

Can Antisperm Antibodies affect Male Fertility after Subinguinal Varicocelelectomy ? A Local Study

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Abstract

Background: Antisperm Antibodies researches emerged in 1899 in a report referring to the ability of sperms to be antigenic in case of injection into a foreign species. In 1900, researchers showed that sperms are also antigenic in case of injection into similar species. Moreover, Wilson (1954) identified ASAB in the partners of infertile male and then observed the association of ASAB with male infertility. In addition, a correlation was found between ASAB and sperm proteomics, and thus researchers specified those ASABs decreasing male fertilities via inhibition of the sperm functions crucial for fertilization.

Objectives: The study aimed at evaluating the effect(s) of varicocelelectomy on the anti-sperm antibody in cases who suffer from varicocele.

Methods: In the present prospective, controlled, clinical research which has been performed during the period from april 2015 to may 2020. Therefore, the blood samples have been randomly provided from 190 males with mean age 33 years (ranging from 20-45 years), with the clinical varicocele who have been indicated for varicocelelectomy. Then, participants have been randomly selected from the urology outpatient clinic of Al-Yarmouk Teaching Hospital in Baghdad, Iraq, and Al-Ramadi Teaching Hospital and private clinic.

Results: In our study, ASA level (27.2%) with the positive serum ASA prior to with those (43.2%) following the operation demonstrated a significant difference statistically ($p = 0.033$). However, such a result has been not confirmed in the patients (27.4%) with positive semen ASA prior to with those (31.3%) following the surgical operation ($p = 0.881$).

Conclusion: According to the results, varicocelelectomy did not have any impact on the semen ASA, despite, but serum antibody enhanced following varicocelelectomy and sperm motility improved. Therefore, varicocelelectomy usefully affected the semen parameters in the infertile men suffering from varicocele.

Keywords: Varicocelelectomy, Anti-sperm Antibodies, Infertility.

Introduction

By definition, varicocele is the incompetent and dilated veins in the pampiniform plexus of spermatic cord. This condition that is one of the causes of male subfertility can be usually corrected by surgery. In fact, it develops in the course of puberty; that is, when exocrine and both endocrine functions of the testicle remarkably enhances, followed by testicular blood flow⁽¹⁾

According to the studies, varicoceles are observed in 35-40% of male with infertility problems and in 15% of males in the general population and leads to abnormal parameters of semen. Researchers have categorized them based on size as small (grade I), medium (grade II) and large (grade III). Findings have shown the large varicoceles as a “bag of worms” under the scrotal skin and a medium varicocele can be easily diagnosed by palpation, in particular, while standing. Finally, the

small varicoceles detected merely via feeling an impulse in scrotum with the Valsalva maneuver or via feeling differences in the size or fullness of the spermatic cord in case of the movement of the patient from the standing state to a supine position. It is notable that majority (90%) of the varicoceles occurs on the left side, and the remainder is bilateral. Isolated right varicoceles suggest intraabdominal or retroperitoneal pathology and merit abdominal and pelvic imaging.⁽²⁾

Low sperm motility (i.e., asthenospermia) is the commonest condition in varicoceles. The first cue of success for treating varicoceles would be improved sperm motility. However, immunological causes have been shown to be involved in more than 10% (4-15%) of cases of infertility.⁽³⁻⁵⁾

Moreover, researchers have observed anti-sperm antibodies (ASAs) in 10% of infertile male and <2% of fertile male.^(6,7)

These antibodies influence the sperms' functions by impairing the sperm penetration into cervical mucus, inhibiting the sperms' capacitation, deficient acrosomal reactions, disorders in the egg fertilization, as well as disruption of the binding of sperms-eggs.

