

Effects of Psychological Status on The Oxidation Parameters of Semen and Blood in Azoospermic Men

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Purpose: In limited number of studies performed concerning the psychological moods of female, and male with the diagnosis of infertility, data related to increased incidence of depression, and anxiety have been reported. The objective of this study is to determine whether azoospermia has any psychological effects on men, and investigate the potential effects of psychological mood on seminal, and plasma oxidative parametres.

Materials and Methods: Twenty-seven patients whose two consecutive semen analyses were reported as pellet –negative azoospermia constituted the azoospermic group, and 30 healthy individuals who applied to the infertility polyclinic with normal seminal parametres comprised the normozoospermic group.

Results: BECK Anxiety scores were significantly higher in the azoospermic group ($P = 0.009$). When compared with the normozoospermic group, higher levels of oxidative parametres, but lower levels of the antioxidative parametre were detected in the azoospermic group ($P < 0.05$). In the azoospermic group, a positive correlation was detected between BECK Anxiety and total oxidant status. Anxiety may increase oxidative parametres in both plasma, and seminal fluid ($r = 0.473, p = 0.026$).

Conclusion: Anxiety may increase oxidative parametres in both plasma, and seminal fluid. Oxidative milieu may impair sperm quality, and affect the success rates of assisted reproductive treatments. The determination of oxidative potential in infertile men, thiol, and proliadase may be used as biomarkers.

Keywords: psychological status; oxidative parametres; azoospermia; infertility

INTRODUCTION

Infertility, defined as the inability to conceive after 1 year of regular unprotected sexual intercourse, affects around 15% of all couples of reproductive age, with about 50% being associated with abnormalities in the male, called male factor infertility^(1,2). One of the causes of male factor infertility is azoospermia. Azoospermia refers to absence of sperm in the ejaculate after centrifugation and microscopic analysis of two semen samples obtained at different time points. This condition affects approximately 1 % of the general male population and 15 % of subfertile men⁽³⁾. The term “oxidative stress” (OS) is defined as an imbalance between the production and the elimination of reactive oxygen species that results in an accumulation of oxidative damage⁽¹⁾. Within the last decades, the knowledge concerning the link between OS and psychiatric disorders has been surging⁽⁴⁾. In limited number of studies performed concerning the psychological moods of female, and male with the diagnosis of infertility, data related to increased incidence of depression, and anxiety have been reported⁽⁵⁻⁷⁾. It has been also reported that psychological conditions trigger the production of reactive free oxygen radicals

leading to disruption of balance between free radicals, and antioxidants with resultant induction of oxidative stress^(8,9). Some biomarkers are used in the detection of oxidative stress including proliadase, and thiol/disulfide homeostasis⁽¹⁰⁻¹²⁾. Prolidase (Pro) is an intracellular enzyme necessary to release proline and hydroxyl-proline from the carboxyl terminus of imidodipeptides which take part in collagen degradation, recycle proline for protein synthesis, matrix remodeling and cell growth⁽⁸⁾. Exposure of proline decreases antioxidant potential, suggesting its role in OS. Exposure of proline decreases antioxidant potential, suggesting that proline induces OS. Statistically significantly higher oxidative status and proliadase activities have been demonstrated in generalized anxiety disorder⁽⁸⁾. Dynamic thiol/disulphide homeostasis was defined as a novel oxidative stress marker by Erel and Neselioglu⁽¹³⁾. Dynamic thiol disulphide homeostasis status has critical roles in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors and cellular signalling mechanisms⁽¹⁴⁾. Moreover, dynamic thiol disulphide homeostasis is being increasingly implicated in many disorders. There is also a growing body of evidence

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demonstrating that an abnormal thiol disulphide homeostasis state is involved in the pathogenesis of a variety of diseases, including diabetes, cardiovascular diseases, cancer, rheumatoid arthritis, chronic kidney disease, Parkinson's disease, Alzheimer's disease, Friedreich's ataxia, multiple sclerosis⁽¹³⁾. The objective of this study is to determine whether azoospermia which is a serious etiological factor of male infertility has any psychological effects on men, and investigate the potential effects of psychological mood on seminal, and plasma oxidative parameters. As far as we may know, this is the first study which investigated the effects of psychological mood on seminal, and plasma oxidative parameters of azoospermic men, and used both proliadase, and thiol/disulphide homeostasis as oxidative markers.

