Research Article

Changes in seminal antioxidant defenses in Iraqi patients with unilateral and bilateral varicocele

Issam Salman Al-Azzawi, MD,FICMS,FEBU
Urology Department , College of Medicine , Al-Mustansiriya University

Date Submitted:15-7-2013
Date Accepted:2-2-214

Address for Correspondence:
Issam Salman Al-Azzawi
Urology Department , College of Medicine , Al-Mustansiriya University

Abstract

Background : The exact pathogenic mechanism by which varicoceles cause impaired testicular function remains poorly understood . One of the possible mechanisms is the generation of seminal oxidative stress. In addition, the impact of bilateral varicocele on spermatogenesis is still poorly studied .

Aims : To study the changes in seminal antioxidant defenses in relation to unilateral and bilateral varicocele, and to correlate them with changes in sperm concentration and total motility.

Patients and methods : A prospective, randomized, controlled study , whereby 60 semen samples were collected and analyzed. Twenty samples were from healthy subjects , having no varicocele (control group), 20 samples from patients with unilateral varicocele and 20 samples from patients with bilateral varicocele . For each semen sample, measurement of the seminal concentration of the antioxidants (Albumin, Glutathione peroxidase and Superoxide dismutase) was done and the seminal concentration of the oxidative stress marker, Malondialdehyde. In addition sperm concentration and total motility were also estimated. Comparison of the results in the 3 groups was done .

Results : Measurement of semen parameters revealed a drop in the mean value of the 3 antioxidants and an increase in the mean value of Malondialdehyde, which was statistically significant in the 2 varicocele groups in comparison with control, and in bilateral varicocele in comparison with unilateral varicocele . Another drop was in the mean sperm concentration and mean percentage of sperm total motility, which was statistically significant in the 2 varicocele groups in comparison with control, but was statistically not significant between the 2 varicocele groups .

Conclusions : Bilateral varicocele causes more significant drop in seminal antioxidant defenses than unilateral varicocele which seems to add a more negative impact on spermatogenesis .

Key words: ***varicocele, oxidative stress, antioxidants, sperm concentration, sperm motility.***

INTRODUCTION

Varicocele is a physical abnormality present in 11.7% of adult men and in 25.4% of men with abnormal semen analysis [1].

Although many men with varicocele are able to father children, there is abundant evidence that varicoceles are detrimental to male fertility, as they are commonly accompanied by decreased testicular volume, impaired sperm quality, and a decline in Leydig cell function [1,2].Varicocele may be responsible for sperm motility defects as well as defects in sperm count and shape [3,4] .

There has been a debate on two issues in varicocele related infertility ; the first one about the impact of the clinical grade and laterality of the varicocele on the magnitude of changes in semen quality , the second one about the mechanism by which varicocele exerts its deleterious effects on testicular function .

It was reported in many studies that men with larger varicoceles present with lower sperm densities, and after varicocelectomy , they show greater relative improvement in semen quality than men with smaller or subclinical varicoceles who present with higher mean sperm densities [5,6]. Other reports stated that bilateral varicoceles may be more damaging to male fertility than unilateral varicoceles [7,8].

Despite a large number of animal and human studies, the exact pathogenic mechanism by which varicoceles cause impaired testicular function remains poorly understood.

Among many theories in this aspect, the generation of reactive oxygen species (ROS) has also been implicated in the pathophysiology of varicoceles [9,10].

A number of studies have demonstrated high levels of seminal oxidative stress (O.S), as evidenced by increased levels of ROS and reduced total antioxidant capacity (TAC) in men with a clinical diagnosis of varicocele, suggesting that sperm dysfunction in varicocele patients may be in part related to O.S [10,11].

Oxidative stress has been shown to affect the integrity of the sperm genome by causing high frequencies of single- and double-strand DNA breaks which are often detected in the ejaculates of infertile men [12,13].

