ORIGINAL ARTICLE

Histopathological Changes that Occur on the Testicular and Penile Tissues Depending on the Treatment of Human Chorionic Gonadotropin: Rat Model

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ABSTRACT

Objective: *To examine the histopathological effects of human chorionic gonadotropin (hCG) treatment on the penile and the testicular tissue in rat model.*

Methods: *The rats of the hCG group (n = 8) were given daily subcutaneous injections of 50 IU of hCG for 15 days (Pregnyl, Organon). Rats of the control group (n = 8) received subcutaneous isotonic saline. All rats were sacrificed at the 1st month after hCG administration. After the received tissue samples were examined, germinal epithelial cell thickness, seminiferous tubule diameter, internal diameter of the tubules, the number of germ cell layers in the testicular tissue, and the diameters of penis, cavernous sinus lumen diameters and collagen tissue amount in the cavernous sinus surrounding were assessed in the sections prepared from the penis.*

Results: *It was detected that there was a decrease in the testis weight, atrophy in the tubules, reduction in spermatogenesis, decrease in the mature spermatocytes, lower mean thickness and the number of cell layers of the germinal membrane in testicular tissue in the hCG group. It was found that the amount of collagen in the penile tissue was significantly higher in the hCG group and the diameters of cavernosal sinus lumens, and diameter of the penis were significantly lower in the hCG group.*

Conclusion: *Human chorionic gonadotropin led to the deterioration in testicular histology and the histological changes in the penile tissue. The degradation in the testicular tissue and these changes formed in the penile tissue may affect the erectile tissue function.*

Keywords: Histopathology, human chorionic gonadotropin, penile, rat, testicular.

INTRODUCTION

Cryptorchidism affects 3%–4% of all full-term male neonates, with the prevalence decreasing to 0.8% by 1 year of age (1). It was revealed that 50% of unilateral cases and two-thirds of bilateral cases are associated with eventual fertility problems (2). Thus, studies to improve fertility have focused on earlier surgical intervention (3, 4) and, more recently, hormonal therapy (5). Hormonal treatment with human chorionic gonadotropin (hCG) may be given initially for cryptorchidism because of reported testicular descent in about 20% of cases (6). Although some studies recommend early hormonal treatment for cryptorchidism (7, 8) at 1500 IU hCG i.m. weekly for 3 weeks (9), others revealed that hormonal treatment may harm the germ cells (10). It is still questionable whether or not early hormonal treatment is safe for germ cells.

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Human chorionic gonadotropin used in the treatment of cryptorchidism may cause the appearance of secondary sex characteristics, such as early epiphyseal closure, pubic hair, genital growth, aggressive behaviour and scrotal hyperpigmentation. However, there are no studies assessing the occurrence of the genital growth. In children who had been treated with hCG, there have not been any studies examining the changes that occur in the penile tissue.

We examined these effects on the rat model since there are no studies examining the histopathological effects of hCG treatment on the penile tissue and the effects on the testicular tissue.

MATERIALS AND METHODS

Wistar albino rats weighing 250 ± 50 g were used in this experimental study. After the approval of the Ethics Committee at our institution (Yuzuncu Yıl University Ethics Committee, Van, Turkey) had been obtained, the care and use of laboratory animals followed. Sixteen 6-week-old male Wistar albino rats were maintained under standard conditions of temperature and 12-hour day/night cycles with food and water *ad libitum* and a constant temperature of 20°C–22°C. They were given free access to tap water. Upon arrival, they were randomized to hCG and control groups (n = 8 each). The rats of the hCG group were given daily subcutaneous injections of 50 IU of hCG for 15 days (Pregnyl, Organon). Rats that served as the control group received subcutaneous isotonic saline (n = 8). All rats were sacrificed by a lethal overdose of sodium pentobarbital (100 mg/kg, ip) to obtain normal, descended, testicular and penile tissue at the 1st month after hCG administration. After the received tissue samples were detected in 10% buffered formalin, the 4 µm sections were taken with the microtome by being embedded into paraffin blocks. The sections were examined in the research microscope by being painted with haematoxylin–eosin stain.

Three sections (proximal, central and distal) were taken from each testis, and in each section, 10 seminiferous tubules were chosen for analysis (*ie* 30 tubules per testis). Spherical or slightly elliptical tubules with the lumen centrally located were chosen because this signified a perpendicular cross-section that would most accurately depict germinal epithelial cell thickness and architecture. Germinal epithelial cell layer thickness was determined by counting the number of epithelial cells from the basement membrane to the lumen at 90°, 180°, 270° and 360°, and mean calculated. The seminiferous tubule diameter (STD), the internal diameter of the tubules and the diameter and the number of germ cell layers were measured. The diameters of penis, cavernous sinus lumen diameters and collagen tissue amount in the cavernous sinus surrounding were assessed in the sections prepared from the penis. Data were analysed by the Mann–Whitney *U*-test with $p < 0.05$ considered to indicate significance.

