ORIGINAL ARTICLE

Role of Glutathione in Male Infertility

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ABSTRACT

Infertility is a worldwide problem and in almost 50% of cases infertility results from abnormality of the male partners. Apart from endocrine disorders, definitive cause and mechanism of male infertility is not clear in many cases. Recent evidence indicates that imbalance between pro-oxidant stress and antioxidant defense plays an important role in the pathogenesis of male infertility. Among the endogenous antioxidant systems, reduced glutathione (GSH) plays a significant role in the antioxidant defense of the spermatogenic epithelium, the epididymis and perhaps in the ejaculated spermatozoa. The current study was therefore designed to evaluate any association that may exist between GSH levels and male infertility. Infertile male patients (having female partners with normal fertility parameters; n=31) and age- matched healthy male fertile control subjects (n=30) were included in this study. In addition to medical history, semen analyses including semen volume, sperm count, motility and morphology were done for each subject. As a measure of antioxidant capacity erythrocyte and seminal plasma GSH concentrations were measured by Ellman's method in fertile and infertile male subjects. The infertile subjects were similar to fertile subjects in terms of age. However, semen volume and sperm count was found significantly lower ($p<0.001$) in infertile males compared with healthy fertile male subjects. Percentage of subjects with abnormal sperm morphology and motility were found higher in infertile group compared with fertile group. The median (range) erythrocyte GSH level did not differ between the two groups (12.62 (0.67-29.82) versus 13.93 (2.10-21.08) mg/gm Hb). However, the seminal plasma GSH level was found markedly suppressed in infertile group (1.64 (0.23-7.50)) compared with fertile group (4.26 (2.32-7.50)) mg/dl ($p<0.001$). In the present study seminal plasma GSH level was found markedly suppressed along with abnormal values for semen volume, sperm concentration and sperm morphology and motility in infertile subjects compared with fertile subjects. This finding indicates that low level of seminal plasma GSH level may be associated with male infertility.

Key Words: Antioxidants, Glutathione, Male Infertility.

Introduction

Infertility is defined as the failure of conception after at least 12 months of unprotected intercourse[1]. Infertility affects at least one couple in six and the commonest single defined cause is sperm dysfunction[2]. Recent evidence has suggested that human semen quality deteriorates by as much as 3% per year[3]. However, we do not have reliable ways to diagnose or treat patients suffering from this distressing problem. In fact, we still lack a clear understanding of the causes of male infertility and of the nature of the defects in sperm cell structure and biochemistry that underlie the loss in fertilizing potential.

Oxidative stress is conventionally defined as an imbalance between pro-oxidant stress and antioxidant defense. A reactive species may be a free radical or a non-radical in structure. There

are three different classes of reactive species relevant in biology and medicine: a. reactive oxygen species (ROS), b. reactive nitrogen species, and c. reactive chlorine species[4]. A battery of different antioxidants normally protects against oxidants[6]. Oxidative stress develops when oxidants outnumber antioxidants and peroxidation products develop, and these phenomena cause pathologic effects[7,8]. In common with all cell types, spermatozoa can defend themselves against oxidative damage. Although the existence and role of catalase in human spermatozoa is controversial[9], they are known to possess two alternative defense mechanisms against superoxide (O2.-) and H2O2, namely superoxide dismutase (SOD) and the glutathione peroxidase/reductase pair[10,11,12]. However, the relative contribution of each of these defense mechanisms in normal men and in men with impaired spermatogenesis remains to be fully elucidated.

Glutathione (L- -glutamyl-L-cysteinylglycine; GSH) is the most abundant non-protein thiol in mammalian cells, being present in concentrations of 0.5-10 mmol/l. 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 sulphydryl (SH) group is a strong nucleophile, and confers protection against damage by oxidants, electrophiles and free radicals[13].

Glutathione has been detected in various concentrations in male reproductive tissues in different species including human being. Substantial quantities of GSH are found in the testis, reproductive tract fluids, and epididymal spermatozoa[14]. Its presence has been shown intracellularly within the sperm as well as extracellularly in the seminal plasma. Glutathione has been found to be low in azoospermics compared with normospermics, and a decrease in seminal plasma and erythrocyte levels of GSH has been shown to cause disruption in the membrane integrity of spermatozoa as a consequence of increased oxidative stress[15]. In addition, deficiency of GSH has been shown to cause instability of the body of the sperm resulting in defective sperm motility[16]. which suggests that low levels of GSH might affect male fertility. However, involvement of GSH with male infertility is still controversial because there are studies that did not find a significant difference of seminal plasma or erythrocyte GSH levels among groups of subjects with different fertility potentials[17,18]. Moreover, no such study has been done in Bangladeshi population. Therefore, it is very important to establish whether GSH level really plays any role in male infertility.

