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IMAGES IN FORENSICS

Testicular morphology in hypogonadotropic hypogonadism after the abuse of anabolic steroids

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Abstract
Hypogonadism in men results from the failure of the testes to produce physiological levels of testosterone and a normal number of spermatozoa due to a disruption of the hypothalamic-pituitary-testicular axis. An example of secondary hypogonadism as a result of anabolic steroid abuse is presented with the case report of a man who committed suicide after a history of aggressive behavior and physical abuse of his wife. The autopsy revealed shrunken testicles, with more than 30% of parenchymatous sclerosis, absent spermatogenesis, and very few Leydig cells detected only by immunohistochemistry. A low-specific immunochemical analysis revealed a very high level of total testosterone (serum 109.8 nmol/L; urine 81.2 nmol/L). A more accurate analysis confirmed an overdose of synthetic anabolic steroids. Doctors in different medical areas should be alerted to chronic abusers of synthetic anabolic steroids among the growing number of recreational athletes.

Keywords Hypogonadotropic hypogonadism · Anabolic steroids · Testosterone · Leydig cells · Erectile dysfunction · Fertility

Case report

A 57-year-old Caucasian man committed suicide with a shot from a pistol (Fig. 1) after killing his wife by a shot from a shot gun. Previously he had worked as a karate coach and his hobby had been practical shooting, thus he had a permit for the pistol. He was known to be dominant in the company of others and had showed signs of excessive aggression, especially towards his wife, who was often physically abused, which had been recorded by the local police. After she had initiated divorce proceedings, he continued to abuse her physically and mentally, even during the night before the murder. Through interviews and evidence collecting it was revealed that he had been regularly injected with anabolic steroids over a lengthy, although unknown, period of time. Furthermore, he was taking Cialis pills (tadalafil – Eli Lilly and Company, Indianapolis, USA), which are used in erectile dysfunction.

External inspection during autopsy revealed that he was very muscular (179 cm, 112 kg) with fairly well-defined secondary sexual characteristics, but there was no data regarding whether they were obviously changed from their previous appearance (Fig. 2). Internal autopsy examination revealed 4 cm thick subcutaneous adipose tissue in the abdominal wall and the testicles were found to be shrunken. The weight of the right testicle (without the epididymis, ductus deferens and tunica vaginalis) was 6.85 g, and the left testicle 6.66 g. The dimensions of the testicles in the longest and shortest axis were: 3.2 × 2.7 cm (right testis) and 3.0 × 2.9 cm (left testis) (Fig. 3). Macroscopically visible degenerative changes with sclerosis of more than 30% of the displayed cut surface in both formalin fixed testicles were apparent (Fig. 4). The remaining parts were of adequate macroscopic morphology, but the histological examination revealed large areas of hyalinization and atrophy of the seminiferous tubules (Fig. 5), with practically absent spermatogenesis. The tubular basement membranes were thickened, and the tubular lumina contained only Sertoli cells (Fig. 6) or were completely obliterated. Only rarely, singular Leydig cells were detected using immunohistochemistry for inhibin (Fig. 7), melan A and calretinin.

Further investigation of his serum and urine confirmed the abuse of anabolic steroids. Primarily, the samples were analyzed using the low-specific immunochemical method. The result for the total testosterone level was very high. In the urine it was 81.2 nmol/L and in the serum it was 109.8 nmol/L. In the laboratory where the analysis was performed, the reference value for total testosterone is 6.7–25.7 nmol/L. Since there is no laboratory

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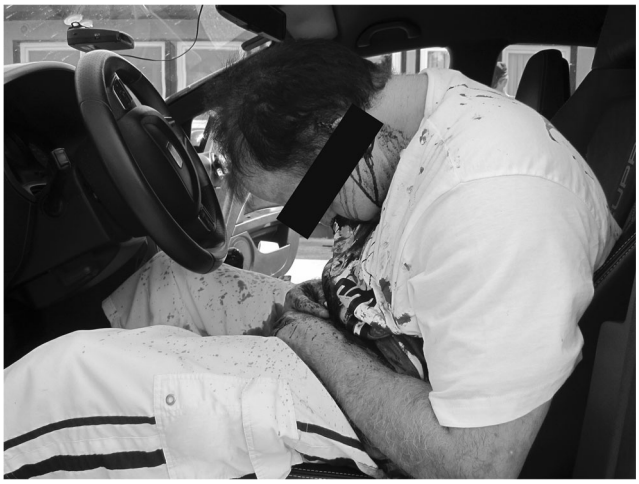

