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Evaluation of antisperm antibodies in infertile men associated with varicocele. *Pre and post varicocelectomy*

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In this prospective study, 27 varicocele associated infertile men undergoing microsurgical inguinal varicocelectomy were included. Varicocele was determined by physical examination and Doppler ultrasound and was categorized by a single examiner with the patient standing as follows: grade 1, palpating an impulse in the scrotum during a Valsalva maneuver; grade 2, tortuous veins palpated without Valsalva maneuver; and grade 3, visible through skin. All other probable causes of infertility, like female factor, were excluded through evaluation including history, physical examination, and laboratory tests including complete blood count, urea, electrolyte, and urine analysis. Hormonal studies (serum follicle-stimulating, luteinizing hormone, and testosterone) were carried out if there was severe oligospermia (density $<10 \times 10^6$/ml) or if there was any indication with regards to history and examination. Semen analysis and antisperm antibodies were measured by the spermMar test performed preoperatively and postoperatively at 6 months. Any risk factors, like history of trauma, torsion, cryptorchidism, vasectomy or vasectomy reversal, genitourinary infection and previous inguinal surgery were also considered. Semen was collected by masturbation into sterile, wide mouthed containers approximately 72 hours after the last abstinence. By the time of liquefaction, the sample was divided for semen analysis and sperm antibodies assay. Semen analysis was performed routinely and using x40 of microscope, Neubauer hemacytometer and papanicolaou staining method. The different parameters of sperm such as motility, density, and morphology were examined. Seminal and serum sperm antibodies [immunoglobulin G (IgG), immunoglobulin A (IgA)] were measured by 2 methods of direct and indirect spermMar test (Ferti Pro NV, Belgium).

In the direct spermMar test, one drop of semen was placed on a microscope slide adjacent to a drop of latex reagent and a drop of antiserum either IgG or IgA. The 3 drops were mixed together with a cover slip and was then used to cover the mixture. After 2-3 minutes, the slide was examined under a microscope and the percentage of motile sperm bound to the latex beads was scored. According to the manufacturer, a score of 40% or more indicates a high probability of immunologic infertility, and a score of 1-39% indicates that immunologic infertility is suspected.

In the indirect spermMar test, blood and seminal plasma were first inactivated by heating at 56°C for 30 minutes and then diluted with 1/4 Ham's F-10 medium containing 10% Albuminar-5 (containing 5% human serum albumin, Blood Research Center, Tehran, Iran). Fresh sperm from a fertile donor washed twice and allowed to swim up in Ham's F-10 medium. Afterwards the sperm concentration was adjusted to 20×10^6 sperm/ml with Ham's F-10. While 100 µl of the suspension of motile donor sperm was incubated with 100 µl of inactivated seminal plasma or serum, which had been diluted in 1/4 Ham's F-10 medium for 1 hour at 37°C. The sperm were then washed 3 times, re-suspended in 50 µl of Ham's F-10 medium, and then tested for membrane bound antibodies similar to that of the direct spermMar test. Data were collected and analyzed using Wilcoxon Signed Ranks and ANOVA test.

The mean age of the patients was 28.7 years (23-42 years), and the mean of the infertility duration was 4.29 years (1-11 years). All had left varicocele (grade one: 2 patients, grade 2: 17 patients, grade 3: 8 patients) and 7 had right varicocele who underwent right varicocelectomy as well. Hormonal studies were performed in 6 patients, all of them had severe oligospermia and negative antisperm antibody. It was normal in 4

patients, but increased follicle stimulating hormone (FSH) was considered in 2. All patients were followed up for 6 months and none of them had recurrent varicocele on physical or Doppler examination.

After varicocelectomy, the sperm density improved in 62% of varicocele patients. The normal morphology were also improved in 66% and sperm motility were 37% of cases. The mean of sperm density was significantly increased ($22.6 \pm 1.9 \times 10^6$ for preoperative and $32.2 \pm 2.8 \times 10^6$ for postoperative, $p<0.01$**)**. The mean of abnormal morphology of sperm was significantly improved (74 ± 18% for preoperative and 53.07 ± 12% for postoperative, $p<0.001$). But the improvement of the mean of sperm motility was not significant (30.8 ± 16.8% for preoperative and 34.8 ± 19% for postoperative).

Preoperatively, none of the patients were highly probable and positive for antisperm antibodies (ASA) (>40%). However, 7 (26%) patients had low probability and positive for ASA (10-40%). They were as follows: 3 (11%) for direct IgA, 2 (7%) for serum indirect IgG, 1 (3%) for direct IgG and 1 (3%) for direct IgA and seminal indirect IgA. During follow-up of the 7 patients, they were grouped according to ASA changes. Antisperm antibodies titer were reduced in 5 patients (group A), increased in 1 patient (group B) and reduced in a particular antibody type but increased in another in 1 patient (group C) within 6 months after varicocelectomy. In group A, sperm count, motility and normal forms improved postoperatively ($p<0.05$). In group B, motility reduced while in group C motility and normal forms was reduced postoperatively **(Table 1).** Twenty patients (74%) were negative for ASA preoperatively. Out of these, 16 (80%) showed an increase in at least one of ASA types to some degrees postoperatively. This increase was significant for serum indirect IgG antibody: 13 out of 16 patients (81%), ($p<0.01$). In the latter cases, comparing with preoperation, sperm density (24 ± 1.86 for preoperative and 30 ± 3.16 for postoperative), sperm motility (28.2 ± 2.6% for preoperative and 37.3 ± 2.1% for postoperative), and high abnormal form of sperm (68 ± 12% for preoperative and 53 ± 5% for postoperative), although none of them were significant.