Another harmful effect of ROS on the sperm is the oxidation of polyunsaturated fatty acids in lipids (lipid peroxidation) leading to damage of sperm membrane [13], and the formation of Malondialdehyde (MDA). The production of this aldehyde is used as a biomarker to measure the level of O.S [14,15].

On the other hand, a combination of antioxidants present in seminal plasma including superoxide dismutase, vitamin C, selenium, transferrin, glutathione peroxidase, albumin and uric acid would protect the sperm by their concentration in normal condition, and when present in balanced formulation. Insufficient levels of these antioxidants may indicate an oxidative stress status and may damage the sperms [10,11,16] .

.This study is conducted to study the impact of unilateral and bilateral varicocele , in a group of Iraqi infertile patients, on the levels of seminal antioxidant defenses and to correlate them with changes in sperm concentration and motility.

PATIENTS AND METHODS

In this prospective randomized controlled study , 60 semen samples were collected and analyzed .

Twenty samples were from healthy volunteers , having no varicocle on clinical examination (control group) and 40 samples were from untreated varicocele patients , attending our urology department of Al-Yarmouk Teaching Hospital .

All subjects included in this study were subjected to history taking, clinical examination (including examination of external genitalia) and seminal fluid analysis . They were all non smokers , having no genital tract infection and have testes size within normal limits, with an age range (20 – 43 year).

The varicocele patients are those with clinically palpable varicocele (grade 2 or 3 by clinical examination) with history of primary or secondary infertility, they were 20 with unilateral left sided varicocele (patient group 1) and 20 with bilateral varicocele (patient group 2).

Semen samples were produced by masturbation (after 3 days of sexual abstinence) and collected into sterile containers .

A conventional semen analysis was performed within one hour of collection ,liquefied semen was centrifuged at 1000 rpm for 6 minutes to obtain the spermatozoa pellet and supernatant , the latter was centrifuged at 3000 rpm for 10 minutes to obtain the seminal plasma .

From the seminal fluid analyses (for the 3 groups), sperm concentration and total motility were recorded according to WHO criteria [17] .

In addition, measurement of the seminal concentration of the antioxidants; albumin ,glutathione peroxidase (GPX) and superoxide dismutase (SOD) was done, and seminal concentration of MDA (as a marker for oxidative stress).

Albumin was determined by using Randox kit and measured in gm /dl .

GPX was determined according to "Bark and Lawrence " method [18].

SOD was determined according to "Beyer and Fridovich " method [19] .

MDA was determined according to "Shahs " method [20].

Then comparison of the results in the 3 groups was done to study the effect of varicocele(being unilateral or bilateral) on the seminal levels of these antioxidant defenses and on sperm concentration and total motility.

For statistical analysis, the Independent student-t-test was used for the difference between means . Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate correlation, >0.7 strong correlation.

Statistical significance was considered whenever the P value was equal or less than 0.05.

RESULT

In this prospective , controlled study, 60 male subjects were randomly and consequently included, their age ranged from 20 to 43 year with a mean of 31.6 ± 6.7 .

There was no significant difference between patient groups 1 and 2 , regarding mean age and clinical grade of varicocele (only patients with grade 2 and 3 varicocele were included).

The mean value of MDA was 6.30 ± 1.93 , 4.78 ± 0.93 and 4.05±0.78 for bilateral varicocele, unilateral varicocele and control groups respectively .There was an obvious rise in the level of MDA from control to bilateral varicocele, with a statistically significant difference in its mean value in both patient groups when compared with control, and between the patient groups themselves , as in table (1)

The mean value of SOD was 5.79 ± 1.13 , 7.98 ± 1.70 and 10.01±1.55 for bilateral varicocele, unilateral varicocele and control groups respectively . There was a steady drop in the level of SOD from control to bilateral varicocele , with statistically significant difference in its mean value in all varicocele patients when compared with control , and between the 2 groups of varicocele , as in table (1)

The mean value of GPX was 21.00 ± 6.46 , 31.29 ± 11.40 and 57.35±13.87 for bilateral varicocele, unilateral varicocele and control groups respectively . There was also an obvious drop in the level of GPX from control to bilateral varicocele , with statistically significant difference in its mean value between the 2 patient groups when compared with control & between the 2 patient groups themselves, as in table (1).

