

Antisperm Antibodies in Undescended Testis

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Abstract

Background: Undescended testis is the commonest genital malformation in boys. Men with a history of undescended testis have a reduced probability of fertility.

Aim of the study: To explore whether the development of anti-sperm antibodies and antitesticular autoantibodies might play a role in inhibiting the future fertility of cryptorchid boys. Histopathological and hormonal assessments were done for those patients.

Patients and methods: The study group consisted of 30 patients with undescended testis who underwent Sub dartous orchidopexy at The AL-Kindi Teaching Hospital and other private hospitals. Orchidopexy and histopathological studies were done for them. Hormonal assessment, antisperm and antitesticular autoantibodies assessment were done for them.

Results: The mean age of the orchiopexy patients was 12 ± 9.4 years. Histopathological study revealed testicular atrophy in 76.6% of the cases. Antitesticular autoantibodies were detected by direct immunofluorescence. Antisperm antibodies and hormonal assay in patients' serum before and after operation illustrated no significant difference.

Conclusions: The late descent of a testicle into the scrotum may impair its development ending in reduced fertility because of histopathological changes (atrophy) already become apparent in the first few months of life. Development of antitesticular autoantibodies may contribute in this process because the ectopic position of testis that damage barrier. Therefore, hormonal and surgical treatments complement each other and should be provided before the child's first birthday.

Keywords: Testis; Sperm; Atrophy; Hormone

Introduction

Undescended testis (UT) is the commonest genital malformation in males. The mechanism that regulates prenatal testicular descent is still unknown; there is evidence that endocrine, genetic, and environmental factors are involved in this process [1]. A birth weight below 2.5 kg and premature delivery are risk factors for maldescent. Placental insufficiency with reduced human chorionic gonadotropin (hCG) secretion [2]. Environmental factors increase the risk of cryptorchidism like persistent organochlorine compounds, monoesters of the phthalates, maternal smoking, and maternal diabetes mellitus [3-5]. Many undescended testicles are accompanied by patency of the vaginal process and like the simultaneous presence of inguinal hernia. A pediatric endocrinological assessment is indicated in order to rule out other syndromes. Detection of testosterone-producing testicular tissue should precede surgical exploration [6]. Thus, hormone therapy is indicated for induction of descent of the retained testicle and stimulation of germ cell maturation and proliferation to contribute to improving fertility.

The complications of undescended testis are twisting of the testicle (testicular torsion), testicular cancer, hypogonadic hypogonadism, low serum level of testosterone hormone, groin mass and infertility; men with a history of undescended testis have a reduced probability of fertility with a low sperm count and generally poorer semen quality than men with normal testicular descent [7]. A normally descended contralateral testis does not compensate this sub fertility. Surgical treatments in boys between the ages of ten months and four years with bilateral cryptorchidism lead to a normal sperm count in 76% of cases compared to 26% in the boys who were operated between the age of 4

and 14 years and this time effect is not so pronounced with unilateral cryptorchidism [8]. This may be due to the impaired spermatogenesis that is characterized histologically in the first months of life by an increasing reduction in the testosterone-producing Leydig cells, delayed onset of spermatogonia, and a quantitatively and qualitatively reduced maturation process of the germ cells [9]. Another cause of infertility in those patients is development of antisperm antibodies (ASA) despite early operation performed between third to ninth months; infertility is not ameliorated [10]. Surgeon is often puzzled by postoperative development of ASA later on [11]. Theoretically, the causes for the development of these ASA in cryptorchidism are a defective blood-testis barrier permitting immunization toward sperm and the barrier is affected by testis non descent and hyperthermia [12,13].

The main of this study was to check up the development of ASA in patients with undescended testis before and after operation, the development of anti testicular autoantibodies and histological study of ectopic testis. Finally, study serum hormonal levels of these patients before and after operation.

