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ORIGINAL ARTICLE

Role of Local Immune Reaction in Primary Obstructive Male Infertility

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Abstract: *Objective:* To find evidence of local immune reaction (cellular and, if possible, humoral) in patients of primary obstructive infertility and to extrapolate its role in the persistence of infertility even after a patent vasoepididymostomy. This was a prospective study in a tertiary hospital setting comparing epididymal histology and epididymal fluid antisperm antibody in men with primary obstructive infertility with those in men with proven fertility. *Material and Methods:* Twenty men with primary obstructive infertility in the study group and five men with proven fertility as controls were included in the study. While performing vasoepididymostomy on such patients, testicular and epididymal tissues were taken, and epididymal fluid was aspirated for estimation of antisperm antibodies. Testicular and epididymal histologies were studied under light microscopy for evidence of cellular immune response to obstruction. Antisperm antibodies in epididymal fluid were assayed by ELISA. *Result(s):* The cellular immune response as assessed by the presence of interstitial inflammatory cells and macrophages in epididymal histology was found in 12/20 patients. Testicular biopsies in all patients were normal. Humoral immune response as assessed by significant titres of antisperm antibodies in epididymal fluid was found in 14/20 patients. Evidence of local immune response (cellular or humoral or both) was found in 17/20 patients. *Conclusion(s):* Men with primary obstructive infertility develop local immune reaction in their epididymis secondary to obstruction. This factor may be responsible for the persistence of infertility even after a patent vasoepididymostomy.
Keywords: Epididymis / Histology / Immunity / Male Infertility

Introduction

After puberty, sperms continue to be produced in the testis and pass into the epididymis as a result of seminiferous tubule contractions of the myoid cells (hormone dependent), fluid build up and pressure and testicular capsule contractions. Epididymis is responsible for sperm maturation and motility and usually two-thirds of the epididymis is needed for sperm transport in the human for normal sperm fertilization capacity. The sperms take approximately two weeks to get through the epididymis ultimately to be stored near the tail portion and in the vas deferens until they are ejaculated. Experimentally it has been shown that epididymal obstruction produces histologic changes in the epididymis and testis besides inducing an immunological response with raised ASA titres in the serum [1-3].

In humans the effect of epididymal obstruction can best be studied in men undergoing vasectomy [4-5]. Study of this group is especially useful since both partners have previously proven fertility. On the basis of such a study, similarities can be drawn between vasectomized patients and those with congenital obstruction to explain the changes that may be responsible for changes in epididymal pathology and sperm morphology.

The extravasation of the highly antigenic sperms or their degradation products into the interstitium brings them into contact with the antibody producing cells. If the exposure is not large enough to reach the lymph nodes, antisperm antibodies will not result in the serum. However, the exposure would be sufficient to induce an immune response locally with its attendant effects on the epididymis and sperms. This local immune response may excite further structural or functional deterioration of the epididymis and the sperms contained within it. These local changes may persist after the obstruction has been bypassed and, along with the production of ASA which possibly could affect the sperm function, may be an important factor influencing fertility. Direct damage due to obstruction has long been implicated as the cause of persistent infertility despite a successful reversal of obstruction. Vasovasostomy by fistula method have a patency rate of 43.7 % but a pregnancy rate only 16.8% [6] and by microsurgical technique while the patency rate is 75.3% and pregnancy rates only 29% [7]. The Vasovasostomy Study Group, the largest multicenter group to assess vasovasostomy efficacy, found that if the interval was less than 3 years from the vasectomy, patency was 97% and pregnancy was 76%. In men more than 15 years from their vasectomies, patency and pregnancy rates fell to 71% and 30%, respectively [8]. Although these effects may be due to the production of ASA or changes in the epididymis, the effect on the epididymal histology or the sperms due to local humoral and cell mediated immune response have never been studied. Actually, the histologic changes reported in obstructive infertility by some authors [9-11] and even after vasectomy reports of inflammatory cell infiltrate with lymphocytes, plasma cells and macrophages, perivasculitis and fibrosis in epididymal histology [12-13] may be due to a local immune response resulting from contact of sperm proteins with local immune competent cells secondary to obstruction to sperm egress [12]. The present study, approved by IEC, presents direct and indirect evidence in the form of cellular and humoral changes of local immune reaction in patients of primary obstructive azoospermia.

