p		0.035	0.000	0.000
	BFR (BIA) (%)	r			0.323	0.663[a]
		p			0.223	0.005
	FFM (kg)	r				0.665[a]
		p				0.005
5	Irisin (ng/mL)	r	-0.140	0.260	-0.278	-0.002
		p	0.592	0.368	0.336	0.994
	BMIp	r		0.670[a]	0.515	0.712[a]
		p		0.009	0.059	0.001
	BFR (BIA) (%)	r			0.351	0.816[a]
		p			0.218	0.000
	FFM (kg)	r				0.516
		p				0.059

[a]$p < 0.01$, [b]$p < 0.05$

BMIp: body mass index percentile, BFR: body fat ratio, BIA: bioelectric impedance analysis, FFM: fat-free mass

Discussion

To the best of our knowledge, this is the first study to evaluate circulating irisin levels according to pubertal stages (stage 2-5) after the onset of puberty in male adolescents. In the literature, there are only four clinical investigations evaluating circulating irisin levels in which the participants consisted of adolescents, but these studies did not specifically interpret the changes according to pubertal stages. These are briefly reviewed below to build up the background for the discussion of the results of our study.

Al-Daghri et al (29) conducted their research with adolescents between 12 and 15 years of age with healthy body weight and found positive relationships between irisin and fasting blood sugar and high-density lipoprotein cholesterol. Circulating irisin levels of female adolescents were found to be higher than male adolescents and, in a multivariate regression analysis for potential confounders, the irisin levels were independently associated with fasting blood glucose levels predominantly in girls which led the authors to conclude that irisin is a predictor of glucose metabolism which has sexually dimorphic effects in adolescence. The participants were not separated according to pubertal stage and the relationship between irisin and puberty was not mentioned.

Blüher et al (22) evaluated irisin concentrations at baseline and follow-up in obese children and adolescents between 7-18 years of age after a yearlong intervention. At baseline, they did not find any significant relationships between irisin levels and age, gender, BMI, or other adipokines. Participants were also classified according to Tanner stages as pre-/early pubertal (stage 1 and 2), pubertal (stage 3 and 4) and post-pubertal (stage 5) and they did not find any evidence for differences depending on pubertal status. However, the pubertal stages of these adolescents were not analyzed separately for males and females, which we believe is not accurate. Overall, circulating irisin levels at baseline increased by 12% after the one year exercise intervention for obesity. In the same study, no correlation was found between BMI standard deviation score and irisin changes.

Jang et al (21) evaluated the relationships between circulating irisin and metabolic profiles and anthropometric indices in adolescents between 12-15 years of age in two groups, one with healthy body weight and one with obesity. They found that circulating irisin was positively correlated with adiposity indices, including percent BFR, fat mass, and the ratio of fat mass to FFM. Again, girls had higher irisin levels

Table 4. The inter-correlations between the parameters studied

		FFM (kg)	BFR (Triceps) (%)	BMIp	Irisin (ng/mL)	Pubertal[a] stage
BFR (BIA) (%)	r	0.137	0.444[b]	0.551[b]	0.060	-0.015
	p	0.334	0.001	0.000	0.672	0.914
FFM (kg)	r		0.446[b]	0.627[b]	-0.012	0.692[b]
	p		0.001	0.000	0.934	0.000
BFR (Triceps) (%)	r			0.702[b]	-0.113	-0.102
	p			0.000	0.361	0.408
BMIp	r				-0.003	0.142
	p				0.980	0.250
Irisin (ng/mL)	r					0.010
	p					0.938

[a]Spearman correlation coefficient, [b]$p < 0.01$.

BMIp: body mass index percentile, BFR: body fat, BIA: bioelectric impedance analysis, FFM: fat-free mass

than boys after adjusting for confounders in the normal-weight adolescents but not in the obese adolescents. In the same research adolescents were further classified as prepubertal (stage 1 and 2), pubertal (stage 3 and 4) and postpubertal (stage 5 and 6) in both normal weight and obese groups but again not differentiating for girls and boys and serum irisin levels did not differ significantly between the groups. They also analyzed the levels of irisin in two groups as pre-menarche or post-menarche in girls and did not find any difference. They found that elevated circulating irisin in adolescents was associated with obesity, whereas irisin increased in adolescents with healthy body weight after exercise but not in the obese group.

Lastly, Reinehr et al (23) investigated irisin and its relation to pubertal status in children and adolescents. Pubertal developmental status was categorized into two groups based on breast and genital stages determined according to Marshall and Tanner (prepubertal, boys with genital stage 1 and girls with breast stage 1; and pubertal, boys with genital stage ≥2 and girls with breast stage ≥2). The irisin concentrations differed significantly between the prepubertal and pubertal children. Analyzing only obese children demonstrated the same findings; the irisin concentrations differed significantly between the obese prepubertal and obese pubertal children.

While evaluating the changes of circulating irisin levels during puberty, it should not be overlooked that irisin is an adipomyokine (10) and its levels may depend on the ratio of body fat and muscle mass (9) which varies with gender and pubertal stage during adolescence. The levels of irisin in girls were already documented to be higher than boys (21,29) and we believe that comparing the irisin levels in the pre-early puberty versus mid-puberty versus post-puberty can only be done accurately by analyzing the data separately for females and males, which is not the case for the above studies (21,22). Relevantly, the study by Reinehr et al (23) has shown that the levels of irisin significantly increase with the onset of puberty in both sexes and the study by Jang et al (21) reported that irisin levels do not change in girls before and after menarche. Thus, we investigated the irisin levels in male adolescents after the onset of puberty, which we believe was a neglected area of irisin-puberty research.

We investigated circulating irisin levels only in boys, keeping the age constant (within 1.5 years) to avoid the confounding effect of age differences and to test the hypothesis that the irisin concentration may vary in the course of sexual maturation. The age 14 years ($>13^{6/12}$ years) was chosen to exclude pre-pubertal boys and to have adolescent boys at different stages of pubertal development since normal puberty in boys would start before $13^{6/12}$ years of age. We also only included healthy boys with normal body weight that had not exercised the day before in order to ensure the only independent variable in grouping the participants of the study was the Tanner stage.

This study evaluated BMI, body fat by two different methods, namely BIA and triceps skinfold thickness and FFM of male adolescents together with irisin concentrations. The significant correlation found between Triceps-BFR and BIA-BFR validated the measurements by different methods. BMI and body fat mass percentages did not differ significantly between pubertal stages, whereas the FFM increased significantly with progressing stages. These results are in concordance with previous studies reporting that the lean body mass changes from approximately 80% of body weight in early puberty to 90% at maturity, which primarily reflects increased muscle mass in male adolescents while the percentage of body fat during puberty decreases from stage 1 to stage 2 and remains unchanged in stages 3, 4 and

5 in males (30,31). Also, in male adolescents, gain in muscle mass reaches its maximum velocity in accordance with the peak height velocity which occurs at Tanner Stage 4 in boys (32) and FFM increase in our study group was found to be maximum from stage 3 to 4. Thus, the size of our study population was large enough to document any physiological changes during pubertal development, if present.

However, we did not find any significant variation in irisin concentrations between pubertal stages. Correlation between irisin levels and BMI, body fat and FFM was investigated for the whole study population and further, separately in each pubertal stage. We did not find any correlations between any of these anthropometric parameters and irisin concentration.

Study Limitations

Serum irisin was measured by a commercial ELISA method, and more reliable levels can be measured by immunoblotting (33) and mass spectroscopy (34), but still, ELISA method allows us to compare between the stages. Mass spectrometry method (34) is sophisticated and not available in every laboratory. It has been demonstrated by the immunoblotting method that the irisin can be measured by binding with the FDNC5 antibody (33). These methods have been preferred in early studies of irisin. In our study, we preferred the ELISA method, which is more widely used.

Conclusion

Although recent findings indicate that irisin levels might differ between prepubertal and pubertal boys, the results of this study suggest that levels do not differ with progressing pubertal maturation in male adolescents.

Ethics

Ethics Committee Approval: The study was approved by the Research Ethics Committee at Hacettepe University (protocol number: GO 16/721-08, date of approval: 24.11.2016).

Informed Consent: Written informed consent was obtained from all participants and their parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Demet Taş, Alkım Akman Öden, Sinem Akgül, Ziya E. Metin, Aslı Pınar, Nuray Kanbur, Design: Nuray Kanbur, Demet Taş, Data Collection or Processing: Demet Taş, Alkım Akman Öden, Ziya E. Metin, Analysis or Interpretation: Nuray Kanbur, Sinem Akgül, Demet Taş, Aslı Pınar, Literature Search: Demet Taş, Writing: Demet Taş, Nuray Kanbur, Sinem Akgül.

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The Incidence and Demographic Distribution of Type 1 Diabetes Mellitus in Children Aged 16 or Younger Between 2000 and 2016 in Cyprus

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What is already known on this topic?

The incidence of type 1 diabetes (T1D) has been reported in various countries. There is no data regarding the incidence rate of T1D in Cyprus

What this study adds?

This study is the first to report the incidence of T1D in the pediatric population from Cyprus. The overall incidence was 11.1/100,000 between 2001 and 2016, which is an intermediate incidence compared to other countries, and did not differ from year to year.

Abstract

Objective: Type 1 diabetes (T1D) is a disease characterized by severe insulin deficiency. In 2008 our group studied the prevalence of diabetes in adults between 20-80 years of age in Cyprus but data regarding this incidence in the pediatric population is lacking. The objective of this study was to report the incidence of T1D among permanent inhabitants aged 16 years or younger between 2001-2016 in Cyprus.

Methods: This study was a retrospective analysis. The patients were mainly evaluated and recorded at Dr. Burhan Nalbantoğlu Hospital, Nicosia. Data was also reviewed from Famagusta Government Hospital, Kyrenia Government Hospital, Near East University Hospital and the Cyprus Turkish Diabetes Association.

Results: A total of 107 subjects were diagnosed as T1D between 2001 and 2016 in the pediatric age group. Forty-nine (45.7%) were girls and 58 (54.3%) were boys. Of these 38.7% were resident in Nicosia, 30.2% Famagusta, 12.3% Kyrenia, 9.4% Guzelyurt and 7.5% Iskele. The proportion of newly diagnosed T1D was highest among children aged 9-12 years (35.5%) followed by children aged 5-8 years (32.7%). Newly diagnosed T1D most frequently presented in March and April. The overall mean incidence rate was 11.1/100,000 between 2001 and 2016. The incidence rates were similar and comparable among the years.

Conclusion: This study is the first to analyze the incidence of T1D in Cyprus. Compared to other countries the incidence rate is intermediate. Our findings are similar to the incidence rates of T1D in South Cyprus and Turkey.

Keywords: Type 1 diabetes, incidence rate, Cyprus

Introduction

Type 1 diabetes (T1D) arises from the autoimmune destruction of pancreatic β-cells leading to a life-long dependence on exogenous insulin (1). The disease most commonly presents in children and adolescents (2). T1D is also the most frequently encountered chronic disease of childhood (3).

The incidence and prevalence of this disorder are not uniform worldwide. A large variability has been reported among different populations. Seasonal variations have

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also been reported. The highest incidence is observed in Scandinavian countries, whereas China and countries close to the equator have lower incidences (4,5,6).

Cyprus is an island located in the Mediterranean region. A study published in 2012 reported the incidence of T1D in South Cyprus. The authors reported that the overall mean incidence rate was 12.46/100,000 between 1990 and 2009. In the first study decade, the mean incidence rate was 10.8/100,000. However, in the second study decade, the mean incidence rate was 14.4/100,000. Thus, the authors concluded that the incidence of T1D was rising in Cyprus (6).

Sardinia is another Mediterranean island which has a higher incidence of T1D than expected from the geographical region. The incidence rate was reported as 38.8/100,000 between 1989 and 1999 (5).

According to a diabetes survey which our group performed in 2008, the prevalence of diabetes between 20-80 years was 11% and prediabetes 18% in Cyprus. However, studies regarding the pediatric population are lacking (7).

In this study, we aimed to calculate the incidence rates of T1D in Cyprus in subjects 16 years of age or younger between 2001 and 2016.

Methods

The study was approved by the Dr. Burhan Nalbantoğlu Hospital Ethical Committee with research number: 026/19. The study was retrospective and did not involve interventions, thus we did not obtain informed consent from the patients or their parents.

Cyprus is divided into five main districts. These are Nicosia, Kyrenia, Famagusta, Iskele, and Guzelyurt. According to data obtained from the obligatory population survey covering the whole of Cyprus, which took place in 2011, a total of 59,315 permanent inhabitants are present between 0-16 years of age. The distribution of this population among districts is seen in Table 1 (8).

Dr. Burhan Nalbantoğlu Hospital, in Nicosia, is the only government hospital which has an endocrinology clinic. Thus, all pediatric patients with hyperglycemia are transferred to this department. Data was collected from this hospital together with records from Girne Government Hospital, Famagusta Government Hospital, Cengiz Topel Government Hospital, and Near East University Hospital. Subjects are advised to register with and provide records to the Cyprus Turkish Diabetes Association, which is a Non-Governmental Organization. The data of the Cyprus Turkish Diabetes Association was used to cross-reference and confirm data collected from hospital records.

T1D was diagnosed according to the International Society for Pediatric and Adolescent diabetes 2018 clinical practice consensus guidelines (9).

Subjects eligible for the study included:

1) Turkish Cypriots and permanent inhabitants in Cyprus;

2) Those with a fasting blood glucose ≥126 mg/dL, low c peptide levels and at least one positive antibody (insulin antibody, islet antibody, glutamic acid decarboxylase antibody);

3) Aged 16 years or younger;

4) Did not have or were not suspected to have type 2 diabetes, monogenic diabetes, neonatal diabetes and other types of secondary diabetes.

Data collected on each case included the gender of the subject, the age of the subject at diagnosis, the year and month of diagnosis and the district in which the subject was resident.

Statistical Analysis

Analysis was performed using the Statistical Package for Social Sciences, version 17 (IBM Inc., Armonk, NY., USA). The incidence was calculated by using the number of cases reported each year by age group (0-4, 5-8, 9-12, 13-16) and sex (male or female). The incidence rates were calculated per 100,000 heads of the population. Comparison of proportions and incidence rates were performed via the χ^2 test. A p value <0.05 was considered statistically significant.

Table 1. Distribution of the <16-year old population across the districts according to the 2011 population survey in Cyprus, together with proportions and incidence rates in these districts

	Population n (%)	Incidence n (%)	Incidence rate (per 100,000)
Nicosia	18.889 (31.74%)	41 (38.3%)	13.5
Famagusta	14.490 (26.67%)	33 (30.8%)	14.2
Kyrenia	13.978 (23.17%)	13 (12.1%)	5.8
Guzelyurt	5.654 (9.33%)	10 (9.3%)	11.1
Iskele	5.392 (9.09%)	8 (7.5%)	9.2
Unknown	-	2 (1.9%)	-
Total	59.315 (100%)	107 (100%)	11.1

Results

According to records, a total of 107 new cases of T1D were identified between 2001 and 2016 in children and adolescents younger than 16. Of these 49 (45.7%) were girls and 58 (54.3%) were boys. The male/female ratio was 1.18:1. The median age of diagnosis was 9 years overall and was the same for both genders. According to the population survey of 2011, the total population of subjects aged 16 or younger was 59,315. Out of these 30,561 were boys and 28,754 were girls. The mean annual incidence rates for boys was 11.86/100000 and for girls 10.65/100000.

The proportion of newly diagnosed T1D was highest among children aged 9-12 years (35.5%) followed by children aged 5-8 years (32.7%). Thus the highest incidence rate was in the 9-12 year-old age group (17.6/100,000) while the lowest incidence by age was found in children younger than or equal to 4 years (5.75/100,000). Proportions and incidence rates according to age groups are seen in Table 2.

The number of cases per year is seen in Figure 1. The overall mean incidence rate was calculated as 11.1/100000 between 2001 and 2016. Between 2001-2005 the mean incidence rate was 11.2/100000; between 2006-2010: 11.4/100000; between 2011-2016: 10.8/100000. The mean incidence rates were statistically similar across the three periods ($p > 0.05$).

The highest population effect of T1D was in Nicosia. However, the highest incidence rate was observed in Famagusta (14.2/100,000) and the lowest incidence rate was observed in Kyrenia (5.8/100,000) The incidence rates of T1D in Kyrenia was significantly lower than the other districts ($p < 0.05$). The incidence rate in Famagusta was similar to that of Nicosia ($p > 0.05$), and higher than that of the other districts ($p < 0.05$) (Table 1).

Newly diagnosed T1D most frequently presented in April and March (14.3%, 13.2% respectively) and higher incidences were generally seen in the winter months compared to the summer months. The monthly distribution according to time of T1D diagnosis is seen in Figure 2.

Table 2. Proportions of newly diagnosed type 1 diabetes mellitus according to age groups

	n	%	Incidence rate/100,000
≤4 years	17	15.9	5.75
5-8 years	35	32.7	15.4
9-12 years	38	35.5	17.6
13-16 years	17	15.9	8.04
Total	107	100	11.1

Discussion

In this study, the incidence of T1D in Cyprus was investigated. The results show that the mean annual incidence is intermediate (11.1/100,000) in children and adolescents aged 16 or younger (10). In Cyprus, there is only one other study of pediatric T1D incidence, which was

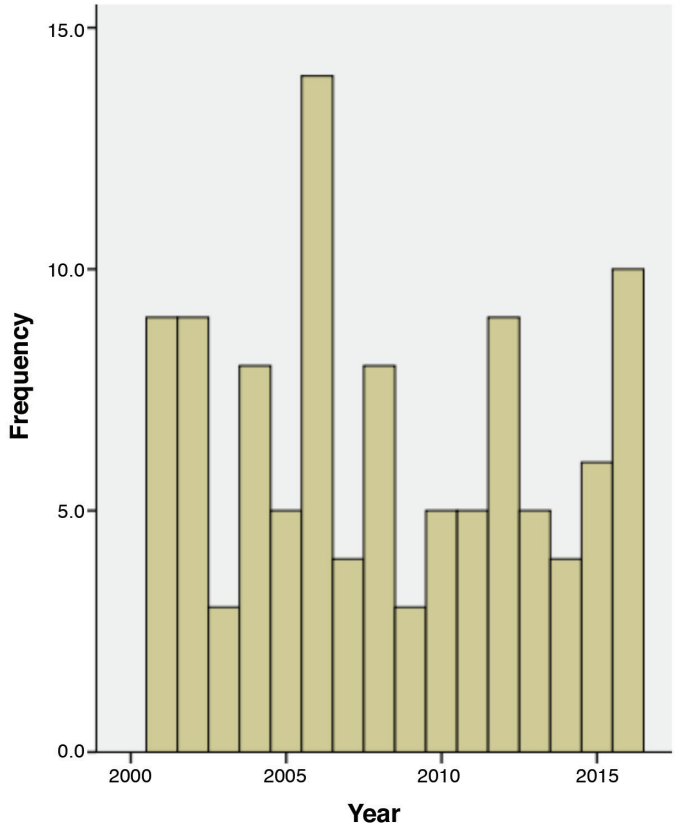

Figure 1. Incidence of type 1 diabetes by years (2000-2016)

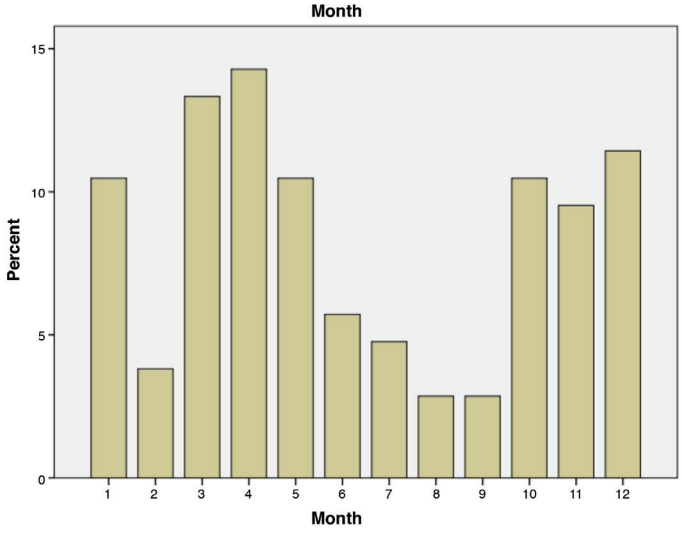

Figure 2. The diagnosis of type 1 diabetes according to the month of diagnosis

undertaken in Southern Cyprus (6). This study is the first population-based report on T1D incidence in the pediatric population of Cyprus.

The study of Skordis et al (6) (2011) analyzed a 20-year data on the incidence of T1D in subjects aged 15 or younger in the Southern Cyprus Territory. In the first decade of the study, the incidence was reported as 10.8/100,000, whereas the incidence was 14.4/100,000 in the second decade. Thus the authors concluded that the incidence of T1D was rising. Our results were not compatible with these findings. We compared the incidence rates between 2001- 2008 and 2009-2016. The mean incidence rate was 11.3/100,000 between 2001-2008 and 11.1/100,000 between 2009-2016. We also report that between 2001-2005 the mean incidence rate was 11.2/100,000; between 2006-2010: 11.4/100000 and between 2011-2016: 10.8/100,000. Thus, the distribution of incidences was balanced among the years.

Cyprus has close relations with Turkey and immigration in both directions is not rare. A prospective study which was published recently in Turkey calculated the mean crude annual incidence of pediatric T1D as 8.99/100,000 between 2013 and 2015 (2). A retrospective study analyzing the incidence of T1D in children 14 years or younger, in Southeast Turkey calculated the mean incidence as 7.2/100,000 (3). A nationwide study involving 17,175 prevalent cases of T1D in subjects younger than 18 years calculated the age-standardized incidence rate as 10.8/100,000 (95% confidence interval: 10.1-11.5) in Turkey (10). Our figures are between those of South Cyprus and Turkey.

Cyprus is an island in the Mediterranean Sea. It has been argued that there is a climate effect on the incidence rate of T1D. We compared our data with other Mediterranean countries having data on the incidence rate of T1D. In Eastern Sicily, the incidence rate of T1D was 10.1/100,000 between 1989 and 1990, and 11.7/100,000 between 1990 and 1994 (4). Our results are similar to the data reported from eastern Sicily.

In Malta, the mean annual incidence rate of T1D was 13.6/100,000 between 1980 and 1987 (11), and 24.68/100,000 between 2006 and 2010 in children aged 14 or younger. The authors calculated a mean annual increase in the incidence of T1D of 21.8% per year (12). The island of Sardinia has also reported high incidence rates; the mean incidence rate was reported as 38.8/100,000 between 1989 and 1999 (13). All these Mediterranean islands are relatively lowly populated which could decrease the reliability of calculated incidence rates.

According to our data, the mean annual incidence rate was slightly higher in boys compared to girls. Previous studies have reported a male predominance in high incidence countries and a female predominance in low incidence populations. A study by Karvonen et al (14) reported that 88% of low incidence populations were predominantly girls, and high incidence countries were predominantly boys. Data from Sardinia report a male/female ratio of 1.4 in children aged 15 or younger (13). Our results were similar to the data reported in Southern Cyprus which reported a male/female ratio of 1.06/1 (6).

The proportion of newly diagnosed T1D was highest in the 9-12 years age group followed by the 5-8 years age group. The lowest incidence rate was found in the 0-4 years age group. These findings are consistent with previous large scale studies. A Multinational study (DIAMOND Project) documented that 10-14 year-old children had the highest incidence rates (14) whereas data from Malta reported the highest incidence rate in children aged 5-9 years (5).

