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COMPARISON OF SINGLE DOSE AND MULTI-DOSE hCG STIMULATION TESTS

TEK DOZ VE ÇOĞUL DOZ hCG UYARI TESTLERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: The human chorionic gonadotropin (hCG) stimulation test is widely used to assess testicular steroidogenesis. This test evaluates Leydig cell function and helps to evaluate testosterone synthesis in testicular enzyme defects, hypogonadism, 46,XY sexual development disorders (DSD), and micropenis. This study will compare testosterone responses to a single dose of 5000 IU/m^2 and three consecutive days of 1500 IU/m^2 hCG regimens.

Materials and Methods: The study evaluated 18 patients who were admitted with micropenis, hypospadias, or undescended testis (Group 1) and 18 patients with a molecular diagnosis of androgen receptor insensitivity (56%) or 5α reductase deficiency (44%) (Group 2). The median (interquartile range; IQR) age of the patients were 1.4 (0.8-4.2) years for Group 1 and 0.7 (0.3-0.8) years for Group 2. Baseline testosterone levels were checked and after a single dose of hCG 5000 IU/m^2 injection into the first group, testosterone levels were observed on day one and day four. In the second group, after the injection of hCG 1500 IU/m^2 for three days, testosterone levels were checked on the fourth day.

Results: There was no significant difference in height, weight, BMI SDS, and baseline testosterone levels between groups. After hCG, stimulation testosterone responses did not vary among the groups. Testosterone levels were increased 40-fold and 40.1-fold on day four compared to the baseline in Group 1 and Group 2. In Group 1, testosterone response was significantly higher on day four compared to day one (p=0.001).

Conclusions: The hCG tests performed with a single dose or multidoses showed that there was no difference between testosterone responses and these two tests were applicable. Since

ÖZET

Amaç: hCG uyarı testi, testis steroidogenezini değerlendirmek için yaygın olarak kullanılmaktadır. Bu test hipogonadizm, 46,XY cinsel gelişim bozukluğu veya mikropenisi olan hastalarda Leydig hücre fonksiyonunu, testise bağlı enzim kusurlarını değerlendirmektedir. Bu çalışmada tek doz 5000 IU/m^2 ve 3 gün 1500 IU/m^2 hCG testlerine testosteron yanıtlarının karşılaştırılması amaçlanmıştır.

Gereç ve Yöntem: Çalışmaya mikropenis, hipospadyas veya inmemiş testisi (Grup 1) olan 18 hasta ile moleküler olarak tanılı androjen reseptör kusuru (%56) ve 5α redüktaz eksikliği (%44) olan 18 hasta (Grup 2) dahil edildi. Hastaların median (çeyrekler aralığı) yaşı Grup 1 için 1,4 (0,8-4,2) yıl, Grup 2 için 0.7 (0,3-0,8) yıl idi. Bazal testosteron düzeyi ölçüldükten sonra ilk gruba tek doz 5000 IU/m^2 hCG uygulanarak testosteron düzeyleri 1. ve 4. gün ölçüldü. İkinci gruba ise 3 gün 1500 IU/m^2 hCG uygulanarak testosteron düzeyleri 4. gün ölçüldü.

Bulgular: Grup 1 ve Grup 2 arasında boy, kilo, vücut kitle indeksi SDS ve bazal testosteron seviyeleri arasında anlamlı bir fark yoktu. hCG uyarısı sonrasında testosteron yanıtlarında da anlamlı fark saptanmadı. Testosteron seviyeleri bazale göre kıyaslandığında 4. günde Grup 1'de 40 kat ve Grup 2'de 40.1 kat artış gösterdi. Grup 1 hastalarında testosteron yanıtı 1. gün ile karşılaştırıldığında 4. günde anlamlı olarak daha yüksek idi (p=0,001).

Sonuç: Tek doz ve çoğul doz ile gerçekleştirilen hCG uyarı testlerinin sonucunda testosteron yanıtları benzer çıkmış olup bu sonuç her iki testin de uygulanabilir olduğunu göstermiştir. 5000 IU/m^2 testi daha az enjeksiyon gerektirdiği ve uygulaması kolay olduğu için Leydig hücre fonksiyonunu değerlendirmede bu test tercih edilebilir. Yeterli testosteron yanıtı için tek doz 5000 IU/m^2

the 5000 IU/m^2 hCG test requires fewer injections and is easy to administer, this test may be preferred to an evaluation of the Leydig cell function. For an adequate testosterone response, the researchers suggest measuring testosterone levels on the fourth day after the single dose of 5000 IU/m^2.

