ORIGINAL RESEARCH

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Assessment of anti-sperm antibodies in couples after testicular sperm extraction

Abstract

Purpose: Testicular spermatozoa can be retrieved successfully by the testicular sperm extraction (TESE) procedure and used for intracytoplasmic sperm injection. Disruption in the blood-testis barrier can lead to the production of antisperm antibodies (ASA). The aim of this prospective study was to investigate the frequency of ASA formation in couples after TESE procedure.

Methods: Thirty-seven couples were included in the study at the Urology Clinic of the Dr. Zekai Tahir Burak Women's Health Training and Research Hospital. History, physical examination, spermiogram, and endocrine profiles were obtained for all male patients. All the male patients in this study had been diagnosed with nonobstructive azoospermia (NOA) and underwent microdissection TESE. Secondary and tertiary cases were also included in the study. Serum samples were obtained from all 74 patients before TESE, and at three and 12 months after TESE. Serum ASA levels were determined. ANOVA was performed for statistical analysis for serum Follicle-Stimulating Hormone (FSH), testosterone and testicular volume. $P < 0.05$ was considered significant.

Results: There were no differences in the testicular volumes, serum FSH and testosterone levels before and after TESE. None of the patients or their partners developed significant levels of ASA as a result of the TESE procedure.

Conclusion: TESE procedure does not cause ASA production in either males or their female partners.

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The first successful pregnancy achieved by spermatozoa obtained by testicular sperm extraction (TESE) took place in 1993 [1]. The combination of intracytoplasmic sperm injection (ICSI) and TESE is now first line treatment in nonobstructive azoospermia (NOA).

Antisperm antibodies (ASA) are found in 9%-12.8% of infertile couples [2]; however, these antibodies are also present in 1%-2.5% of fertile men and 1.4% of fertile women [3]. Antisperm antibodies can impair the fertilizing capacity of human spermatozoa by affecting sperm motility, cervical mucus penetration, gamete fusion, and, potentially, even the first steps of embryo development [4,5]. The formation of antisperm antibodies (ASA) may be a consequence of rupture in the blood-testis barrier. Several etiologic factors for ASA formation have been described, such as genital tract infections, genitourinary trauma, cystic fibrosis, vasectomy, varicocele and inguinal hernia repair [6,7].

The aim of this prospective study was to investigate whether TESE causes ASA formation in either males or females.

Methods

Patients

The study was approved by the local ethics committee and informed consent was obtained from each participant. Forty couples were included in the study between March 2007 and May 2009 at the Urology Clinic of the Dr. Zekai Tahir Burak Women's Health Training and Research Hospital. History, physical examination, spermiogram, and endocrine profiles were determined for all male patients. All the male patients had been diagnosed with NOA and underwent microdissection TESE. Secondary and tertiary cases were also included in the study. Serum samples were obtained for all 74 patients before TESE, and at three and 12 months after TESE. Serum ASA levels were also determined. Three couples were excluded from the study because of previous ASA positivity. Thirty-seven couples (21 primary, 12 secondary and 4 tertiary cases) were, therefore, evaluated.

Surgical approach

Microdissection TESE was performed under local anesthesia according to a procedure reported previously [8].

Serum samples and ASA detection

Blood samples were collected between 09:00 AM and 11:00 AM. The concentration of ASA in serum samples was measured by the anti-spermatozoa antibody ELISA (Enzyme Linked Immunosorbent Assay) from BIOSERV Diagnostics GmbH (Rostock, Germany). ASA levels over 60 IU/ml were accepted as positive. Levels of Ig A, Ig M and Ig G were measured as a combined Ig group.

Statistical analysis

Statistical analysis was performed by Statistical Package for Social Sciences (SPSS) 15 software (SPSS Inc., Chicago, USA). All the data are presented as mean $\pm$ SD. ANOVA analysis was performed for statistical analysis for serum Follicle-Stimulating Hormone (FSH), testosterone, testicular volume and ASA levels. $P < 0.05$ was considered significant.

Results

Pre- and post-operative characteristics of the patients with mean age of 32.8 (± 6.7) years are shown in Table 1. Pre- and post-operative serum FSH and testosterone levels (three and 12 months after TESE) of the patients were not significantly different. There were no differences in the testicular volumes before and after TESE. None of the patients or partners developed significant levels of ASA as a result of the TESE procedure (Table 2). Spermatozoa were found in 15 of 37 (40.5%) patients with NOA.

One patient had a small hematoma that responded to conservative therapy; two patients had prolonged pain (10 days). By the 12th month, one patient had segmental devascularization and the testosterone level of one patient was slightly reduced.

TABLE 1: Characteristics of the male patients (n= 37).