RESULTS

When compared with the control group, a decrease was detected in the testis weight in the hCG group (Table). When the weight difference was histopathologically examined, a clear atrophy was detected in the tubules. In addition, a reduction in spermatogenesis and a clear decrease in the mature spermatocytes were detected (Fig. 1). Consequently, the mean thickness and the number of cell layers of the germinal membrane in the testicular tissue of the hCG group were significantly lower than those of the control group ($p < 0.05$) (Fig. 2). İt was found that the percentage of the open seminiferous tubular lumen in the testicular tissue of all hCG-treated rats was significantly higher than that of the control group ($p < 0.05$). We found that a statistical difference between the mean in the STD in the testicular tissues of hCG-treated rats was significantly higher than that of the control group ($p < 0.05$).

Table: Testes weight and measurement of penis diameters

	hCG group (n = 8)	Control group (n = 8)	*p* value
Testes weight (g)	4375	5425	$p < 0.05$
Penis diameters (µm)	3467	3818	$p < 0.05$

hCG = human chorionic gonadotropin.

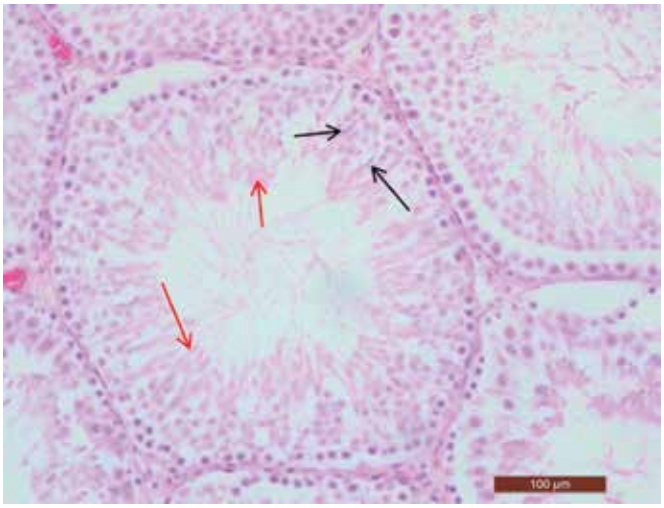

Fig. 1: Decreased spermatids in hCG group testes (black arrows), degenerated spermatogoniums (red arrows). (H&E) Bar = 100 µm.

At the 1st month after hCG administration for 15 days, it was found that the amount of collagen in the penile tissue of all hCG-treated rats was significantly higher than that of the control group ($p < 0.05$). Additionally, we were found that the diameters of cavernosal sinus lumens (Fig. 3A, 3B) and diameters of the penis (Table) of all hCG-treated rats were significantly lower than those of the control group ($p < 0.05$).

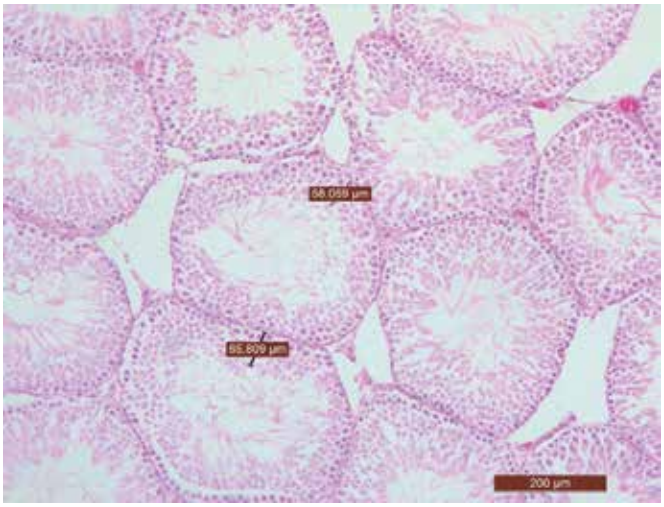

Fig. 2: Germinal membrane thickness in the testicular tubules in hCG group. (H&E) Bar = 200 µm.

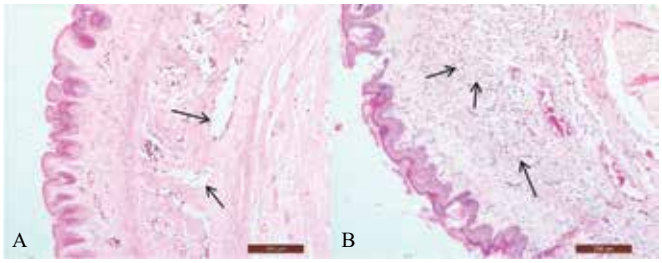

Fig. 3 (A) Cavernous sinuses in healthy penis in the control group (arrows). (H&E) Bar = 200 µm. (B) Narrowed cavernous sinuses in hCG group penis (arrows). (H&E) Bar = 200 µm.