The mean value of Albumin was 0.56±0.21 , 0.68±0.17 and 0.78±0.06 for bilateral varicocele, unilateral varicocele and control groups respectively . The difference in its level , among the 3 groups , was also statistically significant as shown in table (1).

The results of seminal fluid analyses showed the mean sperm concentrations (in million / ml) 18.50±8.83 , 28.79±17.45 and 73.75±16.03 for bilateral varicocele, unilateral varicocele and control groups respectively.

There was a statistically significant difference between control and varicocele patients.

The mean sperm concentration in bilateral varicocele was lower than that of unilateral varicocele, but this difference was statistically not significant , as in table (1).

The mean value of the percentages of total sperm motility was 24.71±12.33 , 37.50±25.17 and 72.30±11.27 for bilateral varicocele, unilateral varicocele and control groups respectively .

Statistically , there was a significant difference in motility between varicocele patients and control group , but the difference in motility between unilateral and bilateral varicocele , though present, was not significant , as in table (1). For sperm concentration ; the correlation was strong in unilateral varicocele, of moderate strength in bilateral varicocele and there was no correlation in the control group . It was a direct correlation with antioxidants and an inverse correlation with MDA , as in table 2 .

For sperm total motility; the correlation was of moderate strength in unilateral varicocele, weak in bilateral varicocele and no correlation in the control group. Again, it was a direct correlation with antioxidants and an inverse correlation with MDA, as in table 3.

DISCUSSION

Varicocele could be presented clinically in different ways ,with possibly different impact on spermatogenesis . It could be subclinical or overt clinically with different grades and it could be unilateral or bilateral .

Many studies conducted about subclinical and clinical varicocele and their role in male infertility [5,6,10,21,22], but the impact of unilateral and bilateral varicocele on male fertility is still poorly studied .

The mystery is not only in the impact of the different clinical presentations of varicocele, but also about the way by which varicocele affects the spermatogenesis (i.e its pathogenesis).

In this study we tried to shed light on the relationship of varicocele, in a group of Iraqi infertile men, with O.S as a possible pathogenesis for the adverse effects on sperm count and total motility. Also we studied the impact of bilateral varicocele on seminal antioxidant levels and correlate them with changes in the sperm count and motility . To our knowledge this is the first study that concern with the pathogenesis of changes in seminal parameters in bilateral varicocele

Table 1. The mean value and range of MDA, SOD, GPX, Albumin, sperm concentration and sperm total motility in the 3 groups

semen parameter	Bilateral varicocele	Unilateral varicocele	Control

Malondialdehyde (MDA)	**6.30±1.93 (2.8-8.5)**	**4.78±0.93 (3.3-6.5)**	**4.05±0.78 (2.5-5.2)**
P value compared to control	0.0001*	0.018*	-
P value compare to unilateral	0.013*	-	-
Superoxide Dismutase (SOD)	**5.79±1.13 (4.0-7.5)**	**7.98±1.70 (5.5-11.1)**	**10.01±1.55 (7.1-13.1)**
P value compared to control	0.0001*	0.001*	-
P value compare to unilateral	0.0001*	-	-
Glutathione Peroxidase(GPX)	**21.00±6.46 (10-33)**	**31.29±11.40 (9-49)**	**57.35±13.87 (40-85)**
P value compared to control	0.0001*	0.0001*	-
P value compare to unilateral	0.007*	-	-
Albumin	**0.56±0.21 (0.29 – 0.90)**	**0.68±0.17 (0.45 – 0.98)**	**0.78±0.06 (0.65 – 0.85)**
P value compared to control	0.0001*	0.017*	-
P value compared to unilateral	0.053*	-	-
Sperm concentration	**18.50±8.83 (0 – 40)**	**28.79±17.45 (10 – 60)**	**73.75±16.03 (40 – 99)**
P value compared to control	0.0001*	0.0001*	-
P value compared to unilateral	0.060	-	-
Sperm total motility percentage	**24.71±12.33 (0 – 40)**	**37.50±25.17 (5 – 75)**	**72.30±11.27 (50 – 90)**
P value compared to control	0.0001*	0.0001*	-
P value compared to unilateral	0.100 بب	-	-
-Data were presented as Mean±SD(Range) ***Significant difference using Students-t-test for two independent means at 0.05 level.**			