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Received June 25, 2012; **Accepted** September 11, 2012; **Published** September 18, 2012

Citation: Mahdi BM, Hasan RM, AL-Kurtas MAM, Ghanim N, Al-Galabi LK, et al. (2012) Antisperm Antibodies in Undescended Testis. J Clin Cell Immunol S10:002. doi:10.4172/2155-9899.S10-002

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Patients and Methods

The study group consisted of 30 patients with undescended testis who underwent subdartous orchidopexy at The AL-Kindi Teaching Hospital and other private hospitals.

The permission of medical ethics committee was obtained for them. The Ethical Committee of the Al-Kindi College of Medicine, Baghdad University and Al-Kindi Teaching Hospital approved the study, and all samples were obtained with informed consent in accordance with the Al-Kindi Teaching Hospital Declaration.

Venous blood samples were drawn from patients before the operation of orchidopexy that include skin inguinal incision, dissection to find the undescended testis, freeing it from surrounding tissues and from the gubernaculum, dissection of the cord to see if there is accompanying inguinal hernia and herniotomy is done, freeing of the cord, incisional biopsy from the testis, creation of subdartos pouch and embedding of the testis in it and closure of the wound. After two to three weeks of follow-up after operation another venous blood samples were obtained from the same patients. Serum was stored at -20°C until analysis for antisperm antibodies by the indirect immunofluorescence test. Sera were diluted 1:10 in buffer and test done using EURO IMMUNE Immunofluorescence kit-GERMANY. The conjugate used was polyclonal antibodies (IgM, IgG and IgA) conjugated with fluorescence material. All slides were evaluated in a blinded testing in order to get correct results.

3 mm sections were done from removed testes using cryostat machine. Direct Immunofluorescence test done using polyclonal (IgG, IgM and IgA) antibodies conjugated with fluorescence material (EURO IMMUNE-GERMANY). All slides evaluated in a blinded testing in order to get correct results.

Hormonal levels assessment (FSH, LH, and Testosterone) in the serum were done before and after operation using VIDAS FSH, LH and Testosterone kits (Bio Merieux France). They are automated quantitative tests for use on the VIDAS instrument for the determination of these hormones in the serum, using the ELFA technique (Enzyme Linked Fluorescence Assay).

Removed testes and testicular biopsies fixed in Bouin solution and embedded in paraffin. Four mm sections were stained with hematoxylin and eosin. A pathologist examined the specimens in a blinded manner. Morphology of tissue was studied to identify atrophy, vascular injury, calcification, edema or inflammation. Atrophy detected macroscopically by smaller in its size and microscopic examination of its histology by many seminiferous tubules have been lost, remaining tubules were atrophied, their basement membrane are hyalinized and thicker. The larger tubules contain Sertoli cells with large pale vesicular nuclei and heavily vacuolated cytoplasm. There are no germ cells. The spaces between the tubules are filled with sheets of hyperplastic interstitial Leydig cells with deeply eosinophilic cytoplasm.

Statistical analysis was done using MiniTab statistical software program 13.20. A P-value ≤ 0.05 was considered significant.

Results

The mean age of the orchidopexy patients was 12 ± 9.4 years (ranging from 1-32 years), median=9 years. Unilateral undescended testis constitutes 73.3% of the cases and the right side is the commonest one (72.7%). The most common location of migrated testis was superficial inguinal ring (80%) as shown in Table 1.

Detection of antisperm antibodies in patients' serum with unilateral undescended testis before and after operation illustrated no significant difference (Table 2). Detection of antitesticular autoantibodies by direct immunofluorescence showed autoantibodies directed against seminiferous tubules and degenerated germinal epithelium (Table 3 and Figure 1). The antitesticular autoantibodies staining is very weak because of low titer of autoantibodies (1:10) in the patient's serum. Hormonal assay (FSH, LH and testosterone) illustrated no significant difference before and after operation (Table 4). Histopathological study revealed testicular atrophy in 76.6% of the cases (Table 5 and Figure 2).