Methods

This study was done in the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. A total of 31 diagnosed infertile male patients (having normal female partner) who attended in the infertility unit of Gynaecology and Obstetrics Department at BSMMU and 30 age-matched fertile healthy control subjects were taken purposively and conveniently by following the inclusion criteria and exclusion criteria. Male subjects were included in this study whose female partners were found normal in terms of fertility parameters. For selecting control group age-matched fertile male having offspring were taken. Those male subjects were excluded from this study who had one or more of the following criteria: acute illness within the last 3 months; chornic illness (e.g. neoplasm, varicocele, trauma, hydrocele, mumps, etc); history of genital tract infections (e.g. urethritis, orchtic etc.) and trauma (e.g. horse riding, cycling etc.); hypogonadism; obstruction of vas deferens; hypospadias; retrograde ejaculation; current history of medication for infertility. In addition, those male subjects were excluded from this study whose female partners had abnormal fertility parameters. This study was approved by the institutional review board and an informed written consent was taken from all subjects.

Five ml of venous blood were collected. 2.5 ml of blood was taken in a heparinzed test-tube for the assay of oxidative stress markers and from the remaining 2.5 ml of blood, serum was separated by centrifugation for hormone analysis. Semen samples were collected from the subjects after 3-4 days of sexual abstinence. Samples were collected in the clinical facility by masturbation into a sterile glass container.20 For internal quality control of semen analysis, spermiograms were carried out by the trained observer, according to the World Health Organization guidelines.21 Spermiograms included semen volume (ml), sperm density (106 per ml), sperm motility (%) and abnormal morphologic features (%).

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 -760C until used for analysis. GSH concentration in semen and erythrocyte was measured by Ellman's method[22]. 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 GSH concentration.

All the data were recorded systematically in a preformed data collection sheet. Data were analyzed by using SPSS 12.0 for Windows. Mann-Whitney U test, unpaired t-test and Z-test were done to find significant difference between groups. Statistical significance was set at $p < 0.05$.

Results

As shown in table 1, fertile and infertile subjects were similar in terms of age. However, semen volume and sperm concentrations were found significantly ($p < 0.001$) lower in infertile subjects compared with fertile subjects (Table 1). Moreover, significantly higher number (percentage) of subjects having abnormal sperm motility and morphology were observed in infertile group compared with fertile group.

Table-I

Parameter	Case (n=31)	Control (n=30)	p-value
Age (years)	34.48±4.59	35.83±4.46	0.25[a]
Semen volume (ml)	2.36±0.96	3.26±0.73	<0.001[a]
Sperm concentration ($x10^6$/ml), Median (range)	19 (5-150)	100 (25 - 180)	<0.001[b]
Sperm motility % (normal / abnormal)	45.2 / 54.8	93.3 / 6.7	0.001[c]
Sperm morphology % (normal / abnormal)	67.7 / 32.3	96.7 / 3.3	0.004[c]

a= unpaired 't' test.
b= Mann-Whitney test
c= 'Z' proportion test

To evaluate antioxidant activity, the levels of GSH in the erythrocytes and seminal plasma were measured both in case and control groups. Median (range) GSH level of erythrocyte was found 12.62 (0.67-29.82) and 13.93 (2.10-21.08) mg/gm Hb in case and control groups, respectively. Thus the GSH level did not differ between infertile and fertile male subjects (Figure 1). On the other hand, median (range) GSH level of seminal plasma was found 1.64 (0.23-7.50) and 4.26 (2.32-7.50) mg/dl in case and control groups, respectively. Thus the seminal plasma GSH level was found significantly ($p < 0.001$) suppressed in infertile males compared with healthy fertile male subjects (Figure 2)