Table 2. The correlation of changes in the seminal levels of antioxidants and MDA with the change in sperm concentration in the 3 groups .

		Sperm concentration		
		Bilateral varicocele	**Unilateral varicocele**	**Control**
Albumin	**r**	0.590*	0.711**	0.144
	P	0.026	0.004	0.545
Malondialdehyde (MDA)	**r**	-0.567*	-0.758**	-0.586**
	P	0.035	0.002	0.007
Superoxide Dismutase (SOD)	**r**	0.650*	0.875**	0.140
	P	0.012	0.000	0.557
Glutathione Peroxidase	**r**	0.609*	0.694**	-0.066
	P	0.021	0.006	0.781
***Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.**				

Table 3. The correlation of changes in the seminal levels of antioxidants and MDA with the change in percentages of sperm total motility in the 3 groups .

		Total Sperm motility percentage		
		Bilateral varicocele	**Unilateral varicocele**	**Control**
Albumin	**r**	0.400	0.581*	0.034
	P	0.157	0.029	0.887
Malondialdehyde (MDA)	**r**	-0.283	-0.635*	-0.122
	P	0.328	0.015	0.610
Superoxide Dismutase (SOD)	**r**	0.353	0.516	0.187
	P	0.216	0.059	0.429
Glutathione Peroxidase	**r**	0.433	0.477	0.034
	P	0.122	0.085	0.888
*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.				

DISCUSSION

Varicocele could be presented clinically in different ways ,with possibly different impact on spermatogenesis . It could be subclinical or overt clinically with different grades and it could be unilateral or bilateral .

Many studies conducted about subclinical and clinical varicocele and their role in male infertility [5,6,10,21,22], but the impact of unilateral and bilateral varicocele on male fertility is still poorly studied .

The mystery is not only in the impact of the different clinical presentations of varicocele, but also about the way by which varicocele affects the spermatogenesis (i.e its pathogenesis).

In this study we tried to shed light on the relationship of varicocele, in a group of Iraqi infertile men, with O.S as a possible pathogenesis for the adverse effects on sperm count and total motility.

Also we studied the impact of bilateral varicocele on seminal antioxidant levels and correlate them with changes in the sperm count and motility . To our knowledge this is the first study that concern with the pathogenesis of changes in seminal parameters in bilateral varicocele .

Alterations in the testicular hemodynamics and microenvironment due to varicocele probably increases reactive oxygen species production in association with decrease in antioxidant capacity and increase in oxidative stress markers (e.g. superoxide and malondiadehyde) [11,23,24].

Smoking & genital tract infection are other possible causes of seminal O.S [13,25] , that's why smokers and those with genital tract infection, were excluded from the study .

All patients with varicocele included in this study have overt clinical varicocele (grade 2 or grade 3), while subclinical varicoceles were excluded because their role in male infertility is controversial [10,22] . There was no significant difference in varicocele size between the 2 groups, which omit the effect of difference in size of varicocele on the results .

Our study included measurement of the levels of 3 antioxidants in the semen ; Albumin, SOD, and GPX which are well known in their vital role for protection of sperms against ROS and maintaining fertilizing capacity [11,26,27,28] . Measurement of MDA concentration in semen was also included, because this byproduct of ROS induced lipid peroxidation is an important biomarker that represent the level of O.S , moreover , recent studies also proved that seminal MDA concentration is negatively correlated with sperm concentration and motility [15,28] .

