

Study The Balance between Reduced – Oxidized Glutathione in Seminal Plasma of Iraqi Subfertile Men

Nuha, Y. AL-Harbi

Biology sciences/Babylon university/Iraq
E-mail:nuhayaerob@yahoo.com

Abstract

Defective sperm function is the most common cause of infertility, and until recently, was difficult to evaluate and treat. Human seminal plasma a natural reservoir of antioxidants that protect spermatozoa from oxidative damages.

Aims: The aim of the present study was to assess the antioxidant status of seminal plasma by contents of reduced glutathione and oxidized glutathione in men with asthenozoospermia compared to normozoospermic males, and their correlations with Seminal parameters.

Semen samples 25 normozoospermic controls and 25 asthenozoospermics were analyzed for physical (sperm concentration, motility, morphology) and chemical (reduced glutathione (GSHr), oxidized glutathione (GSSG)) parameters .

Significance of difference among the groups and coefficient of correlation between the parameters was tested statistically. GSHr level was found to be significantly higher in the normospermic men than the asthenospermic ($p < 0.001$). Levels of GSSG were found to be significantly higher in asthenozoospermic than the normal group ($p < 0.001$). Positive correlation was found between GSHr, GSSG levels and sperm motility ($p < 0.001$, $r = 0.42$; $p < 0.001$, $r = 0.35$). Moreover, it was found to be correlated positively but not significant between GSHr with sperm abnormal morphology ($p = 0.30$, $r = 0.21$). Both GSHr and GSSG showed a negatively but not significant correlation with sperm count ($p = 0.20$, $r = -0.26$; $p = 0.8$, $r = -0.35$). However, the sperm abnormal morphology showed a negative but not significant relationship to seminal GSSG ($P = 0.27$, $r = -0.22$). In the present study seminal plasma GSHr and GSSG levels were found a positive highly significant correlation with sperm motility. This finding indicates that low level of seminal plasma GSHr and high GSSG level in infertile subject, may be associated with male infertility.

Keywords:- Asthenozoospermics men, seminal antioxidants, reduced glutathione level, oxidized glutathione level, sperm parameters.

1.Introduction

Damage induced by endogenously generated reactive oxygen species has been proposed to be a major contributing factor in a variety of human diseases, including male infertility [1]. Male infertility is associated with increased ROS and decreased total antioxidant activity in the seminal plasma. ROS induce nuclear DNA strand breaks. Besides, due to a high polyunsaturated fatty acid content human sperm plasma membranes are highly sensitive to ROS induced lipid. Some studies have shown that infertile men have an impaired seminal plasma non enzymatic antioxidant capacity (also called total antioxidant capacity (TAC), suggesting that a decreased TA may play a pathogenetic role in male infertility [2]. In testis and in seminal fluid, reactive oxygen species (ROS) are produced by spermatogenic cells, spermatozoa, immune cells, mainly neutrophils and high amounts of them can be produced by immature and abnormal spermatozoa and during of inflammation [3]. Hence, human spermatozoa are known to possess all of the major antioxidant defensive systems including catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione reductase (GRD) [4].

One more important antioxidant in human is GSH. It serves as a cofactor for GPX and reacts directly with ROS by its free sulphhydryl groups. When present in extra-cellular space, GSH is able to react directly with cytotoxic aldehydes produced during lipid peroxidation and thus protects the sperm plasma membrane [5,6]. Cellular GSH plays a key role in many biological processes, including the synthesis of proteins and DNA and the transport of amino acids, but notably, it plays a key role in protecting cells against oxidation: the sulphhydryl (SH) group is a strong nucleophile, and confers protection against damage by oxidants, electrophiles and free radicals [7]. Glutathione (GSH) is the most abundant reducing agent found in the body, protecting lipids, proteins and nucleic acids against oxidative damage. GSH combines with vitamin E and Se to form glutathione peroxidase [8]. Tavilani et al [9] indicate a protective role of antioxidant enzymes of seminal plasma against lipid peroxidation of spermatozoa in normozoospermic samples. Since lipid peroxidation leads to loss of motility in human spermatozoa. However, increased malondialdehyde levels, abnormal sperm morphology and higher DNA damage were observed in the cases. The antioxidants superoxide dismutase, catalase and glutathione had a positive association with sperm count and motility while a negative association with the percentage of abnormal morphology was observed [10].