Fig. 1 57-year-old man who committed suicide with a shot from a pistol

in Slovenia where it is possible to determine the specific compounds of synthetic anabolic steroids, which are chemically very similar to endogenous testosterone, a urine sample was sent to a specialized laboratory, which is one of the laboratories in the WADA (World Anti-Doping Agency). The chemical analysis of the urine sample, using chromatographic and mass spectrometric methods, showed the presence of metabolites of synthetic anabolic steroids (Table 1). In addition the isotope ratio analysis was conclusive with the administration of the synthetic testosterone or testosterone precursors. These metabolites and their concentrations were accounted for due to an overdose of synthetic anabolic steroids.

Discussion

Hypogonadism in men is defined as a clinical syndrome that results from the failure of the testes to produce physiological levels of testosterone and a normal number of spermatozoa due to the disruption of one or more levels of the hypothalamic-pituitary-testicular (HPT) axis [1]. Disorders that cause hypogonadism in men could be primary or secondary. Primary failure of the testes could be the result of a genetic disorder (Klinefelter's syndrome), a disorder in development (cryptorchidism), aggressive therapy (chemotherapy or radiation therapy), inflammation (mumps) or trauma. Secondary hypogonadism could be the result of hypothalamic or pituitary disorders that affect the gonadotropic secretion or the action of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) as in genetic, developmental and infiltrative disorders, neoplasms, and anabolic steroid abuse. Different conditions such as aging, eating disorders, hemochromatosis, HIV-infection, glucocorticoid therapy or ethanol, marijuana, and opiates abuse could affect more levels of the HPT axis [2].

Primary and secondary hypogonadism is associated with a low testosterone level and impaired spermatogenesis, but in states of primary hypogonadism LH and FSH levels are elevated while in secondary hypogonadism they are low or normal [1, 2].

The definition of hypogonadism in most studies is related to a low level of total and free testosterone. Only 0.5–3% of the circulating testosterone is free or unbound, therefore the majority is bound to proteins (sex hormone binding globulin (SHBG) and albumin). The threshold of the total testosterone level in serum for the diagnosis of adult male hypogonadism (together with the symptoms of androgen deficiency such as a reduced sexual desire and activity, body hair loss, infertility, shrunken testes, reduced muscle mass and strength, hot flushes and sweats, increased body fat, decreased energy, depressed mood, poor concentration and memory, sleep disturbance) is 11 nmol/L [3, 4].

Fig. 2 Very muscular body with fairly well-defined secondary sexual characteristics

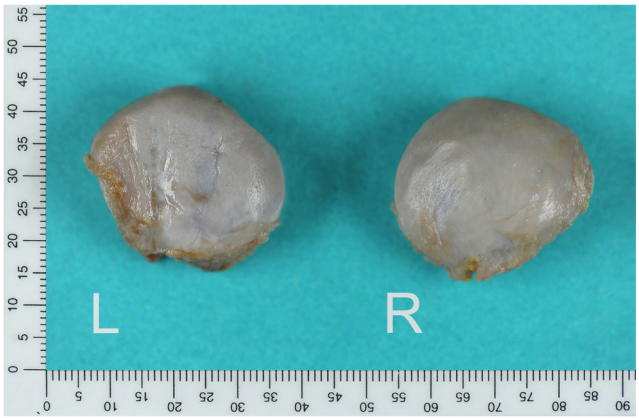

Fig. 3 Secondary hypogonadism with the shrinkage of the left (L) and right (R) testis

The weight of normally developed adult testis in Caucasian men is 21.6 ± 0.4 g (right testis) and 20 ± 0.4 g (left testis). The mean testicular diameter of the longest axis is 4.6 cm (range 3.6–5.5 cm), the shortest is 2.6 cm (range 2.1–3.2 cm). The testicular volume is between 15 to 25 mL [5]. As the model of measuring is not described, it is not known if the data include epididymis, ductus deferens and tunica vaginalis, but we assume that the testes were measured without the other parts which are usually sent together for a pathological biopsy examination.