According to the measurement level of the ASAs prior to varicoectomy and nearly three and six months after that, surgical treatments did not influence the outcomes.⁽⁸⁾

In addition, it is possible that varicoceles are followed by lower or under several conditions greater concentration of ASA. Nonetheless, greater concentration of ASA did not negatively affect the semen parameters.⁽⁹⁾

The mixed agglutination reaction (MAR) test is used to detect antisperm antibodies (useful for asthenozoospermia which can be associated with immunological infertility).⁽¹⁰⁾

Patients and Methods

In this prospective, controlled, clinical study which was conducted during the period from April 2015 to May 2020. Then, we provided the blood samples randomly from 190 males with mean age 33 years (ranging from 20-45 years), with the clinical varicoceles who have been

indicated for varicoectomy. After that, they have been randomly selected from the urology outpatient clinics of Al-Yarmouk Teaching Hospital in Baghdad, Iraq, and Al-Ramadi Teaching Hospital and private clinic.

The exclusion criteria include the following: history of varicoectomy, surgical operation for undescended testes (UDT), testicular detortion or torsion (spontaneously or through operation), testicular biopsies, sexually transmitted diseases, vasectomy, testicular cancer, history of anal sex, testicular trauma, immunosuppressed patients, malnutrition, inguinal herniorraphy, consumption of immuno-suppressive medicines like steroids and cytotoxic and chemo-preventive medicines.

In this step, an urologist diagnosed varicocele via Doppler ultrasound and physical examination (on standing position) according to the following World Health Organization's criteria:

1. grade I, palpation of an impulse in scrotum in the course of a Valsalva maneuver,
2. grade II: tortuous veins, which are palpated without Valsalva maneuver,
3. grade III: visible tortuous vein across the scrotal skin without Valsalva maneuver.⁽³⁾

Patients were assessed by seminal fluid analysis according to (W.H.O) criteria with concentration on sperm total motility, also seminal fluid antisperm antibodies were measured by the SpermMar (sMar) test. We repeated these tests 3 months after surgery which was done by open subinguinal approach.

Semen Analysis done as follows : masturbation has been used to collect the semen samples into sterile, wide-mouthed containers three to four days after abstinence interval. Then, we examined the samples within one hour following ejaculation and divided them for the usual semen analyses, and ASAB assays.

The World Health Organization (WHO) directions and reference values for human semen (11) have been used for semen analysis. Reference values: sperm count (not less than 20 million/ml), volume of the seminal liquid (not less than ≥ 2 ml), motility (not less than 75%), total count of the sperms (not less than 40 million/ejaculate), morphology as well as vitality (in case of the percentage

of viable sperms >75%). Even though experts in the field did not reach an agreement on the morphological classifications, according to a robust criterion, not less than 4% of the sperms must enjoy normal morphologies.

According to the research design, semen analysis has been pre-operatively carried out and three months following the surgical operation based on the standard criteria presented by WHO. Then, we obtained the serum samples preoperatively and postoperatively and kept them at -70°C .

One of the diagnostic kits to identify the anti-sperm anti-bodies of the IgG class in the semen or sera of humans is SpermMar Test IgG. It is possible to perform direct Sperm Mar Test IgG on the un-treated human semen containing motile spermatozoa. Moreover, it is possible to use indirect SpermMar Test IgG on serum.

However, direct Sperm Mar Test IgG would be done via blending the fresh, untreated semen with the latex particles coated with the human's IgG. Then, a mono-specific anti-human IgG anti-serum would be poured into the mixture. Forming agglutinates between the particles and motile spermatozoa demonstrates the existence of IgG anti-bodies on the spermatozoa.⁽¹²⁻¹⁴⁾

All the changes in the titer of seminal ASAB, and in motility of the sperms, pre and post-varicocelelectomy, were stated. As shown by the analyses, the mixed agglutination reaction occurred between latex particles and the motile sperm approved the existence of the sperm antibodies (IgG) on the sperms.⁽¹⁵⁾

Therefore, we collected the outputs of biochemical experiments preoperatively and three months following the varicocelelectomy. Moreover, we employed descriptive statistical testes such as percentage, mean \pm standard deviation (SD) as well as frequency, Mc Nemar's test and paired t-test. Finally, SPSS14 has been used to analyze data and p less than 0.05 has been regarded to be statistically significant.