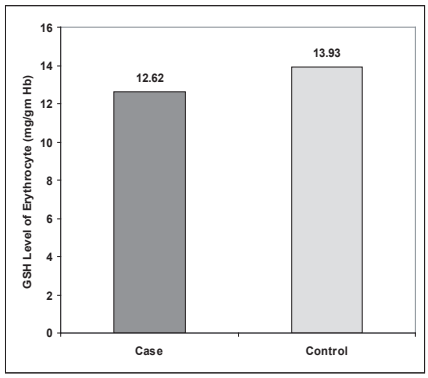

Figure-1: *GSH level of erythrocyte in mg/gm Hb in study subject.*

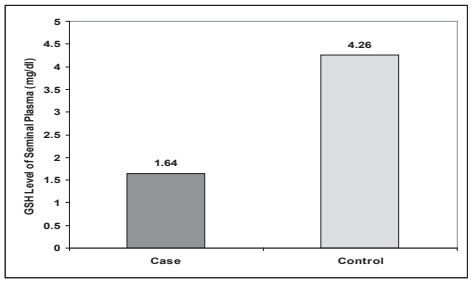

Figure-2: *GSH level of seminal plasma in mg/dl in study subject.*

Discussion

Infertility is a common yet complex problem affecting approximately 15% of couples attempting to conceive a baby. Although infertility problems were attributed to the woman in the past, it is now known that male factors play a role in almost one half of cases. Male fertility depends on the production of normal sperm and the delivery of it to a female partner's 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 vitamin E, GSH, catalase and SOD.[23,24] Vitamin E suppresses lipid peroxidation catalysed by ferrous ion in vitro, and consequently rescues the capacity of ejaculated spermatozoa for sperm-oocyte fusion[25]. The use of other antioxidants, including SOD and GSH, was studied by Griveau and Le Lannou (1994)[24] who examined the ability of SOD and GSH to influence the loss of motility and acrosome reaction rates in spermatozoa prepared by centrifugation. Both GSH and SOD have a protective effect on rates of acrosome reaction and loss of motility over 24 h although only SOD preserves the capacity of the cells to exhibit hyperactivated motility.

In the present study, the antioxidant GSH level in the erythrocyte did not differ between fertile and infertile subjects. However, seminal plasma GSH level was found significantly low in infertile subjects compared with control fertile subjects, which indicates that the antioxidant defense system in the semen was impaired in infertile subjects. Several other studies also clearly showed a decreased GSH level in the seminal plasma of infertile and sub-fertile groups compared with healthy fertile group,26 which is actually similar to our present finding. However, Oschendorf et al. (1998) did not observe a statistically significant difference of seminal plasma GSH level among groups with different fertility potential.[17]

The finding of similar level of GSH in the erythrocyte between fertile and infertile subjects may appear confusing. But this can be explained with the fact that the cell contains many antioxidants other than GSH, and therefore, it may be possible that other antioxidants, which were not measured in the present study, may be altered in response to increased oxidative stress. However, the low levels of GSH in the semen of infertile subjects clearly indicate that the spermatozoa of infertile subjects were exposed to decreased antioxidant level. Moreover, the semen analysis report clearly showed both qualitative and quantitative defects of the sperm obtained from the infertile subjects. The sperm count, sperm morphology and motility, as well as semen volume were all found significantly lower in infertile subjects compared with fertile subjects. Thus it suggests that decreased antioxidant level in the seminal fluid has a negative impact on semen quality. This is a very important finding, and it is well supported by several previously published studies.

Thus the findings of the present study although shows an association between antioxidant level and infertility, the obvious limitation of the present study is that it is unable to confirm whether the low level of antioxidant itself inflicted any damage on the spermatozoa (such as peroxidation of membrane lipids, DNA modification or apoptosis) was not evaluated in the present study due to lack of technical support and funding[27]. At the same time, it is also

important to conduct interventional studies whereby the fertility parameters of the infertile subjects will be evaluated after alleviating oxidative stress with antioxidants.

Conclusion

The findings of the present study show an association with low antioxidant level in the semen and bad quality of spermatozoa of the infertile subjects. Thus we conclude that male infertility may be associated with decreased antioxidant level. However, it cannot be concluded that there is any cause and effect relationship between low GSH level in the seminal plasma and male infertility. This limitation was due to the lack of technical facilities to measure the DNA base modification or apoptosis of the spermatozoa in infertile subjects. Although administration of antioxidants to patients with male infertility has begun to attract considerable interest, adequate randomized controlled trials must be conducted before being given any firm recommendation for clinical practice.

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