Considering the risk factors, antibodies had low probability and positive in 2 out of 3 patients with history of significant scrotal trauma and one out of 2 patients with history of varicocelectomy. Follow-up of these patients showed that the antibody titers were reduced postoperatively.

Bouchot et al,[1] showed that sperm motility and normal head spermatozoa density were significantly increased after varicocelectomy. In our study, sperm density improved in 62% of cases. Mean normal morphology improved in 66% and sperm motility improved in 37%. This is in contradiction with other reports, which is the most common improvement in semen parameters occurring in sperm motility after varicocelectomy (70%).[2] The debate regarding the efficacy of varicocele ligation for improvement of semen parameters is ongoing. Ozen et al[3] evaluated 65 infertile varicocele patients. Antisperm antibody was detected with immunofluorescence method in 24.6% of patients. It had no relationship to varicocele grade. Golomb et al[4] assessed the antisperm antibody with ELISA technique in 32 varicocele infertile patients. It was positive in 90% of patients, in contrast to 41% of control group.

In our study, we found an incidence of sperm-bound immunoglobulin in 26% of infertile varicocele patients and none of them were highly probability and positive with ASA. We did use SpermMar test to evaluate antisperm antibody. SpermMar test has some advantages over immunobead test (IBT).[5] It can be carried out over less motile, unwashed sperm and is more sensitive. Unlike polyacrylamide particles in IBT, latex

Table 1 - Pre and postoperative sperm density, motility and abnormal forms in 7 varicocele infertile patients with low positive antisperm antibody.

Group of patients	Preoperative (mean ± SD)			Postoperative (mean ± SD)		
	Sperm density	Sperm motility (%)	Abnormal form of sperm (%)	Sperm density	Sperm motility (%)	Abnormal form of sperm (%)
Group A (n=5)	$14 \pm 8 \times 10^6$	25 ± (10)	78 ± (10)	$38 \pm 10 \times 10^6$	40 ± (8)*	38 ± (18)*
Group B (n=1)	35×10^6	(35)	(78)	38×10^6	(30)	(79)
Group C (n=1)	40×10^6	(45)	(70)	40×10^6	(35)	(75)

Result of statistical analysis was down only in group A (Wilcoxon Signed Ranks and ANOVA test), *$p<0.05$

particles are the same size in SpermMar kits. SpermMar tests results are reproducible and can be performed by any light microscope even in the office.

Knudson et al[6] reported antisperm antibody levels in 32 varicocele infertile patients, using immunobead test. In his study, 28% had positive immunobead test among whom IgG was found bound to the surface of the sperm in 100% and 86% IgA. Of the 7 patients who were initially antibody positive, 6 (86%) patients remained positive after varicocele ligation. One out of 15 patients who were antibody negative preoperatively became antibody positive postoperatively. These investigators were not able to show any significant difference between pre and post varicocelectomy ASA level.

In our study, ASA was weakly positive in 7 (26%) patients preoperatively and in follow-up it was reduced in 5, increased in one and in the last patient it decreased in that particular antibody type, but increased in another sub-type. These data and other finding[3,4,6] suggest that the incidence of ASA in varicocele infertile patients is still ambiguous, and if it occurs in some cases, varicocelectomy may not always effective. According to the literature, some people believe that men with varicoceles who also have sperm–bound immunoglobulins, have more extensive damage to the seminiferous epithelia than men with varicoceles who lack this finding.[7] In our study, antisperm antibody does not have any harmful effect on semen parameters preoperatively. In this regard, we must consider that none of our patients had high probable antisperm antibody that raises the question of whether the low levels of sperm-bound antibody found in our study, will have significant impact on fertility. Furthermore, after varicocele ligation our patients exhibited the same improvement rate in semen parameters regardless of the preoperative antibody status. Assuming pre and postoperative ASA levels, only increase in serum indirect IgG was significant and revealed no significant effect on sperm parameters. It should be mentioned here that serum antibodies such as IgG are nonspecific and can be falsely positive due to many circumstances like fever, medical or systemic disease, surgery, and major stress. However, none of these states occurred in our patients during follow-up.

Heidenreich et al[8] showed that only vasectomy and previous history of epididymitis could be recognized as risk factors for ASA. In our study, we could find 5 patients with risk factors (3 patients had scrotal trauma wherein 2 of them were ASA positive, among 2 patients who previously had varicocelectomy, one had positive ASA). The ASA level was reduced in all positive cases after varicocelectomy. Like Ozen et al[3] suggested, we did not find any correlation between varicocele grade and pre and postoperative ASA levels. Seven patients underwent bilateral varicocelectomies. The right varicocele added to the left one did not change the semen parameters and ASA titers significantly.

In conclusion, the relationship among varicocele, antisperm antibody and infertility has always been ambiguous. Varicocelectomy may reduce ASA level. This reduction has good quality effect on semen parameters. Also, it may increase ASA level in some patients. This positive conversion has no adverse effect on semen parameters.

Received 15th February 2005. Accepted for publication in final form 7th June 2005.

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