2. Materials & Methods

2.1. Study population : The study was carried out in 50 male partners from couples (range 22-50 years) .The study design included consulting for infertility evaluation in our laboratory of physiology and biochemical, Babylon university, Biology Department.

2.2. Sample collection: Semen was obtained by masturbation technique after at least 3 days of sexual abstinence .Samples were collected into sterile containers for immediate transportation to the laboratory. They were examined immediately after 30 minutes of liquefaction according to WHO guidelines (World Health Organization)[11]. Spermograms included semen volume (ml) , sperm count($10^6/ml$), sperm motility(%),and abnormal morphologic features(%).

2.3. Determination of GSHr and GSSG contents: Semen samples were centrifuged and then seminal plasma was separated for determination of GSH concentrations . For determination of GSH concentrations , a precipitating solution was added to seminal plasma to precipitate all proteins in the sample . After centrifugation, the clear supernatants were stored at -76° until used for analysis. Reduced glutathione(GSHr) in seminal fluid was measured by Ellman's method [12] .In this method, DTNB(5,5-dithiobis(2-nitrobenzoic acid),a disulfide chromogen ,is readily reduced by -Sh groups to an intensely yellow compound .The absorbance of the reduced chromogen is measured at 412 nm by spectrophotometer and is directly proportional to the GSHr concentration. Moreover oxidized glutathione (GSSG) was measured spectrophotometrically by the method of Tannhauser et al [13] calculate the amount and concentration of total disulfide linkage (R-S-S-R) in the sample from the molar extinction coefficient of CNT($4,150M^{-1}cm^{-1}$) .

3. Statical analysis

The differences of mean values of GSHr and GSSG in different groups were evaluated statistically using ANOVA test ($p < 0.001$ was considered statistically highly significant) . The relationship between different parameters was tested by calculating coefficient of correlation (r-value).

4. Results

Student test showed a significant increase of mean GSHr were found in normozoospermics ($3.31 \pm 1.05 \mu mol/l$) compared to asthenozoospermics ($2.18 \pm 1.31 \mu mol/l$ $p < 0.001$) Also, a highly significant increase in GSSG concentration was detected in the seminal plasma of asthenozoospermics compared to the fertile group ($9.69 \pm 5.00 \mu mol/l$ $p < 0.001$) ($5.63 \pm 2.89 \mu mol/l$) (figure 1).

Correlation studies showed high positive association of seminal GSHr, GSSG and to sperm motility ($p < 0.001$, $r = 0.42$; $p < 0.001$, $r = 0.35$)(figure 2). Positive ,but not significant correlation was found between the GSHr and percentage of abnormal morphology ($p = 0.30$; $r = 0.21$) (figure 3). However, negative but not significant correlation was found between GSHr ,GSSG and sperm count ($p = 0.20$, $r = -0.26$; $p = 0.8$, $r = -0.35$) (figure 4),glutathione only oxidized form showed a negative but not significant correlation with abnormal morphology ($p = 0.27$, $r = -0.22$) (figure 3).

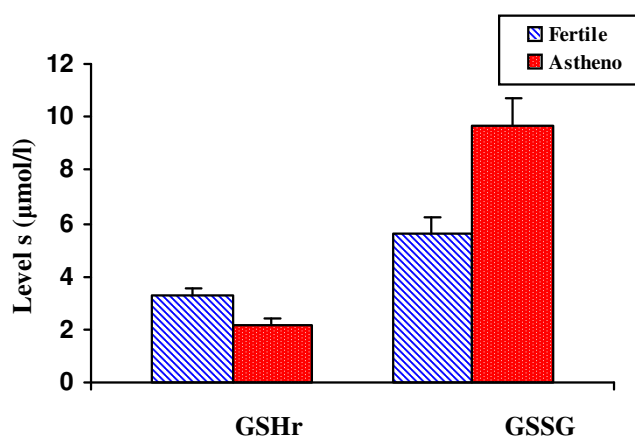


Figure 1. GSHr and GSSG of seminal fluid in µmol/l in study subject.