In our cohort, T1D was most frequently diagnosed in April, followed by March. The study of Skordis et al (6) from Southern Cyprus reported a significantly higher incidence of T1D in cold months (November, December, January, February) compared to hot months (June, July, August, September) in the first decade of the study. Incidence rates in the remaining months were similar to that of the cold months.

The highest mean annual incidence rate was in Famagusta and the lowest rate was in Kyrenia. Both districts have Mediterranean Sea coastlines and the climates of both districts are similar. However, the area of Cyprus is too small to make any conclusions about climate effects and also the number of individuals with T1D are too small to make conclusions about differences in incidence rates among districts. Also due to loss of data, we were unable to record the districts of two subjects which can lead to up to a 2% error rate in this analysis.

Study Limitations

The retrospective format is a study limitation. All subjects are diagnosed and treated at a single center. Thus, the study is strong due to excellent case ascertainment.

Conclusion

In conclusion the incidence of T1D in children aged 16 years or younger is 11.1/100,000 in Cyprus which is intermediate in comparison to other Mediterranean islands. The incidence rate does not appear to be increasing when data are compared between five year periods from 2000 to 2016.

Ethics

Ethics Committee Approval: The study was approved by the Dr. Burhan Nalbantoğlu Hospital Ethical Committee with research number: 026/19.

Informed Consent: The study was retrospective and did not involve interventions, thus we did not obtain informed consent from the patients or their parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Umut Mousa, Hasan Sav, Osman Köseoğluları, Serap Soytaç İnançlı, Neşe Akcan, Rüveyde Bundak, Concept: Umut Mousa, Hasan Sav, Rüveyde Bundak, Design: Umut Mousa, Rüveyde Bundak, Data Collection or Processing: Umut Mousa, Hasan Sav, Osman Köseoğluları, Serap Soytaç İnançlı, Neşe Akcan, Rüveyde Bundak, Ayşe Şahin, Analysis or Interpretation: Umut Mousa, Rüveyde Bundak, Literature Search: Umut Mousa, Writing: Umut Mousa.

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Association of Total and High Molecular Weight Adiponectin with Components of Metabolic Syndrome in Mexican Children

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What is already known on this topic?

Childhood obesity is related to several impaired biochemical parameters, including the concentration of total- and high molecular weight-adiponectin. A low adiponectin concentration was strongly associated with the prevalence of metabolic syndrome.

What this study adds?

The strong inverse correlation between adiponectin levels and biochemical parameters related to carbohydrate metabolism, contribute to the hypothesis that low adiponectin levels are associated with an elevated risk of diabetes. This reinforces the early role of insulin resistance in future vascular events. The circulating concentration of total adiponectin may represent an excellent biomarker to evaluate the risk of metabolic complications in young children.

Abstract

Objective: Childhood obesity linked to metabolic alterations, tend to appear simultaneously with altered adipocytokines, suggesting a role in pathogenetic development. Low circulating level of total and high molecular weight (HMW) adiponectin have been associated with components of the metabolic syndrome (MetS) and could represent an independent risk factor with potential use as a biomarker. To examine the prevalence of MetS in Mexican school children and to investigate the association of total and HMW adiponectin levels with biochemical parameters related to MetS.

Methods: The study included a population of boys and girls, from 8 to 11 years old. Anthropometric and biochemical parameters were evaluated according to weight and MetS status. A correlation analysis was fitted to establish an association between adiponectin concentrations and metabolic indicators.

Results: One-hundred and fifty five children participated (59.4% females) from 8-11 years of age. The prevalence of MetS was of 10.3%. Impaired biochemical parameters, including total and HMW adiponectin, were associated with obesity. The adiponectin level was significantly lower in MetS than in non-MetS subjects (4.5 vs. 5.4 µg/mL). Total- but not HMW adiponectin concentration was negatively correlated with blood pressure, fasting insulin, fasting blood sugar and Homeostatic Model Assessment for Insulin Resistance.

Conclusion: In young children, the total adiponectin level is associated with impaired biochemical parameters of carbohydrate metabolism and could be an excellent early predictor of metabolic complications.

Keywords: Adiponectin, children, insulin resistance, metabolic syndrome, obesity

Introduction

Childhood obesity is a complex disorder, linked to metabolic and clinical abnormalities, such as insulin resistance, dyslipidemia, and hypertension. Various combinations of these impaired metabolic functions, even in children, have been used to define the metabolic syndrome (MetS) (1). The simultaneous occurrence of obesity and impaired metabolic functions demonstrates that the accumulation of adipose tissue is a frequent etiologic basis. Adipose tissue secretes

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numerous physiologically active peptides with properties similar to cytokines, commonly known as adipocytokines, such as leptin, interleukin-6, resistin, and adiponectin. While most of the adipocytokines promote dysregulated metabolism, adiponectin contributes to maintaining energy balance, insulin sensitivity, blood pressure, immunological processes, angiogenesis, fat metabolism, and homeostasis. When adiponectin levels are low, as occurs in central obesity, the risk for metabolic alterations increases in adults, adolescents, and children (2,3,4).

Circulating adiponectin exists as multimers of high-, medium-, and low-molecular-weight (HMW, MMW, and LMW, respectively), with predominantly HMW and LMW isoforms. In adults, low HMW adiponectin concentration reflects metabolic abnormalities related to obesity, insulin resistance, and vascular alterations more specifically than total-adiponectin (5).

Multifactorial disorders, such as MetS, may be affected by characteristics of the study population. In Japanese children, HMW adiponectin was inversely correlated with obesity and insulin resistance (6). Although the Mexican population is a heterogeneous genetic mix, significant heritability for adiponectin and obesity traits substantiate a genetic contribution to circulating cytokine levels in Hispanic children (7,8). Furthermore, the age profile of the population is an important factor related to the pathophysiology of MetS and adiponectin concentration (9). Therefore, this study was designed to investigate the association of total and HMW adiponectin levels with components of the MetS, and its possible role as an early risk marker in young Mexican children.

Methods

Subjects

Children between the ages of 8 and 11 were randomly selected to participate in a cross-sectional study from six representative elementary schools in five districts in a northwestern urban region of Mexico. Schools were selected from lists made available by the Educational Authorities, according to its geographical location. The protocol was presented to the school board, classrooms were selected, and parents were required to sign a written consent form to allow their children to participate. Children without medical therapy, with parental permission and who had fasted, were eligible for the study. The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Autonomous University of Sinaloa, with registration number CONBIOÉTICA-25-CEI-003-20181012. All study procedures were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Volunteers were informed about the aim of the study, and written consent was obtained from their legal guardians.

Anthropometric Variables

Anthropometric variables were measured according to standardized procedures (10). Body weight (BW) was measured with children wearing lightweight clothing and no shoes, to the nearest 0.1 kg using a standardized electronic digital scale (Tanita BC-553; Illinois, USA). Height was measured to the nearest 0.1 cm using a portable stadiometer (Seca-214; Hamburg, Germany) with the head in the Frankfort horizontal plane. Waist circumference (WC) was measured with a non-elastic, flexible measuring tape at the mid-point between the iliac crest and the lower edge of the ribs at the end of a normal expiration. Body mass index (BMI; kg/height in m^2) was calculated and classified according to the age- and gender-specific cut-off points proposed by the World Health Organization (WHO) (11).

Clinical and Metabolic Parameters

Systolic and diastolic blood pressures were obtained from the right arm with the child seated, after rest, using a digital sphygmomanometer and appropriately sized cuff. Venous blood samples were collected in the morning (8:00 to 9:00 am) by direct venipuncture after an overnight (10 to 12 hour) fast. Plasma and serum were separated by centrifugation, aliquoted, and immediately frozen at -80 °C for later analysis. Glucose oxidase method (RANDOX Laboratories Ltd., Antrim, UK) was used to quantify fasting blood glucose levels. Triglyceride (TG), total cholesterol (TChol), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured using an enzymatic colorimetric method (RANDOX Laboratories Ltd., Antrim, UK). Insulin, total- and HMW-adiponectin, were measured by enzyme-linked immunosorbent assay using commercially available kits (ALPCO Immunoassays; NH, USA). Assays were conducted according to recommendations of the fabricant.

Classification of Pediatric MetS

Currently, a standardized definition for MetS exists for adults, but not for children and adolescents. Therefore, modified WHO criteria were applied to diagnose MetS in children (12). This pediatric/adolescent definition requires either insulin resistance, hyperglycemia, or known diabetes plus the presence of two out of three other risk parameters: hypertension (elevated age/gender systolic and/or diastolic blood pressure ≥90th percentile), dyslipidemia (hypertriglyceridemia ≥150 mg/dL or low-serum HDL-C < 39 or < 35 mg/dL in boys and girls, respectively), and central

obesity (age/gender WC ≥90th percentile or BMI ≥95th percentile). The cut-offs for impaired fasting glucose were either ≥100 mg/dL or fasting insulin ≥75th percentile (13). Insulin resistance was defined using the homeostasis model assessment for insulin resistance (HOMA-IR), calculated as the product of the fasting plasma insulin level (μUI/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 (14).

Statistical Analysis

The distribution of data was assessed using the normality test Kolmogorov-Smirnov with Lilliefors correction. Data are presented as the means ± standard deviation. T-test was used for comparison of continuous variables where applicable and by ANOVA with Tukey-Kramer *post hoc* comparison being used to evaluate group differences. For the variables without normal distribution, Kruskal-Wallis test for independent samples, according to BMI classification, was performed. Boys and girls were combined in the same groups because there were no significant sex-related differences in the anthropometric and biochemical data in the obese and non-obese children. Total- and HMW-adiponectin were correlated to anthropometric, biochemical, and clinical parameters using the Pearson or Spearman correlation coefficient. The statistical differences were considered significant at $p < 0.05$. All statistical analyses were performed using the statistical software NCCS v.2007 (15).

Results

The initial population consisted of 294 children, of whom 85 were excluded for not meeting the inclusion criteria or declined to participate, thus the drop out rate was 28.9%. On the day of blood sampling, 44 children were eliminated for not having parental permission or not being fasted, another ten were dismissed for failing to obtain the blood sample because of stress at the moment of sampling. There were no cases of children on medication or kidney disease excluded from the study. This resulted in a study cohort of 155 children (59.4% of females): 75 of healthy weight, 37 overweight, and 43 obese (Figure 1). The prevalence of MetS was 10.3%, according to the modified WHO definition. At the initial analysis, children showed no significant sex-related differences in the anthropometric and biochemical data; therefore, they were combined in the same groups. Characteristics of subjects and comparisons of mean values of clinical and metabolic continuous variables were analyzed according to obesity status (Table 1). Age was similar ($p > 0.05$) between groups. In the obesity group, insulin and adiponectin had statistically higher concentrations ($p < 0.05$), while HDL-C, total- and HMW-adiponectin were lowest. Also, blood pressure was higher in the obesity group.

When comparisons were made according to the presence or absence of MetS (Table 2), there was no difference for age ($p > 0.05$). However, weight, BMI, and WC were different between groups ($p < 0.0001$) with the highest values in the group with MetS. Insulin, HOMA-IR, LDL-C, TG, and blood pressure were significantly higher in the MetS group, while total adiponectin and HDL were significantly lower. Fasting blood glucose concentration, TChol, and HMW-adiponectin did not differ between the two groups.

Absolute values of adiponectin were tested by correlation analysis, and total-adiponectin had a significant negative correlation with anthropometric parameters and biochemical variables related to carbohydrate metabolism but not with those of the lipid metabolism (Table 3). In addition, a significant inverse correlation was found between total-adiponectin and the number of MetS components present. HMW-adiponectin was inversely correlated with weight, BMI, and HDL-C although no other significant correlation was found for the other parameters examined (Table 3).

Discussion

The increase in childhood obesity worldwide is recognized as one of the most severe public health problems. The evidence of the association between childhood obesity and parameters of MetS is increasing (7,16,17). Adipocytokines and genetic background are known to be important in the pathogenesis of MetS (18,19). In the present study, we assessed the impact of childhood obesity and MetS in young

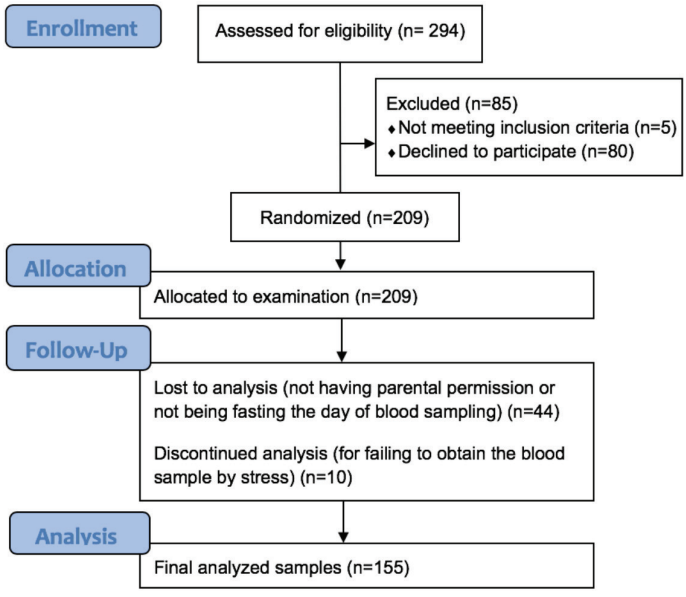

Figure 1. Flow chart of the recruitment stage of the study

Mexican children and its association with total- and HMW adiponectin.

Using the WHO definition, the prevalence of MetS found in this study (10.3%) was higher for the general child population compared with that found in other populations (3% to 8.4%) (20,21,22). However, it is difficult to contrast the prevalence of MetS because modified and non-standard definitions have been used and no globally accepted set of criteria exist for defining MetS in the pediatric/adolescent population. The prevalence of MetS in children and adolescents, based on the National Cholesterol Education Program's Adult Treatment Panel 3 definition, tends to give higher prevalences and has been reported to vary from 4.2% to 18.6%, and in a similarly aged population (7-9 years old) to our study it was 15.8% (23,24,25,26). This increased prevalence highlights the importance of early diagnose of MetS in childhood, to prevent the progression from obesity to insulin resistance, cardiovascular disease, and type 2 diabetes.

The analysis of anthropometric variables according to weight status has confirmed that each component of the MetS worsens with increasing weight, independent of sex (27). As has been reported previously (26,28,29) and was found in our study, several parameters did not show significant differences between overweight and obese children, except for a significantly higher WC in the obese group, which reinforces its importance as a risk indicator. The impaired levels of insulin, HOMA-IR, TG, HDL-C, and total- and HMW-adiponectin in the obese group compared to the normal group confirm the remarkable impact of obesity on metabolic disorders. Compared to normal and overweight children, obese children have a higher prevalence of many components of MetS. This pattern is similar to other studies in obese children and adolescents,

Table 1. Comparison of anthropometric, biochemical, and clinical characteristics of the study participants according to obesity status

Variable	Normal (n = 75) Mean ± SD (min.-max.)	Overweight (n = 37) Mean ± SD (min.-max.)	Obese (n = 43) Mean ± SD (min.-max.)	p value
Sex				
Female (n)	47	19	26	
Male (n)	28	18	17	0.510
Age, years	9.6 ± 0.9 (8-11)	9.8 ± 0.9 (8-11)	9.6 ± 0.9 (8-11)	0.266
Anthropometric variables				
Weight, kg	33.3 ± 5.3[a] (19.9-47.1)	41.5 ± 4.6[b] (31.0-52.4)	52.2 ± 11.4[c] (35.3-84.3)	< 0.00001
Weight z score	-0.66 ± 0.5[a]	0.09 ± 0.4[b]	1.08 ± 1.05[c]	< 0.0001
BMI, kg/m^2	17.1 ± 1.3a (14.2-19.7)	20.5 ± 1.0[b] (18.0-22.9)	25.7 ± 4.6[c] (19.9-49.8)	< 0.00001
BMI z score	-0.71 ± 0.3[a]	0.04 ± 0.2[b]	1.2 ± 1.04[c]	< 0.0001
WC, cm	62.1 ± 5.8[a] (50-76)	69.3 ± 5.1[b] (56.0-80.0)	81.1 ± 10.3[c] (57-104)	< 0.00001
Fasting plasma levels				
Glycaemia, mg/dL	81.3 ± 10.9 (52.7-112.2)	83.1 ± 11.5 (55.1-113.3)	83.7 ± 11.8 (60.1-107.7)	0.539
Insulin, mU/mL	4.8 ± 3.5[a] (0.3-18.2)	8.7 ± 13.3[a, b] (1.3-82.7)	11.4 ± 9.6[b] (0.2-53.5)	< 0.00001
HOMA-IR	1.0 ± 0.8[a] (0.04-4.2)	1.8 ± 2.9[a, b] (0.2-18.0)	2.4 ± 2.0[b] (0.03-11.4)	< 0.00001
Total cholesterol, mg/dL	126.0 ± 38.8 (58.1-297.1)	123.7 ± 42.1 (59.2-221.7)	118.8 ± 37.9 (64.5-240.3)	0.313
HDL, mg/dL	55.3 ± 12.7[a] (15.9-84.6)	57.3 ± 10.9[a] (37.5-82.6)	49.2 ± 12.4[b] (23.4-72.3)	0.015
LDL, mg/dL	124.7 ± 50.5 (42.1-268.9)	126.5 ± 47.9 (48.5-282.5)	146.6 ± 57.9 (58.7-286.0)	0.122
Triglycerides, mg/dL	72.0 ± 38.3[a] (29.1-302.3)	82.1 ± 36.0[a] (36.1-211.7)	111.6 ± 70.7[b] (30.4-364.8)	< 0.001
Total adiponectin, mg/mL	5.8 ± 1.7[a] (2.4-10.9)	5.2 ± 1.6[a, b] (2.7-10.4)	4.6 ± 1.6[b] (1.7-8.5)	0.002
HMW-Ad, mg/mL	3.9 ± 2.0[a] (0.3-9.0)	3.3 ± 1.7[a, b] (1.1-6.5)	2.9 ± 2.0[b] (0.1-8.0)	0.036
Clinical variables				
SBP, mmHg	96.7 ± 9.2[a] (80-115)	101.5 ± 8.6[b] (90-125)	103.5 ± 10.7[b] (80-130)	< 0.001
DBP, mmHg	60.9 ± 5.7[a] (50-80)	63.1 ± 6.2[a] (50-80)	66.6 ± 6.3[b] (60-80)	< 0.0001

BMI: body mass index, WC: waist circumference, HOMA-IR: homoeostasis model assessment for insulin resistance, HDL: high density lipoprotein, LDL: low density lipoprotein, HMW-Ad: high molecular weight adiponectin, SBP: systolic blood pressure, DBP: diastolic blood pressure, SD: standard deviation, min.: minimum, max.: maximum

[a, b, c]Literal different implies statistical differences between groups

Table 2. Comparison of anthropometric, biochemical, and clinical characteristics of the study participants according to the presence or absence of metabolic syndrome

	Without MetS (n = 139, 89.7%) Mean ± SD (min.-max.)	With MetS (n = 16, 10.3%) Mean ± SD (min.-max.)	p value
Age, years	9.6 ± 0.9 (8-11)	9.8 ± 0.7 (8-11)	> 0.05
Anthropometric variables			
Weight, kg	38.2 ± 8.2 (19.9-68.8)	60.3 ± 10.9 (39.9-84.3)	< 0.0001
Weight z score	-0.21 ± 0.76	1.8 ± 1.0	< 0.0001
BMI, kg/m^2	19.3 ± 3.1 (14.2-29.4)	28.2 ± 6.3 (23.9-49.9)	< 0.0001
BMI z score	-0.21 ± 0.69	1.8 ± 1.4	< 0.0001
WC, cm	66.9 ± 8.8 (50.0-94.0)	87.1 ± 8.9 (72.0-104.0)	< 0.0001
Fasting plasma levels			
Glycaemia, mg/dL	82.1 ± 11.2 (52.7-113.3)	84.9 ± 12.0 (64.4-107.6)	> 0.05
Insulin, mU/mL	6.7 ± 8.7 (0.2-82.6)	15.4 ± 6.9 (8.3-32.8)	< 0.001
HOMA-IR	1.4 ± 1.9 (0.03-18.0)	3.2 ± 1.4 (1.7-7.4)	< 0.0001
Total cholesterol, mg/dL	123.5 ± 37.8 (58.1-297.0)	122.9 ± 51.8 (76.8-240.3)	> 0.05
HDL, mg/dL	55.5 ± 11.9 (15.9-84.6)	42.2 ± 12.5 (23.4-64.2)	< 0.0001
LDL, mg/dL	127.5 ± 48.4 (42.1-282.6)	163.7 ± 75.3 (67.6-286.0)	< 0.01
Triglycerides, mg/dL	76.4 ± 38.2 (29.1-302.3)	163.8 ± 80.7 (73.5-364.9)	< 0.0001
Total adiponectin, mg/mL	5.4 ± 1.7 (2.2-10.9)	4.5 ± 1.5 (1.7-6.8)	< 0.05
HMW-Ad, mg/mL	3.4 ± 1.9 (0.08-9.0)	3.5 ± 2.1 (1.3-8.0)	> 0.05
Clinical variables			
SBP, mmHg	98.8 ± 9.7 (80-125)	107.5 ± 8.4 (100-130)	< 0.001
DBP, mmHg	62.4 ± 6.2 (50-80)	67.8 ± 6.3 (60-80)	< 0.01

MetS: metabolic syndrome, BMI: body mass index, WC: waist circumference, HOMA-IR: homoeostasis model assessment for insulin resistance, HDL: high density lipoprotein, LDL: low density lipoprotein, HMW-Ad: high molecular weight adiponectin, SBP: systolic blood pressure, DBP: diastolic blood pressure, SD: standard deviation

Table 3. Pearson's correlation analysis between total adiponectin, high molecular weight-adiponectin and anthropometric, clinical, and biochemical parameters in the study cohort

Variable	Total adiponectin	p value	HMW adiponectin	p value
Weight, kg	-0.377	< 0.0001	-0.189	< 0.05
BMI, kg/m^2	-0.340	< 0.0001	-0.198	< 0.05
WC, cm	-0.310	< 0.001	-0.101	> 0.05
Glycaemia, mg/dL	-0.280	< 0.001	-0.043	> 0.05
Insulin, mU/mL	-0.171	< 0.001	-0.085	> 0.05
HOMA-IR	-0.175	< 0.001	-0.077	> 0.05
Total cholesterol, mg/dL	0.028	> 0.05	0.116	> 0.05
HDL-C, mg/dL	0.113	> 0.05	0.171	< 0.05
LDL-C, mg/dL	0.015	> 0.05	-0.002	> 0.05
Triglycerides, mg/dL	-0.076	> 0.05	0.120	> 0.05
SBP, mmHg	-0.116	> 0.05	-0.066	> 0.05
DBP, mmHg	-0.136	> 0.05	0.027	> 0.05
No. of MetS components	-0.279	< 0.001	-0.109	> 0.05

HMW: high molecular weight, BMI: body mass index, WC: waist circumference, HOMA-IR: homoeostasis model assessment for insulin resistance, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, HMW-Ad: high molecular weight adiponectin, SBP: systolic blood pressure, DBP: diastolic blood pressure, MetS: metabolic syndrome

in which low serum adiponectin levels were associated with markers of MetS, such as hyperglycemia, hyperinsulinemia, high blood pressure, and dyslipidemia (2,25,30).

When comparing our cohort, stratified by the presence or absence of MetS, there is a suggestion that the prevalence of MetS increases directly with BMI. Similar to Turkish and Portuguese children with MetS and obese Italian children, in our study, no differences were observed in TChol level, suggesting that this indicator is less critical in this age group (24,31,32). However, in Korean children, an association between non-HDL cholesterol and MetS has been reported (33).