Material and Methods

Twenty patients of primary obstructive infertility were studied under a fixed protocol. The selected patients were azoospermic with clinically palpable vas deferens and epididymis. None of the patients had a history or clinical evidence of infection, trauma or surgery; none had undergone vasectomy. They underwent fine needle aspiration cytology (FNAC) of both testes, and patients with active spermatogenesis were included in the study. Other selection criteria were that the wife should be normal and that the vas should be patent. Patency of the vas deferens was confirmed intraoperatively by injecting 20 ml of normal saline into the vas at the

proposed site of anastomosis and asking the patient whether he had any desire to micturate. Patients with fructose absent in the semen and those with serum FSH levels more than two and one half times the normal value were excluded. However, during the course of the study certain criteria were relaxed; some patients with FNAC of the testes showing impaired spermatogenesis and those with raised FSH levels were also included. The selected patients underwent unilateral vasoepididymal anastomosis. Patients who had undergone vasoepididymostomy on one side were reoperated only after a gap of at least one year. Peroperative testicular and epididymal tissues were transported in Bouin's fluid to the Pathology department. Smears of epididymal fluid were also made for cytology. Epididymal fluid was aspirated with a micropipette and diluted with 0.5 ml. of normal saline for the analysis of antisperm antibody.

Five controls were also studied. The testes and epididymis were obtained at autopsy of subjects who had died from roadside trauma. All of these were of proven fertility. Testicular and epididymal wedge biopsies were taken from these cases. Epididymal fluid was also aspirated for cytology and also for the analysis of antisperm antibody. Testicular and epididymal tissue specimens were processed for paraffin block preparation, five micron sections were taken were observed under microscope for grade of spermatogenesis [14] and for evidence of local immune reaction on the basis of morphological characteristics of the cells and by immunohistochemical techniques. Peroperatively, on incision of the epididymis, the inspissated seminal fluid coming out was taken on a few slides and smears were made. These smears were fixed in difluorotetrachloroethane and sperm morphology studied using Papanicolaou staining procedure [15]. Epididymal fluid collected in the syringe was analyzed for antisperm antibody by ELISA technique utilizing a commercially available Varelisa Sperm Antibodies Enzyme Immunoassay kit. Varelisa Sperm Antibodies is an indirect noncompetitive enzyme immunoassay for the semi-quantitative and qualitative determination of lgG, IgM and lgA antibodies directed to spermatozoa surface antigen. The wells of a micro titer plate are coated with spermatozoa surface antigen. Antibodies specific for sperm present in the patient sample bind to the antigen. In a second step the antigen-antibody complex reacts with an enzyme labeled second antibody (Enzyme Conjugate) which leads to the formation of an enzyme labeled antigen-antibody sandwich complex. The enzyme label converts added substrate to form a colored solution. The rate of color formation from the chromogen is a function of enzyme conjugate complexed with the bound antibody and thus is proportional to the initial concentration of the respective antibodies in the patient sample. The significant ELISA was absorbance (OD) value > 0.328 as mentioned in the kit manual. Statistical analysis was done using the Student's *t*-test.

Results

The epididymis was not found to be distended in two patients, was only mildly distended in four cases and had obvious distension containing yellow inspissated material in the remaining cases. Discharge on incising the epididymis was negligible in seven cases and moderate to free flowing in the remaining cases.

Spermatogenesis in testicular biopsy specimen was assessed by grading of spermatogenesis and by quantitation of mature spermatids per seminiferous tubule [16]. All thirteen cases with Grade 1 (normal) spermatogenesis were seen to have > 30 spermatids per tubule. All four cases with Grade 2 spermatogenesis were seen to have < 30 mature spermatids per tubule. All cases of Grade 3 (one) and Grade 4 (two) spermatogenesis had no mature spermatid per tubule. Thus there is a good correlation between grading of spermatogenesis and quantitation of mature spermatids per tubule with > 30 mature spermatids per tubule indicating normal spermatogenesis and < 30 mature spermatids per tubule indicating some degree of hypospermatogenesis. In our study, thirteen out of twenty patients (65%) had Grade 1 (normal) spermatogenesis.

Histology of the epididymis in this study demonstrated dilated epididymal ducts in twelve out of twenty cases (60%), epithelial flattening with focal or diffuse loss of cilia in eleven cases (55%) and pigment in epithelial cells in eight cases (40%). Sperm ingestion by epithelial cells could not be demonstrated. Intraluminal macrophages were present in eleven cases (55%) and intraluminal sperms in thirteen cases (65%). The coexistence of sperms and macrophages in the ducts was statistically significant (p = 0.02). Out of eleven cases in which intraductal macrophages were present, eight (72.72%) showed sperm ingestion, ten (90.9%) showed vacuolation and five (45%) showed pigment. (Table 1; Figures 1-4)