We have two major findings in our study; the first one is the significant decrease in the 3 antioxidant defenses and increase in the O.S marker (MDA) in the semen of all our patients with varicocele compared with control group . This clear varicocele related seminal O.S status in our patients supports other studies that reached to the same findings [10-12,24,28-30].

Moreover , all our varicocele patients (in the 2 groups) had a significant drop in sperm concentration and total motility in comparison with control, which was also in agreement with other studies [1-4,23].

When we correlated this decrease in the seminal antioxidant defenses with the changes in sperm

concentration and motility, we found a strong correlation for the sperm concentration, and a correlation of moderate strength for sperm total motility . This my confirm the impact of varicocele related seminal O.S on spermatogenesis , which was concluded in other studies [2,24,30].

The second major finding (which is unique to our study) is that, in patients with bilateral varicocele, there was a further significant decrease in all the 3 seminal antioxidants and increase in MDA in comparison with unilateral varicocele . This means that bilaterality in varicocele may play a similar role to that of the increase in grade (size) of varicocele, in increasing the seminal O.S status .

There was a further drop in sperm concentration and total motility in patients with bilateral varicocele , compared with unilateral varicocele, but it was statistically not significant , and when we correlated these changes with the changes in seminal antioxidant levels in this group we found a correlation of moderate strength for sperm concentration and a weak correlation for sperm total motility.

In conclusion, patients with varicocele have a significant drop in the seminal antioxidant defenses (Albumin , SOD and GPX) and an increase in the O.S marker (MDA) which was highly correlated with the drop in their sperm concentration and total motility .

Bilateral varicocele causes more significant drop in seminal antioxidant defenses, than unilateral varicocele which seems to add a more negative impact on spermatogenesis, that was more apparent on sperm concentration.

Measurement of seminal antioxidant levels and O.S marker level , may be used in the future in clinical practice , to predict the improvement in fertility after varicocelectomy .

REFERENCES

1. World Health Organization . The influence of varicocele on parameters of fertility in a large group of men presenting to infertility clinics. *Fertil Steril*, 1992; 57:1289 -93 .
2. Pasqualotto FF . Semen profile, testicular volume and hormonal levels in infertile patients with varicoceles compared with fertile men with and without varicoceles. *Fertil Steril*, 2005; 83:74–77 .
3. Gorelick JI, Goldstein M. Loss of fertility in men with varicocele . *Fertil Steril* , 1999; 59 : 613 – 6 .
4. Male infertility best practice policy committee of the American urological association , practice committee of the American society for reproductive medicine . Report on varicocele and infertility. *Fertil Steril* , 2004; 82 : 142 -5.
5. Steckel J , Dicker AP, Goldstein M . Influence of varicocele size on response to microsurgical ligation of spermatic veins. *J Urol* , 1993; 149:769-771.
6. Giagulli VA, Carbone D . Varicocele correction for infertility: which patients to treat?. *Int J Androl*. 2010 ; 34 (3): 236–41.
7. Cockett AT, Takihara H, Iwamura M et al . Pathophysiology of clinical varicoceles in infertile men . *Int J Urol* , 1998; 5 : 113 – 115 .
8. Paul J. Turek . Are bilateral varicoceles more damaging to fertility ? . *Urology Times* ,2001; 29 (1): 9.
9. Marmar JL .The pathophysiology of varicocele in the light of current molecular and genetic information. *Hum Reprod Update* , 2001; 7: 461-72 .
10. Chen SS, Chang LS, Wei YH . Oxidative damage to proteins and decrease of antioxidant capacity in patients with varicocele . *Free Radic Biol Med*, 2001; 30 : 1328 – 34 .
11. Mostafa T,Anis TH, El-Nashan A, et al . Varicocelectomy reduces reactive oxygen species levels and increases antioxidant activity of seminal plasma from infertile men with varicocele . *Int J Androl*. 2001; 24(5): 261-5.
12. Zini A, Dohle G . Are varicoceles associated with increased deoxyribonucleic acid fragmentation ? . *Fertil Steril*, 2011; 96(6): 1283-7.
13. Comhaire FH , Dhooge W, Mahmoud A . A strategy for the prevention of male infertility . *Verh-K-Acad-Geneeskd-Belg* , 1999 ; 61 : 441 – 52 .
14. Nair V,O' Neil CL, Wang PG, et al . Malondialdehyde . Encyclopedia of Reagents for Organic Synthesis . John Wiley & Sons, New York , 2008 .
15. Del Rio D, Stewart AJ, Pellegrini N . A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress . *Nutr Metab Cardiovasc Dis*. 2005; 15 (4): 316–28.
16. Sharma RK, Pasqualotto FF, Nelson DR, et al . The reactive oxygen species – total antioxidant capacity score is a new measure of oxidative stress to predict male infertility . *Hum Reprod* , 1999 ; 14 : 2801 – 7 .
17. World health organization . WHO Laboratory manual for the examination and processing of human semen . 5th edn . *WHO* , 2010 .
18. Bark FR , Lawrence AR . Liver necrosis and lipid peroxidation in the rat as the result of paraquat and diquat administration . *J.clin .invest* ,1980; 65: 1024 -1037.
19. Beyer WF , Fridovich I . As saying for superoxide dismutase activity : some large consequences of minor in condition. *Analy .biochem* ,1987 ;161: 559 -566.
20. Shah SV, Walker PD . Evidence suggesting a role for hydroxyl radical in glycerol –induced acute