Regarding biochemical parameters, total-adiponectin had the strongest inverse correlation respect to HMW adiponectin, with glycaemia, followed by number of MetS components, HOMA-IR and insulin concentration. Similar findings have been described, where total adiponectin had a significant inverse relation with HOMA-IR and obesity, and its low concentration was an essential determinant of insulin sensitivity and HDL in children and may predict type 2 diabetes (7,29,32,34,35). No significant correlation was found for biochemical parameters related to fat metabolism (e.g., TG, HDL-C, LDL-C and TChol), probably due to the young age of the population and the pathophysiology of MetS (24,36). Longitudinal studies showed that blood pressure and TG decreased when HOMA decreases, independently of changes in BW, supporting the hypothesis that insulin resistance is the central abnormality contributing to these cardiovascular risk factors and development of atherosclerosis and MetS (24,29). The studies found that insulin resistance, or its accomplice, hyperinsulinemia could precede to dyslipidemia, enhancing the output of very-LDL and raising TG; this lipid overload in muscle is diverted to the liver, promoting fatty liver and atherogenic dyslipidemia (37). These mechanisms affecting lipid metabolism could be at an early stage in our young population where, instead, we observed impaired glucose homeostasis as the principal affliction (38). These results obtained in the present study, support the early observations about the need to include insulin resistance, as proposed in the WHO criteria, for the diagnosis of MetS in children (23,31,39,40).

A protective role of adiponectin is evident early in life and compromised in youth-onset obesity, and low concentrations could be considered a risk factor (7,32,34). It has been suggested that low levels of adiponectin are involved in the association between childhood obesity and adult atherosclerosis (41). In the present study, total- and HMW-adiponectin were decreased in obese children and correlated with anthropometric variables (weight and BMI). However, whereas Total-adiponectin correlated with several biochemical parameters, HMW-adiponectin only correlated with HDL-C. Previous studies have found that sub-fractions of adiponectin have different biological effects, but their degree of association may vary according to the characteristics of the population, such as the different age groupings included in the studies (42,43). Adiponectin levels decline with age in association with changes in sex hormones and growth factors. Among growing youth, total fat mass is the primary determinant of adiponectin concentrations, and the age effect is mostly a result of increased fat mass with increased age (44,45). Consistent with the above, changes in total- and HMW-adiponectin levels in childhood obesity is different to that in elderly obese patients (46). Therefore, the relationship between adiponectin and the biochemical parameters of dyslipidemia may not be established until puberty (47).

Besides, the association of HMW adiponectin with MetS indicators seems to be influenced by adiposity (48). In obese prepubertal children, HMW adiponectin shows a closer relationship with the improvement of carbohydrate metabolism parameters than with body fat content. Other studies confirm that the relationships of plasma adiponectin with a favorable lipid profile depend on adiposity and that central obesity plays a significant role in the relationships of adiponectin with TG. These findings may mean that adiponectin may not necessarily play a favorable role in lipid metabolism, and it might have multiple effects on this metabolic process based on the underlying condition. Different studies have demonstrated that adiponectin concentrations have ethnic variance and were lower in Asian as compared to African-American children, were positively related to insulin sensitivity and HMW-adiponectin was not superior in predicting metabolic variables (49,50,51). Our data indicate that, in the context of the MetS in Mexican children, HMW-adiponectin might not have the same degree of relevance. Hence, the relationships between adiponectin levels and anthropometric and biochemical indicators in children appear to be independent of sex and influenced by ethnicity and lifestyles associated with modernization. We suggest that the genetic backgrounds of cohorts should also be considered in future studies and body composition analysis should be more detailed in order to investigate the relevance of adiponectin in pathogenesis of pediatric MetS.

Study Limitations

Limitations of our study are mostly due to the limited sample size and its cross-sectional nature. However, our findings are consistent with the idea that ethnic differences influence the distribution of adiponectin isoforms and their relationship with metabolic parameters.

Conclusion

Childhood obesity is related to several impaired biochemical parameters, including the concentration of total- and HMW-adiponectin. A low adiponectin concentration was related closely to the prevalence of MetS. The strong inverse correlation between adiponectin levels and biochemical parameters related to carbohydrate metabolism, contribute to the hypothesis that low adiponectin levels are associated with an elevated risk of diabetes. The absence of correlation between total- and HMW-adiponectin and fat metabolism indicators could be explained by the young age of the study population. Furthermore, it reinforces the importance of early insulin resistance in development of the MetS and possibly future vascular events. Therefore, circulating concentration of total adiponectin may represent an excellent biomarker to evaluate the risk of metabolic complications in young Mexican children. Additionally, a consensual pediatric definition of MetS is needed in order to better compare between studies and populations, and adequate screening and evaluation of children at risk or with MetS.

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Ethics

Ethics Committee Approval: The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine, Autonomous University of Sinaloa, with registration number CONBIOÉTICA-25-CEI-003-20181012.

Informed Consent: Volunteers were informed about the aim of the study, and written consent was obtained from their legal guardians.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Javier A. Magaña Gomez, Design: Carla E. Angulo Rojo, Data Collection or Processing: Daniela Moreno-Mascareño, Analysis or Interpretation: Gisela Duarte de la Peña, Javier A. Magaña Gomez, Literature Search: Daniela Moreno-Mascareño, Carla E. Angulo Rojo, Writing: Daniela Moreno-Mascareño, Carla E. Angulo Rojo, Javier A. Magaña Gomez.

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The Effects of Prehospital Care on Outcome in Pediatric Diabetic Ketoacidosis

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What is already known on this topic?

Despite the guidelines on initial management of pediatric diabetic ketoacidosis (DKA) significant variations (intravenous fluids and insulin therapy) can be observed in the prehospital setting or peripheral health care facilities.

What this study adds?

This is the first study exploring the low utilization rate of prehospital emergency medical services for children with DKA. Notably inappropriate fluid type/dose and insulin were administered in centers for primary/secondary care. Patients who received inappropriate initial management were more likely to develope complications.

Abstract

Objective: Despite the guidelines, significant variations can be encountered in initial therapy for pediatric diabetic ketoacidosis (DKA) in the prehospital setting. These variations occur mostly in fluid administration, insulin dosing, route of administration, and other aspects of the initial resuscitation and stabilization. The aim was to identify the effect of transport care on outcomes in children with DKA admitted to the emergency department (ED).

Methods: Patients admitted to a tertiary-care pediatric ED between 2015-2019 with a diagnosis of DKA were retrospectively identified. Details of pre-pediatric ED care, including transport modality, patient demographics, clinical features, laboratory evaluation, fluid therapy, insulin dosing, and short-term outcome were recorded.

Results: The study cohort included 147 episodes of DKA in 136 patients aged 9 months-21 years. Emergency Medical Service (EMS) transported only 37.4% of cases. EMS utilization rate was significantly higher ($p = 0.003$) in severe cases, most of whom were >10 years ($p = 0.04$). During transport 85% received intravenous fluid bolus. Use of fluids other than normal saline was significantly higher when transport time was >30 minutes ($p = 0.001$). Acute kidney injury and cerebral edema developed in 21.7% and 7.4% of episodes, respectively. These complications were more common in the EMS transport group. Pediatric intensive care admission rate was also higher in the EMS compared to the non-EMS group ($p = 0.01$)

Conclusion: Parents did not call the ambulance for most cases although a higher complication rate occurred in EMS patients. EMS providers and referral facilities should improve their knowledge of pediatric DKA.

Keywords: Diabetic ketoacidosis, prehospital care, diabetes mellitus, insulin, pediatric transport

Introduction

Diabetic ketoacidosis (DKA) is one of the serious acute complications of type 1 diabetes mellitus (T1DM). DKA occurs at the onset of diabetes in half of patients (1,2). The annual rate of DKA in pediatric T1DM is 6-8%, with a case fatality rate ranging from 0.15% to 0.31% in developed countries. However, recent data from developing countries has shown that the mortality rate in children with DKA was 6-24% (3,4,5).

The principles of management of DKA in the pediatric population include optimization of: 1) volume status;

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2) hyperglycemia and ketoacidosis; 3) electrolyte abnormalities; and 4) potential precipitating factors. Although, the management of these patients should be organized in comprehensively equipped or tertiary hospitals, the initial interventions performed before/during transport influence the final outcome. Thus some concerns remain about pre-hospital care of DKA in children (6). Despite the guidelines and recommendations concerning the optimal type and amount of intravenous (iv) fluid in the initial resuscitation of DKA, significant variations of initial iv fluid treatment occur in clinical practice. Similar inappropriate interventions including sub-optimal insulin dosing, route of administration, and other aspects of the initial resuscitation and stabilization, which are provided before transfer, have been reported (7).

This is, to the best of our knowledge, the first study exploring the utilization rate of prehospital Emergency Medical Services (EMS) for children with DKA, and the effect of transport modality-provided care and the association of these with prognosis. Interventions performed before referral and en route, administered fluid type and dose, insulin dosing and route of administration were also investigated.

Methods

Study Design

This is a retrospective cohort study conducted in the Emergency Department (ED) of Ege University Children's Hospital between 1st January 2015 and 31st May 2019. The Ege University Local Ethical Committee (18-7/8) approved this study.

Definition and Treatment Protocol

DKA was defined based on the International Society for Pediatric and Adolescent Diabetes (ISPAD) clinical practice consensus guidelines (8). According to this guideline, DKA is defined by the presence of all of the following; hyperglycemia (blood glucose > 200 mg/dL), metabolic acidosis (venous pH < 7.3 or serum bicarbonate < 15 mEq/L) and ketosis (blood beta-hydroxybutyrate > 3 mmol/L or moderate-large urine ketones). Our institutional management protocol for DKA was consistent with the ISPAD guidelines. The severity of DKA was structured into three groups: mild (pH = 7.2-7.29), moderate (pH = 7.1-7.19), and severe (pH < 7.1).

For children with moderate and severe acidosis, initial resuscitation of 20 mL/kg of isotonic sodium chloride solution (0.9%), for mild cases and 10 mL/kg fluid was administered over 60 minutes. Following the initial fluid resuscitation, continuous, low-dose iv insulin infusion rate of 0.05 U/kg/hr was administered for children who were younger than 5 years old, and 0.1 U/kg/hr rate was used for children older than 5 years. Any exceptions to these recommendations for fluid administration (type/dosing) or insulin dosing during transport in EMS were defined as an inappropriate fluid or insulin therapy.

Study Population and Data Collection

All patients admitted to our ED with DKA were included in the study. The data collection form included information on: transport modality (ambulance or not); patient demographics; clinical features; laboratory evaluation; administered resuscitation therapy (fluid type and volume, insulin type and dose) en route; and stabilization treatment in the ED. We also reviewed the medical records of all hospitalized patients to identify any subsequently developed complications, such as acute kidney injury (AKI) and/or cerebral edema (CE), and to collect outcome data during their hospital stay.

Statistical Analysis

Statistics Package for the Social Sciences, version 22.0 software (IBM Inc., Chicago, IL, USA) was used for statistical analysis. Continuous data are represented by the mean and standard deviation or median and interquartile range (IQR), as appropriate. Categorical variables are expressed by frequency and cross tables. The chi-square test (or Fisher's exact probability test) were used to compare demographics. Mann-Whitney U or t-test was performed for two independent groups, as appropriate. Values of $p < 0.05$ were regarded as statistically significant.

Results

During the study period medical care was given to 192 endocrine emergencies in our ED. Most of them were DKA (163/192; 84.9%) involving 150 individual patients (Figure 1). We excluded 16 episodes in 14 patients due to missing data. The final analysis was performed for 147 episodes of DKA in 136 patients. Sixty-one percent were female and the mean age was 11.1 ± 4.7 years (range 9 months to 21 years). Table 1 summarizes the demographic characteristics of patients in the study.

For most episodes, caregivers or parents did not choose to use ambulance transfer to the ED for their children (62.6%). EMS transported slightly more than one-third of this cohort (37.4%) (Table 1). The most common EMS transfers (43/55, 78.2%) were performed for patient referral from secondary care hospitals. EMS brought seven (12.7%) episodes from the field, and the remaining referrals were two (3.6%) from tertiary care ED and three (5.4%) from the primary care

physician office. Patients who arrived at the ED without using EMS were more likely to have been sent from the primary care physician office (37.5%) (Table 1).

Tachycardia, dehydration, vomiting and altered mental status (Glasgow Coma scale ≤14) were more common in the EMS transported group when compared with the non-ambulance group. Comparisons of the clinical features in patients brought with or without EMS are shown in Table 1.

More than half of episodes (55.7%) were mild and only 13.6% were severe DKA (Figure 1). The comparison of patients' laboratory findings between the severity groups of DKA are shown in Table 2. The mean pH was 6.90 (range was 6.70-7.07) in the severe group. EMS utilization rate was significantly higher in severe cases and most of the severe cases were adolescents (older than 10 years) ($p < 0.001$, $p = 0.04$ respectively) (Table 3).

Nearly half of the patients (42.8%) present with DKA at the time of diagnosis. The other most common causes of DKA presentation were; insulin omission (34.1%), insulin pump dysfunction (14.9%) and precipitating factors such as infections (8.2%). The proportion of children with new-onset T1DM and severe DKA was higher in the adolescent group. Although inappropriate fluid use was higher in patients under five years of age, complications were more common in patients older than five years (Table 4).

Most patients who were brought by EMS (45/55; 81.8%) received iv fluid bolus and the most common administered fluid (84.4%) was normal saline (0.9% NaCl) during the transport. Only a minority of episodes received inappropriate fluid type (such as 0.9% NaCl + 10% Dextrose, 0.45% NaCl + 10% dextrose or only 10% dextrose) (15.6%). Inappropriate fluid dosing was the most common mistake encountered (66.7%) (Figure 2).

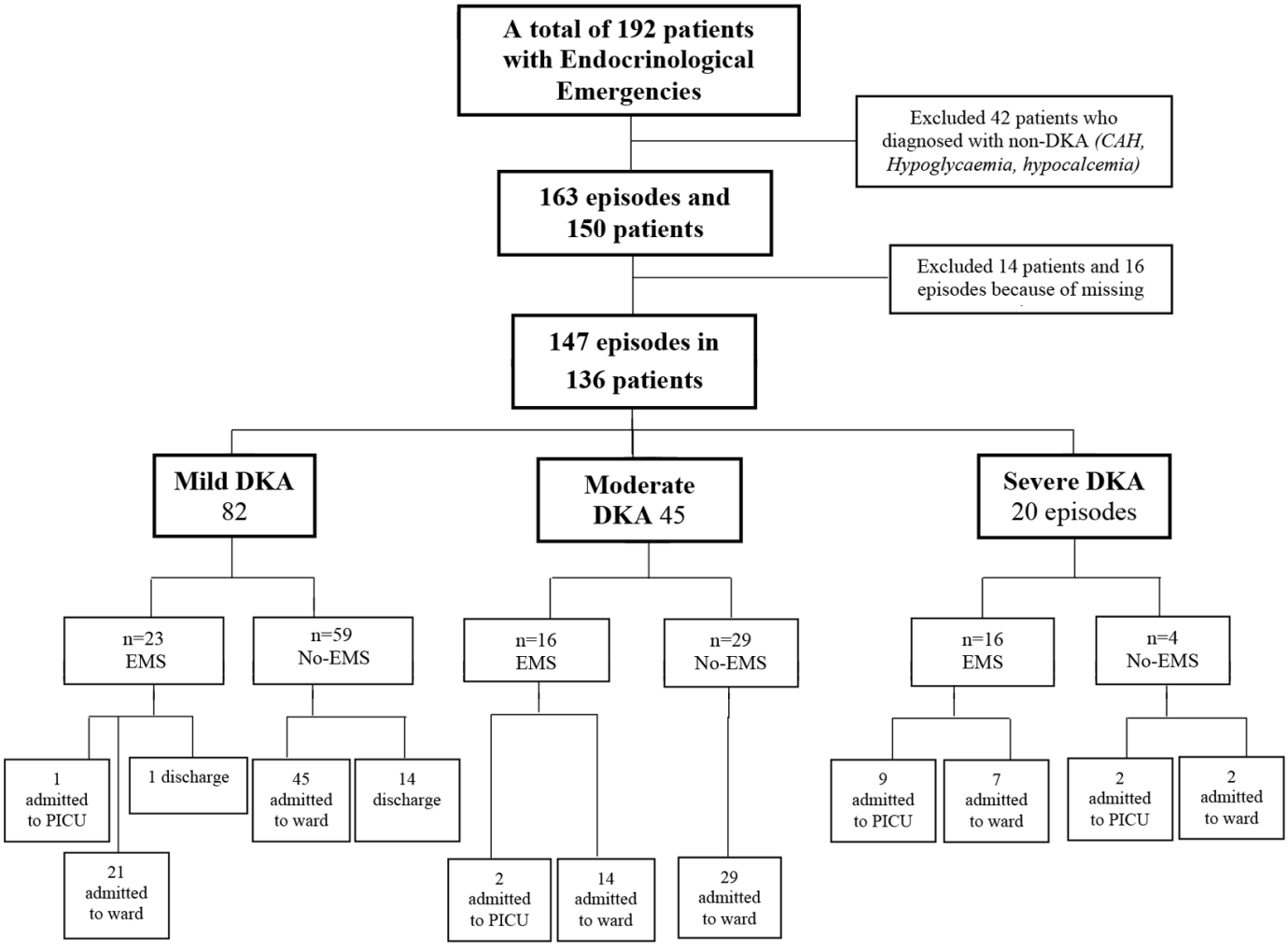

Figure 1. Distribution of episodes recruited in the study period

DKA: diabetic ketoacidosis, CAH: congenital adrenal hyperplasia, PICU: pediatric intensive care unit, EMS: Emergency Medical Services

Inappropriate initial fluid doses and insulin treatments were associated with EMS transport ($p < 0.001$ and $p = 0.009$, respectively) (Table 3).

The EMS transfer group (39/55; 70.9%) arrived within one hour at the ED and only 16 patients' transport duration lasted more than one hour. The rate of inappropriate fluid use was significantly higher when the transport time lasted more than 30 minutes ($p = 0.001$).

Table 1. The patient characteristics and clinical features of episodes

Patient characteristics	
Age (mean ± SD)	11.1 (±4.7)
< 5 years of age, n (%)	22 (14.9)
Number of adolescent (> 10 years), n (%)	51 (34.6)
Girls:Boys ratio	1.6:1
Referral center	
EMS, n (%)	55 (37.4)
Home	2
Field	5
Primary care hospital	3
Secondary care hospital	43
Tertiary care hospital	2
Other	0
No EMS, n (%)	92 (62.6)
Home	49
Field	0
Primary care physician	33
Secondary care hospital	6
Tertiary care hospital	0
Other	4
Transport time, h (mean, min.-max.)	1.1 (0.25-3.2)
Clinical features (EMS/not EMS) (n)	
Tachycardia	22/42
Dehydration	22/41
Polydipsia	10/30
Vomiting	14/25
Polyuria	10/28
Kussmaul breathing	13/15
Loss of weight	8/19
Abdominal pain	7/16
Altered mental status	9/4
DKA causes, n (%)	
New onset T1DM	63 (42.8)
Insulin omission	50 (34.1)
Disfunction of insulin pump	22 (14.9)
Infections	12 (8.2)

T1DM: type 1 diabetes mellitus, DKA: diabetic ketoacidosis, SD: standard deviation, EMS: Emergency Medical Service, min.: minimum, max.: maximum

In total 19 episodes received insulin therapy following fluid resuscitation. Although appropriate continuous, low-dose [begin with 0.05 (< 5 years) to 0.1 U/kg/h], iv insulin infusion was performed for only six episodes, 13 episodes received inappropriate insulin therapy (11 subcutaneous; two subcutaneous and iv).

The median (IQR) length of stay in the ED was 2.1 (1.0-4.0) hours. AKI and CE developed in 21.7% and 7.4% of patients, respectively. These complications more likely to develop in moderate and severe DKA groups (Table 5). In addition, PICU admission rate was significantly higher in severe DKA who were transported by EMS, although there was no significant difference between EMS utilization and complications in severe DKA ($p < 0.001$ and $p = 0.317$, respectively) (Table 6). One hundred and seventeen episodes (80.2%) were admitted to the ward, and 14 to PICU. The rate of PICU admission was also higher in EMS when compared to the non-EMS group ($p < 0.001$) (Figure 1). No significant association was found between the patients who received inappropriate interventions when compared with patients who received appropriate interventions during EMS with either PICU admission rate or complications.

Discussion

The present study demonstrates several issues of concern regarding the prehospital management of pediatric DKA before referral and transfer to a tertiary care ED. Calling an ambulance (from the field to hospital) and using it for inter-hospital transport is the recommended response for patients with DKA in Turkey. There are several reasons for this. It is the most common emergency in pediatric T1DM and is still the major cause of hospitalization, morbidity, and mortality (1,2,9). Most morbidities and deaths due to DKA take place out of the hospital, presumably from

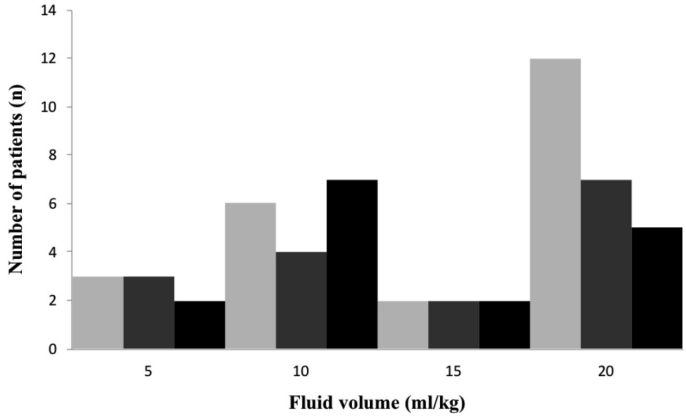

Figure 2. Administered intravenous fluid bolus volume (ml/kg) based on diabetic ketoacidosis severity

Table 2. Comparison of patients' laboratory findings between the severity groups of diabetic ketoacidosis

	DKA groups					
	Mild		Moderate		Severe	
Laboratory findings	**EMS (n = 23)**	**No-EMS (n = 59)**	**EMS (n = 17)**	**No EMS (n = 28)**	**EMS (n = 15)**	**No EMS (n = 5)**
Bedside glucose, mg/dL Mean (± SD)	398 (± 75)	402 (± 109)	373 (± 125)	366 (± 80)	341 (± 107)	435 (± 67)
Venous pH Mean (± SD)	7.26 (± 0.38)	7.27 (± 0.23)	7.14 (± 0.02)	7.15 (± 0.03)	6.9 (± 0.1)	6.9 (± 0.12)
Serum bicarbonate, mmol/L Mean (± SD)	14.4 (± 3.9)	16.6 (± 4.3)	9.7 (± 2.5)	9.7 (± 2.1)	5.6 (± 2.1)	5.7 (± 0.1)
Serum lactate, mmol/L Mean (± SD)	1.6 (± 0.99)	1.7 (± 0.7)	1.4 (± 0.9)	2.3 (± 1.1)	2.2 (± 1.2)	2.0 (± 0.4)
Serum sodium, mmol/L Mean (± SD)	133.9 (± 2.7)	132.3 (± 3.1)	132.4 (± 3.1)	133.0 (± 3.4)	132.6 (± 4.1)	136 (± 5.6)
Serum corrected sodium, mmol/L, Mean (± SD)	136.9 (± 3.4)	141.9 (± 5.9)	137.6 (± 4.4)	137.9 (± 3.2)	139.4 (± 1.3)	138.1 (± 2.7)
Serum potassium, mmol/L Mean (± SD)	4.6 (± 0.6)	4.3 (± 0.4)	4.3 (± 0.7)	4.3 (± 0.7)	4.5 (± 0.7)	3.7 (± 0.2)

DKA: diabetic ketoacidosis, SD: standard deviation, EMS: Emergency Medical Service, min.: minimum, max.: maximum

Table 3. The association between diabetic ketoacidosis severity, appropriate treatments (fluid and insulin), complications, Pediatric Intesive Care Unit admission rates with Emergency Medical Service utilization

	EMS (n = 55)	No EMS (n = 92)	Total (n)	p
DKA severity				
Mild	23	59	82	
Moderate	17	28	45	**0.000**
Severe	15	5	20	
Appropriate initial fluids treatment (type/dose)§				
Yes	12	91	103	**0.000**
No	33	1	34	
Appropriate insulin treatment (type/dose)ϕ				
Yes	3	4	7	**0.009**
No	12	0	12	
Complications				
CE	8	3	11	**0.039**
AKI	13	19	32	
PICU admission rate (%)	21.8	2.2	9.5	**0.000**

DKA: diabetic ketoacidosis, EMS: Emergency Medical Service, CE: cerebral edema, AKI: acute kidney injury, PICU: pediatric intesive care unit.