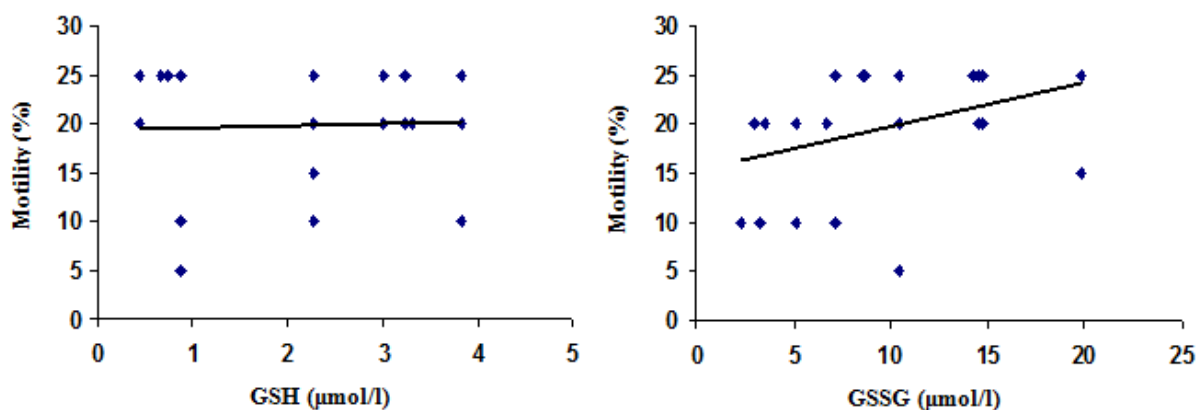


Figure 2. Correlation between seminal plasma reduced glutathione ,oxidized glutathione and sperm motility percentage.

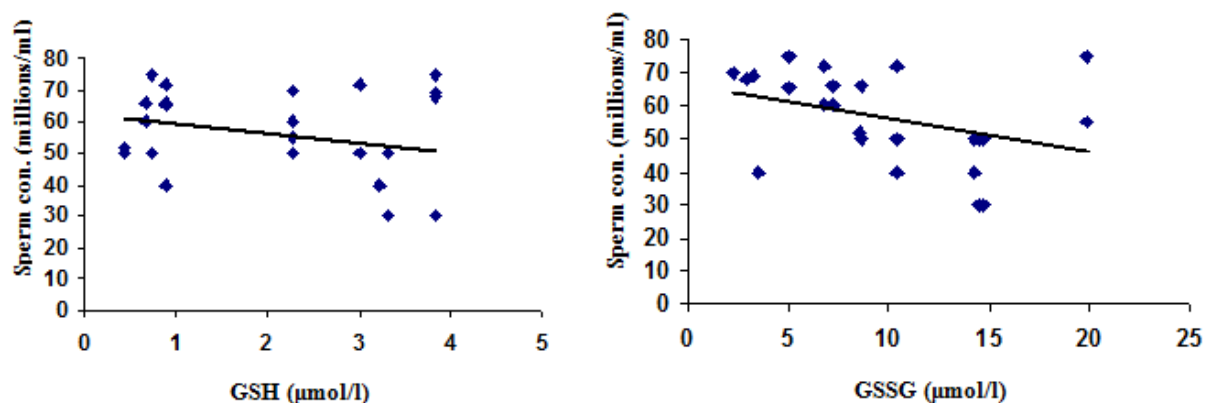


Figure 3. Correlation between seminal plasma reduced glutathione ,oxidized glutathione and abnormal sperm morphology.