Results

As mentioned earlier, the participants' mean age equaled 33 years (ranging from 20-45 years) and 78% (155) have been married. Nearly 84% (158) of the cases experienced uni-lateral varicoceles, 104 of them with grade II varicoceles and 16% (32) with the bi-lateral varicoceles, 20 of whom had grade II varicocele. Furthermore, 65% of the cases exhibited grade II varicoceles and 5% showed grade I and 30% exhibited grade III varicoceles. Finally, 134 patients (70.5%) had undergone surgery for treating infertility and 56 (29.5%) due to scrotal or inguinal pain.

Next, 3 major parameters of the semen analysis (SA) preoperatively and postoperatively have been compared, showing a remarkable improvement in the parameters for the sperm motility ($p < 0.001$) and sperm count ($p < 0.005$); however, the obtained result has been not the case for the impaired sperm morphologies in the postoperative interval ($p = 0.59$) (see Table 1).

Table-1: Seminal Fluid Analysis before and after operation(Subinguinal Varicocelelectomy.

	Before operation	After operation
Vol.(ml)	3+- 0.05	3.5+-0.7
Viscosity %	76.6	68.3
Sperm count(million/ml)	33.8+-19	38+-15
Sperm motility % of immotile No.	48(70)	27(50)
Sperm morphology % of immotile No.	15(25)	7(12)
Time of liquefaction in minute	15-30	15-30
Semen pH	7.35+-0.11	7.35+-0.11

Correlation of the semen analysis to the indications for surgery (pain, infertility, other) has been confirmed by the results. As seen, one of the 3 sperm indices; that

is, motility, morphology and count has been impaired in 86% of the cases operated for treating infertility. Additionally, 2 of the indices have been impaired in 54% of the cases (Table-2).

Table-2: Antisperm Antibodies percentage (sperm- and serum-bounded) before and after operation.

Antisperm Antibody %	Before operation	After operation
Sperm- bounded (in semen)	27.4%	31.3%
Serum- bounded	27.2%	43.2%

The presence of ASA in semen implies the fact that it is also positive in serum, though it considerably declined following the surgical operation ($p = 0.033$). Moreover, varicocelelectomy elevated the serum ASA remarkably ($p = 0.033$).

Even though a majority of the cases have shown to be negative for ASA in both sera and semen prior to and following the operations for ASA, after surgical operation did not show any remarkable significance between the married or single cases.

Discussion

In general , about 50% of infertility cases are due to a male factor.(16)

Dealing with the causes of male infertility & pathogenesis , is a very complex issue , and was the subject of research for decades .

One of the studies addressed ASA measurement prior to the surgical operations and three and six months after that in 81 cases with a mean age of 28.7 years. According to the results, 21 of 81 cases exhibited weak positive ASAs prior to the surgery. Following the surgery, ASA declined in 15 cases and enhanced in 3 cases. Moreover, it has been negative in 61 cases prior to the operation. Additionally, 48 cases exhibited relatively greater ASA concentration following the surgery, though such an enhancement has been not significant statistically. Hence, the researchers referred to the possible reduction of ASA concentration by varicocelelectomy.

This research results matched with the findings reported in one of the studies in the field in terms of preoperative and postoperative sperm count; however, it does not match in terms of the sperms' morphologies and motility prior to and following the varicocelelectomy procedure .⁽¹⁷⁾

Evaluating the semen analysis with regard to the indications for varicocelelectomy demonstrated that sperm motility is the most impaired indicator in cases experiencing surgery because of pain or infertility. ⁽¹⁷⁾

According to this research, 16% of the cases experienced bi-lateral varicocelelectomy so that the percentage equaled 45% in infertile males. Moreover, a significant difference existed between both groups with the normal and impaired semen analysis and uni-lateral with bi-lateral varicocelelectomy ($p = 0.001$).