MATERIALS AND METHODS

Fifty seven patients who applied to infertility polyclinic of our hospital between January 2017 and June 2017 because of infertility and voluntarily participated in the trial were included in the study. Twenty-seven patients whose two consecutive semen analyses were reported as pellet -negative azoospermia constituted the azoospermic group, and 30 healthy individuals who applied to the infertility polyclinic with normal seminal parameters comprised the normozoospermic group. Approval of the local ethics committee was obtained for the study (date of the approval: 05.01.2017 and registration #:01-18). The patients were informed about the study in detail, and then their undersigned consent forms were obtained.

Exclusion criteria of the study were as follows: presence of significant leucospermia, age > 45 years, BMI > 30 kg/m², presence of varicocele, epididymo-orchitis, testicular torsion, testicular trauma, and tumour, smokers, and use of pentoxifylline, vitamin preparations and antidepressants.

In addition, participants were asked to fill in Turkish version of the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). The BDI is composed of 21 questions that are scored on a four-point Likert scale (0-3 points). The BAI was used to assess the severity of clinical anxiety during the past week. It is a self-reported inventory containing 21 items on a four-point scale. Beck Anxiety Inventory, and Beck Depression Inventory classify the severity of depression, and anxiety as follows: 0-13 pts, absence of depression or anxiety; 14-19 pts, mild; 20-28 pts, moderate, and 29-63 pts, severe depression, and anxiety⁽¹⁵⁾.

For the investigation of infertility after 3 days of sexual abstinence, the patient ejaculated into a sterile container by masturbation without using lubricating gel. One drop from collected semen samples was dropped on Macler camera and examined under microscope at 20× magnification, and sperm counts, motility and leucocyte counts were determined. Besides, semen sample was spread on a slide, properly stained and dried before examining sperm morphologies under microscope at 100 × magnification. Based on the results of spermogram according to WHO 2010 criteria, the patients were categorised in azoospermic and normozoospermic groups. The remaining semen samples were centrifuged at 3 000 × g for 15 min and stored at -80°C till oxidative parameters were analysed. Besides, venous blood samples were drawn from the participants after 12 hr of fasting and centrifuged at 4 000 × g for 10 min, and the

separated serum portions were stored at -80°C till oxidative parameters (TAS, TOS) were analysed.

Measurement of total oxidant status

Seminal plasma TOS levels were analysed using commercial Rel Assay kits developed by Erel. For the measurement of TOS levels, as described in the operating principles of the test, colorimetric method based on oxidation of ferrous ion to ferric ion by oxidant molecules contained in the samples was used. The results were expressed in $\mu\text{mol H}_2\text{O}_2/\text{L}$ ⁽¹⁶⁾.

Measurement of total antioxidant status

Seminal plasma TAS was analysed using commercial Rel Assay kits developed by Erel. This method developed by Erel is fully automated system and measures total antioxidant capacity (TAC) of the body against potent free radicals. Measurement of TOS levels is based on decolourisation of coloured ABTS cationic radical as a result of reduction by all antioxidant molecules contained in the sample in proportion with total concentration of antioxidant molecules⁽¹⁷⁾. As a calibrator, Trolox which is a water-soluble analogue of vitamin E is used. The results were expressed as mmol Trolox Equivalent/L.

Measurement of oxidative stress index

Oxidative stress index of the samples is calculated as the ratio of total oxidant levels of samples to total antioxidant levels of the samples and expressed as percentages⁽¹⁷⁾. Before calculation mmol unit of TAS is converted to the unit of micromole as performed for TOS test.