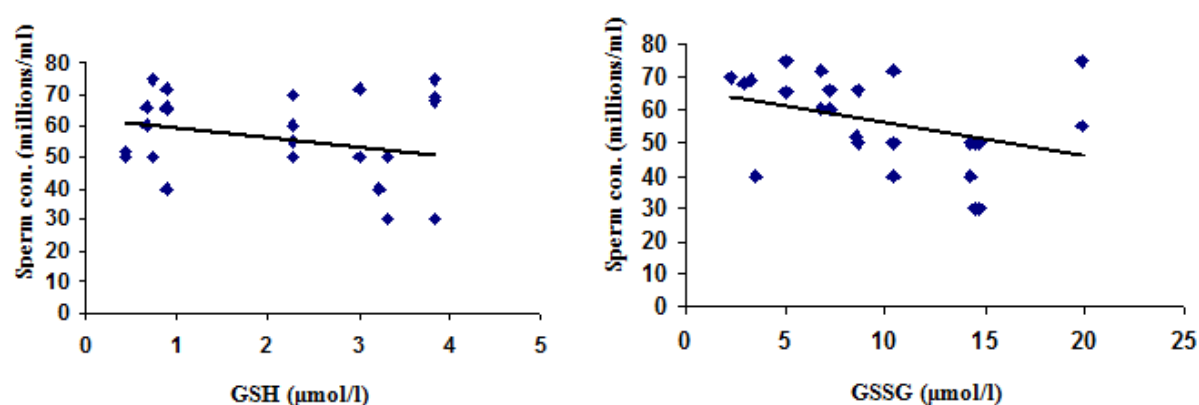


Figure 4. Correlation between seminal plasma reduced glutathione ,oxidized glutathione and sperm concentration.

Discussion

Male fertility depend on the production of normal sperm and the delivery of it to a female partners reproductive tract .Most commonly , Male infertility arises when the man is unable to produce or deliver fully functioning sperm. The potential role of antioxidants in ameliorating such damage has begun to be examined with studies involving reduce glutathione and oxidized glutathione [14,15].

Results showed that a significantly lower seminal reduced glutathione (GSHr) in asthenospermic men compared to normospermic men and highly significant elevated in seminal GSSG in infertile males compared with fertile male subjects ,Which corroborated with the findings of [16] found reduced GSHr showed significant elevation in seminal plasma of controls . However, GSSG was lightly increased in asthenospermic men than normospermics .Furthermore , like Chaudhari etal.,[17] who observed of reduced glutathione were found to be

significantly higher in normozoospermic than the asthenozoospermic group ($p < 0.01$). Moreover, it was found to be correlated positively with all seminogram parameters. and [18] Who showed seminal plasma GSH level was found significantly ($p < 0.001$) suppressed in infertile males compared with healthy fertile male. other studies could not observe any difference in seminal GSH concentration between fertile and infertile men [19,20].

In the present study, showed highly significant and a positive correlation in seminal plasma GSHr and GSSG to sperm motility. Again the results of the present study was in accordance with previous study that showed also highly significant and positive correlation between seminal GSHr, GSSG and sperm motility [21,22], Which are regarded as the most important criteria for normal fertilizing ability of the spermatozoa. Also, the present study observed a positive but not significant association of seminal GSHr to sperm abnormal morphology.

Aerobic metabolism of human sperm produces various reactive oxygen species (ROS), Which are potentially harmful to the sperm plasma membrane with its high content of polyunsaturated fatty acids [23,24]. The toxic lipid peroxides are known to cause various impairments of the sperm cell, such as membrane damage and decrease in motility [25],[26]. GSH deficiency may render the mid-piece unstable, resulting in defective morphology and motility [27,28].

The results of the present investigation showed that lower GSHr and higher GSSG levels in seminal plasma were associated with a lower quality of sperm count, however higher GSSG levels were a negative but not significant association with sperm abnormal morphology. These findings were compatible with that observed by [16,21]; [29,30] noted a significant and negative correlation among seminal GSSG and the percentage of abnormal morphology. This result provides evidence that GSSG in seminal plasma seem to protect the quality of sperm cell membrane and morphology [31].

Conclusion

Decreasing seminal plasma antioxidants levels could have significant role in etiology of impaired sperm function. Others have found that both deficient and excessive concentrations of antioxidants are deleterious for the sperm and others have shown the effect of antioxidants on some sperm parameters but not all of them. In this investigation, I found seminal plasma GSHr and GSSG levels is closely related to sperm motility, and the decreased GSHr level and increased GSSG level in seminal plasma may be one of the causes of male infertility.

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