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Antisperm Antibody is not Associated with Infertile Males with Epididymal Cyst

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Abstract

Objective: The purpose of this study was to investigate the level of antisperm antibodies (ASA) in infertile men with epididymal cyst (EC).

Materials and Methods: A total of 42 infertile men with unilateral EC diagnosed by scrotal color Doppler ultrasonography were enrolled in the study. Semen samples were analyzed and ASA levels were measured by direct immunobead test for ASA types IgG and IgA according to the World Health Organization (WHO) laboratory manual (2010). Leukocyte was determined using a myeloperoxidase staining method.

Results: Of these 42 infertile men with EC assessed for ASA-IgG, only two cases (4.8%) were respectively present 12% and 18% motile spermatozoa binding to immunobeads in sperm samples. None of these cases satisfied the clinical positive level for ASA-mediated infertility according to the WHO criteria, whilst relatively abnormal sperm parameters were revealed in partial cases. ASA-IgA was not detected in all cases. Nine (21.4%) cases with EC showed that leukocytes were increased in their samples.

Conclusions: The significant incidence of ASA was not found in infertile men with EC, which suggests that EC would have not an increased risk for the production of ASA. But increased leukocytes in semen of the cases would be noted for their treatment

Keywords: Epididymal cyst; Antisperm antibody; Infertility; Sperm; Leukocyte

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Introduction

Epididymis is the organ for sperm maturation, transport, and storage. Under physiological conditions, the epididymal tubule microenvironment plays a critical role in the protection of sperm [1]. Thus changes in structure and function of epididymis can directly affect the status of sperm in the epididymis.

Epididymal cysts (ECs) have been reported in 20%-40% of asymptomatic individuals, with 29% having more than one cyst [2]. Congenital EC is widely accepted as a cystic neoplasm of Wolffian origin, although infection, obstruction, hormonal disorders, and trauma have been proposed as etiological factors of EC [3]. In recent years, EC is being recognized to be more prevalent than expected due to the widespread use of scrotal color Doppler ultrasonography. It is reported that 14.4% in studies of males of all age patients had asymptomatic EC [4]. Moreover, clinical investigations indicated that EC would be related to male infertility [5, 6]. Several contributing factors have been proposed to explain the EC-associated infertility, such as epididymal duct obstruction, tubular atrophy, and changes of epididymal microenvironment, which can lead to abnormal sperm parameters [7-9].

The EC is lined by columnar epithelium and contains serous fluid [7]. Previous studies showed that the fluid of EC contained spermatozoa [8, 9]. Additionally, local production of pro-inflammatory cytokines, such as IL-6, IL-8 and TNF-α were observed in EC [10]. These pathological features of EC indicated that the development of EC might involve epididymal tubules, and the disruption of blood epididymal barrier (BEB) might exist due to EC. This would provide a possibility for sperm antigens to escape from the epididymal tubule into systemic circulation and thereby to activate epididymal autoimmune response and induce the production of antisperm antibody (ASA).

ASA is associated with decreased male fertility potential [11]. ASA-mediated immunologic infertility is ascribed to be one of the important mechanisms by which infertility is mediated in humans [12]. The ASA, generated from epididymal autoimmunity to sperm surface antigens, has also been found to be associated with male infertility [13]. In addition, breaches in the BEB are considered to be one of the causes which will result in the production of ASA [14]. Due to EC may have a negative effect on epididymis, it is necessary to evaluate the relationship between EC-associated male infertility and ASA.

This study was conducted to detect the ASA levels in infertile males with EC by using the direct immunobead test (D-IBT), aiming to clarify whether the EC would be a risk factor for inducing the production of ASA in such cases.

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Methods

Study subjects

A total of 42 infertile males (range 24-48 years, mean age 32.8±5.5 years) who visited infertility clinics at the First Affiliated Hospital of Jinan University, China were enrolled in the study. EC was diagnosed by scrotal color Doppler ultrasonography and all cases were unilateral EC. Inclusion criteria for this study were normal testes, normal hormonal profile, and without a history of radiotherapy, chemotherapy, chronic illness, or medication.