	Preoperative	3 months after TESE	12 months after TESE	F	P value
Serum FSH (IU/l) Mean ($\pm$ SD)	15.4 $\pm$ 2.63	16.1 $\pm$ 2.59	16.4 $\pm$ 2.15	1.603	0.206
Serum total testosterone (ng/dl) Mean ($\pm$ SD)	4.1 $\pm$ 0.78	3.9 $\pm$ 0.72	3.7 $\pm$ 0.68	1.927	0.151
Testicular volume (ml) Mean ($\pm$ SD)	9.8 $\pm$ 1.29	9.5 $\pm$ 1.35	9.2 $\pm$ 0.94	2.467	0.090

TABLE 2: Serum ASA levels

	Before TESE	3 months after TESE	12 months after TESE	F	P value
Male patients (Mean±SD; IU/ml) (n= 37)	31.14±10.47	33.16±12.37	35.24±2.10	1.755	0.178
Female patients (Mean±SD; IU/ml) (n= 37)	27.05±11.44	29.18±12.18	31.22 ±10.75	1.217	0.300

Discussion

Testicular spermatozoa can be retrieved successfully by the TESE procedure and used for ICSI in cases of NOA [9]; however, a decrease in testosterone, and the development of testicular scars and devascularization after conventional TESE have been reported [10,11]. Recently, micro TESE has been shown to have high testicular sperm retrieval rates and a low incidence of postoperative complications [8].

The blood-testis barrier is formed by the continuous layer of Sertoli cells and separate sperm and its precursors from the immune system [12]. Antisperm antibodies may be produced when this blood-testis barrier is breached, allowing sperm antigens to elicit an immune response. Any disruption in the blood-testis barrier can lead to the production of ASA. Vasectomy, acute epididymitis, cryptoorchidism and testicular trauma may all lead to production of ASA [13-15]. Couples with ASA have a higher incidence of impaired sperm motility, abnormal sperm agglutination and unexplained infertility [16-18]. ASA may impair sperm fertilizing ability and is a serious factor which may prevent the success of various fertilization techniques. ICSI seems to be successful in overcoming this problem [4].

Histopathological alterations in the damaged testis are manifested by a markedly increased number of lymphocytes and macrophages in the interstitium, autoantibody production, different degrees of germ cell degeneration and sloughing, resulting in aspermatogenesis and atrophy of the seminiferous tubules [19,20]. Recently, testicular tissue antigens have proven crucial for the study of male infertility due to infection and inflammation. A study by Fijak and colleagues, designed to induce experimental autoimmune orchitis (EAO) in rats, showed that a number of proteins were upregulated, including heat shock proteins 60 (Hsp60) and 70 (Hsp70), disulphide isomerase ER-60, alpha-1-anti-trypsin, heterogeneous nuclear ribonucleoprotein H1 (hnRNP H1), sperm outer dense fibre major protein 2 (ODF-2) and phosphoglycerate kinase 1. ODF-2, a testis-specific protein, was identified as a major target for immune attack [21]. Heterogeneous nuclear ribonu-

cleoproteins were first described as a major group of chromatin-associated RNA binding proteins. Disulphide isomerase ER-60 is localized in the lumen of the endoplasmic reticulum, is enriched in secretory cells, and is responsible for the rearrangement of both intra-chain and inter-chain disulphidebonds in proteins in order to form native quaternary structures. R-60 and Hsp-70 were also identified by ASAs from infertile men [22].

Because immunological infertility is a rare occurrence, there are relatively few studies on this issue. Literature reveals few studies where the immunologic response has been evaluated after different sperm recovery techniques. Steele *et al.* showed that none of the patients undergoing percutaneous biopsy developed ASA [23]. Westlander *et al.* also found that testicular sperm aspiration did not cause ASA formation [24]. There are a limited number of studies in which ASA after TESE was evaluated. Harrington *et al.* performed 20 open testicle biopsies, in addition to 31 percutaneous testicle biopsies, and none of these patients exhibited ASA at six months of follow up [25]. Komori *et al.* compared conventional TESE and micro TESE and reported that neither group produced ASA. ASA was measured by a sperm immobilization assay in that study [26].

In contrast to other published studies, this study also included the partners of the patients and some patients had undergone recurrent TESE. The etiology of sperm immunity in human females is unknown, but several possible mechanisms have been proposed, including cross-reactivity with microbial antigens and interferon gamma-mediated potentiation of the anti-sperm immune response in women whose male partners have sperm autoantibodies in their semen [27]. A number of clinical studies as well as in animal models, have shown that ASA, in the sera of infertile women, caused low fertilization rates and poor embryo quality. Shibahara *et al.* found that women with positive ASA levels have lower pregnancy rates than a control group because ASA in women may cause damage to human embryos during early stage development *in vivo* [28]. Similarly, Taneichi *et al.* reported cases where ASA worsened embryo quality and fertilization rates [29]. In rats, ASA

in plasma has also been shown to have an inhibiting effect on embryo development [30].

Conclusion

After the 12-month follow up, neither male patients that had undergone TESE nor their female partners developed ASA. Thus, the TESE procedure does not cause ASA production in couples; and further studies addressing immunological causes of infertility are needed.

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