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Original Article

The ghrelin and orexin activity in testicular tissues of patients with idiopathic non-obstructive azoospermia

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Abstract The aim of the present study is to evaluate the presence of ghrelin and orexin in the testicular tissue of patients who have undergone microscopic testicular sperm extraction (micro-TESE) due to idiopathic non-obstructive azoospermia. Seventy azoospermic cases were included in this study; serum hormone levels were measured and genetic investigations were performed. The patients were divided into two groups: micro-TESE (+) and micro-TESE (−). The number of Leydig cells and stained cells in the seminiferous tubules were counted under a light microscope, and we analyzed ghrelin and orexin activity. The relationship between serum hormone levels and ghrelin and orexin distributions in testicular tissue was evaluated according to micro-TESE results. While sperm was found in 33 cases (47.1%), micro-TESE was negative in 37 cases (52.9%). Peptide hormone activity in testicular tissue was higher in micro-TESE (+) cases. However, interstitial orexin ($p = 0.038$) and ghrelin ($p = 0.002$) activity showed statistically meaningful differences. Many different peptides, genes, and other

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unknown mechanisms play important roles in testicular function. In particular, the peptides orexin and ghrelin may play regulatory roles in testicular function in humans.

Introduction

Spermatogenesis is a complex process controlled by hormonal and genetic factors. While follicle stimulating hormone (FSH) and testosterone are well-known regulators of sperm production, it has been suggested that several other paracrine and genetic factors can also affect spermatogenetic activity.

Ghrelin, a 28-amino-acid peptide, is primarily expressed in the hypothalamus and has been found to affect growth-hormone secretion [1,2]. It has also been observed to play important roles in the stimulation of food intake and the development of adipose tissue, with central neuroendocrine activity [3–5]. Recently, ghrelin expression and activity have been described in experimental and human studies [6–8]. Ghrelin expression has been shown in Leydig cells *in vitro*, and it has been reported that ghrelin activity in testicular tissue is inversely correlated with testosterone levels [9]. Although it has been suggested that ghrelin is an endocrine, paracrine, and autocrine regulatory substance in human testes, there are no studies on ghrelin activity and spermatogenesis in the literature.

Orexins, which are hypothalamic neuropeptides, were first identified in the lateral hypothalamic area and found to be related to feeding behavior [10,11]. Two different orexins are derived from the 130-amino-acid prepro-orexin: orexin A (Ox-A), with 33 amino acids, and orexin-B (Ox-B), with 28. Both affect their own specific receptors, called OX1R and OX2R, respectively. In experimental studies, OX1R has been described in the genitourinary system, especially in Leydig and Sertoli cells, seminiferous tubules, epididymis, and vesicle seminalis [12–14]. However, the relationship between orexin expression and steroidogenesis/spermatogenesis in humans is unknown.

The aim of the present study is to evaluate the presence of ghrelin and orexin activity in the testicular tissues of patients who have undergone microscopic testicular sperm extraction (micro-TESE) procedures due to idiopathic non-obstructive azoospermia (NOA). Ghrelin and orexin expressions were investigated in different parts of human testicular tissue, including the Leydig cells, Sertoli cells, and seminiferous tubules. Additionally, their relationships with spermatogenetic activity and their correlations with serum gonadotropins and sex steroid levels were examined.

Material and methods

The Local Ethics Committee approved all procedures, and all subjects provided written informed consent before being enrolled in the study. A total of 242 idiopathic NOA patients were evaluated with detailed anamnesis and physical examinations. Serum hormone levels, including FSH, luteinizing hormone (LH), total testosterone, and estradiol (E_2), were measured. Peripheral karyotype and chromosome Y micro-deletions were evaluated in all cases. Patients with hypogonadotropic hypogonadism, a history of cryptorchidism/orchidopexy, mumps orchids, non-specific orchids, radiotherapy/chemotherapy, or any type of genetic abnormality were excluded from enrollment. Therefore, a total of 70 cases were included in the study.

Micro-TESE and sperm retrieval

Micro-TESE was performed under general anesthesia using an operating microscope. Microdissection was performed according to the previously described technique of Schlegel [15]. Seminiferous tubules were examined under the operating microscope (25 × magnification) after longitudinal tunical incision. Enlarged tubules were dissected and removed, then minced in sperm-wash solution in a petri dish. A droplet of tissue suspension was placed on a glass slide and investigated under a light microscope at 400 × magnification. This procedure was repeated on both testicles until spermatozoa were detected. The tunica albuginea was closed with 5/0 polypropylene, and the scrotum was closed with 4/0 Vicryl. Preoperative antibiotic prophylaxis was administered in all cases.

If no spermatozoa were observed during the operation, testicular samples were prepared using a mini-gradient method and collagenase was performed for 1 h. Later, all samples were placed in ICSI dishes for spermatozoa collection. Based on micro-TESE results, the patients were divided into two groups: micro-TESE positive (+) and micro-TESE negative (−).

Histopathology and orexin/ghrelin staining

The specimens were fixed with Bouin's solution for 12 h at room temperature. The tissues were prepared in an automatic tissue processor (Shandon Excelsior ES, Thermo Scientific, Cheshire, UK) using ascending concentrations of formaldehyde, ethanol, xylene, and paraffin wax. The tissues were evaluated histochemically with hematoxylin and eosin (H&E) staining and immunohistochemically using ghrelin and OX antibodies. Sections of 3 μm were taken from paraffin blocks that were previously coated with poly-L-lysine. Specimen slides were then deparaffinized with xylene for 10 min. Hydrogen peroxide, phosphate-buffered saline (PBS), and a nonspecific blocking reagent (Ultra V block: ScyTek Laboratories, Logan, Utah, USA) were applied as antigen retrieval solutions. Rabbit anti-human ghrelin (polyclonal) (PHOENIX, Burlingame, CA, USA) and rabbit anti-human OX-A (polyclonal) (PHOENIX) antibodies were then localized at room temperature for approximately

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