Keywords: Disorders of sex development, hCG stimulation test, testosterone, 46,XY

hCG uygulandıktan sonra 4. gün testosteron düzeylerini ölçmeyi önermekteyiz.

Anahtar Kelimeler: Cinsiyet gelişim bozukluğu, hCG uyarı testi, testosteron, 46,XY

INTRODUCTION

Testosterone is the main product of steroidogenesis, and it is produced by Leydig cells. At birth, following the perinatal decline, the hypothalamic-pituitary-gonadal (HPG) axis is active until 3-6 months of extrauterine life and then lies dormant until the onset of puberty (1). The HPG axis reactivates after the pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. This reactivation stimulates the secretion of luteinizing hormones (LH) which leads to testicular testosterone production at the onset of puberty (1). Testosterone and its metabolites drive pubertal development of sexual characteristics and sex differentiation of male patients. Therefore, the evaluation of testosterone concentration and its precursors is helpful to identify the disorders of testicular function and development (1).

The human chorionic gonadotropin (hCG) is a glycoprotein which is produced by the human placenta. hCG is usually obtained from the urine of pregnant women (uhCG), however, a recombinant hCG preparation (rhCG) is also produced (2, 3). hCG activates the LH/hCG receptor on Leydig cells and stimulates sex steroid secretion (2). The hCG stimulation test has been used frequently for the evaluation of testicular steroidogenesis. This test determines Leydig cell function and is used for the evaluation of testicular enzyme deficiencies in hypogonadism, bilateral nonpalpable gonads, micropenis, infertility, 46,XY disorders of sex development, and delayed puberty (4-6). Nevertheless, many different hCG test protocols are reported particularly in terms of the number of injections, application doses, and blood drawing time. Therefore, this study wanted to investigate hCG test protocols with three days 1500 IU/m^2 and single dose 5000IU/m^2, and to define the criteria for a normal response and improvement of the procedure.

MATERIALS AND METHODS

In this study, 36 prepubertal boys were enrolled (Tanner stage I), aged between 0.02-10.4 years old. Eighteen patients were presented with hypospadias, cryptorchidism, or micropenis but a diagnosis had not been obtained yet (Group 1). In Group 1 the hCG stimulation test was performed with one intramuscular injection of hCG 5000 IU/m^2 given early in the morning. Testosterone levels were measured immediately before the injection and then 24 and 96 hours after hCG injection (Group 1).

Another 18 boys had a molecularly verified diagnosis of 5α reductase deficiency (n=8) or androgen insensitivity syndrome (n=10) and received 3 daily 1500 IU/m^2 hCG injection. Blood samples for testosterone were taken just before the hCG injection and then 24 hours after the last injection (Group 2).

Anthropometric measurements of the patients for height and weight were taken by the same auxologist. Body mass index (BMI) was calculated with the formula: BMI = weight (kg)/height (m^2). Standard deviation scores (SDS) of these measurements were calculated using national data (7). Haycock formula was used to calculate the body surface area (BSA).

All blood samples were measured at the same endocrine laboratory using the same methods. Plasma T levels were measured by electrochelumiscence (ECLIA) (Cobas Roche Diagnostics, Mannheim, Germany).

The study protocol was approved by the local Ethics Committee (Date: 17.07.2020, No: 985). Written informed consent was obtained from all parents.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows 21.0 was used for statistical analysis. The distribution between variables was evaluated with the Shapiro–Wilk test. Comparisons were made between single versus multiple doses, and in Group 1 testosterone 24 hours versus 96 hours after injection. Nonparametric tests were used to compare the study variables. The Mann-Whitney U test was used to compare differences between the two independent groups. The Wilcoxon rank-sum test was performed to compare paired repeated testoterone levels in the groups. The Friedman's two way analysis of variance (ANOVA) by ranks test was used to compare the baseline, first day and fourth day testosterone levels within the same groups. Data presented as median [interquartile range (IQR)]. p value <0.05 was accepted as statistically significant.