§Appropriate initial fluids treatment (type/dose): For children with moderate and severe acidosis, initial resuscitation of 20 mL/kg of isotonic sodium chloride solution (0.9%), for mild cases 10 mL/kg fluid was administered over 60 minutes.

ϕAppropriate insulin treatment (type/dose): Low-dose intravenous (iv) insulin infusion rate 0.05 U/kg/hr administered for children who is younger than 5 years old, and 0.1 U/kg/h rate used for children older than 5.

Table 4. Comparison of clinical features, interventions and complications between the age groups

	Age groups			p
	0-5 years (n)	5-10 years (n)	> 10 years (n)	
Severe DKA	2	7	11	0.121
New-onset T1DM	12	18	33	**0.000**
Use of ınappropriate fluids	5	8	21	**0.000**
Mistaken insulin use	2	4	7	0.903
Complications	4	7	32	**0.000**

DKA: diabetic ketoacidosis, T1DM: type 1 diabetes mellitus

Table 5. The association of diabetic ketoacidosis severity and Emergency Medical Service utilization between the rate of complications

	Complications			p
	No (n, %)	AKI (n, %)	CE (n, %)	
Severity				
Mild	73 (89)	8 (9.7)	1 (1.7)	0.645
Moderate	25 (55.5)	14 (31.1)	6 (13.4)	**0.000**
Severe	6 (30)	10 (50)	4 (20)	**0.000**
EMS utilization				
Yes	34 (61.8)	13 (23.6)	8 (14.6)	0.065
No	70 (76.1)	19 (20.6)	3 (3.3)	

EMS: Emergency Medical Service, CE: cerebral edema, AKI: acute kidney injury

Table 6. The comparison between the rate of complications, pediatric intesive care unit admission and transport modality in severe diabetic ketoacidosis group

	Complications			p	PICU admission (n, %)	p
	No (n, %)	AKI (n, %)	CE (n, %)			
EMS	5	8	3		11 (73.3)	
No EMS	1	2	1	0.317	1 (20)	**0.000**
Total	6	10	4		12 (60)	

EMS: Emergency Medical Service, CE: cerebral edema, AKI: acute kidney injury, PICU: pediatric intesive care unit

severe dehydration and acidosis, which can be treated by iv fluids and insulin. If the primary goals of the management of DKA are performed appropriately and earlier in the clinical course, morbidity and mortality are clearly shown to be reduced (5). Unfortunately, this study shows that most children (62.6%) who have DKA do not access ambulances as their first medical contact. The reason for the low rate of EMS utilization is not clear and warrants further investigation. This is the first Turkish study to examine in detail the nature of the request for EMS in children with DKA. Since no previous study has published ambulance transport rates to hospital, our results suggest that patients preferred to use primary care physicians or secondary care hospitals and we believe that this may explain the higher rates of non-ambulance transport observed in this study.

The incidence of DKA as the first presentation of new-onset T1DM has a large variability from country to country. Although, the DKA incidence of new-onset T1DM has been decreasing in European countries, such as Austria (34%), Germany (21.1%), Finland (22.4%), Denmark (17.9%), Italy (41.9%) and France (43.9%); this range was between 80-88% in African countries (1,10,11). In our country, this incidence was previously reported to be 33-55% (12,13). In the current cohort of patients,this rate was similar rate to the previous Turkish studies and to the reports from France and Italy. Precipitating factors such as infections, alcohol abuse, and insulin dose omission were the remaining main causes of DKA in diagnosed T1DM (14). Unlike in developed countries, where infection is the most common precipitating factor for DKA, insulin disruption/omission was the major precipitating factor for DKA in the studied patients (34.8%) (14).

The severity of the episodes in the previous studies was reported as mild/severe in 33% and 9%, respectively (13,15). In the current cohort, the severe DKA rate was 14.6% which is similar to data from Germany (16%), France (14.8%) and Italy (11.2%) and less than reports from Poland (22.5%) and Saudi Arabia (26.1%). This difference may be explained by lower parental educational achievement.

In previous studies, a young age, especially less than two years, and low accessibility to medical care were identified as risk factors for DKA at T1DM diagnosis (11,15,16,17,18). This association of young age and DKA at diagnosis may be explained by worse beta cell dysfunction, more aggressive diabetes and delayed detection of diabetic symptoms occurring more frequently in young children. It has been shown that children less than five years of age are at higher risk of metabolic decompensation at initial presentation (19). However, some studies indicated that informing the parents about diabetes symptoms decreased the risk of DKA at T1DM diagnosis in young children (20). In contrast to these studies, the present study found more frequent and more serious DKA episodes in children aged >10 years old. Children and adolescents at this age have likely escaped parental control. Thus, detection or reporting of symptoms may be delayed. Similar to our findings, a recent study from New Zealand reported an increased risk for DKA at age around 11 years (21). This may depend on better awareness in parents who have children <5 years and that adolescents do not recognize symptoms.

The management of DKA in any setting, both for patients with newly or previously diagnosed T1DM, can be divided into four physiologic principles which are restoration of fluid volume, inhibition of lipolysis, correction of electrolyte abnormalities and correction of acidosis. Delayed, insufficient or inappropriate treatment is a potential risk for developing complications of DKA (22). The timing of fluid therapy as an initial treatment has a considerable effect on the outcome of DKA and it is recommended that it should be given within the first hour (23). Since the majority of patients spend the first time when treatment of DKA could be given in the ambulance, if they use it, they should receive the initial therapy en route. Despite all the guidelines and recommendations this study has highlighted several concerns about the prehospital management of DKA (6). Although, only one-third of these cohort patients were brought by ambulance to the ED, there was still inappropriate fluid dose and insulin used for DKA during ambulance transport. The incidence of severe DKA and complications of kidney injury, CE and PICU admission rate were significantly higher in patients transported with EMS. The high rate of complications and morbidity associated with DKA in EMS-transported patients was related to both transport facilities and the severity of DKA. We believe that the majority of referring physicians from secondary care hospitals and prehospital healthcare providers attending to these children lacked the clinical experience to manage DKA. The uncorrected hypovolemia in our cohort may have

resulted in complications. Since most severe patients were in the EMS group this would also explain the difference in the rate of developing complications. Since our study was conducted retrospectively and the sample size was small, further, well-designed prospective studies with a large sample size would be needed to clarify the situation.

There are many factors that contributed to the development of complications in the non-EMS group. Since the most common referral place in this group was the home, parents of children with T1DM should be advised to use ambulance transport when bringing their children with DKA to the ED. The second most common referral center in the non-EMS group was primary care and we believe that feedback should be made to primary care physicians for transferring these patients by EMS with an appropriate management protocol.

Study Limitations

There are several limitations to the present study. The retrospective design of our study is the most crucial limitation which may have led to selection bias. In addition the single-center experience with a small sample size cohort were also limitations. Well-designed larger, prospective, multi-center studies are needed to explore and explain this causal relationship.

Conclusion

The severity, complications, PICU admission and morbidity associated with DKA in our study was higher than that reported from developed countries. The root causes for the above were lack of parental education concerning DKA, inappropriate transport type and inappropriate therapy with fluid and/or insulin, and delayed management due to lack of clinical experience and facilities for managing DKA in the primary/secondary health-care facilities. This is compounded by transport problems associated with referral hospitals and lack of follow-up and continuum of care among known diabetics.

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Ethics

Ethics Committee Approval: The Ege University Local Ethical Committee (18-7/8) approved this study.

Informed Consent: Informed consent was taken from the patient's parents.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Caner Turan, Ali Yurtseven, Damla Gökşen, Eylem Ulaş Saz, Concept: Caner Turan, Eylem Ulaş Saz, Design: Damla Gökşen, Eylem Ulaş Saz, Data Collection or Processing: Caner Turan, Elif Gökçe Bülbül, Analysis or Interpretation: Caner Turan, Damla Gökşen, Eylem Ulaş Saz, Literature Search: Caner Turan, Elif Gökçe Bülbül, Damla Gökşen, Eylem Ulaş Saz, Writing: Caner Turan, Damla Gökşen, Eylem Ulaş Saz.

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Soluble CD40 Ligand Levels in Children with Newly Diagnosed Graves' Disease

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What is already known on this topic?

Graves' disease (GD) is believed to result from a complex interaction between genetic background, environmental factors, and the immune system. Soluble CD40 ligand (sCD40L) might be involved in the evolution of many autoimmune diseases and may have diagnostic and therapeutic implications.

What this study adds?

To our knowledge, this is the first study to assess serum sCD40L concentrations in children with newly diagnosed GD. High concentrations of sCD40L were found in children with newly diagnosed GD compared to healthy controls and there was a correlation between sCD40L and thyroid stimulating hormone receptor antibodies and thyroid volume which may suggest a biologically active role for sCD40L in GD.

Abstract

Objective: Soluble CD40 ligand (sCD40L) is elevated in various autoimmune disorders, which may have diagnostic and therapeutic implications. The aims of the current study were to evaluate serum sCD40L concentrations in children with newly diagnosed Graves' disease (GD) and to correlate its levels with patients' clinical and laboratory parameters.

Methods: This study included 48 children with newly diagnosed GD and 48 healthy children. Serum thyroid-stimulating hormone (TSH) (TSH, fT4 and fT3), TSH receptor antibodies (TRAbs), high sensitivity C-reactive protein (hsCRP) and sCD40L levels and thyroid volume were measured.

Results: Compared to control subjects, children with GD had higher thyroid volume standard deviation scores (SDS) ($p = 0.001$), and higher levels of hsCRP ($p = 0.001$), TRAbs ($p = 0.001$) and sCD40L ($p = 0.001$). Significant correlations were found between sCD40L and age ($p = 0.01$), thyroid volume SDS ($p = 0.001$), hsCRP ($p = 0.01$) and TRAbs ($p = 0.001$). In multivariate analysis, sCD40L concentrations were correlated with TRAbs [odds ratio (OR) = 3.1, 95% confidence intervals (CI): 2.2-2.7, $p = 0.001$] and thyroid volume SDS (OR = 2.1, 95% CI: 1.2-2.7, $p = 0.001$).

Conclusion: This preliminary study has evidence of high concentrations of sCD40L in children with newly diagnosed GD and a correlation between sCD40L and both TRAbs and thyroid volume, which may indicate a biologically active role for sCD40L in the pathogenesis of GD.

Keywords: Graves' disease, soluble CD40 ligand (sCD40L), thyroid hormone, thyroid volume

Introduction

Graves' disease (GD), the most common cause of spontaneous thyrotoxicosis, is believed to result from a complex interaction between genetics, environmental factors, and the immune system (1). GD is mediated by autoantibodies against the thyroid stimulating hormone (TSH) receptor (TRAbs) that bind to and activate TSH receptors, thus stimulating thyroid hormone synthesis, secretion and thyroid cell growth (2). Cluster of differentiation

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40 ligand (CD40L) is a trimeric transmembrane protein of the tumor necrosis family and was originally identified on the cells of the immune system (3). It binds to CD40, which is mainly expressed on antigen-presenting cells and B cells although it is present on other types of cells such as thyroid follicular cells (4). After cellular binding, the surface-expressed CD40L is then cleaved and/or released over a period of minutes to hours generating a soluble fragment (sCD40L), which retains full biological activity. It has number of immune functions that include cell-to-cell interactions, antigen presentation and pathogen capture (5). CD40-sCD40L interaction has an emerging role in the evolution of some autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis and mixed connective tissue disease (6). Little is known about the role of sCD40L in GD (7). This study was conducted as a preliminary evaluation to estimate the serum concentrations of sCD40L in a group of children with newly diagnosed GD and its relationship to patients' clinical and laboratory variables.

Methods

Patients

This is a cross-sectional case-control study involving children and all were newly diagnosed before the start of medical treatment. They were consecutively recruited over a period of two years from 2015 to 2017 and all were attending the Pediatric Endocrinology Clinic of Children's Hospital, Assiut University, Assiut, Egypt. The diagnosis of GD was based on the presence of clinical manifestations of hyperthyroidism, low serum levels of TSH, high serum levels of free thyroxine (fT4), free triiodothyronine (fT3), and high titers of thyrotropin receptor antibodies (TRAbs) (8). Excluded from the study were those with: systemic or other immune-medicated diseases; subclinical hyperthyroidism; previous GD relapse; Graves' ophthalmopathy; toxic adenoma; toxic multinodular goiter; and cases coming from iodine deficient areas. Healthy children recruited from the general population and matched for age, gender, pubertal status, and socioeconomic status (SES) were also included as control subjects for statistical comparison. The inclusion criteria for the control group were: demonstration of normal serum TSH and fT4; negative antithyroid antibodies; and no past or family history of thyroid disease.

Methodology

All participants underwent detailed medical histories and clinical examinations with special emphasis on age at onset of GD and its duration. Anthropometric measurements (height and weight) and vital signs were recorded. Body mass index (BMI) was calculated using the standard formula: BMI = weight (kg)/height (m)^2. BMI was expressed as standard deviation (SD) scores (SDSs) to normalize for age and sex (9) using national growth reference data (10). Blood pressure was recorded and expressed as SDS to normalize for age and sex (11). Pubertal development was assessed by Tanner staging (12). Thyroid volume was estimated using ultrasonography (7.5-MHz linear array transducer) (GE Healthcare Bio-Systems, Milwaukee, WI, USA). Thyroid volume values were obtained by calculating the volumes of both lobes as follows: Lobe (mL) = Length x width x depth (mm) x 0.479. Thyroid volume was expressed as SDS on the basis of published references values for age and gender (13,14). Imaging data were reviewed by the same pediatric radiologist, who was blinded to the biological data.

Laboratory Investigations

Blood samples were obtained at 8.00 a.m. after an overnight fast for estimation of serum concentrations of TSH, fT4, and fT3 (Immulite™ 2000 Third Generation, Diagnostic Products Corporation, Los Angeles, CA., USA). The reference ranges for thyroid hormones were as follows: TSH = 0.4-4.0 mU/L, fT4 = 10.0-26.0 pmol/L, and fT3 = 3.5-5.5 pmol/L. The coefficients of variations (CV) for thyroid hormones were as follows: TSH = 5.0 and 5.1% at concentrations of 4.0 and 10.0 mU/L, respectively; fT4 = 6.5% at concentrations of 10.0 pmol/L; and fT3 = 8.9% at concentrations of 3.5 pmol/L. Serum TRAb levels were measured with a 3rd generation TBII assay (TRAb3rd) using the automated Cobas electrochemiluminescence analyzer (Elecsys, Roche Diagnostics GmbH, Penzberg, Germany). The cut-off value for positive concentration of TRAbs was 1.75 IU/L. The serum concentration of high sensitivity C-reactive protein (hsCRP) was measured using an hsCRP enzyme-linked immunoabsorbent assay (ELISA) kit (catalog no. E29-056; Immunospec Corp., Canoga Park, CA, USA). Measurement of serum sCD40L levels was performed using a specific ELISA (Biosource Int., CA, USA) according to the manufacturer's instructions. The intra-assay and interassay coefficients of variation for sCD40L were 5.00% and 6.30%, respectively, with a sensitivity of 0.067 ng/mL, The reference range for sCD40L level was 0.16-10 ng/mL (15).

Ethical Consideration

The protocol of the study was carried out in accordance with the Declaration of Helsinki ethical principles for medical research involving human subjects. The study was approved by the Ethical Committee of Assiut University (approval number: 10/2018) and informed consent and assent were obtained from all participants or their parents/guardians for younger children before inclusion in the study.

Statistical Analysis

All statistical analyses were carried out using Statistical Package for the Social Sciences, version 18.0 (IBM Inc., Chicago, IL, USA). Quantitative variables were presented as means ± SDs, and qualitative variables were presented as percentages. The Kolmogorov-Smirnov test was used for assessing normality of data distribution. Comparisons between parametric and non-parametric values were performed using a two-tailed Student's t-test and Mann-Whitney U tests, respectively. Categorical variables were compared using the chi-square or Fisher's exact tests. Correlations between sCD40L and clinical, and laboratory variables were performed using Pearson's correlation coefficient test. Multivariate analysis was used to determine the factors that were significantly associated with elevated sCD40L concentrations. The odds ratios (ORs), 95% confidence intervals (95% CI) and significances were calculated. For all tests, values of $p < 0.05$ were considered statistically significant.

Results

The study included 48 children, 34 girls (70.1%) and 14 boys (29.2%), with a mean age of 14.4 ± 3.6 years (range: 11-18 years) with a new diagnosis of GD. Compared to 48 age, sex and SES matched healthy children, patients had significantly lower mean BMI SDS ($p = 0.01$) and higher mean heart rate ($p = 0.01$). Patients also had significantly higher mean hsCRP and sCD40L concentrations ($p = 0.001$ for both) (see Table 1). Patients' sCD40L levels had significant positive correlation with age ($r = 0.319$, $p = 0.01$), thyroid volume SDS ($r = 0.564$, $p = 0.001$), hsCRP ($r = 0.323$, $p = 0.01$) and TRAbs concentrations ($r = 0.632$, $p = 0.001$) but not with fT3, fT4, or TSH concentrations (see Table 2). Multivariate analysis showed that sCD40L concentrations were significantly correlated with TRAbs (OR = 3.1, 95% CI: 2.2-2.7, $p = 0.001$) and thyroid volume SDS (OR = 2.1, 95% CI: 1.2-2.7, $p = 0.001$).

Discussion

The current study has demonstrated that sCD40L levels were significantly higher in children with GD compared with controls ($p = 0.001$). Moreover, sCD40L correlated positively with TRAbs concentration, which remained significant after regression analysis (OR = 3.1, 95% CI: 2.2-2.7, $p = 0.001$). Mysliwiec et al (7) reported that sCD40L levels were elevated in adult patients with GD compared to control subjects, although the difference did not reach statistical significance. Experimental studies have shown in vitro that increased sCD40L concentrations were associated with adhesion molecules and monocyte chemoattractant protein-1 release, impaired migration of endothelial cells and O2 generation in monocytes (16) which suggested that that sCD40L played an important role in the regulation of autoimmune and inflammatory responses, which in turn are likely to be involved in the pathogenesis of GD (7). Blockade of the CD40-CD40L pathway with BI 655064 in

Table 1. Clinical and laboratory characteristics of the patient and control groups

Characteristics	Patients (n = 48)	Controls (n = 48)	p value
Female/male	34/14	32/16	NS
Age (years)	14.4 ± 3.6	15.4 ± 3.6	NS
BMI SDS	-0.37 ± 1.06	0.30 ± 2.16	< 0.01*
Heart rate (beat per minute)	113 ± 13	98 ± 8	< 0.01*
Systolic BP SDS	0.72 ± 03	0.63 ± 0.2	NS
Diastolic BP SDS	0.37 ± 0.1	0.32 ± 0.05	NS
TSH (mIU/mL)	0.061 ± 0.02	1.95 ± 0.9	< 0.001*
fT4 (pmol/l)	35.8 ± 9.3	13.32 ± 2.55	< 0.001*
fT3 (pmol/l)	13.4 ± 4.4	4.22 ± 2.1	< 0.001*
TRAbs (IU/L)	16.32 ± 4.65	0.7 ± 0.7	< 0.01*
Thyroid volume SDS	3.2 ± 0.9	0.3 ± 0.1	< 0.001*
hsCRP (mg/L)	329 ± 20.5	67.9 ± 12.8	< 0.001*
sCD40L (ng/mL)	16.2 ± 3.5	3.66 ± 1.2	< 0.001*

*Significant difference.

Data are means ± standard deviation.

BMI-SDS: body mass index standard deviation score, TSH: thyroid stimulating hormone, fT4: free thyroxine, fT3: free triiodothyronine, TRAbs: thyroid stimulating hormone receptor antibodies, sCD40L: soluble CD40 ligand, hsCRP: high-sensitivity C-reactive protein, NS: non-significant

Table 2. Correlation between soluble CD40 ligand and the other parameters in children with Graves' disease

Parameter	r value	p value
Age (years)	0. 319	**< 0.01***
BMI SDS	-0.204	NS
Heart rate (beat per minute)	0.119	NS
Systolic BP SDS	0.123	NS
Diastolic BP SDS	0.125	NS
Thyroid volume SDS	0.564	**< 0.001**
TSH (µIU/mL)	-0.212	NS
fT4 (pmol/L)	0.135	NS
fT3 (pmol/L)	0.199	NS
hsCRP (mg/L)	0.323	**< 0.01***
TRAbs (IU/L)	0.632	**< 0.001***

*Significant difference.

BMI-SDS: body mass index slandered deviation score, TSH: thyroid stimulating hormone, FT4: free thyroxine, FT3: free triiodothyronine, TRAbs: thyrotropin receptor antibodies, NS: non-significant

rheumatoid arthritis patients with insufficient response to methotrexate-IR resulted in marked improvement in clinical and biological parameters (17,18), suggesting that the CD40-CD40L pathway might prove to be a target for novel therapeutic strategies for autoimmune diseases.

CRP is an acute-phase protein associated with systemic inflammation. In this study, the circulating levels of hsCRP were significantly higher in children with GD than in the control children. Furthermore, the hsCRP levels were positively correlated with sCD40L levels ($r = 0.323$, $p = 0.01$). These findings are consistent with those of previous studies (19,20), that showed increased systemic inflammation in adult patients with GD.

Interestingly age was significantly associated with sCD40L concentration ($r = 0.319$, $p = 0.01$). This is in agreement with El-Asrar et al (21) who reported significant positive correlation between sCD40L levels and age in a cohort of children with type 1 diabetes mellitus. On the other hand, Cholette et al (22) reported that sCD40L levels are high at birth and remain significantly higher throughout childhood than sCD40L concentrations in adults. Future research may help to answer questions regarding the underlying reasons for developmental changes in sCD40L serum levels.

Thyroid volume SDS was significantly higher in children with GD compared with control children ($p = 0.01$). Furthermore, a significant positive correlation between sCD40L levels and thyroid volume SDS was demonstrated that reminded significant after regression analysis (OR = 2.1, 95% CI: 1.2-2.7, $p = 0.001$) suggesting a direct causal relationship between sCD40L and thyroid volume. Previous studies indicated that increased levels of sCD40L may reflect a greater degree of T cell infiltration of the thyroid gland in patients with GD as the degree of surface CD40 expression was shown to closely correlate with intensity of lymphocyte infiltration, in addition to the direct thyroid growth-stimulating role of sCD40L that may result in diffuse goiter (7).