renal failure . *Am .J. physiol* . 1989; 256(24) : 438-443.
21. Mostafa T . Seminal plasma reactive oxygen species- antioxidants relationship with varicocele grade . *Andrologia*. 2012; 44(1): 66-9.
22. Dhabuwala CB, Hamid S, Moghissin KS . Clinical versus subclinical varicocele : improvement in fertility after varicocelectomy . *Fertil Steril*, 1992 *;* 18: 564 – 565 .
23. Schoor RA, El hanbly SM, Niederberger C . The pathophysiology of varicocele – associated male infertility . *Curr Urol Rep* , 2001; 2 : 432 – 6 .
24. Ricardo Miyaoka , Sandro C. Esteves . A Critical Appraisal on the Role of Varicocele in Male Infertility . *Advances in Urology* . 2012; 497-595.
25. Saleh RA, Agarwal A, Sharma RK , et al . Effect of cigarette smoking on levels of seminal oxidative stress in infertile men : a prospective study . *Fertil Steril* , 2002 ; 78 : 491 – 9 .
26. El zanaty S, Erenpreiss J, Becker C . Seminal plasma Albumin : origin and relation to the male reproductive parameters . *Andrologia* , 2007 ; 39: 60 – 5 .
27. Garrido N . Relationship among standard semen parameters, glutathione peroxidase/glutathione reductase activity, and mRNA expression and reduced glutathione content in ejaculated spermatozoa from fertile and infertile men . *Fertil Steril.* 2004; 82 :1059-66.
28. Cathy KN, Ajay KN, Ashok Agarwal . Varicocele and male infertility; part 2 pathophysiology of varicoceles in male infertility . *Hum Reprod Update*, 2001 ; 7 : 473 – 81 .
29. Fatma Atig . Altered Antioxidant Status and Increased Lipid Peroxidation in Seminal Plasma of Tunisian Infertile Men ; *Int J Biol Sci* 2012; 8(1):139-149.
30. Barbieri ER, Hidalgo ME, Venegas A, et al . Varicocele –associated decrease in antioxidant defenses . *J Androl* , 1999; 20 : 713 – 7 .