ORIGINAL ARTICLE

Immunological and Hormonal Profile of Infertile Pakistani Males With Varicocele

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ABSTRACT

Aims: To compare the levels of serum FSH, Testosterone in infertile men with and without varicocele and to detect the Antisperm antibodies (ASA) in the serum of infertile men with and without varicocele.
Study Design &Settings: It is a Cross sectional prospective study the laboratory work was done at Physiology Department, University of Health Sciences, Lahore.
Methods: This Cross sectional study was conducted on ninety patients that fulfilled the inclusion criteria. The patients were chosen from the infertile couples reporting to the Combined Military Hospital Lahore and General Hospital Lahore for infertility.
Detailed history was taken meticulous physical examination and semen analysis of was performed on all the subjects. Scrotal ultrasound was done to confirm varicocele. Serum FSH, testosterone and antisperm antibody determination were carried out by ELISA.
Results: Varicocele was found in 35 out of 99 infertile men by physical examination and confirmed by Scrotal Ultrasound. The infertile men with varicocele had higher levels of serum FSH than infertile men without varicocele. Levels of testosterone were lesser in subject with varicocele. All (99) infertile males with and without varicocele were subjected to ASA test. It was observed that out of 35 infertile men with varicocele1 was positive, while 3 out of 64 men without varicocele were positive for ASA.
Conclusion: Our results suggest no relationship of varicocele with abnormal serum FSH & Testosterone levels and presence of ASA in infertile men with varicocele.
Keywords: Varicocele, Anti-sperm antibodies, Serum Testosterone, FSH, Infertile men.

INTRODUCTION

Internationally one out of every six couples reports in infertility clinics for treatment of infertility [1], [2]. Among these 40-50% are due to male factors, 25 % cases are due to female , 20% are due to a combination of female and male factors while 10% have un-explained infertility[3,4]. The prevalence of varicocele is 25%in men with abnormal semen analysis[5]. In varicocele, there is enlargement and dialatation of veins of the pampiniform plexus in the spermatic cord[6]. In infertile men it is the commonest cause of infertility and occurs in approximately 30% to 81% of infertile men [7]. Several theories have been presented to explain the impairment of fertility by varicocele [8]. These theories include increased sperm DNA damage[9] increase in apoptosis[8], tissue hypoxia, oxidative stress degenerative changes in the seminiferous tubule, and decreased production of Fas protein resulting in immunological infertility [10]. In Varicoceles, seminal plasma antioxidant activity diminishes and sperm reactive oxygen species (ROS) production increases[11]. Excessive reactive oxygen species (ROS) can cause damage to DNA, proteins, and cellular lipids by inducing oxidative changes[11]. Surgical varicocelectomy decreases ROS production, increases levels of seminal plasma antioxidants and improves seminal parameters[11]. Presence of antibodies and deranged hormonal levels are blammed for decrease motility in infertility due to varicocele[12]. Hormonal imbalance and sperm autoimmunity affect each other and work in close association[13]. Antisperm antibodies are found in 11% of infertile men and its prevalence is 23.8% to 31% among infertile men with varicocele[14]. ASA decreases sperm motility hence inability to penetrate the cervical mucus resulting in reduced fertilizing potential[15]. Testosterone a steroid hormone, is essential for initiation and maintenance of spermatogenesis[16]. Moreover, it plays a key role in the development/maintenance secondary sexual characteristics of males[17]. Decrease levels of testosterone were found in infertile men having poor seminal parameters and varicocle[17]. Hence this study was designed to detect the hormonal levels and presence of ASA in infertile men with and without varicocele.

MATERIALS AND METHODS

This study was conducted on 99 subjects selected from Combined Military Hospital and Lahore General

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Hospital. The subjects were 35 infertile males with varicocele aged between 20-40 years, and 64 age matched infertile males without varicocele. Serum samples were taken from all subjects and kept at -80c until assay was performed. Participants were instructed to come for Semen sample collection after a restraint of three days from sexual intercourse. After collection in sterile containers semen was allowed to liquefy at 37 c for 30 minutes. Semen analysis was performed within one hour of sample collection. Physical examination of all subjects was performed before doing Scrotal Ultrasound

Assay of serum: All serum samples were analyzed for levels of FSH, testosterone and ASA by an enzyme linked immunosorbent assay (ELISA).

Testicular ultrasonography was performed on all subjects.

Inclusion Criteria: Male subjects with abnormal semen parameters having primary infertility exceeding 2 years duration.