On examination of the relationship between other markers of thyroid function and sCD40L there was a no correlation between sCD40L and either fT3 or fT4 concentration. This is in agreement with Yamamoto et al (23), who reported the same finding in adult patients with GD. Despite the possible important role of sCD40L in the pathogenesis of GD (24), it appears that high serum concentrations of sCD40L are associated with the presence of goiter but not with elevated thyroid hormone levels. However, further studies are needed to clarify the role of sCD40L in relation to the thyrotoxic activity of GD.

Study Limitations

The cross-sectional survey and the small number of subjects represent the major limitations of this study. As such, it is not possible to conclude whether higher sCD40L levels are directly involved in the pathogenesis of GD or just a consequence of the immune-mediated process.

Conclusion

This preliminary study has evidence of higher concentrations of sCD40L in children with newly diagnosed GD. There was also a strong positive correlation of sCD40L with both TRAbs and thyroid volume, which may suggest a biologically active role for sCD40L in the pathogenesis of GD.

Ethics

Ethics Committee Approval: The study protocol was approved by the Local Ethics Committee of Assiut University Children Hospital, Assiut, Egypt (approval number: 10/2018).

Informed Consent: Written informed consent was obtained from the parents of all participants.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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CASE REPORT

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Catecholamine-induced Myocarditis in a Child with Pheochromocytoma

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What is already known on this topic?

The clinical presentation of pediatric pheochromocytoma and paragangliomas (PPGLs) is highly variable. Classic symptoms of catecholamine excess include headaches, diaphoresis and palpitations which may be episodic in nature. The most common symptom in children is sustained hypertension. Excessive catecholamine may stimulate myocytes and cause structural changes, leading to life-threatening complications ranging from stress cardiomyopathy (CM) to dilated CM. Catecholamine-induced myocarditis is a rare clinical manifestation seen in adult patients with pheochromocytoma.

What this study adds?

To our knowledge, no pediatric case presenting with myocarditis has been reported in the literature. Our patient was diagnosed with myocarditis as the first symptom without the expected signs and symptoms due to catecholamine elevation. Even if there are no signs and symptoms of catecholamine elevation, functional PPGLs may lead to CM.

Abstract

Pheochromocytomas and paragangliomas (PPGLs) are rare neuroendocrine tumors. The clinical presentation of pediatric PPGLs is highly variable. In cases with pheochromocytoma (PCC), excess catecholamine may stimulate myocytes and cause structural changes, leading to life-threatening complications ranging from stress cardiomyopathy (CM) to dilated CM. Herein, we report the case of catecholamine-induced myocarditis in a child with asymptomatic PCC. A 12-year-and-2-month-old male patient with a known diagnosis of type-1 neurofibromatosis was brought to the emergency department due to palpitations and vomiting. On physical examination, arterial blood pressure was 113/81 mmHg, pulse was 125/min, and body temperature was 36.5 °C. Laboratory tests showed a leucocyte count of $12.8x10^3$ µL/L and a serum C-reactive protein level of 1.1 mg/dL (Normal range: 0-0.5). Thyroid function tests were normal, while cardiac enzymes were elevated. Electrocardiogram revealed no pathological findings other than sinus tachycardia. The patient was diagnosed with and treated for myocarditis as echocardiography revealed a left ventricular ejection fraction of 48%. Viral and bacterial agents that may cause myocarditis were excluded via serological tests and blood cultures. Blood pressure, normal at the time of admission, was elevated (140/90 mmHg) on the 5th day of hospitalization. Magnetic resonance imaging revealed a 41x46x45 mm solid adrenal mass. The diagnosis of PCC was confirmed by elevated urinary and plasma metanephrines. The patient underwent surgery. Histopathology of the excised mass was compatible with PCC. It should be kept in mind that, even if there are no signs and symptoms of catecholamine elevation, CM may be the first sign of PCC.

Keywords: Pheochromocytoma, myocarditis, neurofibromatosis type-1

Introduction

Pheochromocytomas (PCC) and paragangliomas (PPGLs) are rare neuroendocrine tumors. The prevalence of PPGLs among children with hypertension is 1.7% (1). A PCC is a catecholamine-producing paraganglioma (PGL) of adrenal medulla origin. PGLs are tumors originating from sympathetic or parasympathetic paraganglia. The average age at admission for pediatric PPGLs is 11-13 years, and

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they are more common in males with a ratio of 2:1 (2). The clinical presentation of pediatric PPGLs is highly variable. The classic symptoms of catecholamine excess include headaches, diaphoresis and palpitations, which may be episodic in nature. This triad of the disease is present in about 54% of patients (3). The most common symptom in children, present in 60-90% of cases, is sustained hypertension (2).

Features of PCC are summarized by the "Rule of 10s": 10% is malignant, 10% is extra-adrenal, 10% is bilateral, and 10% is hereditary. However, up to 80.4% of PCC in children are hereditary (4). PPGLs may be a part of hereditary syndromes, such as multiple endocrine neoplasia type 2A or 2B, Von Hippel-Lindau syndrome and neurofibromatosis type 1 (NF1) (1,2).

In cases with PCC, excess catecholamine may stimulate myocytes and cause structural changes, leading to life-threatening complications ranging from stress cardiomyopathy (CM) to dilated CM. Catecholamine-induced myocarditis is an infrequent clinical manifestation seen in adult patients with PCC (5).

Case Report

A 12-year-and-2-month-old male patient was brought to the emergency department due to palpitations that started at night together with repeated vomiting; ten episodes of vomiting were reported. It was learned that the patient had been followed up in the neurology clinic with the diagnosis of NF1, had no other complaint and did not use any medication. On physical examination, arterial blood pressure was 113/81 mmHg, pulse was 125/min, body temperature was 36.5 °C, weight was 30.5 kg (-1.86 standard deviation (SD)], and height was 137.9 cm (-1.87 SD). There were extensive cafe-au-lait spots, including in the lumbosacral and gluteal regions and covering the left thigh, and a 10x10 cm non-tender lumbar soft tissue lesion. The testicular volumes were 4/4 mL. There was no consanguinity between his parents. Laboratory tests showed a white blood count count of $12.8x10^3$ µL/L and a serum C-reactive protein concentration of 1.1 mg/dL [normal range (NR): 0-0.5]. Thyroid function tests were normal, while cardiac enzymes were elevated; troponin 1: 3.6 ng/mL (NR: 0-0.04), Pro-brain natriuretic peptide: 6730 ng/L (NR: 0-125), creatine kinase muscle B: 43 U/L (NR: 0-24). Electrocardiogram (ECG) revealed no pathological findings other than sinus tachycardia. The patient was diagnosed with and treated for myocarditis as echocardiography (ECHO) revealed a left ventricular ejection fraction (LVEF) of 48%, and mild mitral and aortic insufficiency. Serologic tests for the commonest viruses associated with CM, including Adenovirus, Coxsackie group B, Parvovirus, Herpes Simplex virus, Epstein-Barr virus, Rubella, and Human Immunodeficiency virus, and blood cultures were negative. Since blood pressure that was normal at the time of admission had become elevated (140/90 mmHg) by the 5th day of hospitalization, the patient underwent Doppler ultrasound and subsequent abdominal magnetic resonance imaging (MRI). The MRI indicated that a 41x46x45 mm solid mass lesion, which had heterogeneous but diffuse contrast enhancement, was located between the liver and the anterior upper pole of the right kidney, displaced the liver to the anterior, and was heterogeneous hypointense in the T1A series and heterogeneous hyperintense in the T2A series (Figure 1A, 1B). Due to the combination of hypertension and an adrenal mass, a PCC was suspected and the relevant investigations were performed. 24-hr urine metanephrine was 13124 µg/L (NR: 50-250), 24-hr urine normetanephrine was 4987 ng/mL (NR: 84-422), plasma metanephrine was 136 ng/mL (NR: <90), adrenocorticotropic hormone was 21 pg/mL, and cortisol was 26 µg/dL. The diagnosis of PCC

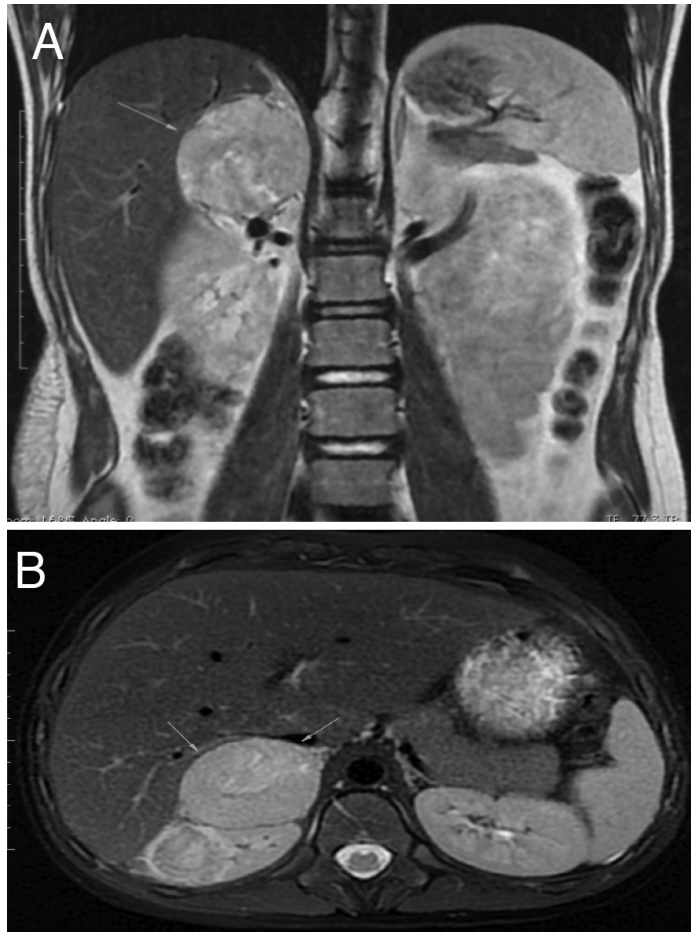

Figure 1. A, B) Abdominal magnetic resonance imaging findings: 41x46x45 mm solid mass lesion (green arrows), which had heterogeneous but diffuse contrast enhancement, was located between the liver and anterior upper pole of right kidney, displaced the liver to anterior

was confirmed by elevated levels of urinary and plasma metanephrines. Gallium-68-dodecanetetraacetic acid tyrosine-3-octreotate (^{68}Ga-DOTATATE) positron emission tomography indicated a 40x55x45 mm mass with well-defined smooth margins between the upper pole of the right kidney and posteromedial of the right lobe of the liver. ACE inhibitor (Enalapril) and furosemide treatment initiated for the patient with the diagnosis of myocarditis were terminated. The patient was started on doxazosin treatment and subsequently on amlodipine for PCC. Doxazosin therapy was initiated at 1 mg/day, the dose was increased with blood pressure monitoring, and then, the calcium channel blocker amlodipine was added at 0.05 mg/kg/day. Blood pressure was brought under control (lowered below the 95th percentile) with both drugs at 10 mg/day. The patient underwent surgery once the LVEF increased to 76%. A high-sodium diet was recommended before the surgery. A saline infusion was initiated the night before the surgery and continued during the surgery for volume expansion. Blood pressure monitoring was performed intraoperatively. No hypotension was observed during and after the excision of the mass. There was no complication during or after the surgery. Pathological findings of the excised mass were compatible with PCC (Figure 2). Histological and immunohistochemical analyses confirmed the diagnosis of PCC.

Discussion

PCC-related CM is frequently associated with stress CM, such as ampulla or Takotsubo, in which there are ST segment changes on ECG and left ventricular apical ballooning. It has been reported with dilated and hypertrophic CM and more rarely with myocarditis in adult patients (5). To our knowledge, no pediatric case presenting with myocarditis has been reported in the literature. As catecholamine-related CM is reversible, early diagnosis and PCC resection are very important, and delayed diagnosis may lead to irreversible cardiac remodeling and death (5).

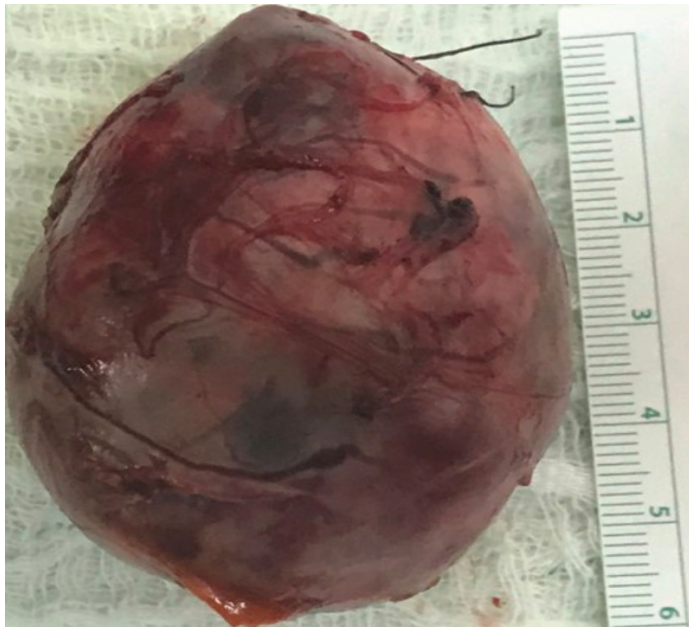

Figure 2. Excised mass: 6x5x4.5 cm nodular lesion

Our patient was diagnosed with myocarditis as the first symptom without the signs and symptoms typical of catecholamine elevation. The clinical presentation of functional PPGLs depends on differences in catecholamine secretion and release, as well as on individual patient sensitivities to catecholamines (6). Furthermore, patients with large tumors exhibit fewer symptoms because of metabolic degradation of most of the catecholamines produced leading to a clinical picture of relatively lower circulating free catecholamines but high urinary excretion of catecholamine metabolites (7). The large tumor diameter in our patient may be another factor in the absence of evident catecholamine-related symptoms.

At admission and during the early days of the first hospitalization, the patient's normal blood pressure was attributed to the low ejection fraction due to myocarditis. Hypertension is reported in 65% of patients with PCC-related CM, and the classic triad of the disease (headache, palpitations, and diaphoresis) is reported in only 4%. The diagnosis of PCC-related CM is usually delayed due to atypical presentations in most of the patients (5).

Catecholamines create a positive inotropic effect by regulating cardiac functions at low concentrations but lead to the following harmful effects at high concentrations (8): epinephrine or norepinephrine activates protein kinase A by binding to B2 receptors and through cyclic adenosine monophosphate (cAMP) to produce an increased contractile response. Increased cAMP induces free radical formation, expression of stress hormone genes, and apoptosis. Excessive catecholamine levels cause functional hypoxia due to increased contractility, decreased blood flow due to coronary spasm, mitochondrial dysfunction caused by excess free fatty acids, and cardiomyocyte damage due to excess intracellular calcium. The catabolism of catecholamines proceeds by two major pathways regulated by monoamine oxidase and catechol-ortho-methyl transferase. When these enzymes become saturated and the concentration of circulating catecholamines is excessive, auto-oxidation mechanisms may be initiated, which leads to the formation of oxidized catecholamines (8).

Our patient had widespread cafe-au-lait spots and a plexiform neurofibroma; he had been followed up in the

neurology department with the diagnosis of NF1. NF1 is an autosomal dominant disorder, which emerges as a result of *de novo* germline mutations in approximately half of the patients. The incidence of PCC among patients with NF1 has been reported to be between 2.9-14.6% (9,10). On the other hand, somatic NF1 mutations were detected in 25% of sporadic PPGLs (11). Considering the low prevalence and slow growth of PPGLs, it has been recommended that asymptomatic patients with NF1 should be screened every three years, starting from 10-14 years of age, and biochemical tests for PPGL should be performed before elective surgical procedures in patients with NF1 (9).

Conclusion

In conclusion, even if there are no signs and symptoms of catecholamine elevation, PCC-related CM may arise. PPGLs should be considered during the evaluation of non-ischemic, non-valvular CM, even if there are no signs of catecholamine excess. Making an accurate diagnosis in the early period will protect these patients from life-threatening complications.

Ethics

Informed Consent: A written informed consent was obtained from the patient's family.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Emrah Şenel, S. Ahmet Uçaktürk, Emine Azak, İbrahim İlker Çetin, Eda Mengen, Pınar Kocaay, Concept: S. Ahmet Uçaktürk, Design: S. Ahmet Uçaktürk, Data Collection or Processing: S. Ahmet Uçaktürk, Analysis or Interpretation: S. Ahmet Uçaktürk, Literature Search: S. Ahmet Uçaktürk, Eda Mengen, Pınar Kocaay, Writing: S. Ahmet Uçaktürk.

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CASE REPORT

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A Rare Etiology of 46,XY Disorder of Sex Development and Adrenal Insufficiency: A Case of MIRAGE Syndrome Caused by Mutations in the *SAMD9* Gene

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What is already known on this topic?

The recently described MIRAGE syndrome, which has autosomal dominant inheritance, is a very rare form of syndromic adrenal hypoplasia.

What this study adds?

Here, we presented the first syndromic adrenal hypoplasia case which was diagnosed with MIRAGE syndrome in Turkey.

Abstract

Adrenal hypoplasia is a rare congenital disorder. In spite of biochemical and molecular genetic evaluation, etiology in many patients with adrenal hypoplasia is not clear. MIRAGE syndrome is a recently recognized congenital disorder characterized by myelodysplasia, infection, growth restriction, adrenal hypoplasia, genital phenotypes, and enteropathy. Here we present a case of MIRAGE syndrome due to a heterozygous missense variant (c.2920G > A; p.E974K) mutation in the sterile alpha motif domain-containing protein-9 *(SAMD9)* gene. This report describes the first MIRAGE syndrome patient in Turkey.
Keywords: Adrenal hypoplasia, 46,XY disorder of sex development, MIRAGE syndrome

Introduction

Normal gonadal differentiation and sex development depend on the meticulous choreography and synchrony of a network of endocrine, paracrine, and autocrine signaling pathways, reflecting the actions and interactions of specific genes, transcription factors and hormones. Perturbations of this intricate network of gene regulation and gene expression governing fetal gonadal development result in disorders of sex development (DSD). These disorders are congenital and involve a spectrum of abnormalities in which the chromosomal, genetic, gonadal, hormonal or anatomical aspects of the sex are atypical (1). DSD patients have been grouped according to karyotype: 46,XY DSD, 46,XX DSD and sex chromosome DSD (1). However, due to the complexities of chromosomal and gonadal development, some diagnoses can be included in more than one of the three major categories. The number of genes identified as being involved in sex development continues to increase. Nevertheless, despite many recent genetic advances, the specific molecular etiology of the genital ambiguity in an individual cannot always be identified.

The recently described MIRAGE syndrome (OMIM# 617053), which has autosomal dominant inheritance, is a very rare form of syndromic adrenal hypoplasia. Its prevalence is < 1/1000000 and the six core characteristic features are myelodysplasia, recurrent invasive infections, growth restriction, adrenal hypoplasia, genitalia anomalies, and enteropathy. Additional associated features are variable and include prematurity, chronic lung disease, developmental delay, dysmorphism and central nervous system anomalies (2,3).

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The cause of the syndrome is germ line, heterozygous *SAMD9* variants that usually occur de novo leading to gain of function mutations in *SAMD9*. The cytogenetic location is 7q21.2, which is the long (q) arm of chromosome 7 at position 21.2. This gene encodes a sterile alpha motif domain-containing protein and is widely expressed (expressed in 208 organs). The protein product localizes to the cytoplasm and may play a role in regulating cell proliferation and apoptosis (2,3). *SAMD9* is likely to act as a growth repressor expressed in endothelial cells, and to lesser degree in fibroblasts. Pathogenic variants in the *SAMD9* gene consequently result in excessive growth-restricting activity intrinsic to the protein (4).

We present here a case of MIRAGE syndrome due to a heterozygous missense variant (c.2920G>A; p.E974K) mutation in the *SAMD9* gene.

Case Report

The patient who was five months old presented to our hospital with fever, lack of oral intake, vomiting, and diarrhea. Due to a diagnosis of adrenal insufficiency, the patient was referred to Pediatric Endocrinology Unit after being managed by the inpatient clinic of the infectious diseases department. The medical history revealed that the patient, the third live-born among five pregnancies of a healthy 32-year-old mother, was prematurely born by cesarean section in the 31st gestational week with a birth weight of 930 grams (<3rd percentile) with severe intrauterine growth retardation. The parents were nonconsanguineous and there was no family history of adrenal insufficiency. It was reported that the patient required resuscitation followed by endotracheal intubation due to postnatal respiratory difficulty and was treated with surfactant due to respiratory distress syndrome. In addition the patient medical history included mechanical ventilation for six weeks and intravenous immunoglobulin (IVIG) treatment, which was started for thrombocytopenia that was detected during the follow-up. Steroid therapy and oral salt supplementation were started after the patient was diagnosed with adrenal insufficiency after skin hyperpigmentation was observed on the fifteenth postnatal day. Table 1 shows the results of hormone profile evaluation at 15 days of age and at five months of age. The patient was discharged from an external medical center after four months of treatment in the intensive care unit. At the age five months old, the initial physical examination showed a weight of 3850 grams (<3rd percentile), height of 57 cm (<3rd percentile) and a head circumference of 36 cm (3rd percentile). The patient could not support the

Table 1. Hormonal results of the patient

	Age 15 days	Normal range	Age five months	Normal range
Glucose (mg/dL)	71	70-100	86	70-100
Sodium (mmol/L)	133	136-145	138	136-145
Potassium (mmol/L)	5.33	3.5-5.1	4.14	3.5-5.2
ACTH (pg/mL)	>1250	6-48	92.2	6-48
Cortisol (mcg/dL)	2.7	2.8-23	2.93	2.8-23
Aldosterone (ng/dL)	3.63	19-141	2.21	5-90
Plasma renin activity (ng/mL/h)	15.14	11-167	3.93	2.35-37
TSH (mIU/mL)			7.9	0.9-7.7
Free T4 (ng/dL)			1.02	0.75-1.49
Testosterone (ng/dL)			23	75-400
FSH (mIU/mL)			0.69	0.16-4.1
LH (mIU/mL)			0.36	0.02-7
DHT (ng/dL)			24.35	*
Androstenedione (ng/dL)			18	6-68
17-OHP (ng/dL)			38	**
AMH (ng/mL)			141	39.1-91.1

ACTH: adrenocorticotropic hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, T4: thyroxine, TSH: thyroid-stimulating hormone, AMH: anti-Mullerian hormone, DHT: dihydrotestosterone, 17-OHP: 17-hydroxyprogesterone.

*DHT decreases rapidly during the first week, then increases to 12-85 ng/dL between 30-60 days. Levels then decrease gradually to prepubertal values by seven months (prepubertal children <3 ng/dL).