RESULTS

The median age of patients were 1.4 (0.8-4.2) years for Group 1 and 0.7 (0.3-0.8) years for Group 2. The ages of the patients in Group 1 were higher than Group 2. Accordingly, patients in Group 1 had a higher BSA than Group 1. The characteristics of the patients are presented in Table 1. Baseline testosterone levels were prepubertal in

four in Group 2 were significantly higher than testosterone levels of day one in Group 1 (p<0.001). None of the patients developed side effects related to the hCG test. Comparisons of hCG tests are given in Table 2 and Table 3. Figure 1 shows the median testosterone levels of both groups.

Table 1: Characteristics of patients in Group 1 and in Group 2. Data presented as median (interquartile ranges).

	Group 1 (5000IU/m², single dose) n=18	Group 2 (1500 IU, 3 days) n=18	p
Age (years)	1.4 (0.8-4.2)	0.7 (0.3-0.8)	**0.002**
Weight SDS	-0.6 (-1.4-0.4)	0.02 (-1.1-0.7)	0.613
Height SDS	-1.1 (-2.1 and -0.4)	-0.3 (-1.5-0.2)	0.273
BMI SDS	0.1 (-0.7-1.1)	-0.2 (-0.9-0.5)	0.273
BSA	0.5 (0.4-0.6)	0.4 (0.3-0.5)	**0.01**
Diagnosis n (%)			
Androgen insensitivity syndrome	NA	55.6	
5α reductase deficiency		44.4	

SDS: standard deviation score, BMI: body mass index, BSA: body surface area, NA: not available

Table 2: Comparison of hCG tests. Data presented as median (interquartile ranges)

|	Group 1 (n=18) 5000 IU/m², single	Group 2 (n=18) 1500 IU/m², 3 days	p*
Testosterone (ng/mL)			
Baseline	0.03 (0.03-0.03)	0.2 (0.03-0.8)	**0.025**
Day 1	0.4 (0.2-1.7)	-	-
Day 4	3.3 (1.9-5.3)	4.9 (2.8-6.1)	0.152
Increase (fold)			
Baseline/Day 4	40 (41.6-179.3)	40.1 (3.4-171.8)	0.143
Baseline/Day 1	14 (5.6-57.7)	-	-

*: Mann–Whitney U test

all patients. In Group 1, after the injection of 5000 IU/m², testosterone levels increased to 0.4 (0.2-1.7) ng/mL and 3.3 (1.9-5.3) ng/mL on day one and day four, respectively. Testosterone level on day one was significantly higher than baseline testosterone levels (p=0.005). In addition, testosterone levels on day four were significantly higher than the baseline (p<0.001). In Group 2 the stimulated testosterone level was 4.9 (2.8-6.1) ng/mL and was significantly higher according to the baseline levels (p<0.001). Peak testosterone levels of Group 1 and Group 2 did not differ (p=0.152) however, both were significantly higher than their baseline values. Testosterone levels increased 40-fold in Group 1 and 40.1-fold in Group 2 compared to the baseline (p=0.142). The testosterone levels of day

Table 3: Comparison of hCG tests in the groups

	Group 1 p	Group 2 p
Testosterone (ng/mL)* Baseline-Day 1-Day 4	<0.001	-
Testosterone (ng/mL)** Baseline-Day 1	0.005	-
Testosterone (ng/mL)** Baseline-Day 4	<0.001	<0.001
Increase testosterone** Day 1-Day 4	0.005	-

*: Friedman's two-way analysis of variance by ranks, **: Wilcoxon signed-rank test
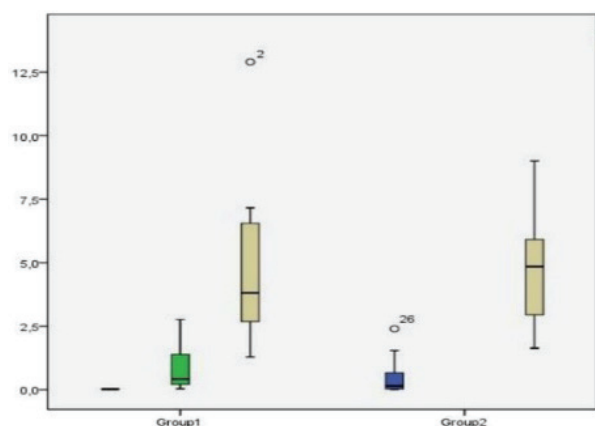
Figure 1: Median testosterone levels of Group 1 and Group 2

DISCUSSION

This study compared a single dose of 5000 IU/m^2 and three consecutive days of 1500 IU/m^2 hCG regimens. Peak testosterone levels in both tests were found to be similar. In Group 1, the testosterone level was significantly higher on day four than day one. As a result, a 5000 IU/m^2 hCG injection and blood drawn on day four was found more pratical than the other options.