As testosterone is mainly produced by testicular endocrine cells called Leydig cells which could be either singly or in groups in the supporting tissue in the interstitial spaces between the seminiferous tubules [6] each cause which could diminish the number of Leydig cells would disturb the physiological levels of the androgenic hormones and change the androgenic status [7]. Different causes (dysgenetic hyalinization, hormonal deficit, ischemia, obstruction, inflammation, and physical or chemical agents) could cause hyalinization of the seminiferous tubules with a significant reduction in the number of ortotopic cells and testicular shrinkage. A disrupted HPT axis and hormonal deficit, even

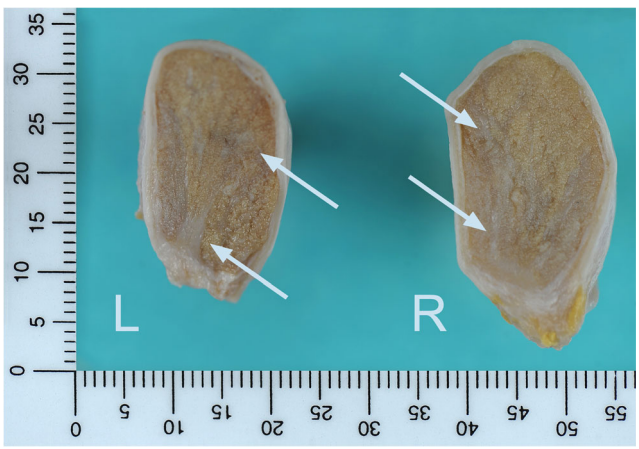

Fig. 4 Cross-section of the left (L) and right (R) testis with degenerative changes (arrows)

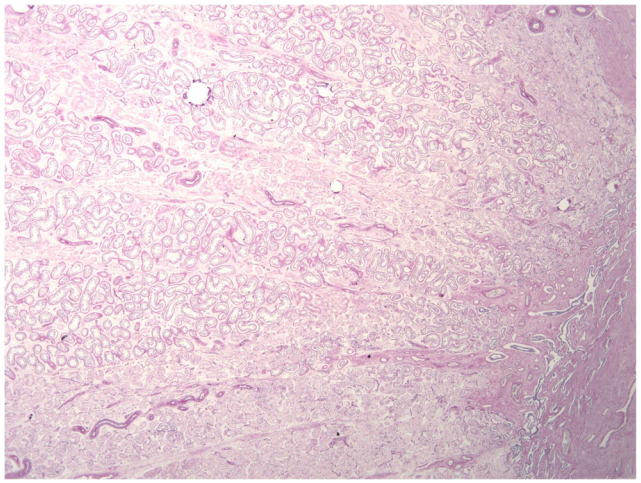

Fig. 5 Low power view giving the impression of an irregular band-like atrophy of testicular parenchyma with at least 30% fibrosis. Rete testis at the right (hematoxylin and eosin, ×10)

after the chronic abuse of anabolic steroids, causes diffuse tubular hyalinization with a diminished number of Sertoli and germ cells, peritubular myofibroblasts, and Leydig cells, which could disappear as hyalinization progresses [5].

Testosterone and its synthetic derivatives were primarily used for therapeutic purposes because of the androgenic and anabolic effect. In recent decades, professional athletes and bodybuilders have included these substances in their training programs mainly because of anabolism (stimulation of the protein synthesis and increasing muscle size and bone metabolism). This approach has become gradually more popular among recreational bodybuilders and power athletes. Some of them use these substances just for aesthetic purposes [8]. Today, the production of anabolic steroids is growing and it is directed towards designing steroids with a prevalence of the anabolic effects over the androgenic ones.