**Levels increase after the first week to peak values ranging from 40-200 ng/dL between 30 and 60 days. Values then decline to prepubertal range before one year (1-10 years: 3-90 ng/dL).

head and frontal bossing was present. External genital examination showed a pubic hair Tanner stage 1 with testes 1 ml bilaterally, in the middle and proximal portions of the inguinal canal. His stretched penile length was 2.0 cm. The patient was reported to receive 30 mg/m^2/day hydrocortisone therapy. During follow-up, diarrhea was resistant to therapy and there was enteropathy that indicated colitis. The patient also had thrombocytopenia and/or anemia that required recurrent transfusions. IVIG therapy for thrombocytopenia was again administered. As the patient could not tolerate oral intake, nutritional support was provided by nasogastric tube feeding. For respiratory distress, intermittent oxygen was supplied by nasal cannula. Combined antibiotic treatment was applied for recurrent attacks of infection and sepsis. A stress dose of IV hydrocortisone (100 mg/m^2/day) was administered. Serum electrolytes were normal. Therefore, fludrocortisone acetate and sodium chloride were not administered. Table 1 presents the results of laboratory tests for adrenal insufficiency and DSD in the patient. Scrotal ultrasonography imaging; the right testis, which was measured as 9x8x4.5 mm (vol: 0.2 cc), was localized in the middle portion of the right inguinal canal, while the left testis, which was measured as 6x8x8 mm (vol: 0.2 cc), was observed in the proximal portion of the left inguinal canal. Surrenal ultrasonography imaging; there was no imaging evidence that indicated the presence of adrenal glands, so bilateral adrenal hypoplasia was assumed. The peripheral chromosomal analysis resulted in a 46,XY karyotype. The presence of adrenal insufficiency, adrenal hypoplasia, the anomaly of the genitalia, resistant diarrhea, invasive infections and recurrent thrombocytopenia with episodes of anemia were compatible with MIRAGE syndrome. The results of bone marrow aspiration biopsy for delineating the etiology of thrombocytopenia, anemia and neutropenia were not compatible with myelodysplastic syndrome (MDS). The cytogenetic investigation of bone marrow revealed a 45,XY, -7 [4]/46,XY [3] karyotype. This result was described as mosaic monosomy 7. Therefore, *SAMD9* sequencing was performed and identified a heterozygous missense variant c.2920G > A; p.E974K mutation in exon 3 in the *SAMD9* gene. As the patient developed intolerance to oral intake due to vomiting and diarrhea, the decision to cease enteral feeding and to start total parenteral nutrition was made. The patient was admitted to the intensive care unit upon worsening of the general condition with tachypnea, tachycardia, and fever. Hypotension, decrease in respiratory sounds and coagulopathy developed during the follow-up and the patient was lost due to multisystem organ failure.

Result

In light of these clinical findings, the case was diagnosed as MIRAGE syndrome. Full gene sequencing of the patient was performed. In the patient, a heterozygous one base change (c.2920G > A) leading to a missense mutation p.E974K (p.Glu974Lys) was indentified in the *SAMD9* gene. The variant was previously reported to be pathogenic with gain-of-function effect in patients with MIRAGE syndrome (2,5). This variant was not found in public SNP databases (dbSNP136, 1000 genomes, the NHLBI Exome Sequencing Project Exome Variant Server, or The Exome Aggregation Consortium). *In silico* prediction methods, SIFT and Clinvar, indicated that the mutation would be pathogenic.

Genetic analysis of parents could not be performed to confirm the *de novo* nature of the variant because the parents' blood samples were not available. The family was requested to attend for blood sample collection, but they did not come.

Written informed consent was obtained from the patient's family for publication of the case.

Discussion

Adrenal hypoplasia is a rare, congenital and life-threatening disease. Patients with adrenal hypoplasia are clinically classified into two categories: the first is without any extra-adrenal features (non-syndromic adrenal hypoplasia), and the second category is with such features present (syndromic adrenal hypoplasia).

The genes responsible for the former category include those that code for the corticotropin receptor *(MCR2)* or its accessory protein *(MRAP)*, *DAX1* transcription factor *(NR0B1)*, nicotinamide nucleotide transhydrogenase *(NNT)* and mitochondrial thioredoxin reductase (TXNRD2). The syndromic category includes four different forms which are AAA syndrome (*AAAS* mutations), IMAGE syndrome (*CDKN1C* mutations), MIRAGE syndrome (*SAMD9* mutations), and a syndrome with *MCM4* mutations (2).

New advances in molecular genetics technologies have led to the identification of various rare gene defects in patients with primary adrenal insufficiency. However, 20-60 % of patients with primary adrenal insufficiency remain genetically undiagnosed (6). Previous molecular genetic studies in MIRAGE syndrome have specifically targeted patients with adrenal insufficiency. The first two studies of MIRAGE syndrome were reported by studies from Japan and the United Kingdom and 94 % of patients with *SAMD9* variant from these two reports had adrenal insufficiency (2,3).

MIRAGE syndrome was first described by Narumi et al (2016) (2) in 11 patients that showed strikingly similar phenotypes, including prenatal and postnatal moderate to severe growth restriction. The presence of skin hyperpigmentation, even before the onset of salt-losing symptoms in these patients, led to a suspicion of adrenal insufficiency and adrenal hypoplasia was detected via ultrasonography in seven patients. The extent of neurodevelopmental effects varied among patients as four patients out of eight, who survived the first year of life, did not have head support and any speech. Out of the seven patients with the 46,XY karyotype, underdevelopment of the genitalia with microphallus, cryptorchidism and hypospadias was observed in six; and one of the patients had complete female external genitalia at birth. During the early toddler years, all of the patients had thrombocytopenia and/or anemia that required transfusions which spontaneously resolved. Serious invasive infections, such as sepsis, meningitis and fungal infections were observed at all times; six patients died before the age of two years, mainly due to invasive infections. Two patients, who were diagnosed with chromosome 7 mosaic monosomy and developed MDS died due to complications. The MDS in these patients developed at two and three years of age. Heterozygote *SAMD9* gene mutations were detected in all of the patients (2).

The clinical features of our case were similar to previously described patients' findings. Although mosaic monosomy 7 was detected in our patient, MDS did not develop. However, our patient died soon after presentation due to invasive infections and thus it was not possible to establish any evidence of future MDS development. The relationship between MIRAGE syndrome and MDS is complex. MDS is a heterogeneous disease, characterized by clonal hematopoiesis, the proliferation of ineffective blood cells and an increased risk of acute leukemia. In half of the patients with MDS, there are chromosomal abnormalities that most commonly include interstitial or complete deletion of chromosome 7 (7,8).

SAMD9 is known to be a potent and widely expressed growth repressor (9,10). The first described *SAMD9* mutation responsible for human disease was the homozygote p.K1495E variant that caused familial normophosphatemic tumoral calcinosis (9). Cells with *SAMD9* mutations are characterized by structural and functional variations in the endosomal system (2). The propensity of cells to overcome the growth restriction of mutant *SAMD9* protein, somatic monosomy 7, 7q deletion or even somatic deletion-nondisjunction mutations are observed in MIRAGE patients with or without any evidence of MDS (2,3). In spite of the loss of the whole of chromosome 7, cells with loss-of-function mutations would likely gain a survival advantage over mutated cells with growth restriction. A similar "aneuploidy adaptation" mechanism in disordered cells has been reported in a mouse model with fumarylacetoacetate hydrolase deficiency (11). The first evidence of the adaptation-by-aneuploidy mechanism in humans by deletion of chromosome 7 in *SAMD9* mutation carriers was reported by Narumi et al (2016) (2).

Gonadal differentiation has a significant effect on gender development in human embryos. Understanding the developmental biology and embryology of the urogenital system is crucial to categorization and definition of the molecular basis of the disease and, if possible, the treatment of an individual patient. Sexual differentiation refers to the process through which male or female phenotype develops. Throughout the first two months of human gestation, both sexes develop in the same way. The gonads, internal genital ducts and external genital structures all develop from bipotential embryologic tissues. Each cell in the developing gonad has the potential to differentiate into either a testicular or ovarian cell.

The gonads are derived from intermediate mesoderm. In humans, between the fourth and sixth gestational weeks, the urogenital ridges develop as paired protrusions of the coelomic epithelium (mesothelium). The gonads, adrenal cortex, kidney and reproductive tract originate from the urogenital ridges. Several genes are necessary for the development of the bipotential gonad. Due to their origin as part of the developing urogenital system, ovaries and testes are initially located high in the abdomen near the kidneys. One of the earliest morphologic changes is increased proliferation and size of developing 46,XY gonads. 46,XY DSDs include disorders of testicular development, disorders of androgen synthesis and action, replacing and expanding the former category of male pseudohermaphroditism, and XY sex reversal (12).

The *SAMD9* gene is expressed in many tissues including the adrenal gland and gonad. Mutations of this gene occur with many disorders including effects in these tissues. Histological examination of placenta tissues obtained from patients with MIRAGE syndrome, performed in order to reveal DSD mechanisms, have revealed characteristic placental villous deterioration. Mutated *SAMD9* proteins have potent growth-restricting capacity, and thus they can directly cause systemic growth restriction and testicular hypoplasia. Additionally, *SAMD9* variants also affect the placenta, resulting in poor vascular supply and suboptimal human chorionic gonadotropin (HCG) stimulation. Testicular hypoplasia and insufficient HCG stimulation result in lack of testosterone synthesis. The assumption that the coexistence

of the two mechanisms, the direct effect by a pathogenic variant and the indirect effect by placental insufficiency is a plausible mechanism leading to a serious clinical phenotypes (13).

Although the inheritance mode is autosomal dominant, the fact that less than 25% of all reported patients are female suggests that MIRAGE syndrome might be overlooked in girls. Female patients with 46,XX karyotype do not show any external genitalia abnormalities although ovarian dysgenesis is present histologically (2). In all of the 15 patients with 46,XY karyotype, there were external genital anomalies that ranged from hypospadias to full female phenotype (13). In addition, the early diagnosis in the current case was mainly due to the 46,XY DSD of our patient. It is possible that female patients without external genital anomalies may die before any definitive diagnosis is reached.

There is a broad spectrum of phenotype variation related to *SAMD9* (14). These variations can pose difficulties in the clinical diagnosis of MIRAGE syndrome. However, some features are notable. Adrenal insufficiency seems to be a relatively consistent feature and was the reason for the identification of the largest cohort described to date. Previous reports underline the features of the systemic disorder and, in particular, the high rates of death in MIRAGE syndrome. In order to improve the outcome, an early diagnosis that might lead to appropriate medical intervention is required. This case of MIRAGE syndrome, with a previously identified p.E974K mutation, emphasizes the dysmorphology and other findings that might assist in the earlier detection of this disorder.

Conclusion

MIRAGE syndrome is difficult to diagnose correctly in patients with syndromic adrenal hypoplasia due to various genetic etiologies and overlapping clinical and biochemical features. This is the first report from Turkey of syndromic adrenal hypoplasia which was diagnosed with MIRAGE syndrome.

Ethics

Informed Consent: A written informed consent was obtained from the patient's family.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Eda Mengen, Aynur Küçükçongar Yavaş, Concept: Eda Mengen, Design: Eda Mengen, Data Collection or Processing: Eda Mengen, Aynur Küçükçongar Yavaş, Analysis or Interpretation: Eda Mengen, Literature Search: Eda Mengen, S. Ahmet Uçaktürk, Writing: Eda Mengen.

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CASE REPORT

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A Novel Missense Mutation in Human Receptor Roundabout-1 *(ROBO1)* Gene Associated with Pituitary Stalk Interruption Syndrome

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What is already known on this topic?

Pituitary stalk interruption syndrome (PSIS) is a rare, congenital anomaly of the pituitary gland characterized by pituitary gland insufficiency, thin or discontinuous pituitary stalk, anterior pituitary hypoplasia, and ectopic positioning of the posterior pituitary gland. The underlying genetic etiology for the vast majority of cases remains to be determined.

What this study adds?

Heterozygous mutations of the human Receptor Roundabout-1 *(ROBO1)* gene were recently shown to be responsible for PSIS. Here, we used an exome sequencing approach to reveal a novel missense mutation (c.1690C > T, p.Pro564Ser) in *ROBO1* gene in a 4 year boy with with PSIS, The mutation was carried by his mother, whose pituitary magnetic resonance imaging showed also an abnormality.

Abstract

Pituitary stalk interruption syndrome (PSIS) is characterized by the association of an absent or thin pituitary stalk, an absent or hypoplastic anterior pituitary lobe and an ectopic posterior pituitary (EPP) lobe. The causes of this anatomical defect include both genetic and environmental factors. Molecular genetic defects have been indentified in a small number of patients with PSIS. A 4-year-old boy presented with hypoglycemia and hyponatremia associated with growth hormone, thyroid stimulating hormone, and adrenocorticotropic hormone deficiencies. The patient had right sided strabismus. magnetic resonance imaging images showed pituitary hypoplasia, EPP and absent pituitary stalk. A novel Receptor Roundabout-1 *(ROBO1)* missense mutation (c.1690C > T, p.Pro564Ser) that may contribute to the disorder was found in this patient and his mother, who also exhibited pituitary abnormalities.

Keywords: Receptor Roundabout-1 gene, pituitary stalk interruption syndrome, combined pituitary hormone deficiency, missense mutation

Introduction

Pituitary stalk interruption syndrome (PSIS) is a rare disorder due to the blocked transportation of hormones from the hypothalamus to the pituitary. The estimated incidence of this disorder is 0.5/100,000 births (1,2). Patients with PSIS are characterized by a combination of specific pituitary hormone deficiencies. There are typical findings evident on cranial magnetic resonance imaging (MRI), including interrupted or thin pituitary stalk, absent or ectopic posterior pituitary, and anterior pituitary hypoplasia (3,4). The diagnosis of this syndrome mostly depends on MRI imaging in combination with classical clinical and laboratory findings.

Despite intensive research into PSIS, the etiology remains unknown in 95% of cases, although genetic causes are suspected. In the human, mutations in *LHX4, OTX2, HESX1, SOX3, PROKR2, GPR161* and *CDON* have been postulated to be associated with PSIS. Recently, mutations in the Receptor Roundabout-1 *(ROBO1)* gene have been reported in five patients with PSIS (5), confirming its genetic association with PSIS.

Here, we report a case of PSIS with multiple anterior pituitary deficiencies and the classical triad of MRI findings, in which whole exome sequencing (WES) analysis identified a novel heterozygous mutation in the *ROBO1* gene. This mutation is also carried by his mother, who also had abnormal pituitary

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function and short stature, thereby providing strong circumstantial evidence for the association between this variant and the familial pituitary abnormalities.

Case Report

The patient was a 4-year-old boy who was admitted to our hospital with an episode of generalized tonic-clonic seizures. The episode was not associated with fever or any sign of infection. He was born fullterm at 39 weeks gestation and delivered with normal birth parameters (weight 2860 g: 10th-25th centile, length 50 cm: 50th centile). Psychomotor development was normal. At presentation the height of the patient was 97 cm (< 3th centile) and his weight was 16 kg (10th-25th centile). Findings on physical examination were unremarkable apart from right strabismus (Figure 1). The penis was 2 cm (stretched) and the testicular volume was 1 mL bilaterally. The patient was the first child of non-consanguineous Chinese parents. The father was healthy and of normal stature (170.5 cm, 25th-50th centile), while the mother had a short stature (146 cm, < 3th centile). No further details of the mother's medical history were available. His maternal grandmother also reported short stature and strabismus.

The patient was found to have severe hypoglycemia with a blood glucose concentration of 0.92 mmol/L (normal, 3.3-5.5) and severe hyponatremia with a blood sodium (Na) of 117 mmol/L, (normal, 135-155). Hormone concentrations and biochemical parameters measured during hypoglycemia were as follows: serum insulin 0.9 mU/L, serum cortisol 0.55 ug/dL (normal, 6.2-19.4l), adrenocorticotropic hormone (ACTH) 9.8 pg/mL, urine ketone bodies were negative, plasma lactate 1.8 mmol/L (normal, < 2) and serum ammonia 52.2 mol/L (normal, < 80). The levels of creatinine kinase, creatine kinase MB fraction, organic acids, amino acids, acylcarnitines and free carnitine in plasma were normal. Blood gas analysis was pH 7.44, PCO_2 29.6 mmHg, HCO_3 22.4 mmol/L, BE -0.6 mmol/L. No abnormalities were detected on complete blood count. Hemoglobin A1c was 5.6% (normal, 4-6%). Growth hormone (GH) deficiency was attributed to this patient after insulin tolerance test and L-dopa test, with a peak GH of 0.3 and 0.05 ng/mL, respectively. Insulin like growth factor-1 (IGF-1) was 25 ng/mL (normal, 66-427) while prolactin was in the normal range. In addition to GH deficiency, he was diagnosed with central hypothyroidism [free T4, 8.6 pmol/L (normal, 10.8-20), thyroid stimulating hormone (TSH): 0.489 ulU/mL (normal, 0.8-5)]. Luteinizing hormone (LH) was 0.13 IU/L and follicle-stimulate hormone (FSH) 0.7 IU/L. The karyotype was 46,XY. Echocardiogram, electroencephalogram (EEG) and video-EEG showed no abnormalities. Cranial MRI revealed a small anterior pituitary gland, absent pituitary stalk and an ectopic posterior lobe (Figure 2). A diagnosis of PSIS was made based on these clinical and laboratory findings. The patient presented with combined pituitary

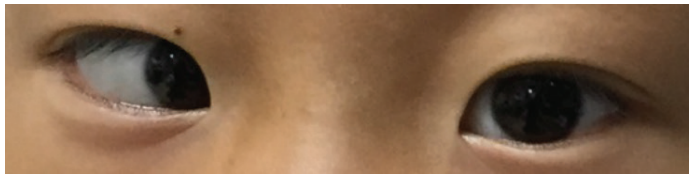

Figure 1. Image of the patient showing right strabismus

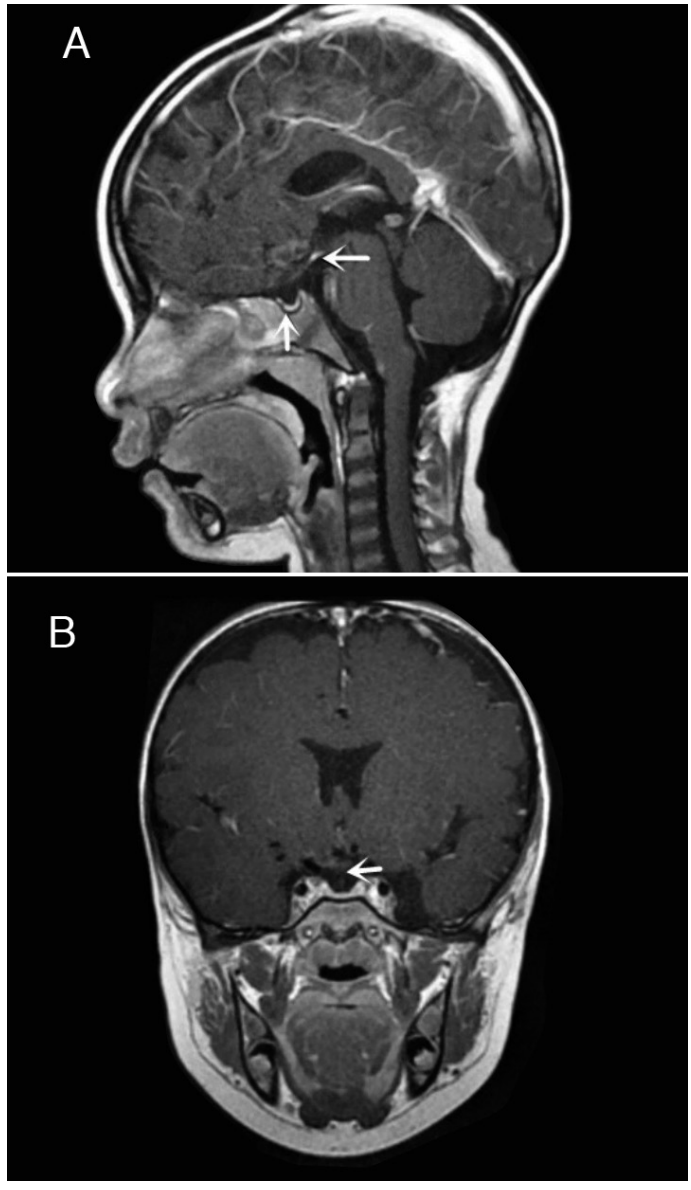

Figure 2. Sagittal and coronal magnetic resonance imaging of the pituitary confirming pituitary stalk interruption syndrome

(A) Sagittal view. The small anterior pituitary (vertical arrow) and the posterior lobe was localized at the hypothalamic region (horizontal arrow). (B) Coronal view. The pituitary stalk is absent

hormone deficiency (CPHD) including GH deficiency, central hypothyroidism and central adrenocortical insufficiency. He was then treated with saline and hydrocortisone and a good response to this was obtained with stabilized blood sugar and blood Na concentrations. Subsequently, thyroxine (LT4) and GH replacement therapy were started.

Genetic Analysis

DNA samples obtained from the family were sequenced to identify the causal gene using WES. DNA was isolated from peripheral blood using DNA Isolation Kit (Bioteke Corporation, AU1802, Wuxi, China). Genomic DNA samples (1 μg) were fragmented into 200-300 bp portions using a Covaris Acoustic System (Covaris, Woburn, MA, USA). The DNA fragments were then processed by end-repairing, A-tailing, adaptor ligation and a four-cycle pre-capture polymerase chain reaction amplification, after which all exons and the 50 bp bases in their adjacent introns were captured by SeqCap EZ Med Exome Enrichment Kit (Roche, Madison, WI, USA). Post-capture amplification and purification was performed on the DNA library and then sequenced on an Illumina HiSeq X Ten platform (Illumina, San Diego, CA, USA) mannually. The raw data produced were then filtered and aligned with the human genome reference (hg19) using the BWA Aligner (http://bio-bwa.sourceforge.net/) and variants were identified using NextGene V2.3.4 software (Soft genetics, LLC, State College PA, USA). The data had a 151.24× mean read depth and about 97.95% of the targetbases were covered at 20× average read depth. The filtered variants were then annotated by using NextGene V2.3.4 and the laboratory's own scripts to get related information, including the conservation of nucleotide bases and amino acids, prediction of the biological functions, frequency in normal populations (compared with 1000 Genomes, ExAC, dbSNP database and local specific databases), and the data from HGMD, Clinvar and OMIM. The potential effect of the variants were predicted by SIFT and Polyphen-2 (6,7,8). All variants of pathogenicity were interpreted according to the American College of Medical Genetics standards and categorized (9). The *ROBO1* gene has three transcripts recorded in the National Center for Biotechnology Information, of which NM_002941.3 was used as the reference sequence. Potentially pathogenic variants were verified using Sanger sequencing.

WES data filtering identified a heterozygous c.1690C>T, p.Pro564Ser variant (RefSeq: NM_002941.3; Chr3: 78717393) in the *ROBO1* gene (Figure 3). Segregation studies revealed that the mother was also a carrier of the same mutation. This rare sequence variant was further predicted to be "probably damaging" with a score of 0.999 in Polyphen-2 and "damaging" with a score of 0.01 by SIFT. Multiple amino acid sequence alignments showed that p.Pro 564 is highly conserved in human, Chimps (Pan troglodytes), mice (Mus musculus), zebra fish (Danio rerio), frogs (Xenopus tropicalis) and chickens (G gallus) (Table 1).