Exclusion Criteria

1. Male having infertility for less than a year.
2. Subjects having recent history of trauma or surgery of the genitourinary tract.
3. Subjects having intra-abdominal malignancy and chronic diseases.

Statistical analysis: Values represent the mean SD, statistical evaluation was done with Student's t-test and Chi-square test. $P < 0.05$ was considered statistically significant. Statistical analyses were performed with SPSS version 21.0.

RESULTS

The mean age of infertile men as shown by age distribution was 30 years. All infertile men (99) underwent physical examination and scrotal USG, 35 subjects were found to have concomitant varicocele, while 64 males did not have varicocele. Serum FSH levels were found to be in normal range in all 99, however infertile men with varicocele had higher FSH levels but the difference was not significant (p value 0.06) between two groups (Table 1). In all the 99 males serum Testosterone levels were found to be in normal range (4 – 8 ng/ml). However estimates in relevance to varicocele were found to be lower but did not fall beyond the normal range (Table 2). The difference in serum testosterone level between two groups is not significant (p value 0.07). All (99) infertile males were subjected to ASA test. It was observed that ASA were found only in one infertile man with varicocele, while 3 out of 64 men without varicocele were positive for ASA (Table 3). The difference between the groups was not significant (p value 0.66).

Table 1: Comparison of serum FSH in infertile men with and without varicocele

Variables Varicocele	Infertile Male (n=99)	
	Positive (n=35)	Negative (n=64)
FSH levels	5.5 – 6 miu/ml	3 – 6 miu/ml
Mean ± SD	4.75 ± 0.16	4.5 ± 0.97

P value 0.065

Table 2: Comparison of Serum Testosterone levels in infertile men with and without varicocele.

Variables Varicocele	Infertile Male (n=99)	
	Positive (n=35)	Negative (n=64)
Testosterone level	7 - 8ng/ml	4 - 8ng/ml
Mean ± SD	7.5 ± 0.32	6.0 ± 1.30

P value 0.07

Table 3: Comparison for Presence of ASA among infertile men with and without Varicocele.

Subjects (n=99)	ASA Present	ASA Absent
Infertile men with Varicocele (n=35)	1	34
Infertile men without varicocele (n=64)	3	61

Chi-Square = 0.66 Non-Significant

DISCUSSION

Multiple factors play role in pathogenesis of varicocele which is considered the most common cause of male infertility[7]. Testicular damage or autoimmune process causes inhibition of spermatogenesis and leads to infertility in the males between 25 to 45 years of age[3]. In the current study, 35(35%) males have varicocele. Similar results (30-40%) are shown in study conducted by Mohammad[7]. In the current study sperm concentration was decreased in 16 subjects while 19 subjects have sperm motility defects in infertile men with varicocele. A Study conducted by Mohammad also showed similar results [7].Apoptosis in testis with varicocele may be the cause of decrease in sperm concentration [10]. As far as the hormonal evaluation of these infertile subjects is concerned FSH levels were comparatively higher in subjects with varicocele but within normal range. These higher levels of FSH in infertile subjects with varicoeles might be due to decrease in testicular volume, sperm concentration and sperm motility in these subjects. Testosterone levels are not statististically different in two groups, however some studies reported that varicoceles causes lydig cell dysfunction and subsequent decrease in testosterone biosynthesis [13, 18]. Certain studies showed that varicocele has a negative influence on the Leydig cell function thus indirectly decreasing testosterone production[18]. It is repoted that testosterone has a pivotal role in reproductive function as well as morphological development and in

males[18] while other suggested that testosterone hormone has no connection with sperm concentration [19]. In the current study, ASA were found in only one infertile males with varicocele while they were present in three infertile men without varicocele. Multiple studies reported that varicocele-associated infertility has little or no relevance with ASA[16, 17]. A lot of contradiction exists in literature about varicocele induced ASA formation. The study conducted by Ishikawa showed no improvement in semen parameters after varicolcelectomy in men with or without ASA[18]. Patients with a history of mumps, or either a fresh varicocele or a history of varicocele had statistically significant lower levels of MAR antisperm antibodies (ASAs) than patients with no such conditions[16]. As there is little evidence about association of varicocele with male infertility in local population therefore, furthur studies should be conducted on a larger sample of local infertile male to further explore this association.

CONCLUSION

Estimates of Serum Testosterone were found to be lower while Serum FSH were higher in infertile men with varicocele but both fell within the normal range. A higher number of infertile men without varicocele were positive for ASA as compared to infertile men with varicocele.