Blood sampling and measurement of dynamic thiol/disulphide homeostasis

Venous blood samples were drawn in tubes containing ethylenediaminetetraacetic acid and serum samples were immediately separated by centrifugation at 1500 g for 10 min. Samples were coded and stored at -80°C. Serum dynamic thiol/disulphide homeostasis was determined with a recently developed spectrophotometric method described by Erel and Neselioglu using an automated clinical chemistry analyser (Roche, cobas 501, Mannheim, Germany)⁽¹³⁾. Sodium borohydride is the essential agent in this method that is used to reduce dynamic bonds to functional thiol groups. To prevent extra reduction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), unused sodium borohydride is removed by formaldehyde addition. The total thiol content of the sample is measured using a modified Ellman reagent. Subtracting native thiol content from total thiol gives twice the disulphide amount. Having found the disulphide amount, the disulphide/total thiol ratio, disulphide/native thiol ratio and native thiol/total thiol ratio can be calculated.

Determination of Prolidase Activity

Serum was diluted 40-fold with 2.5 mmol/L Mn²⁺, 40 mmol/L Tris-HCl buffer (pH 8.0) and pre incubated at 37°C for 2 hr. The reaction mixture containing 30 mmol/L Gly-Pro, 40 mmol/L Tris-HCl buffer (pH:8.0), and 100 μL of preincubation serum in 1 mL was incubated at 37°C for 30 min. Adding 0.5 mL of 20 % trichloroacetic acid solution then stopped the incubation reaction. The supernatant was used for measurement of proline by the method proposed by Myra et al.⁽¹⁸⁾, which is a modification of Chinard's method.⁽¹⁹⁾ Intra-assay coefficient of variation of the assay was 3.8%.

Table 1. Study subgroup participants' demographic, anxiety - depression scores and semen parameters

Variables	Azoospermic group, n = 27 (Mean ± SD)	Normozoospermic group, n = 30 (Mean ± SD)	p Value
Mean age (years)	29,4 ± 5,7	30,4 ± 6,4	0.535
Education (years)	9,1 ± 4,45	10,5 ± 5,10	0.546
Body Mass Index (BMI)	25,4 ± 5,8	26,5 ± 4,6	0.469
Duration of marriage (years)*	4 (8)	3,5 (3,7)	0.887
Duration of infertility (years)*	4,2 (1,9)	---	NA
Volume*, ml	1,4 (1)	1,6 (1)	0.605
Concentration**(x10 ⁶ /mL)	0	34,6 ± 37,9	NA
Motility** (%motile sperm)	0	42,9 ± 30,0	NA
Morphology** (%normal sperm)	0	8,3 ± 6,7	NA
BAI** score	20,4 ± 10,1	7,9 ± 5,3	0.003
BDI score *	11 (22)	10,5 (14,2)	1.000

Abbreviations: BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory

*Median (IQR), **Mean ± standard deviation (SD)

Statistically significant *p* values were written as bold

Statistical analysis

Statistical analyses were performed using SPSS Version 15 (SPSS Inc. Chicago USA) software program. Distribution of numerous variables was evaluated using Shapiro-Wilk test. Parametric tests were used for data with normal distribution. For intergroup comparisons parametric Student's *t*-test, and nonparametric Mann-Whitney *U* test were used. For correlation Spearman's correlation analysis was employed. The results were expressed as mean ± standard deviation, median (interquartile range); and *p* < .05 was accepted as the level of significance.

RESULTS

Demographic and BDI, and BAI data are shown in **Table 1**. The groups were similar in mean age, education, body mass index (BMI), duration of marriage and infertility, and volume of semen. A significant intergroup difference was not found concerning BECK D scores (*P* = 1.000). BECK A scores were statistically significant higher in the azoospermic group (*P* = 0.009). In both azoospermic, and normozoospermic groups, any statistically significant correlation did not exist between BECK A (*r* = 366, *p* = 0.079; *r* = 366, *p* = 0.079 and D scores (*r* = 103, *p* = 0.631; *r* = 180, *p* = 0.400), with duration of marriage, and the infertility duration.