DISCUSSION

Cryptorchidism is the most common disorder of sexual differentiation in men (11). While 89% of patients with untreated bilateral cryptorchidism develop azoospermia, only 32% of those treated with gonadotrophins do so (12). Hormonal treatment of cryptorchidism was introduced 84 years ago, and the first hormone used was hCG (13). Human chorionic gonadotropin is a polypeptide hormone produced by the human placenta, with an α-subunit that is almost identical to that of follicle-stimulating hormone, thyroid-stimulating hormone and luteinizing hormone (LH), so its action is similar to that of LH. A meta-analysis of 4524 cases of cryptorchid testes showed that hCG treatment has a success rate of 19% compared to a placebo effect of 4% (6).

Recently, Schwentner *et al* showed that neoadjuvant hormonal treatment with gonadotropin-releasing hormone improves the fertility index in prepubertal cryptorchidism (14). In contrast to these studies, it was shown that histological findings indicated a reduction in the number and maturation of germ cells in cryptorchid infants treated with combined hormonal therapy (7). Cortes *et al* reported that hormonal treatment given for testicular descent may harm the germ cells (10). It was also reported that unsuccessful hCG treatment resulted in lower spermatogonia per tubule in boys with cryptorchidism.

In the rat testis, germ cell death occurs through apoptosis, characterized by internucleosomal fragmentation of DNA, and this is regulated by androgens and gonadotrophins. In cryptorchid boys, germ cell apoptosis was assessed after unsuccessful hCG treatment at simultaneous orchidopexy and testicular biopsies. There were more apoptotic germ cells in the contralateral scrotal than in the undescended testis; both interstitial cells and germ cells were affected, and the germ cells undergoing apoptosis were exclusively spermatogonia. Germ cell apoptosis was increased in both scrotal and inguinal testes, returning to the initial level 1 month after the hCG treatment, suggesting that hCG withdrawal increases germ cell apoptosis in the human testis (15).

Because of increasing serum testosterone levels during hCG treatment by about 150- to 200-fold, followed by a decrease to low prepubertal levels within a few weeks, it was assumed that the increase in germ cell apoptosis after hCG treatment most likely reflects the androgen withdrawal effect. Thus, normal development of the testis is disrupted by the hCG treatment, possibly through the mechanism of increased apoptotic germ cell death. Consequently, it was proposed that because of poor treatment success rates and shown side effects, the potential hazards of hCG treatment on the testis should be critically re-evaluated (16).

The rat model appears particularly useful in a study evaluating testicular histology, because developmental parameters in rats are similar to those in humans. Kaya *et al* showed that gonadotropin therapy deteriorated the seminiferous tubular histology of testes in rats (17). However, their results showing detrimental effects of hCG do not conflict with those of previous series (10, 15, 16). The mean percentage of the open seminiferous tubular lumen in testicular tissues of all hCG-treated rats was found to be significantly higher than that of

control rats. Similarly, mean thickness and the number of cell layers of the germinal membrane were significantly lower in testicular tissues of all hCG groups at the 1st month. In a study conducted by Karaman *et al*, they reported that these histological changes that the hCG made in the testicles were reversible and dose dependent (18).

When we looked at the literature, the number of the studies showing the effect of hCG on the normal testis tissue is limited and also the results are controversial. When compared to the control group, we found the decline in testicular weight in the hCG group. When the weight difference was histopathologically examined, a clear atrophy was detected in the tubules and connected to it. In addition, a reduction in spermatogenesis and a clear decrease in the mature spermatocytes were detected. Accordingly, the mean thickness and the number of cell layers of the germinal membrane in the testicular tissue of the hCG group were significantly lower than those of the control group ($p < 0.05$). We found that the statistical difference between mean STD in testicular tissues of hCG-treated rats was significantly higher than that of the control group ($p < 0.05$). The percentage of the open seminiferous tubular lumen in the testicular tissue of all hCG-treated rats was significantly higher than that of the control group. According to all of our findings, we observed that hCG had the negative effects on the testicular tissue. Our findings related to the histological changes formed in rat testis due to the use of hCG have supported the findings of Kaya *et al* (17).

When we examined the literature, there was no study simultaneously examining the effect of hCG on the penile tissue and its effects on testicular tissue in rat model. In our study, at the 1st month after hCG administration for 15 days, it was found that the amount of collagen in the penile tissue of all hCG-treated rats was significantly higher than that of the control group ($p < 0.05$). Additionally, diameters of cavernosal sinus lumens and diameters of the penis of all hCG-treated rats were found to be significantly lower than that of the control group ($p < 0.05$). It was observed that there were the collagen tissue growth around the cavernous sinus and accordingly the contraction the lumens, and as a result the shrinkage in the diameter of the penis. These results make us think that the erectile tissue function changes may become due to the use of hCG, and it may affect the erectile function during puberty.

In this study, we saw that hCG led to the deterioration in testicular histology and the histological changes in the penile tissue. In order to be able to clearly assess the impact on erectile tissue function of the degradation in the testicular tissue and these changes formed in the penis tissue, further more long-term studies are needed, including the biochemical parameters together.

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