REFERENCES

1. Autin C. When should we start getting worried if baby is not coming? Rev Med Brux.2008 ; 29: 346-50.
2. Cahill DJ, Wardle PG. Management of infertility. BMJ 2002; 325: 28-32.
3. Mehta RH, Makwana S, Ranga GM, Srinivasan RJ, Virk SS. Prevalence of oligozoospermia and azoospermia in male partners of infertile couples from different parts of India. Asian J Androl 2006; 8: 89-93.
4. Ikechebelu JI, Adinma JI, Orie EF, Ikegwuonu SO. High prevalence of male infertility in southeastern Nigeria. J Obstet Gynaecol 2003; 23: 657-9.
5. Chang Jin Yoon, Hyun Jun Park, and Nam Cheol Park. Reactive Oxygen Species in the Internal Spermatic and Brachial Veins of Patients with Varicocele-Induced Infertility Korean J Urol. 2010 May; 51(5): 348–353. Published online 2010 May 19. doi: 10.4111/kju.2010.51.5.348
6. Choi WS, Kim TB, Paick JS, Kim SW. Factors related to improvement or normalization of semen parameters after microsurgical subinguinal varicocelectomy. Korean J Urol. 2009; 50:39–45.
7. Mohammed A, Chinegwundoh F. Testicular varicocele: an overview. Urol Int 2009; 82: 373–9.
8. Gürdal M, Kireççi S, Huri E, Karaman I, Türkeri L. Correlation between duration of varicocele and apoptosis in testicular tissue in an experimental model. Urology. 2008;72:933–936. [PubMed]
9. Griveau JF, Le Lannou D. Reactive oxygen species and human spermatozoa: physiology and pathology. Int J Androl. 1997;20:61–69. [PubMed]
10. Koksal IT, Usta M, Orhan I, Abbasoglu S, Kadioglu A. Potential role of reactive oxygen species on testicular pathology associated with infertility. Asian J Androl. 2003;5:95–99. [PubMed].
11. Mostafa T, Anis TH, El-Nashar A, Imam H, Othman IA. Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele. Int J Androl. 2001;24:261–265. [PubMed]
12. Djaladat, Hooman MD; Mehrsai, Abdolrasol MD; Rezazade, Modjtaba PHD; Djaladat, Yasaman MD; Pourmand, Gholamreza MD Varicocele and Antisperm Antibody: Fact or Fiction? Southern Medical Journal: January 2006 - Volume 99 - Issue 1 - pp 44-47
13. R. Kiran and T. G. Shrivastav, “A correlation study between steroid hormone levels and anti-sperm antibodies in serum and seminal plasma of men with or without reduced sperm motility,” Journal of Endocrinology and Reproduction, vol. 1, pp. 31–35, 2007.
14. V. A. Bozhedomov and O. V. Teodorovich, “Epidemiology and causes of autoimmune male infertility,” Urologiia, no. 1, pp. 35–44, 2005. View at Scopus
15. Hala I. Al-Daghistani,[1] Abdul-Wahab R. Hamad,[2] Muna Abdel-Dayem,[3] Mohammad Al-Swaifi,[3] and Mohammad Abu Zaid Evaluation of Serum Testosterone, Progesterone, Seminal Antisperm Antibody, and Fructose Levels among Jordanian Males with a History of InfertilityBiochemistry Research International Volume 2010, Article ID 409640, 8 pages doi:10.1155/2010/409640
16. E. Veräjänkorva, M. Laato, and P. Pöllänen, “Analysis of 508 infertile male patients in south-western Finland in 1980–2000: hormonal status and factors predisposing to immunological infertility,” European Journal of Obstetrics Gynecology and Reproductive Biology, vol. 111, no. 2, pp. 173–178, 2003.
17. F. Lombardo, L. Gandini, A. Lenzi, and F. Dondero, “Antisperm immunity in assisted reproduction,” Journal of Reproductive Immunology, vol. 62, no. 1-2, pp. 101–109, 2004
18. T. Ishikawa and M. Fujisawa, “Varicocele ligation on free testosterone levels in infertile men with varicocele,” Archives of Andrology, vol. 50, no. 6, pp. 2004;443–448.
19. Takada S, Tsujimura A, Ueda T, et al. Androgen decline in patients with nonobstructive azoospemia after microdissection testicular sperm extraction. Urology. 2008;72(1):114–118