The mother, who has short stature, also carried the same *ROBO1* variant. She then underwent endocrine evaluation which showed an ACTH of 38.8 pg/mL, cortisol of 8.1 ug/dL,

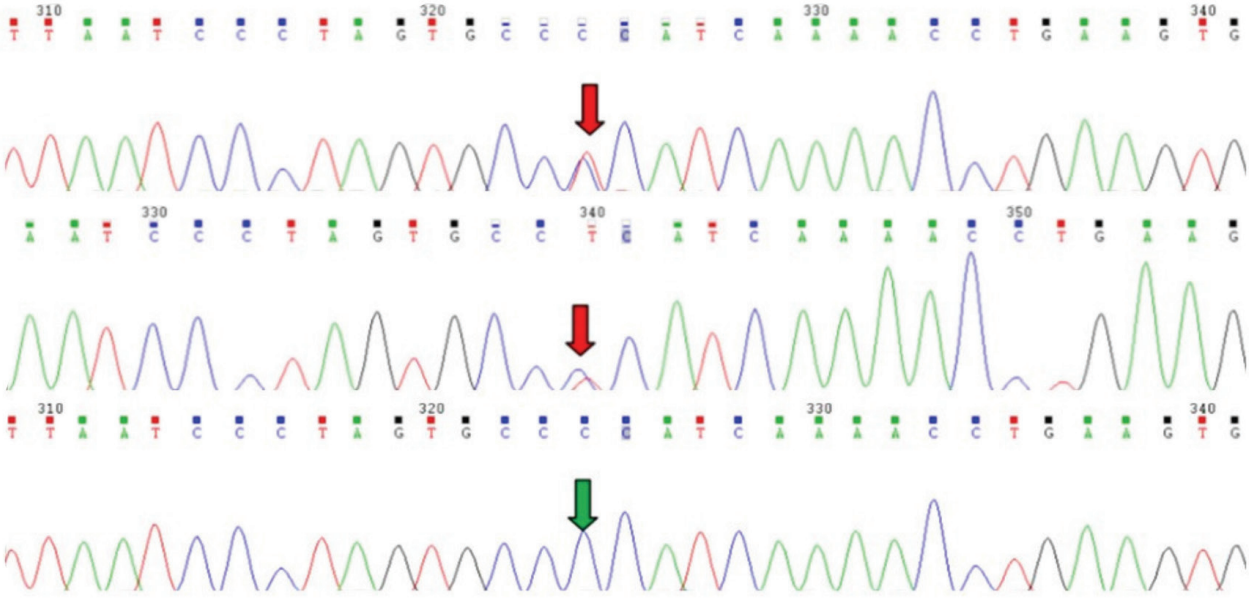

Figure 3. Sanger sequencing results of the family

The heterozygous c.1690C>T, Pro564Ser *ROBO1* mutation is found in the patient (top) and his mother (middle). The father (bottom) has no mutation. The red arrows show the mutation

IGF-1 of 188 ng/mL (115-307 ng/mL), FT4 9.4 pmol/L, TSH: 2.16 ulU/mL, LH: 2.97 IU/L, FSH: 5.34 IU/L and E2 of 67 pg/mL. Her pituitary MRI showed a thin pituitary stalk with hypoplasia of the adenohypophysis (Figure 4).

Discussion

The patient reported here had right strabismus and CPHD in GH, ACTH and TSH. His mother, who also carried the variant, had abnormal pituitary function, short stature but normal eye structure while the patient's maternal grandmother had short stature and strabismus. Though DNA was not available from the deceased grandmother, this may reflect phenotypic variability in this family. The phenotypic variability found in the patient and his mother could be due to the impact of other genes in pituitary development or gene-environment interactions (10), and is similar reports of the missense variants involving *HESX1* and *LHX4* genes (11), of which the heterozygous variants are characterized by highly variable phenotypes amongst family members.

To date, several etiological factors have been proposed for PSIS, and there is good evidence for a polygenic etiology. *HESX1, LHX4, OTX2, SOX3*, and *PROKR2* have been reported to be associated with PSIS (12,13,14). In 2017, five unexplained PSIS cases including two famial cases identified one nonsense, one missense and one frameshift mutation (all heterozygous) in the *ROBO1* gene by WES (5) and this report was the first to identify novel heterozygous frameshift, nonsense and missense variants (p.Ala977Glnfs*40, two affected sibs; p.Tyr1114Ter, sporadic case and p.Cys240Ser affected child and paternal aunt) in *ROBO1* gene (Table 2) (15). Of these five cases, three showed isolated GH deficiency and the other two presented with combined GH and TSH deficiencies.

Table 1. Alignment of amino acid sequences encoded by the *ROBO1* gene from different species

Species	Aa alignment
Human	RPTDPNLIPSA**P**SKPEVTDVSRNT
P troglodytes	RPTDPNLIPSA**P**SKPEVTDVSRN
M musculus	RPTDPNLIPSA**P**SKPEVTDVSKN
D rerio	RPTDPNLIPSA**P**SKPDVTDVSRT
X tropicalis	RPTDPNLIPSA**P**SKPE
G gallus	RPTDPNLIPSA**P**SKPEVTDVSRN

The P residue is highlighted in **bold** for each sequence

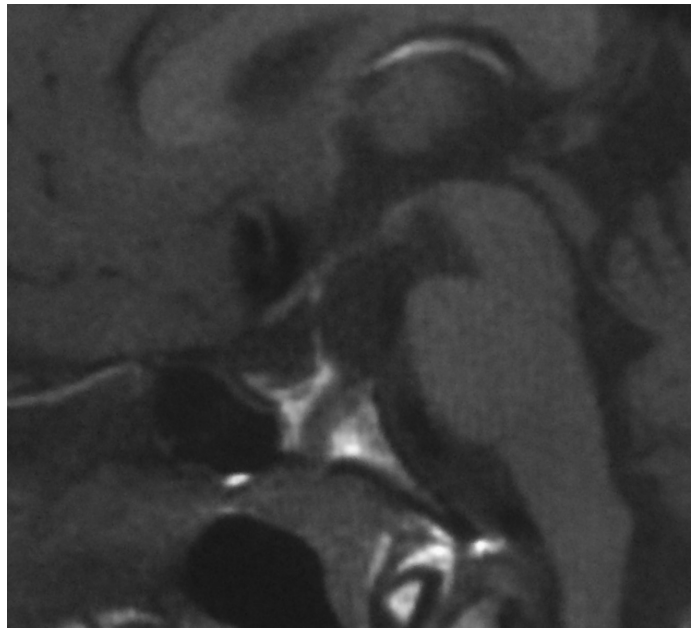

Figure 4. Magnetic resonance imaging (MRI) image from the patient's mother. Her pituitary MRI showed a thin pituitary stalk and hypoplasia of the adenohypophysis

Table 2. Clinical and genetic features of patients with *ROBO1* mutations in pituitary stalk interruption syndrome

Case#	M/F	Mutation	Eyes	Pituitary function	Clinical finding
1 (5)	F	c.2928_2929delG	Strabismus	Isolated GH deficiency	NA
2 (5)	M	c.2928_2929delG	Strabismus	Isolated GH deficiency	NA
3 (5)	M	c.3450G > T	Ptosis	Isolated GH deficiency	NA
4 (5)	F	c.719G > C	Strabismus	Combined GH and TSH deficiencies	NA
5 (5)	F	c.719G > C	-	Combined GH and TSH deficiencies	Cardiomyopathy
6 (15)	M	c.1342 + 1G > A	Strabismus	Combined GH, TSH, PRL, ACTH, LH/FSH deficiencies	psychomotor developmental delay, severe intellectual disability, sensorineural hearing loss, characteristic facial features
Present case	M	c.1690C > T	Strabismus	Combined GH, TSH, ACTH deficiencies	Micropenis

GH: growth hormone, TSH: thyrotropin, PRL: prolactin, ACTH: adrenocorticotropic hormone, LH: luteinizing hormone, FSH: follicle-stimulating hormone, NA: not avalible, M: male, F: female

#Ref. 5, 15

Dateki et al (15) identified a novel homozygous splice site mutation in *ROBO1* (c.1342 + 1G > A) in a 5 year-old boy. The patient had CPHD, psychomotor developmental delay, severe intellectual disability, sensorineural hearing loss, strabismus, and characteristic facial features. Their findings suggest *ROBO1* gene as one of the potential causative genes of PSIS.The clinical phenotype of the patients harboring the *ROBO1* mutation varied in terms of the ocular and endocrine manifestations. In our report, by using next generation sequencing (NGS) technology, we identified a maternal missense mutation (c.1690C > T, p.Pro564Ser) in the *ROBO1* gene in a case diagnosed with PSIS and CPHD. This variant was predicted to be possibly pathogenic by Polyphen-2 and SIFT. Multiple amino acid sequence alignments showed that p.Pro 564 is highly conserved across various species including primates, other mammals birds and fish. All these findings suggest that this variant could play an important role in disease causation. Other patients who harbor *ROBO1* mutations have been reported to share some phenotypic features with the present patients. Four cases presented with strabismus and one case presented with ptosis. These data suggest that mutations in *ROBO1* contribute to ocular anomalies. Cardiomyopathy was seen in one patient and one patient had psychomotor developmental delay. More cases are needed to elucidate the relationship between genotype and phenotype. The *ROBO1* and its ligand Slit are known to influence axon guidance and central nervous system patterning in both vertebrate and nonvertebrate systems (16). Missing expression of *ROBO1* could lead to ectopic differentiation of forebrain neurons. The chemo repulsive ligand Slit and its receptors of the ROBO family are expressed in the developing and adult brain (17) and are crucially involved in the formation of midline commissures. Slit2 and Slit1/2 double knockout animals display defects in corticothalamic and thalamocortical targeting, callosal and hippocampal commissure projections (18) and defects in the formation of the optic chiasm. Calloni et al (19) described a 9-year-old boy with severe intellectual disability, absence of the transverse pontine fiber, thinning of the anterior commissure and corpus callosum, and compound heterozygous variants in the *ROBO1* gene. These findings strongly suggest that human *ROBO1* variants could result in neurodevelopmental disorders. Our patient was subsequently found to exhibit a wide range of symptoms, including classic CPHD and right strabismus. Thus the *ROBO1* gene may be one of the potential causative genes for PSIS and CPHD. Bjorke et al (20) showed that Slit signaling is necessary to inhibit the initiation of oculomotor neuron development. Oculomotor axons at the midline crossing are characterized by an axon-like process that forms from the cell body as a secondary axon. It may be possible that this repolarization is subject to ROBO regulation. Overall, the introduction of NGS technology in the diagnostic workflow will lead to the identification of novel genetic determinants in pediatric patients with pituitary defects (21,22). There is mounting evidence that ROBO variants are associated with ocular as well as pituitary abnormalities.Additional examples of *ROBO1* variants and clinical PSIS cases are needed to explore the function of *ROBO1* and its effect on human embryogenesis and organogenesis.

Ethics

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

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Ziqin Liu, Design: Xiaobo Chen, Data Collection or Processing: Ziqin Liu, Analysis or Interpretation: Ziqin Liu, Literature Search: Xiaobo Chen, Writing: Ziqin Liu.

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CASE REPORT

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Hypoprolactinemia as a Clue to Diagnosis of Mild Central Hypothyroidism due to *IGSF1* Deficiency

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What is already known on this topic?

IGSF1 deficiency has been recently found to be an X-linked cause of central hypothyroidism (CeH). Additionally patients may present hypoprolactinemia, obesity, transient partial growth hormone deficiency, delayed adrenarche, normal timing of testicular enlargement but delayed testosterone rise in puberty resulting in delayed adolescent growth spurt, and adult macro-orchidism.

What this study adds?

We present an asymptomatic boy with mild CeH due to a novel mutation of *IGSF1* gene. He mostly had low normal FT4 levels while PRL was undetectable. If he had not had his PRL levels measured most probably diagnosis would be missed.

Abstract

Loss-of-function mutations of *IGSF1* are an X-linked cause of central hypothyroidism (CeH) and hypoprolactinemia. A boy who is now 15.2 years old presented at the age of 7.69 years for evaluation of obesity. Previous thyroid function evaluation suggested CeH [FT4 0.6 ng/mL, thyroid-stimulating hormone (TSH) 2.2 mIU/L] but his physician took no action. At presentation he was clinically and biochemically euthyroid, prepubertal and obese. Serum prolactin (PRL) was undetectable. Biochemistry was normal except for mild hypercholesterolemia, total cholesterol 198 mg/dL. Subsequently FT4 and TSH levels fluctuated between 0.72-0.95 ng/dL (normal 0.8-2.0) and 1.94-5.77 mIU/L (normal 0.3-5.0), respectively. Sequencing of *IGSF1* gene revealed a novel genetic change c.3805C > T in exon 19; substitution of amino acid Arginine at position 1269 with a premature «stop» codon resulting in an altered protein product. The patient additionally presented delayed adrenarche, low height velocity that resolved spontaneously and normal pubertal onset associated with increased FSH levels. At 14 years-of-age, while the patient was at Tanner stage 4, PRL levels became detectable, rising gradually to 2.3 ng/mL at last examination. Thyroxine replacement therapy resulted in decrease in total cholesterol 103 mg/dL. A high index of suspicion for the disorder is needed since several measurements of thyroid function may be required for CeH to be disclosed. The patient's normal FT4 levels and normal intelligence would have resulted in a missed diagnosis if the serum PRL levels had not been measured. This case highlights the importance of measuring PRL in a boy with low normal FT4 and normal TSH levels.

Keywords: Central hypothyroidism, hypoprolactinemia, *IGSF1*

Introduction

Loss-of-function mutations of the immunoglobulin superfamily, member 1 *(IGSF1)* gene have been recently described as an X-linked cause of congenital central hypothyroidism (CeH) (1), with an estimated prevalence of 1/100000 (2). CeH is the hallmark of the disorder, however, patients additionally may present with hypoprolactinemia, transient partial growth hormone (GH) deficiency (GHD), normal timing of testicular enlargement but delayed testosterone rise in puberty resulting in delayed adolescent growth spurt, and adult macro-orchidism (3). The *IGSF1* gene resides on the X-chromosome and thus its mutations affect mainly males, although female heterozygous carriers may present with CeH (3). The prevalence of low FT4 in female carriers is reported to be 18% (4). The *IGSF1* gene encodes an immunoglobulin superfamily glucoprotein of the plasma membrane and the *IGSF1* protein was

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observed in somatotropes, thyrotropes, and lactotropes of anterior pituitary, whereas it was absent in gonadotropes or corticotropes. Moreover, the *IGSF1* protein is predominantly expressed in testis, muscle, heart and pancreas.

We present a boy with mild CeH due to a novel mutation of the *IGSF1* gene. Additionally, the patient presented with undetectable prolactin (PRL) levels, that proved to be the clue to diagnosis.

Case Report

A boy of Greek descent, who is currently 15.2 years old, presented to our pediatric endocrinology clinic at the age of 7.69 years for obesity evaluation. He is the first child of unrelated parents, born after normal delivery with normal body weight and length. Developmental milestones were achieved at a normal age. During the preschool years he had normal height velocity but increase in body weight. Thyroid function tests (TFT) ordered by his pediatrician, at 3 and 4 years-of-age, were compatible with CeH (FT4 0.5 ng/mL, thyroid-stimulating hormone (TSH) 2.2 mIU/L and FT4 0.65 ng/mL, TSH 1.8 mIU/L, respectively), however, no action was taken. His parents and siblings (a girl and twin boys currently 13 and 9.5 years old, respectively) are healthy. Mother did not breast-feed any of her four children because of inadequate milk production.

At presentation, the patient's height standard deviation score (HSDS) was 122.5 cm (HSDS -0.55). He was prepubertal and euthyroid, with no typical symptoms of hypothyroidism such as fatigue, constipation, or bradycardia. His weight was 35.1 kg (WSDS 1.67), body mass index (BMI) 23.4 kg/m^2 (BMI-SDS 2.89). The thyroid gland was non-palpable. School performance was reported as very good. Target height (TH) SDS was + 1.1.

TFT showed FT4 1.0 ng/dL (0.8-2.0), TSH 1.98 mIU/L (0.3-5.0), PRL < 0.7 ng/mL (3-18), insulin like growth factor 1 (IGF1) 126 ng/mL (110-565) and bone age was 6.7 years. Biochemistry was normal except for a mild increase in total cholesterol 198 mg/dL (< 170), high-density lipoprotein (HDL)-cholesterol 68 mg/dL (> 40), low-density lipoprotein (LDL)-cholesterol 123 mg/dL (< 129) and triglycerides 36 mg/dL (< 150). During the next two years there was fluctuation of FT4 levels between 0.72-0.95 ng/dL, of TSH levels between 1.94-5.77 mIU/L, whereas PRL was always undetectable. Thyrotropin releasing hormone (TRH) test showed a normal TSH response, 0':3.44 mIU/L, 30':14.73 mIU/L, 60':11.71 mIU/L, and an abnormal PRL response 0': < 0,4 ng/mL, 30': 1.7 ng/mL, 60': 0.9 ng/mL. Basal PRL levels became detectable at 1.7 ng/mL at the age of 14 years, at Tanner stage 4, increasing slightly to 2 ng/mL and 2.3 ng/mL at the age of 14.7 years and 15.2 years, respectively. Thyroid ultrasonography revealed a hypoplastic thyroid gland, total thyroid volume 2.1 mL and 2.2 mL at 9 and 15.2 years-of-age, respectively.

At 9.8 years, due to low height velocity (Figure 1), a GH stimulation with glucagon was performed that showed a

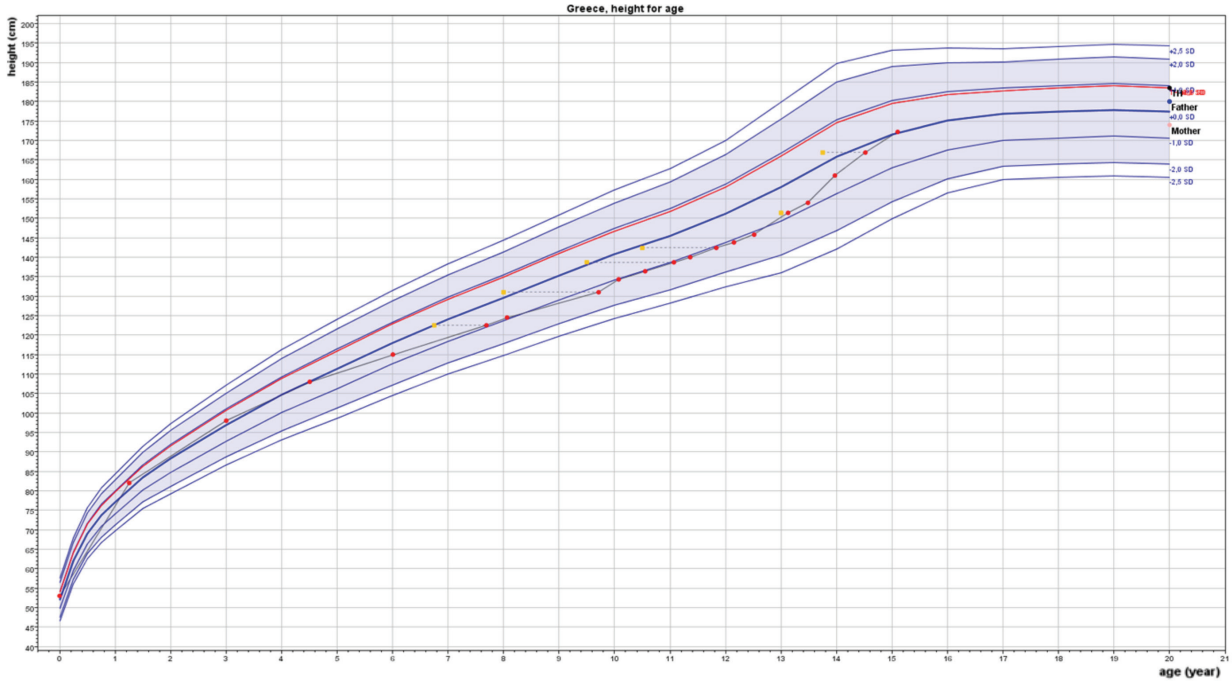

Figure 1. Progression of height. Height velocity normalized spontaneously after the age of 10 years. Squares denote bone ages. Arrow depicts initiation of L-thyroxine treatment

subnormal peak level of serum GH 4.7 ng/mL and normal peak serum cortisol 25.7 μg/dL (normal > 18 μg/dL). Serum IGF1 was 116 ng/mL (normal 110-565). Brain MRI showed a normal pituitary. Soon afterwards the boy presented with spontaneous normalization of height velocity and we therefore suspended further testing of the GH axis. At the age of 11 years thyroxine replacement was started, his FT4 being a little below normal 0.7 ng/dL, which resulted in undetectable TSH. Normalization of FT4 had no substantial difference in the boy's general well-being nor in his growth parameters (height, BMI), however it was associated with a substantial decrease in lipid levels, total cholesterol 103 mg/dL, HDL-cholesterol 51 mg/dL, LDL-cholesterol 45 mg/dL, triglycerides 37 mg/dL.

The boy entered puberty at 12 years of age. FSH levels were increased at 9.3 to 11.4 mIU/mL, during prepuberty and early puberty, whereas LH levels were normal. At last examination, at the age of 15.2 years serum FSH was still increased (15.3 mIU/mL) with normal testicular volume (TV) of 18 mL. Testosterone levels were < 10 ng/mL until TV 12 mL. At last examination testosterone levels were 451 ng/dL, being low normal for a TV of 18 mL. Moreover, the boy presented with delayed biochemical adrenarche [serum dehydroepiandrosterone sulfate (DHEAS) being 12 ng/mL at the age of 7.8 years, 16.1 ng/mL at the age of 9.9 years, 36 ng/mL at the age of 11.8 years and 75 ng/mL at 12.5 years of age] and delayed pubarche at 13 years of age. The patient also exhibited a delayed onset of pubertal growth spurt, at about 13 years of age when TV was 13.5 mL.

Height velocity is normal, as are serum IGF1 levels at 316 ng/mL (152-540), and predicted adult height is within TH.

TFT of his mother showed normal levels of T4 6.24 μg/dL, TSH 2.97 mIU/L, as well as PRL 8.5 ng/mL. His twin brothers also had normal TFT and PRL, brother 1: FT4 1.34 ng/dL, TSH 2.80 mIU/L, PRL 3.6 ng/mL and brother 2: FT4 1.44 ng/dL, TSH 2.87 mIU/L, PRL 4.5 ng/mL.

Molecular Analysis

Analysis of *IGSF1* gene revealed a genetic change, c.3805C > T in exon 19 (Figure 2), that resulted in substitution of amino acid Arginine at position 1269 with a «stop» codon and the production of an altered protein product. This genetic change has not been reported previously in patients with CeH. We also performed analysis of the gene in the boy's mother and sister. His mother was found to carry the same mutation as the proband, but no mutation was found in his sister. *IGSF1* gene analysis was not performed in his brothers because of normal thyroid function in both of them.

Discussion

We identified a novel *IGSF1* nonsense mutation in a Greek patient with congenital CeH. The molecular defect observed in our patient (p.Arg1269X) prematurely truncates the *IGSF1* protein at the end of the 12th Ig loop in the extracellular portion of the C-terminal domain. The *IGSF1* protein includes 12 Ig-like domains in two sets of five and seven motifs separated by a linker region, followed by a transmembrane domain and a short cytoplasmic tail (5). The N-terminal segment undergoes translational proteolysis while the C-terminal is expressed extracellularly at the plasma membrane. The precise molecular role of *IGSF1* remains unclear.

To date, more than 30 distinct mutations have been described including missense, nonsense, frameshift and whole gene deletions (6,7) that lead to loss of protein function. All but one of the reported mutations are located in the C-terminal domain of the protein and impair *IGSF1* trafficking from the endoplasmic reticulum to the plasma membrane. There is no clear genotype-phenotype correlation, while variation in the extent of hypothyroidism or the other clinical features, even within families, has been reported (8,9).