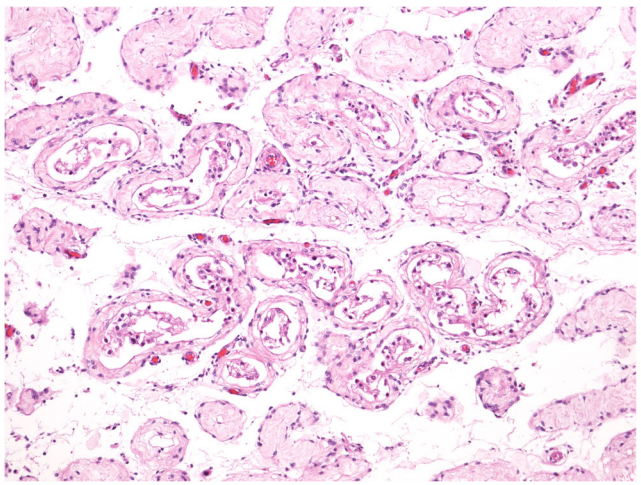

Fig. 6 Atrophic seminiferous tubuli, populated by Sertoli cells only, surrounded by hyalinized and obliterated tubular structures (hematoxylin and eosin, × 100)

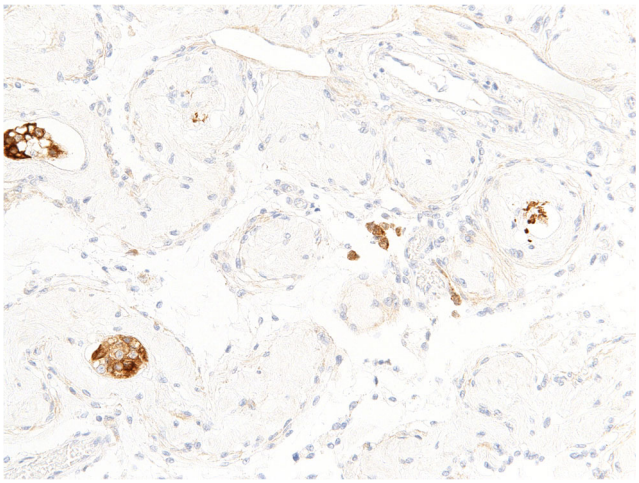

Fig. 7 Only a few Leydig cells are recognizable in the interstitium of the atrophic testis (inhibin, ×200)

Of course hypertrophy of the skeletal muscle is not the only effect. Other side effects could affect the cardiovascular system (hypertension, arrhythmia, ventricular dysfunction), blood (erythrocytosis), liver (hepatic peliosis, cholestasis, neoplasms), and kidneys (renal failure secondary to rhabdomyolysis and diffuse membranoproliferative glomerulonephritis). Chronic abuse of anabolic steroids could trigger aggressiveness, mood swings, depression, and even psychosis [8].

Androgenic side effects are acne, alopecia, low urinary tract symptoms attributable to prostate enlargement, lower libido, erectile dysfunction, and hypogonadotrophic hypogonadism with diminished fertility. However, this state is reversible because the HPT axial inhibition is transient after the discontinuation of anabolic steroids for several months [9, 10]. Even Leydig cells, which have morphological anomalies and a decreased number during chronic abuse, tend to proliferate after discontinuation of anabolic steroids but remain below the regular counts, even after a longer period [11]. Therefore, persistent androgenic side effects of anabolic steroids abuse cannot be ruled out [8].

In our case, a differential diagnostic consideration due to the man's aggressive behavior combined with signs of diminished spermatogenesis could also point to the possibility of Klinefelter syndrome, which shares some characteristics with the presented case. However, the man had fathered two children and the morphology of testicular hyalinosis stained by van Gieson Weigert elastic stain showed that the amount of elastic fibers was not decreased, as would be expected in a dysgenetic tubular hyalinization [5], this, in addition to the almost extinct Leydig cells, confirmed our diagnosis.

Table 1 Metabolites of the synthetic anabolic steroids confirmed in urine

Metabolite	Concentration (ng/mL)
Metandienone	2500
Nandrolone	2200
Mesterolone	16,000
Dehydrochloromethyltestosterone	230

Conclusion

The spread of a sports culture, that uses a variety of tools and substances, to the growing number of recreational athletes, also has drawbacks, because among them there are those who try to achieve their goals at any cost. Because they are uninformed about the harmful effects of the selected shortcut, such as taking synthetic anabolic steroids, these users suffer from temporary or permanent psychosomatic changes, often seeking help; initially from general practitioners, but later from urologists, internists, psychiatrists or others who should be alerted to the negative side of a "healthy sporting life".

Compliance with ethical standards

Ethical approval No ethical approval was required.

Conflict of interest The author declares that he has no conflict of interest.

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