Table-1: Histologic characteristics of the epididymis in the study group

Features in epididymal histology	**No (n=20)**	**%**
Epithelial flattening with loss of cilia	11	55
Pigment in epithelial cells	08	40
Ductal dilatation	12	60
Ductal sperms	13	65
Ductal macrophages	11	55
Vacuolation	(10)	(90.9)
Sperm ingestion	(08)	(72.7)
Pigment	(05)	(45.7)
Epithelial breach with sperm extravasation	07	35
Interstitial macrophages	07	35
Interstitial lymphocytes, plasma cells etc	09	45
Evidence of cellular immune reaction in interstitium	12	60

Figure-1: Photomicrograph of the epididymis of a patient in the control group showing normal epididymal ducts with orderly layering of nuclei and cilia on the luminal surface (H&E x100)

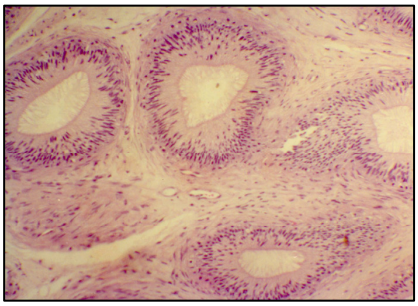


Figure-2-: Photomicrograph of the epididymis of a patient in the study group showing markedly dilated ducts containing sperms and macrophages (H&E x60)

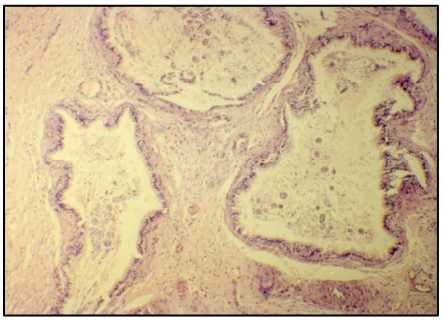


Figure-3: Photomicrograph of the epididymis of a patient in the study group showing sperm extravasation with epithelial breach (H&E x250)

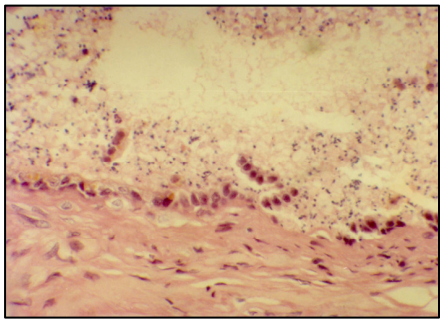
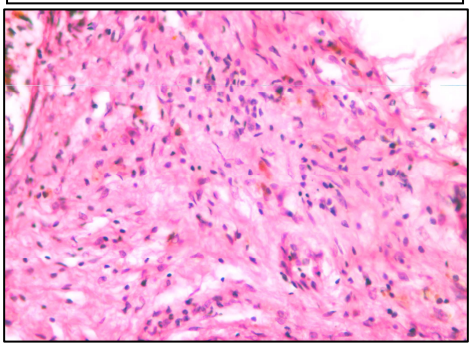


Figure-4: Photomicrograph of the epididymis of a patient in the study group showing sperm extravasation with interstitial inflammatory cell infiltration (H&E x400)



The present study strongly suggests the occurrence of local immune reaction in the epididymis consequent to obstruction since extravasation of sperm with epithelial breach was seen in seven cases (35%) and inflammatory cell infiltration (lymphocytes, plasma cells, macrophages etc.) into the interstitium was seen in twelve cases (60%). There is a significant correlation ($p = 0.01$) between presence of interstitial inflammatory cell infiltration and sperm ingestion by ductal macrophages. The above mentioned changes in the epididymal histology were not found in of the controls.

Study of epididymal fluid cytology revealed sperms to be present in thirteen out of twenty cases (65%); this had a statistically significant correlation with grade of spermatogenesis ($p= 0.04$). Macrophages were seen in the epididymal fluid in eleven cases (55%), six of these eleven cases (55.5%) showed sperm ingestion by macrophages. Antisperm antibodies in epididymal fluid were evaluated by ELISA. Fourteen cases (70%) showed significant ELISA. Since dilatation of epididymis was obvious in only fourteen cases with minimal discharge on incising the epididymis in seven cases, it is possible that insufficient fluid was available for estimating ASA titres in the remaining cases. None of the five controls showed a significant ELISA.

Thus, in this study, seventeen out of twenty cases (85%) had one or both evidences of local immune reaction. None of the five controls (0%) had any evidence of local immune reaction. The testicular and epididymal histology was normal and there was no evidence of inflammatory cell infiltrate in the interstitium.