This research findings are compatible with several researches not all investigations assessing the ASA semen and serum in the pre- and post-operative intervals. For example, Ozen et al. ⁽¹⁸⁾ evaluated 60 infertile cases suffering from varicoceles and identified ASA through immuno-fluorescent in 24.6% of the cases. In addition, Golombo et al.'s ⁽¹⁹⁾ study assessed ASA via ELISA in 32 infertile cases suffering from varicoceles. Results have shown a positive ASA in 90% of the cases compared to 41% of the controls.

In their research, Knudson et al. ⁽²⁰⁾ utilized immuno-bead test to report the level of ASA in 32 infertile cases suffering varicoceles. The present research showed

28% of the patients with the positive immunobead test findings, out of which IgG has been bound to the sperms' surfaces in 100% and IgA in 86% of the cases. Therefore, investigators have been not capable of showing a significant difference between pre- and post-varicocelectomy ASA concentration.

According to Djaladat et al.'s ⁽¹⁷⁾ study, 26% ASA positivity has been observed prior to the surgery; however, six months following the surgical operation, concentration of ASA declined in 15 and enhanced in 3 cases whereas 3 other cases showed no changes. Moreover, semen analysis indicated the improvement in the sperms' count and morphology ($p < 0.05$) but motility did not improve ($p = 0.02$). Therefore, the researchers stated that even though varicocelectomy can decline the concentration of ASA and improve the semen indices, it does not have any consequences for the quality of the semen indices.

It has been found that patients (71%) with the positive serum ASA postoperatively also exhibited a positive semen.

A significant difference ($p = 0.033$) has been observed by comparing the cases (27.2%) with the positive serum ASA prior to with the cases (43.2%) following the surgery; however, such a result has been not observed for patients (27.4%) with the positive semen ASA before with the cases (31.3%) after the operation ($p = 0.881$).

A comparison between those cases with the developed ASA following a surgical operation and the cases with a negative ASA following the surgery demonstrated that just motility ($p = 0.035$) significantly differed amongst the 3 semen indices. Moreover, a comparison of the cases with the positive seminal vs. serum ASA reflected a significant ($p < 0.001$) difference in the percentage of the impaired sperm motility. Nevertheless, the observed differences have been not significant according to the sperms' morphologies or count. Put differently, varicocelectomy may result in the improved sperm count as well as morphologies, though motility would be impaired in case of followed by a positive ASA. Even though researchers have presented numerous etiologies for the ASA occurrence, just acute epididimitis and vasectomy associated to the considerable ASA enhancement.

Conclusion

In the infertile male population, presence of ASA was higher in those patients with varicocele. A positive ASA in sera or semen must not be regarded an issue because of its slight effect on the sperms' motility. With regard to a considerable improvement in the sperms' motility following the varicocelectomy procedure, it is not necessary to abandon such a surgical operation in high-risk cases with a positive ASA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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References

- 1- Jack W. McAninch, Tom F. Lue. "Smith And Tanagho's GENERAL UROLOGY". 19th ed. Ch.44; ;(2020). PP.722
- 2- Philip M. Hanna, Thomas J. Guzzo, S. Bruce Malkowics, et al. "PENN Clinical Manual of Urology". 2nd ed. Ch.21; ;(2014). PP.688-689
- 3- Rogenio A, Lobo A, Daniel R, Richard JR, Pavlson M. Role of immunology in infertility. In: Lobo A, Mishell DR, Paulsen RJ, Shoupe D, editors. Infertility, contraception and reproductive endocrinology. Massachusetts: Blackwell Science; (1997). pp. 675–9.
- 4- Bronson RA, O'Connor WJ, Wilson TA, Bronson SK, Chasalow FI, Droesch K. Correlation between puberty and the development of autoimmunity to spermatozoa in men with cystic fibrosis. *Fertil Steril.*; (1992).58(6):1199–204.
- 5- Flickinger CJ, Baran ML, Howards SS, Herr JC. Sperm autoantigens recognized by autoantibodies in developing rats following prepubertal obstruction of the vas deferens. *J Androl.*; (1996).17(4):433–42.
- 6- De Almeida M, Soumah A, Jouannet P. Incidence of sperm-associated immunoglobulins in infertile men with suspected autoimmunity to sperm. *Int J Androl.*; (1986).9(5):321–30.
- 7- Heidenreich A, Bonfig R, Wilbert DM, Strohmaier WL, Engelmann UH. Risk factors for antisperm antibodies in infertile men. *Am J Reprod Immunol.*;