The hCG stimulation test is the gold standard test to determine Leydig cell function. In recent years, assays like Insl3, anti-mullerian hormone (AMH), and inhibin B were identified for the evaluation of male reproductive system (8). However, the hCG stimulation test remains a key step in identifying the testicular hormone secretion. Testosterone responses of hCG stimulation tests help to differentiate bilateral nonpalpable gonads, hypogonadotropic hypogonadism, and constitutional delay of puberty from anorchia and some enzymatic defects of testicular steroidogenesis (9).

Nevertheless, poor standardization of hCG test protocols, lack of normative values, and different biochemical methods for steroid hormone measurements are some of the restrictions of hCG stimulation tests (2). hCG is a polypeptide hormone and has similar structure and action with LH because it interacts with the same receptor. In clinical practice, hCG is used when LH activity is needed. hCG has a long plasma half-life and stimulates Leydig cell steroidogenesis through the activation of LH/hCG receptors (10, 11).

In recent years, a recombinant hCG (rhCG) has been defined (250 μg/ampule; ~6500 IU). When compared with uhCG (5000 IU, single dose) the testosterone responses did not differ. Peak testosterone values were reached 72 hours after injections, and the increase over baseline was significantly high in both groups (12). In another study, a single dose application of 6500 IU rhCG was given to unilateral and bilateral cryptorchid boys and testosterone levels were increased from 10 ng/dl to 247.8±135.8 ng/dL and 253.6±128.0 ng/dL: respectively, 7 days after the injection. These studies suggest that rhCG is a favourable test to replace the urinary hCG test for the evaluation of Leydig cell steroidogenesis (3). However, it is more expensive, but rhCG can be used in countries in which uhCG is unavailable (3).

There are a few hCG stimulation tests reported in the literature. Different forms of hCG tests were performed and discrepancies of serum testosterone responses were reported. Previously, 5000 IU/1.7 m^2 single dose hCG was administered to prepubertal boys and their testosterone levels were measured before the injection and thereafter at 0.5, 1, 2, 3, 6, 12, 24, and 36 hours, and then daily for 6 days. The early response (at approximately 2-4h) of serum testosterone was absent in all boys, whereas the late response was constant at 2-5 days (13). Ishii et al. performed the hCG test with a dose of 3000 IU/m^2 for three consecutive days and gave a cut-off testosterone level of 1.1 ng/mL for patients who needed further evaluation and hormone replacement treatment was required (14). In another experiment, the peak testosterone level was reached on the third day and the maximal response was maintained through the fifth day, in normal adult males injected with 10.000 IU of hCG (15). In another study, hCG was applied three consecutive days and the daily dose was adjusted according to the age (1 year old, 500 units; 1-10 years, 1000 units; >10 years, 1500 units) and testosterone levels were measured 24 hours after the final injection. The study found the positive predictive value of the hCG test to be 89% and the negative predictive value of the hCG test to be 100% (16). Kolon et al. investigated 77 prepubertal boys and the hCG dose was applied according to weight (100 IU/kg) or BSA (5000 IU/1.7 m^2). Serum testosterone levels were measured at 72 and 96 hours after the injection. The researchers recommended that a single weight or BSA based hCG dose with testosterone measurement after three or four days was cost effective, practical, and reliable in testicular evaluation (17). Great variability in dosing schedules of hCG tests exist and Table 4 lists some of the previously recommended hCG protocols from the literature (13-20).

The main limitations of this study were the absence of a healthy control group and a small number of patients because this was a single center study for a rare disease. Secondly, a genetic analyses could not be obtained from all the patients so Group 1 patients did not have a certain diagnosis. However, most studies of hCG stimulation tests did not have a molecularly diagnosed patient cohort.

CONCLUSION

This study investigated the testosterone response after one and three daily doses of hCG protocols in prepubertal boys. Testosterone response in both groups was significantly high according to baseline values. Therefore, the hCG test is a reliable method for the evaluation of testosterone biosynthesis. Peak testosterone levels of both groups did not vary significantly. When a comparison of the easy applicability, practicality, and economy of both tests was done, it is suggested that performing a 5000 IU/m^2 rather than the 1500 IU/m^2 three day protocol would be the best method.

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