Discussion

Obstruction to the outflow of the sperms is an important cause of primary infertility in males**.** The commonest site of obstruction is the distal part of the epididymis or the proximal part of vas deferens [17]. The obstruction results most frequently due to vasectomy or may be due to congenital, infective or traumatic causes. The resultant effects following vasectomy have been well documented both in human subjects and experimentally. After vasectomy, epididymal distension and sperm granuloma formation result from raised intraluminal pressure [1, 4-5]. The sperm granuloma is a dynamic structure and a site of much spermatozoal phagocytosis by its macrophage population. Intraluminal phagocytosis may explain why some reproductive tracts become depleted of spermatozoa [4]. In many species, spermatozoa in the obstructed ducts are destroyed by intraluminal macrophages, and degradation products, rather than whole sperm, are absorbed by the epididymal epithelium. The epididymal environment is required for the final maturation of spermatozoa and the acquisition of normal motility and fertilizing ability. A report suggest that these epididymal functions may be impaired in some men after vasectomy due to a disturbance of epididymal physiology producing morphologic abnormalities of the sperm tail thus resulting in persistent infertility after vasectomy reversal by vasovasostomy [18]. In other types of obstructive infertility also effect on the epididymis in human subjects has been documented although sperm granulomas have never been reported. [9-11]

Clinically antisperm antibodies are found in the serum in 3% to 12% of men who undergo evaluation for infertility as compared to 2% in the normal fertile population [19]. Obstruction leading to the production of antisperm antibodies is best studied in vasectomized and subsequently vasovasostomized men. In this group both partners have previously proven fertility. Following vasectomy the prevalence of ASA is 34% to 74% and they persist in 38% to 60% following successful vasovasostomy [20]. Production of ASA after obstruction of the epididymis has also been reported in experimental animals.[2,21] After vasectomy ASA are produced as a result of granuloma formation at the site of vasectomy or proximal to it [22]. These sperm granulomas are sites of spermatozoal phagocytosis by its macrophage population which initiate the immune response.[4,22] The production of ASA results from the degradation spermatozoal products which may leak at the breached epididymal sites [23] or which may be produced in larger quantities than to be overcome by the immunological suppressor barrier [4,22,24]. ASA may result in the serum, seminal fluid or on sperm surface either as a result of transport of spermatozoal degradation products by the macrophages to the regional lymph nodes, breakdown of the blood-testis / blood-epididymis barrier due to trauma or infection with escape of sperm antigens directly into the microcirculation, or, by production of antibodies against infective agents like Chlamydia which cross react with spermatozoa [20]. Surprisingly, the association of ASA with obstructive azoospermia due to congenital causes is not yet well established with conflicting data being reported in literature [20]. A report shows that antisperm antibodies are present in the serum of men with epididymal occlusion whether of infective or congenital origin [20].

The incidence is higher when occlusion is more distal. Sperm agglutinating antibodies in serum were found to be present in 5% of patients with pathological obstruction, negative in patients with a patent vasoepididymostomy and positive in 17% of those with an unsuccessful vasoepididymostomy. The four patients who impregnated their wives after vasoepididymostomy were antibody negative [20].

Even after obstructing the flow, sperms continue to be produced. As a result epididymal dilatation with breach in the epithelium and extravasation have been reported previously [9,11]. Extravasation of the highly antigenic sperms and their exposure to the immunologically active cells within the epididymis interstitium could possibly excite a local immune response resulting in local damage and producing ASA [12].

Immunologically the testis is a privileged site with the blood-testis barrier morphologically and humorally separating the germ cell population from the interstitial tissue[23]. In obstructive infertility, the testis is affected directly as a result of epididymal dilatation due to obstruction with consequent hypospermatogenesis possibly due to pressure atrophy of the seminiferous tubules when the rate of sperm production exceeds the rate of sperm absorption [2]. It has even been suggested that obstruction may result in resorption of sperms within the epididymis and a feedback mechanism operates which decreases the rate of spermatogenesis [21]. In the present study there was a good relation between grading of spermatogenesis and quantitation of mature spermatids per tubule. Thus, thirteen out of twenty patients (65%) had Grade 1 (normal) spermatogenesis. The other 35% with variable degrees of hypospermatogenesis may be having primary testicular insufficiency or may have obstruction as a contributory factor. This study does not establish the effect of obstruction on the degree of spermatogenesis. Following vasectomy in humans and in experimental animals, changes in the testis attributable to an immune alteration have been reported [22, 25-27]. However, the effect of the possible immune response to obstruction was not evident in testicular histology in the present study with the interstitium being free from any inflammatory cell infiltration.