Semen analyses

When compared with the normozoospermic group, higher levels of oxidative parameters namely prolidase, TOS, and OSI, but lower levels of the antioxidative parameter (ie. TAS) were detected in the azoospermic group (*P* < 0.05) (**Table 2**).

In the normozoospermic group, the correlation between semen parameters (volume, sperm counts in one millilitre, sperm motility, and morphology), and TAS, TOS,

OSI, prolidase, BECK A and D values was examined. Statistically significantly negative correlations were detected between prolidase, and sperm counts (*r* = -566, *P* = 0.009), motility (*r* = -526, *P* = 0.017), and morphology (*r* = -545, *P* = 0.013), and also between OSI, and sperm counts (*r* = -650, *P* = 0.002). A significant correlation was not detected between other parameters.

Serum analyses

Levels of serum prolidase, TOS, OSI were higher in the azoospermic group relative to normozoospermic group (*P* < 0.05), but antioxidant parameters (TAS, native thiol, total thiol and disulphide) were lower than those of the normozoospermic group (*P* < 0.05) (**Table 3**). In the azoospermic group, a positive correlation was detected between BECK A and TOS (*r* = 473, *P* = 0.026). A negative correlation was detected between BECK A and plasma prolidase levels (*r* = 493, *P* = 0.017).

DISCUSSION

As a generally accepted corollary, major sources of seminal oxidative stress are leucocytes, and defective sperm cells. In our previous study⁽¹⁾, contrary to our prediction during planning of the study, we found higher oxidative stress, but lower antioxidative parameters in seminal plasma of azoospermic men devoid of leukocytes, and spermatozoa in comparison with normozoospermic men. As a possible cause of this condition, we reported that azoospermia which is a serious etiological factor of infertility exerts psychological effects on men, and subsequently higher levels of plasma oxidative parameters increasing with stress pass through seminal vesicles, and prostate into seminal fluid leading to enhanced oxidative stress, but lower antioxidative parameters in seminal plasma of azoospermic men. However we did not analyze the psychological mood of azoospermic men in our previous study. Therefore in the

Table 2. Seminal plasma oxidative/antioxidative parameters in azoospermic and normozoospermic groups.

	Azoospermic group, n = 27	Normozoospermic group, n = 30	p Value
Prolidase* (U/l)	36.4 (25,2)	21.7 (12,2)	0.019
TAS**, mmol Trolox Equivalent/L	0.94 ± 0.33	1.30 ± 0.32	0.001
TOS*, μmol H ₂ O ₂ /L	18.7 (3,5)	15.3 (4,0)	0.037
OSI**	1.99 ± 0.65	1.23 ± 0.52	0.000

Abbreviations: TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index

Table 3. Serum oxidative/antioxidative parameters in azoospermic and normozoospermic groups.

	Azoospermic group, n = 27	Normozoospermic group, n = 30	p Value
Prolidase*	8.8 (9.1)	6.5 (1.9)	0.032
TAS, mmol Trolox Equivalent/L	0.87 ± 0.28	1.13 ± 0.35	0.010
TOS*, µmol H ₂ O ₂ /L	16.7 (3.3)	14.0 (2.7)	0.003
OSI*	1.9 (1.6)	1.25 (0.6)	0.001
Native thiol (µmol/L)	403.9 ± 79.4	475.4 ± 86.8	0.007
Total thiol (µmol/L)	460.5 ± 79.5	549.2 ± 93.9	0.002
Disulphide (µmol/L)	28.3 ± 9.4	36.3 ± 13.5	0.029
Disulphide/native thiol ratio	7.3 ± 2.7	7.9 ± 3.2	0.476
Disulphide/total thiol ratio	6.3 ± 2.1	6.7 ± 2.3	0.496
Native thiol/total thiol ratio	87.4 ± 4.2	86.5 ± 4.6	0.495

Abbreviations: TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index

*Median (IQR), Mean ± SD

present study we aimed to evaluate psychological mood of the azoospermic men, and investigate the correlation between psychological mood, and oxidative parameters of blood, and seminal fluid.