IGSF1 is expressed in thyrotrope cells of the anterior pituitary. *IGSF1*-deficient male mice have reduced serum TSH and decreased pituitary Trhr mRNA levels (1), while others have shown that the principal impairment is attenuated TRH actions in pituitary thyrotropes (10). Garcia et al (11), in a patient with severe congenital CeH due to complete deletion of the *IGSF1* gene, described markedly decreased TSH bioactivity, poor response to TRH stimulation and decreased TRHR expression. Our patient showed a normal

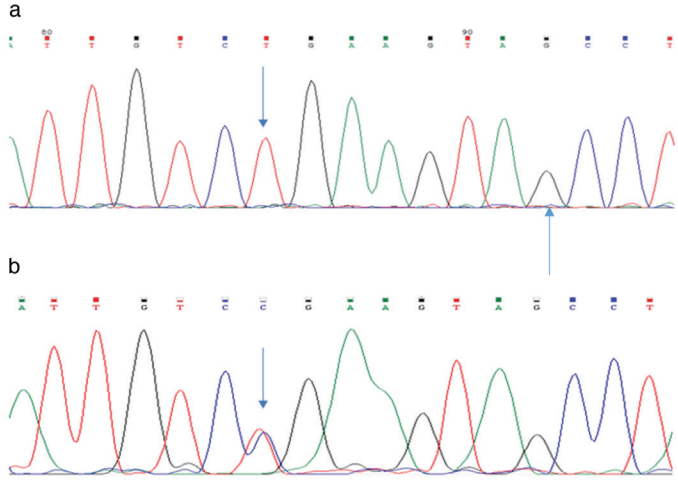

Figure 2. Sequencing of the *IGSF1* gene showing the c.3805C > T (p.Arg1269Ter) genetic change in exon 19: (a) patient, (b) mother

TSH response to TRH stimulation, suggesting impaired endogenous TRH action. Moreover, he had a hypoplastic thyroid gland, a finding observed in 74% of *IGSF1* deficient patients (4).

IGSF1 protein is detected in pituitary lactotropes, however PRL deficiency is present in about 67% of *IGSF1*-deficient patients (3). No explanation for normal PRL levels has been given. Our patient had undetectable serum PRL, and very poor PRL response to TRH stimulation suggesting pituitary dysfunction. However, basal PRL levels became detectable at the age of 14 years showing a gradual increase. It remains to be seen whether PRL will normalize as the child grows older.

Increased birth weight or length is observed in a substantial number of patients (12). 67% of *IGSF1*-deficient male children were classified as overweight and 21% as obese (4), being in accord with the phenotype of our patient. It is unclear how these metabolic alterations are related to *IGSF1* deficiency.

Children with *IGSF1* deficiency present with disharmonious pubertal development, that is pubertal onset at a normal age but delayed testosterone increase occurring at an advanced TV. In adult life testosterone levels are usually low or low normal. Late adolescent and adult patients commonly present with macro-orchidism, however, TV may be normal (13) or increased from the prepubertal years. Our patient entered puberty at a normal age. At onset of puberty the patient's basal FSH levels were increased, LH concentrations were normal for pubertal onset and showed a normal progression according to pubertal status, whereas testosterone levels in early puberty were low for TV but normalized as puberty progressed. It is not clear what causes the disharmonious pubertal development in these patients.

Patients with *IGSF1* deficiency have been reported to present with delayed adrenarche (14). Our patient presented with the marker of biochemical adrenarche, that is serum DHEAS ≥40 μg/dL, after the age of 12 years and pubarche at the age of 13 years. The median age of pubic hair development for Greek boys is 11.2 years (15).

Transient partial GH deficiency has been reported in a subset of patients with *IGSF1* deficiency. It is not clear why our patient exhibited growth deceleration, although subnormal GH secretion, low normal IGF1 levels and the delayed bone age might suggest transient GH deficiency that resolved before adolescence. The period between 6 and 11 years of age in boys constitutes the juvenile phase of growth characterized by growth deceleration relative to the preceding childhood phase and by increase of adrenal androgens (adrenarche) (16). Based on the very low DHEAS levels of our patient during this period we can speculate that low adrenal androgens may exaggerate the normal growth-decelerating pattern of the juvenile period. Normalization of height velocity, which occurred prior to thyroid hormone substitution, might be attributed to the gradual increase of adrenal androgens

Conclusion

In conclusion, we present a male patient with CeH and PRL deficiency due to a novel mutation of the *IGSF1* gene. Additionally, he presented with obesity, disharmonious puberty, and delayed adrenarche which are all features of the *IGSF1* syndrome. The patient had mostly low normal FT4 levels, thus PRL deficiency was the clue to diagnosis. Most reported cases of CeH due to *IGSF1* deficiency are symptomatic necessitating L-thyroxine replacement. We believe, however, that a significant number of patients are undetected because symptoms may be absent or subtle. Diagnosis is important for genetic consultation, since no clear genotype-phenotype correlation is observed, even within the same family. Furthermore, with TSH-based neonatal congenital hypothyroidism screening programs neonatal diagnosis will be missed and definitive diagnosis is likely to be delayed. Children of female carriers and female children of male patients should be screened in neonatal life for FT4 and TSH levels. This case highlights the importance of determining PRL levels in a boy with low normal FT4 and normal TSH levels.

Ethics

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

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Anastasios Papadimitriou, Anna Papadopoulou, Kleanthis Kleanthous, Concept: Anastasios Papadimitriou, Anna Papadopoulou, Design: Anastasios Papadimitriou, Anna Papadopoulou, Vassiliki Papaevangelou, Data Collection or Processing: Kleanthis Kleanthous, Dimitrios Papadimitriou, Analysis or Interpretation: Anastasios Papadimitriou, Anna Papadopoulou, Literature Search: Kleanthis Kleanthous, Dimitrios Papadimitriou, Writing: Anastasios Papadimitriou, Anna Papadopoulou, Vassiliki Papaevangelou.

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

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Keywords: Central hypothyroidism, euthyroid sick syndrome, abetalipoproteinemia

Dear Editor,

I read the article of Soylu Üstkoyuncu et al (1) entitled "Novel *MTTP* Gene Mutation in a Case of Abetalipoproteinemia with Central Hypothyroidism" in Journal of Clinical Research in Pediatric Endocrinology with great interest. The diagnosis of abetalipoproteinemia was suspected in a 9-month-old patient with chronic diarrhea, failure to thrive, decreased subcutaneous adipose tissue, acanthocytosis, elevated liver function test, and decreased triglyceride and total cholesterol levels. Genetic analysis revealed a novel disease-causing variant in *MTTP*. In addition, because of mild-low free T4, normal free T3 and normal thyroid stimulating hormone (TSH) findings, central hypothyroidism was considered and L-thyroxine (12.5 mcg/day) was started and increased to 37.5 mcg/day at follow-up. Other pituitary hormones and pituitary imaging have been reported to be normal.

Central hypothyroidism is the failure of thyroid hormone synthesis associated with insufficient TSH stimulation. This occurs due to anatomical or functional disorders in the pituitary (secondary hypothyroidism) or hypothalamus (tertiary hypothyroidism) (2). Low free T4 and low/normal TSH levels would indicate central hypothyroidism. However, in hypothalamic disorders, slightly elevated TSH levels may be detected, as in subclinical or mild primary hypothyroidism cases (2). In the presented case, low free T4 and normal TSH level were considered to indicate central hypothyroidism. However, lack of TSH suppresion during follow-up with (even increasing) L-thyroxine suggests that the diagnosis of central hypothyroidism is questionable (2). Alexopoulou et al (3) demonstrated that mean TSH levels in treated central hypothyroidism patients were 0.2 ± 0.5 mU/mL. Furthermore, normal (presented as low) baseline and high stimulated levels of cortisol of this patient suggest that thyroid abnormality may be associated with critical disease, not central hypothyroidism. In addition, thyroid function test results similar to central hypothyroidism may be seen in severe disease states, but this condition usually returns to normal with the improvement of the clinical picture (4). The authors report that normal free T3 level in the patient helped ruling out euthyroid sick syndrome, however, it has also been shown that thyroid hormone measurement results in immunoassay methods commonly used in our country are less reliable due to assay interference (5). Liquid chromatography-mass spectrometer method has been reported to offer superior specificity over the immunoassays for determination of thyroid hormones (5). Finally, if a diagnosis of central hypothyroidism is being reported to be associated with abetalipoproteinemia, sequencing of candidate genes are needed as well.

In addition, serum phosphate level (3.15 mg/dL) was considered normal according to adult reference range. However, this level is low compared to the appropriate age group (3.8-6.5 mg/dL) and warrants further assessment .

In conclusion, with limited data presented, the diagnosis of central hypothyroidism is suspicious in this patient and it would have been more appropriate to monitor thyroid function tests without treatment. There are various adaptive changes (such as hypothalamus-pituitary suppression) to reduce metabolic rate in severe disease states, which makes it difficult to diagnose central hypothyroidism. Taking all together, thyroid function tests should not be evaluated in cases with severe illness unless there is a strong suspicion of hypothyroidism.

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NOTES

BH tedavisi

işte bu kadar kolay[1,2*]

Saizen® Likit*,
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Saizen® Likit* 12 mg/1,5 ml kullanıma hazır enjeksiyonluk çözelti içeren kartuştur.[2]

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

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

SAİZEN 12 mg/1,5 mL enjeksiyonluk çözelti içeren kartuş. Steril. Her bir kartuş 1,5 mL çözeltide 12 mg somatropin içerir. Enjeksiyonluk çözelti mL başına 8 mg somatropin içerir. **Terapötik Endikasyonlar:** Çocuklarda ve ergenlerde: Çocuklarda endojen büyüme hormonu salgısının olmaması veya azlığı ile meydana gelen büyüme geriliği. /Kromozom analizleri ile teyid edilmiş, gonadal disgenezili kızlarda büyüme geriliği (Turner sendromu)./ Kronik böbrek yetmezliğinden ötürü prepubertal çocuklarda büyüme geriliği (CRF)./ Doğum ağırlığı ve/veya boyu –2 SD altında olan, gestasyonel yaşa göre küçük doğmuş (SGA) ve 4 yaş ya da üzeri itibariyle büyümeyi yakalayamamış (son yıl içinde HV SDS < 0) kısa çocuklarda büyüme bozukluğu (mevcut boy SDS < -2,5 ve ayarlanmış parental boy SDS < -1). Yetişkinlerde: Yetişkinlerde, büyüme hormonu eksikliğini ölçen tek dinamik test ile teşhis edilen belirgin büyüme hormonu eksikliğinin replasman tedavisi. Hastalar aşağıdaki kriterlere uymalıdır: Çocuklukta başlayan: Çocukluk esnasında büyüme hormonu eksikliği teşhis edilmiş hastalar, tekrar test edilmelidir ve SAİZEN ile replasman tedavisine başlamadan önce hipofiz hormonu eksiklikleri teyid edilmelidir./ Yetişkinlerde başlayan: İzole GH yetmezliğinde, 2 dinamik test ile, ve hipofiz veya hipotalamus hastalığına bağlı durumlarda diğer hipofiz ön lob hormonlarından en az birinin (prolaktin hariç) eksikliği durumunda ise 1 dinamik test ile yetmezliğin kanıtlanması halinde eksikliği yerine koymak amacıyla kullanılır. **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 tavsiye edilmektedir: Çocuklarda ve ergenlerde: Endojen büyüme hormonu salgısının yetersiz olduğu büyüme geriliği gösteren hastalarda: Subkutan uygulama ile günde 0,7-1,0 mg/m² vücut yüzey alanı ya da günde 0,025-0,035 mg/kg vücut ağırlığı. / Gonadal disgeneziden ötürü büyüme geriliği gösteren kızlarda (Turner sendromu): Subkutan uygulama ile günde 1,4 mg/m² vücut yüzey alanı ya da günde 0,045-0,050 mg/kg vücut ağırlığı.Turner sendromlu hastalarda non-androjenik anabolik steroidlerle birlikte tedavi büyümeye cevabı artırabilir. / Kronik böbrek yetmezliğinden ötürü büyüme geriliği gösteren prepubertal çocuklarda (CRF): Subkutan uygulama ile günde 1,4 mg/m² vücut yüzey alanı ya da günde 0,045-0,050 mg/kg vücut ağırlığı. / Gestasyonel yaşa göre küçük doğmuş kısa çocuklarda büyüme geriliği (SGA): Tavsiye edilen günlük doz, subkutan uygulama yoluyla 0,035 mg/kg vücut ağırlığı (ya da 1 mg/m2/gün)'dır. Uygun yetişkin boya ulaşınca ya da epifizler kapanınca tedavi kesilmelidir. 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 (kızlarda) 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, İnsulin-benzeri Büyüme Faktörü 1 (IGF-1) değerleri ile kontrollü şekilde ayarlanmalıdır. Önerilen en fazla büyüme hormonu dozu nadiren 1,0 mg/gün değerini aşar. Genellikle en düşük etkili doz uygulanmalıdır. Yaşlılarda veya kilolu hastalarda, daha düşük dozlar gerekli olabilir. **Uygulama Şekli:** SAİZEN enjeksiyonluk çözeltinin uygulanması için kullanma talimatında ve seçilen oto enjektör (iğnesiz cool.click oto enjektörler veya easypod oto enjektör) ile birlikte verilen kullanma kılavuzundaki talimatlar takip edilmelidir. Hedeflenen easypod kullanıcıları ağırlıklı olarak 7 yaşından büyük çocuklar ve erişkinlerdir. Bu cihazların çocuklar tarafından kullanımı daima yetişkin gözetiminde yapılmalıdır. Böbrek/karaciğer yetmezliği: Pozolojі 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 malign tümor bulgusu varlığında kontrendikedir. Tedaviye başlamadan önce, aktif intrakranial tümörler inaktif olmalı ve antitümör tedavi tamamlanmalıdır. Tümör büyümesine dair bir bulgu varlığında tedavi kesilmelidir. Proliferatif veya preproliferatif diyabetik retinopati durumunda Somatropin kullanılmamalıdır. Açık kalp ameliyatı, karın ameliyatı, çoklu kaza travması, akut solunum yetmezliği, ya da 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 ekstrakranial neoplazili hastalar hekim tarafından dikkatle ve düzenli aralıklarda kontrol edilmelidir. İntrakranial tümöre bağlı sekonder büyüme hormonu eksikliği olan hastalar, altta yatan hastalığın herhangi bir ilerleme ya da yineleme durumu için sık sık muayene edilmelidir. 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 meninjiyomlar olmak üzere intrakranial tümörler, ikincil neoplazmlar içinde en yaygın olanıdır. SAİZEN, beraberinde büyüme hormonu eksikliği teşhisi konulmadığı sürece, büyüme geriliği olan, genetik olarak Prader-Willi sendromu olduğu doğrulanmış pediatrik hastaların uzun dönem tedavisi için endike değildir. 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 kısıtlı sayıda ölsemi vakaları rapor edilmiştir. Bununla beraber, predispoze faktörlerin yokluğunda büyüme hormonu kullananlarda lösemi insidansının arttığına dair bir bulgu yoktur. Somatropin, insülin duyarlılığını azaltabileceğinden dolayı, hastalar glukoz intoleransı yönünden gözlenmelidir. Diabetes mellitusu 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 baş ağrısı, görsel problemler, bulantı ve/veya kusma durumlarında, papilödem için funduskopi önerilir. Papilödem varsa, iyi huylu intrakranial basınç artışı (psödotümor serebri) tanısı düşünülmeli ve uygunsa SAİZEN tedavisi kesilmelidir. Halen intrakranial hipertansiyonlu hastalarda klinik kararı vermede rehberlik edecek yeterli kanıt yoktur. Büyüme hormonu tedavisine tekrar başlanırsa, intrakranial hipertansiyon semptomları için dikkatli gözlem gerekir ve intrakranial hipertansiyon tekrarlarsa tedavi kesilmelidir. Nadir görülmesine rağmen, özellikle karın ağrısı gelişen çocuklarda olmak üzere somatropin ile tedavi edilen hastalarda pankreatit düşünülmelidir. Skolyozun somatropin ile tedavi edilen bazı hasta gruplarında (örneğin Turner sendromu) daha sık olduğu bilinmektedir. Ayrıca, herhangi bir çocukta hızlı büyüme skolyozun ilerlemesine neden olabilir. Somatropinin skolyozun insidansını veya şiddetini arttırdığı gösterilmemiştir. Tedavi sırasında skolyoz belirtileri izlenmelidir. 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. Femur başı epifizlerinde kayma, genellikle büyüme hormonu eksikliği ve hipotiroidizm gibi endokrin bozuklukları ve büyüme sıçramaları ile ilişkilidir. 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, ekleımle 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 (tedavi başlamadan önce 1 yıl süreyle asidoz, hiperparatiroidizm ve beslenme kontrolunu içerir) saptanmalı ve büyüme hormonu tedavisi esnasında sürdürülmelidir. Tedavi böbrek nakli esnasında kesilmelidir. 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 nigricans) oral glikoz tolerans 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 dozaj azaltılmalıdır. Akut kritik hastalık gelişen tüm hastalarda, büyüme hormonu ile tedavinin olası yararları; olabilecek potansiyel riskler düşünülerek değerlendirilmelidir. Büyüme hormonu replasmanının başlatılması inaktif kortizonun kortizole dönüştürülmesini sağlayan bir enzim olan 11ß-hidroksisteroid dehidrogenaz, tip 1 (11ß-HSD1) aktivitesini azaltarak bazı hastalarda ikincil adrenal yetmezliği ortaya çıkarabilir ve glukokortikoid replasmanı gerekebilir. Glukokortikoid replasman tedavisi alan hastalarda somatropinin başlatılması kortizol eksikliğinin ortaya çıkmasına neden olabilir. Glukokortikoid dozunun ayarlanması gerekebilir. Somatropin alan bir kadın oral östrojen tedavisine başlarsa, serum IGF-1 seviyelerini normal yaşa uygun aralıkta tutmak için somatropinin dozunun artırılması gerekebilir. Tersine, somatropin kullanmakta olan bir kadın oral östrojen tedavisini bırakırsa, büyüme hormonu fazlalığı ve / veya yan etkilerin artmasından kaçınmak için somatropin dozunun azaltılması gerekebilir. Lipoatrofiyi önlemek için enjeksiyon farklı yerlere yapılmalıdır. Yetişkinlerde büyüme hormonu eksikliği ömür boyu süren ve tedavi edilmesi gereken bir durumdur. Ancak 60 yaş üstü hastalarda ve uzun süreli kullanımlarla ilgili sınırlı deneyim bulunmaktadır. SAİZEN her dozunda 1 mmol (23 mg)'dan daha az sodyum ihtiva eder, yani aslında "sodyum içermez". Biyoteknolojik ürünlerin takip edilebilirliğinin sağlanması için uygulanan ürünün ticari ismi ve seri numarası mutlaka hasta dosyasına kaydedilmelidir. **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. Büyüme hormonu kortizonun kortizole dönüşmesini azaltır ve daha önceden keşfedilmemiş santral hipoadrenalizmi ortaya çıkartabilir veya düşük glukokortikoid replasman dozlarını etkisiz hale getirebilir. Oral östrojen replasmanı alan kadınlarda, tedavi hedefine ulaşmak için daha yüksek dozda bir büyüme hormonu 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. Gebelerde yapılmış klinik veri mevcut değildir. Hayvanlarda somatropin içeren ürünlerle ilgili yürütülen reproduktif 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. **İstenmeyen Etkiler:** Hastaların %10'a kadarı enjeksiyon yerinde kızarıklık ve kaşıntı yaşayabilir. Yetişkinlerde büyüme hormonu replasman tedavisi esnasında sıvı retansiyonu beklenir. Ödem, eklem şişmesi, artralji, miyalji ve paraesteziler 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 yan 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 delesyonu olan hastalar dışında, büyümede yavaşlama ilişkilendirilmemiştir. Büyüme hormonu gen kompleksinin delesyonuna bağlı boy kısalığı gibi nadir görülen durumlarda, büyüme hormonu ile tedavi büyümeyi geriletен antikorları indükleyebilir. Büyüme hormonu ile tedavi edilmiş veya edilmemiş büyüme hormonu eksik çocuklarda bazı lösemi vakaları rapor edilmiştir. Bununla beraber, predispoze faktörlerin yokluğunda büyüme hormonu kullananlarda lösemi insidansı arttığına dair bir bulgu yoktur. İstenmeyen etkiler, ciddiyetleri açısından azalan sıra içinde yer almaktadır. Bilinmiyor: Lokal veya yaygın aşırı duyarlılık reaksiyonları; Çok seyrek: Hipotiroidizm; Yaygın: Yetişkinlerde: Sıvı tutulması: periferal ödem, sertlik, artralji, myalji, paraestezi; Yaygın olmayan: Çocuklarda: Sıvı tutulması: periferal ödem, sertlik, artralji, myalji, paraestezi; Bilinmiyor: İnsülin rezistansı, hiperinsülinizm ve nadiren hiperglisemi ile sonuçlanabilir; Yaygın: Baş ağrısı (izole), karpal tünel sendromu (yetişkinlerde); Yaygın olmayan: İdiopatik intrakranial hipertansiyon (benign intrakranial hipertansiyon), karpal tünel sendromu (çocuklarda); Bilinmiyor: Pankreatit; Çok seyrek: Femur başı epifiz kayması, ya da femur başının avasküler nekrozları; Yaygın olmayan: Jinekomasti; Yaygın: Enjeksiyon yeri reaksiyonları, Lokalize lipoatrofi (enjeksiyon yeri değiştirilerek önlenebilir). **Doz Aşımı ve Tedavisi:** Önerilen dozları aşmak yan etkilere sebep olabilir. Doz aşımı hipoglisemi ve sonrasında hiperglisemiye yol açabilir. Bundan başka, somatropin doz aşımı sıvı retansiyonuna sebep olabilir. **Saklamaya Yönelik Özel Tedbirler:** Raf ömrü 18 aydır. Kullanım sırasındaki kimyasal, fiziksel ve mikrobiyolojik stabilite, birbirini takip eden 7 gün 25°C'de veya altında olabilecek şekilde, 2°C-8°C sıcaklıkta ise toplam 28 gün olarak gösterilmiştir. Kullanılmayan SAİZEN kartuşu 2°C-8°C arasında buzdolabında saklanmalıdır. Dondurmayınız. Işıktan korumak için orijinal ambalajında saklayınız. İlk enjeksiyondan sonra, SAİZEN kartuşu ya da içinde SAİZEN kartuşu bulunan easypod oto enjektör maksimum 28 gün süresince 2°C-8°C arasında buzdolabında saklanmalıdır. Bu süre boyunca, en fazla birbirini takip eden 7 gün süresince, 25°C altında veya buzdolabı dışında tutulabilir. SAİZEN kartuşu art arda 7 gün boyunca buzdolabının dışında saklandığında, yeniden buzdolabına konulmalı ve ilk enjeksiyon tarihinden itibaren hesaplanacak şekilde 28 gün içinde kullanılmalıdır. Easypod oto enjektör kullanılırken, kartuş cihazın içinde tutulmalıdır. Cool.click iğnesiz oto enjektör, daima SAİZEN kartuşundan ayrı bir şekilde ve buzdolabının dışında saklanmalıdır. Kullanılmış kartuşu ışıktan koruyunuz. **Ruhsat Sahibi:** Merck İlaç Ecza ve Kimya Tic.A.Ş. Atatürk Mh. Ertuğrul Gazi Sk. Metropol İstanbul Sit. C2 Apt. No: 2A/20, Ataşehir/İstanbul Tel: 0 216 578 66 00 Fax: 0 216 469 09 22 **KDV dahil Perakende Satış Fiyatı:** 649,21 TL (02.09.2019). **Ruhsat Numarası:** 2019/160 İlk **Ruhsat Tarihi:** 02.04.2019 **KÜB Onay Tarihi:** 02.04.2019. Reçete ile satılır. Daha geniş bilgi için firmamıza başvurunuz.

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

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