Discussion

The late descent of a testicle into the scrotum may impair its development. Reduced fertility is the main risk of primary cryptorchidism even after treatment due to histopathological changes in the first few months of life. In our study, unilateral undescended testis constitutes 73.3% and the rest was 26.6% bilateral and 80% were located in the superficial inguinal ring and 76.6% of them were associated with hernia. Other study found that bilateral cryptorchidism was 45% and the rest was unilateral [10]. Cendron et al. documented the position of testis at the time of surgery and found that position at tubercle represent 42% and at inguinal canal was 12% [14]. Ductal abnormalities, hernias (patent processus vaginalis), and testicular maldevelopment are more common in patients with abdominal testes [15].

Other important thing that puzzled surgeon is development of antisperm antibodies (ASA) after treating cryptorchidism postoperatively [16]. Theoretically, there are many reasons for serum ASA production in cryptorchidism like maldeveloped cryptorchid testis primarily has a defective blood-testis barrier permitting immunization toward sperm, or whether the barrier is affected by testis nondescent and hyperthermia [17] or from orchidopexy. In our study, we did not find a significant difference in ASA production in the serum before (10%) and after (16.6%) orchidopexy by indirect immunofluorescence

Data	No.	%
Unilateral	22	73.3
Right	16	72.7
Left	6	27.2
Bilateral	8	26.6
Position		
Abdominal	6	20
Superficial inguinal ring	24	80
Association with		
Hernia	23	76.6
Pain	0	0
Torsion	0	0
Abnormality with genitourinary symptoms	0	0

Table 1: The demographic data of patients with undescended testis.

	ASA positive unilateral No. %	ASA negative Unilateral and bilateral No. %	P-value
Before operation	3 10	27 90	0.226
After operation	5 16.6	25 83.3	

Table 2: Antisperm antibodies of patient's serum with undescended testis before and after orchidopexy by indirect immunofluorescence test.

	No. %	P-value
Antitesticular autoantibodies Positive (Unilateral and bilateral)	16 63.3	0.606
Antitesticular autoantibodies negative	14 46.6	
Total	30 100	

Table 3: Antitesticular autoantibodies by direct immunofluorescence test.

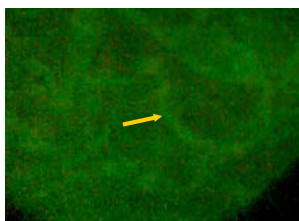


Figure 1A: Patient biopsy

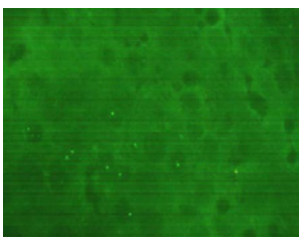


Figure 1B: Positive control

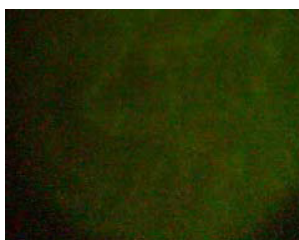


Figure 1C: Negative control

Figure 1: Antitesticular auto antibodies by direct immunofluorescence showed autoantibodies directed against Seminiferous tubules and degenerated germinal epithelium (arrow).

test. This was in agreement with other studies [18]. Mirilas et al. [19] showed that intra-abdominal testicular stay and orchidopexy do not elicit autoimmune response to sperm and ASA are probably due to other reason like testicular heat or harmful orchidopexy trauma technique and cross reactions to other epitopes, or a combination of these. Other cause to development ASA due to focal cryptic obstruction of testicular seminiferous tubules or other obstructive anomalies of sperm pathway associated with cryptorchidism could be responsible for serum ASA in operated cryptorchids [20].

Detection of antitesticular antibodies by direct immunofluorescence test done on testicular biopsy in both unilateral and bilateral showed that 63.3% of the patients had these autoantibodies directed against Seminiferous tubules and degeneration of germinal epithelium. These autoantibodies may be the cause of atrophy, infertility and development of ASA later on. These autoantibodies develop because the ectopic testis is intra abdominal and there is a break to blood testis barrier.