Multiple number of studies which investigated the correlation between infertility, and psychological state of both men, and women have been published. In a meta-analysis, Greil et al. analyzed the correlation between infertility, and psychological distress, and indicated that infertility is generally related to psychological distress which may be both the cause, and outcome of infertility⁽⁷⁾. The authors also emphasized that the duration of infertility, and treatment should be taken into consideration in studies which would be performed concerning infertility, and psychological distress. Very limited number of studies have investigated psychological mood in azoospermia which is a serious etiological cause of infertility. Akbal et al. reported increase in symptoms of erectile dysfunction, anxiety, and depression in azoospermic men in whom TESE could not find any viable spermatozoa relative to normozoospermic men⁽²⁰⁾. In recent studies, Castro et al. demonstrated presence of a correlation between azoospermia, and neuropsychiatric diseases⁽²¹⁾. Bak et al. reported that patients with the established diagnosis of nonobstructive azoospermia which signifies absence of sperm production had experienced intense panicky feelings with increased tendency to insomnia, anxiety and depression.⁽²²⁾ In our present study, we found statistically significantly higher anxiety scores in azoospermic men when compared with the normozoospermic group. While depression scores were not so high in azoospermic men. Levels of anxiety did not correlate with the duration of marriage, and disease in our study, relatively shorter average duration of marriage, and disease in the azoospermic group may be the reason why we couldn't find any correlation between these parameters. In groups with longer duration of infertility, more realistic outcomes may be obtained.

It is already known that oxidative parameters increase, while antioxidative parameters decrease in both plasma, and seminal fluid of infertile men. In our study, we found increases in oxidative parameters (TOS, OSI, prolidase), but decreases in antioxidative parameters (TAS, thiol) in plasma, and seminal fluid of azoospermic men. Despite contradictory arguments made in many studies, as is the case with multiple number of diseases including anxiety, and depression, prolidase has been associated with increased oxidative stress, and decreased antioxidant levels.⁽²³⁾ It has been estimated

that this oxidative effect is caused by release of proline mediated by the action of prolidase. In support with the study which detected higher levels of prolidase in anxiety, we found a positive correlation between BECK-A scores and levels of TOS, and prolidase in the plasma of azoospermic men. Besides, prolidase and increased oxidative stress negatively affected sperm counts, motility, and morphology. Ozcan et al. reported the presence of a negative correlation between prolidase activity on the wall of the varicocele, and sperm counts in azoospermic men with varicocele.⁽²⁴⁾ They indicated that this condition might be the result of an oxidative milieu secondary to increased levels of prolidase in the spermatic vein, and duct. In the same study, they reported lack of any correlation between sperm motility, and enzymatic activity of prolidase.

Thiol is a novel biomarker which can be measured only in blood, and thiols are antioxidant buffers which balance both intracellular, and extracellular oxidative processes.⁽²⁵⁾ In accordance with decreased antioxidative potential in infertile men, we found lower thiol levels in azoospermic infertile men when compared with normozoospermic individuals. Though any relevant study has not been performed in infertile men, some studies have demonstrated antioxidative contribution of thiol levels to oxidative processes. Tokgöz et al. detected marked decreases in native, and total thiol levels in the sera of the patients who had undergone prostate biopsies⁽¹²⁾. They also indicated that these decreases might be related to acute oxidative stress due to the procedure of prostate biopsies. Kozanhan et al. performed a study with health care professionals who had been exposed to anesthetic gases, and reported decreased thiol, but increased disulfide levels⁽¹¹⁾. They stated that these changes stemmed from oxidative effects of anesthetic gases, and thiol/disulfide balance might be used as a biomarker in the diagnosis of oxidative stress.

CONCLUSIONS

Infertility is an important health problem which may induce neuropsychiatric disorders as anxiety. Anxiety may increase oxidative parameters in both plasma, and seminal fluid. Oxidative milieu may impair sperm quality, and affect the success rates of assisted reproductive treatments. We think that in the determination of oxidative potential in infertile men, thiol, and prolidase may be used as biomarkers in infertility.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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