- (1994).31(2):69–76.
- 8- Sinisi AA, Di Finizio B, Pasquali D, Scurini C, D'Apuzzo A, Bellastella A. Prevalence of antisperm antibodies by SpermMARtest in subjects undergoing a routine sperm analysis for infertility. *Int J Androl.*; (1993).16(5):311–4.
 - 9- Oshinsky GS, Rodriguez MV, Mellinger BC. Varicocele-related infertility is not associated with increased sperm-bound antibody. *J Urol.*; (1993).150(3):871–3
 - 10- John Reynard, Simon Brewster, Suzanne Biers. *OXFORD HANDBOOK OF UROLOGY*. 3rd ed. Ch.12; (2013). PP.556;
 - 11- Marc GO. Surgical management of male infertility and other scrotal disorders. In: Walsh PC, Retik AB, Vavghao ED, Wein AJ, editors. *Campbell's Urology*. Philadelphia: W.B. Saunders;. (2002) .. 1571–7
 - 12- BOETTCHER B., HJORT T., RUMKE Ph., SHULMAN S. and VYAZOV O.E. (eds.): Auto and iso-antibodies to antigens of the human reproductive system. 1. Results of an international comparative study of antibodies to spermatozoa and other antigens detected in sera from infertile patients deposited in the WHO Reference bank for reproductive immunology. *Acta Pathol Microbiol Scand*, , 258 (suppl.) : 1-69 STEDRONSKA J. and HENDRY W.F.: The value of the mixed (1977).
 - 13- antiglobulin reaction (MAR-Test) as an addition to routine seminal analysis in the evaluation of the subfertile couple. *Am. J. Reprod. Immunol.*, (1983)., 3 : 89-91
 - 14- VERMEULEN L. and COMHAIRE F.H.: Le test aux particules de Latex, et le test spermatotoxique selon Suominen : simplification et nouveauté dans l'arsenal du diagnostic immunologique. *Contraception Fertilité-Sexualité*, , (1983).11 (suppl.) : 381-384.
 - 15- Rajah SV, Parslow JM, Howell RJ, Hendry WF. Comparison of mixed antiglobulin reaction and direct immunobead test for detection of sperm-bound antibodies in subfertile males. *Fertil Steril.*; (1992).57(6):1300–3,
 - 16- Agarwal A, Mulgund A, Hamada A, Chyatte M. A unique view on male infertility around the globe. *Reproductive Biology and Endocrinology.* ; (2015) 13(1). 22
 - 17- Djaladat H, Mehraei A, Rezazade M, Djaladat Y, Pourmand G. Varicocele and antisperm antibody: fact or fiction? *South Med J.*; (2006).99(1):44–7.
 - 18- Ozen H, Asar G, Gungor S, Peker AF. Varicocele and antisperm antibodies. *Int Urol Nephrol.*; (1985).17(1):97–101,
 - 19- Golomb J, Vardinon N, Homonnai ZT, Braf Z, Yust I. Demonstration of antispermatozoal antibodies in varicocele-related infertility with an enzyme-linked immunosorbent assay (ELISA) *Fertil Steril.*; (1986).45(3):397–402
 - 20- Knudson G, Ross L, Stuhldreher D, Houlihan D, Bruns E, Prins G. Prevalence of sperm bound antibodies in infertile men with varicocele: the effect of varicocele ligation on antibody levels and semen response. *J Urol.*; (1994).151(5):1260–2.