The hypothalamus is the integrative center of the reproductive axis and receives messages from both the central nervous system and the testes to regulate the production and secretion of gonadotropin releasing hormone (GnRH) which appears to be essential for stimulating the production and release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) from anterior pituitary and are secreted episodically in response to the pulsatile release of GnRH. LH and FSH both bind to specific receptors on the Leydig cells and Sertoli cells within the testis. Testosterone, the major secretory product of the testes, is a primary inhibitor of LH secretion in males. Isolated increased levels of FSH constitute an important, sensitive marker of the state of the germinal epithelium [21].

Hormonal assay regarding FSH, LH and Testosterone showed no significant difference before and after operation in our study because 73.3% of our patients were unilateral undescended testis. Rusnack et al. demonstrated that males with unilateral undescended testes were more likely to have higher sperm density and normal hormone profile, while those with bilateral undescended testes had severe oligospermia and abnormal hormonal parameters [22]. Other studies of hormonal profiles have documented different levels of inhibin B, FSH and LH levels. Patients with bilateral undescended testes have significantly lower sperm concentration and inhibin B levels, while showing higher FSH and LH levels than those with unilateral undescended testis [23].

As a means of determining the maturation status of the testicle,

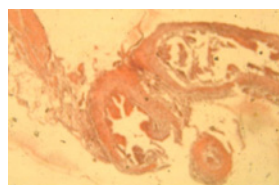


Figure 2A: Patient biopsy

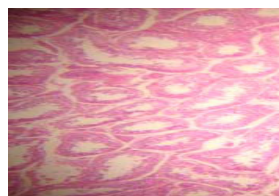


Figure 2B: Positive control



Figure 2C: Negative control

Figure 2: Histopathological study showed testicular atrophy.

Hormones	Before operation	After operation	P-value Mann-Whitney
FSH mIU/ml			
Lower limit	2.2	12.1	0.3827
Upper limit	12.4	13.1	
Median	7.0	12	
LH mIU/ml			
Lower limit	0.33	3.5	0.6625
Upper limit	10.53	6.33	
Median	7.8	5.4	
Testosterone mIU/ml			
Lower limit	0.37	4.3	0.0809
Upper limit	3.2	5.5	
Median	2.3	4.9	

Table 4: Hormonal assay of undescended testis before and after operation.

	No.	%
Vasculitis	0	0
Atrophy	23	76.6
Calcification/microlithiasis	0	0
Interstitial edema	5	16.6
Granuloma	0	0
Poly morphonuclear cells inside seminiferous tubules	4	13.3

Table 5: Frequency of histological lesions in operated testis.

the simultaneous presence of testicular and ovarian tissue in one germ cell, dysgenesis, atrophy or tumor is suspected, intraoperative testicular biopsy is done. In our study, histopathological study of testicular biopsies showed atrophy in 86.6% of cases. Perrelli et al. [24] mentioned that degree of histological damage was directly proportional to the age of the patients. The complete lack of germ cells at the time of surgery is an important predictor of future fertility in the boy with cryptorchidism. Testicular biopsy should be performed at the time of orchidopexy to identify those who would benefit from LHRH treatment after the procedure. The LHRH analogs induce replication and differentiation of germ cells that enhance the chance of fertility [25].

Conclusions

The late descent of a testicle into the scrotum may impair its development ending in reduced fertility because of histopathological changes (atrophy) already become apparent in the first few months of life. Development of antitesticular autoantibodies may contribute in this process because the ectopic position of testis that break blood testis barrier. Therefore, hormonal and surgical treatments complement each other and should be provided before the child's first birthday.

Recommendations

The number of patients is too small. Further study is needed with a large number of samples.

Acknowledgments

We would like to thank all patients who helped us and the staff of histopathology department in Al-Kindy Teaching Hospital and laboratory staff in Endocrinology center.

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This article was originally published in a special issue, **Clinical, Cellular & Molecular Biology of Autoimmune Disorders** handled by Editor(s). Dr. Abdul Rahman Asif, George-August-University, Germany