The epididymis is considered to be the most likely source of antibody secretion. It is densely populated with lymphocytes and macrophages [23]. Normally, epididymal epithelial lining prevents the extravasation of sperms or their degradation fragments into the interstitium, thus blocking a direct interaction between antibody producing cells and sperm proteins. A breach in the epididymal epithelium would lead to a direct contact between sperm antigens and immune effector cells. The secretion of antibodies by the epididymis would, therefore, be increased as suggested by the presence of inflammatory cells including plasma cells, in the interstitium of these cases [9]. These antisperm antibodies secreted locally in the epididymis would act on the already disintegrating spermatozoa and further impair their function and maturation. It is possible that the cell mediated immune response also acts on the epididymal tissue producing irreversible histological changes, which impair the function of the epididymis, in turn impairing sperm maturation. Consistent to the reports in the literature, this study demonstrated dilated epididymal ducts (60%),

epithelial flattening with focal or diffuse loss of cilia (55%) and pigment in epithelial cells (representing remnants of ingested sperms) (40%). Sperm ingestion by epithelial cells could not be demonstrated. Intraluminal macrophages (55%) and intraluminal sperms (65%) represented the important function of the macrophages in scavenging the sperms in the obstructed epididymis. The intraductal macrophages demonstrated sperm ingestion, vacuolation and pigment. This correlates well with Phadke's observation that phagocytosis of spermatozoa in case of obstructive azoospermia was chiefly intraluminal [9].

Following vasectomy, dilatation of epididymal ducts with sperm extravasation due to obstruction has also been reported [9]. Inflammatory cell infiltrate with lymphocytes, plasma cells and macrophages, perivasculitis and fibrosis in epididymal histology have been demonstrated following vasectomy similar to the findings in our study [12]. Hargreave et al attribute the histologic changes in these reports to be due to a local immune response resulting from contact of sperm proteins with local immune competent cells secondary to obstruction to sperm egress [12]. Study of epididymal fluid cytology revealed sperms to be present in 65% and macrophages in 55% with more than half of them showing sperm ingestion. In comparison, Phadke observed macrophages in fifty two out of hundred cases (52%) and sperm ingestion in forty seven out of these fifty two cases (90.4%).[9]

The reports in literature about epididymal histology do not take into account the possibility of a local immune reaction. The present study strongly suggests the occurrence of local cell mediated immune reaction in the epididymis consequent to obstruction since extravasation of sperm in the interstitium with epithelial breach was demonstrated to be associated with inflammatory cell infiltration (lymphocytes, plasma cells, macrophages) into the interstitium. The above mentioned changes in the epididymal histology were not found the controls.

Various reports have focused on the possible role of ASA with the fertilization process by way of interfering with sperm functions involved in the fertilization process. However, the actual role of ASA is not yet sufficiently known, because conflicting data have been produced. In the present study, antisperm antibodies in epididymal fluid were evaluated by ELISA. Fourteen out of twenty cases (70%) showed significant ELISA (OD value > 0.328) for antisperm antibodies. None of the five controls showed a significant ELISA. It has been shown that men with infertility have antisperm antibodies in serum and seminal fluid more often than a control population with recent fertility [22]. Studies of the immunologic consequences of vasectomy indicate that the sperm antibodies when they appear in the ejaculate following vasovasostomy, are likely to affect the sperm function, suggesting that they are clinically important [28-30]. However, there is little evidence that suggests a cause/effect relationship between ASA and abnormality of principal semen parameters (sperm count, motility and morphology) although a recent report of meta-analysis on the effects of immunosuppressive corticosteroid treatment of male infertility revealed that treatment reduced the level of antisperm antibodies, improved sperm motility and sperm count, and increased conception rate [31].

Presence of antisperm antibodies as determined by ELISA (humoral immune response) or the presence of interstitial inflammatory cell infiltration in epididymal histology (cell mediated immune response) has been considered as evidence of local immune reaction. In this study, seventeen out of twenty cases (85%) had one or both evidences of local immune reaction. None of the five controls (0%) had any evidence of local immune reaction.

Our observations support the view that long standing obstruction to the sperm egress leads to back pressure which causes epithelial breach in the epididymis. This leads to a break in blood- seminal fluid barrier, which causes stimulation of the local immunological processes. Thus, the persistence of infertility in patients with obstruction to the egress of sperms even after a patent anastomosis could be due to the abnormal morphology or impaired function of the spermatozoa caused by locally secreted antisperm antibodies.

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