Semen analysis

Semen samples were collected by masturbation after 3-7 days of sexual abstinence. After liquefaction of semen, semen analysis was performed according to the World Health Organization laboratory manual (WHO, 2010). Sperm morphology was examined by the Papanicolaou-staining method, and leukocyte was determined using a myeloperoxidase staining technique [15].

ASA test

ASA level in semen samples was detected by the D-IBT according to the WHO protocol [15]. Briefly, 5μl washed sperm suspension was placed on a glass slide and 5μl of each type of immunobeads (IgG and IgA, Irvine Scientific, California, USA) were added to the sperm suspension and mixed well, and then a cover slip was placed on each of the mixtures. After incubated for 10 min at room temperature, the slide was examined with a phase-contrast microscope. The percentage of motile spermatozoa with one or more attached immunobeads was recorded. More than 50% motile spermatozoa with one or more attached immunobeads were considered immunologic infertility according to the WHO criteria [15]. In our laboratory, 10-49% motile spermatozoa with immunobeads in sperm samples were deemed to be suspicious positive [16].

Results

ASA in sperm samples of infertile males with EC

Of the 42 infertile men with EC assessed for ASA-IgG, only two cases (4.8%) were respectively present 12% and 18% motile spermatozoa binding to immunobeads in sperm samples. None of these cases satisfied the clinical positive level for ASA-mediated infertility according to the WHO criteria. With regard to ASA-IgA, all cases were devoid of binding immunobeads.

Semen analysis in infertile males with EC

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In these 42 infertile men with EC, sperm concentration was 57.8±36.4×10^6sperm/ml, sperm motility was 53.6±11.5%, and abnormal sperm morphology was 94.6±3.8%. According to the lower reference limit of the WHO manual [15], 7 cases (16.7%) had low sperm concentration (11.2±3.1×10^6sperm/ml), 15 cases (35.7%) had low sperm motility (30.6±5.7%), and 22 cases (52.4%) showed high abnormal sperm morphology (98.5±1.1%).

Nine cases (20.4%) had higher leukocyte levels (3.8±1.3×10^6/ml) than the reference limit (1.0×10^6/ml) of the WHO manual [15]. Moreover, no clinical symptom of infection such as epididymitis or prostatitis was found.

Discussion

In this study, none of the infertile males with EC reached the 50% diagnostic criteria of ASA-mediated infertility. Only 2 cases were respectively detected 12% and 18% motile spermatozoa with immunobeads, contributing to a sub-positivity rate of 4.8% in the study. Therefore, such low incidence and titre of ASA could not be contributed to the pathogenesis of EC-associated immune-infertility.

Previous investigations demonstrated that the fluid of EC was found to contain a few motile and nonmotile spermatozoa [10, 17]. The presence of sperm in EC could be related to the disruption of epididymal tubules. It is supposed that sperm antigens in EC may leak from epididymal tubules into systemic circulation, and then activate the autoimmune response to induce the production of ASA. Moreover, inflammatory cytokines were present in substantial concentrations in the EC [10]. Cytokines are messengers of the immune system, which can activate lymphocytes and induce immune response. In this study, the results showed that EC was not involved in the production of ASA. Three possible causes could be considered about this paradox: (1) EC is benign cystic lesion and has well-defined wall. Tissue destructions of epididymis caused by infiltration of malignant tumors may not exist in EC which appears as a benign lesion. Hence, the BEB was not damaged, and sperm antigens can not escape from the epididymal tubules into systemic circulation. (2) Under certain circumstances, the BEB could be damaged by EC. When disruption in the BEB was slight, sperm antigen accumulations might fill up the damage site. Thus very little quantity of sperm antigens could be induced to release into the systemic circulation. Such a low concentration of sperm antigens, autoreactive T cells and B cells are presumed to maintain ineffective and therefore show no activation in response to sperm antigens [18, 19]. Consequently, the autoimmune reaction might be suppressed in infertile males with EC. (3) Interstitial tissue of epididymis comprises a loose connective tissue, macrophages and lymphocytes are frequently observed within the interstitial tissue [18, 20]. Macrophages and lymphocytes are responsible for phagocytosis of excess sperm antigens, and could subsequently inhibit autoimmune response [21]. Furthermore, the possibility that BEB may be severely damaged in infertile males with EC could not be excluded. Under this condition, a large amount of sperm antigens were released into the systemic circulation. The administration of antigen in high does can induce specific unresponsive states, and peripheral tolerance known to be high

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zone tolerance (HZT) is supposed to established [22]. Thus the autoimmune response may be suppressed in these patients. Herein, an additional mechanism of peripheral tolerance termed clonal ignorance may be engaged in the absence of ASA as well [23].

Abnormal sperm parameters can contribute to lower fertilizing potential, leading to male infertility. In this study, incidences of the lower reference limit of the WHO criteria for sperm motility, count, and abnormal morphology in infertile males with EC were 30.6%, 35.7%, and 52.4%, respectively, which indicated that poor sperm parameters existed in some infertile men with EC. Several mechanisms involved in the EC-associated sperm parameters abnormalities have been reported [7-9]. Among these mechanisms, it is widely accepted that the size of EC exerts a significant pressure on the epididymis, in that micro-surgical removal of EC showed most significant improvement of sperm motility [24]. As the acquisition of sperm motility during passing through the epididymis, EC could have negative effects on regulation of sperm motility in epididymidis, resulting in decrease in sperm motility after ejaculation. It was noted that most cases had a high percentage of abnormal sperm morphology. We reasoned that defects in sperm morphology cannot be excluded before the formation of EC in these cases due to the shaping of sperm head occurring in spermiogenesis. On the other hand, EC would change epididymal secretion and absorption that result in the disturbance of sperm maturation in the epididymis. Thus EC could influence on sperm morphology such as nuclear chromatin degeneration, neck or mid-piece cytoplasm retention, and tail coiling, resulting in more spermatozoa with abnormal morphology. In addition, because of EC and its evolving, the pressure of EC on the epididymis would affect the epididymal blood supply and then cause epididymis atrophy, which could change epididymal physiological and anatomic integrity, leading to impaired epididymal functions and abnormal sperm parameters. Therefore, EC would probably, at least in part, play a certain role in the pathogenesis of EC-associated infertility.

Generally, the presence of leukocyte in semen may reflect infection of the reproductive tract. In this study, increased leukocyte levels in the samples were observed in 20.4% cases. However, the origin of leukocyte in semen for those cases was unclear, since no obvious clinical symptom of infection was found in them. The etiology of the epididymal cysts is uncertain. Previous study indicated that local production of pro-inflammatory cytokines like IL-6 and IL-8 were involved in the formation of EC [10]. Additionally, the close association of IL-6 and IL-8 with seminal leukocyte was found [25, 26]. It seems reasonable to assume that leukocytes in semen of infertile males with EC could be associated with the pro-inflammatory cytokines. On the other hand, most cases were examined leukocyte in the normal reference range, which indicated that the formation of EC in these patients might not be caused by infection. Although the significance of leukocyte in semen remains a matter of debate, leukocyte is a frequent finding in semen of infertile males, even in the absence of inflammatory [27]. As leukocyte in semen was linked to decreased sperm quality [28, 29], therefore, increased leukocyte levels in semen should be treated effectively.

In this study, some potential limitations should be considered. Firstly, this study confined to infertile men with EC only, owing to the lack of the control group of fertile males with EC.

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Secondly, the development of EC was not assessed, whilst this was an essential factor that would influence on ASA production under certain conditions. Thirdly, the sample sizes were relatively small, and more cases are needed to further evaluate aspects on EC-associated infertility.

In conclusion, the significant incidence of ASA was not found in the infertile men with EC, which suggests that EC may be not a risk factor for the production of ASA. Increased leukocytes in semen were found in part of the cases with EC, and this phenomenon should be noted in clinical treatment. More investigations on the pathogenesis of infertile men with EC will further help to understand the EC-associated infertility and increase the clinical treating efficacy.

Conflict of Interest

None declared.

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