

Evaluation of Seminal Plasma Antioxidants and Serum Male Hormones Status in Infertile Patients with Unbalanced Chromosomal Abnormalities

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Received date: October 30, 2017; Accepted date: January 20, 2018; Published date: January 27, 2018

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Abstract

Background: Male infertility appears to be a major clinical problem among men of reproductive age in all societies. Idiopathic male infertility is considered to be a multifactorial disorder affected by genetic, environmental, and hormonal factors. Oxidative stress seems to stand out as one of the underlying mechanisms. In this context, we aimed to evaluate seminal plasma antioxidants SOD (superoxide dismutase), GPx (glutathione peroxidase), CAT (catalase) and zinc levels, hormone levels, and semen parameters in fertile donors and patients with unbalanced chromosomal abnormalities.

Methods: Semen samples from 119 patients with unbalanced chromosomal abnormalities and 30 fertile men were analyzed according to World Health Organization guidelines (2010). All patients underwent a measurement of testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Seminal plasma antioxidants activities of SOD, GPx, CAT and zinc concentration were measured using colorimetric methods.

Results and conclusion: Hormonal analysis showed statistically significant increase of FSH and LH levels in all patients ($p < 0.001$). However, a significant decrease of serum testosterone level was observed in patients compared to fertile group ($p < 0.05$). Seminal antioxidant system evaluation revealed a significant decrease of CAT, GPx, SOD and zinc activities in infertile group compared to fertile group ($p < 0.05$). Our study showed that patients with unbalanced chromosomal abnormalities had significant alterations of antioxidant system and a significant perturbation of hormonal levels which interfere with fertility. These alterations may be due to a loss or derangement of chromosomal segments containing genes involved in the regulation of these systems.

Keywords: Zinc; Hormonal level; Antioxidant enzymes; Chromosomal aberrations

Introduction

Male infertility appears to be a major clinical problem among men of reproductive age in all societies. Infertility of unknown origin, also termed idiopathic infertility, is of great concern because its pathophysiology remains undetermined [1]. Idiopathic male infertility is considered to be a multifactorial disorder affected by genetic, environmental, and hormonal factors [2]. Although the molecular basis of idiopathic infertility has not been clearly described, oxidative stress seems to stand out as one of the underlying mechanisms [1,3].

In fact, seminal plasma contains small amounts of reactive oxygen species (ROS), such as hydroxyl radicals (HO), superoxide anions (O_2^-), and hydrogen peroxides (H_2O_2), which are constantly generated by spermatozoa in response to both external and internal stimuli [4]. Low ROS levels are essential in vital processes, including intracellular messaging leading to proliferation like immunity, and defense against microorganisms [5]. On the other hand, excessive ROS production lead to oxidative stress, resulting in decreased sperm motility, viability, and increased midpiece sperm defects that impair sperm capacitation and acrosome reaction [6]. Human spermatozoa are rich in polyunsaturated fatty acids (PUFA), and therefore are susceptible to

ROS attack [7]. To counteract the harmful effects of ROS, seminal plasma possess a wide range of antioxidant enzymatic and non-enzymatic defensive factors, including SOD, GPx, CAT, zinc, glutathione (GSH), beta-carotene, Vitamin A, ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) [8].

Therefore the present study was undertaken to assess seminal antioxidant enzymatic activities, seminal zinc level and hormonal levels in both fertile and infertile group.

Subjects and Methods

Patients and control samples

Our study was carried out on 119 patients consulting for infertility in our laboratory of Cytogenetic and Reproductive Biology at Farhat Hached Hospital in Sousse, Tunisia. All patients were selected based on their karyotype.

In fact, these patients consult our laboratory for a constitutional karyotype in the context of a family survey of male infertility. After cytogenetic analyses and based on the karyotype results, were reconvened the patients presented an unbalanced (number or structure) chromosomal aberrations.

All these patients had a conjugal life for at least one year without any contraception. The medical history was reviewed for all cases. Subjects under any medication or antioxidant supplementation are not included in the study. In addition, we excluded all subjects with varicocele, genital infection, leukospermia or any diseases. Smoking or alcoholic men were either excluded from the study because of their high seminal levels of ROS that may decrease sperm antioxidant activities. For the comparative study, 30 healthy men with normal semen profiles and proven fertility were recruited. All subjects in this group had a normal 46,XY karyotype.

This protocol was approved by the local ethics committee, and all patients and controls had previously given informed consent for the study.

Semen analysis

Semen samples were collected by masturbation into sterile cups following 3 days of sexual abstinence. After liquefaction, total sperm motility, concentration and viability were evaluated according to the World Health Organization guidelines (2010) [9]. Sperm viability was assessed using the eosin test, and sperm concentration was determined with Thoma cell counting chamber. Sperm morphology was evaluated using a Spermascor. At least 100 spermatozoa per patient were examined at $\times 100$ magnification according to David's modified classification [10]. The fresh semen was then centrifuged at 2000 rpm for 10 min. The supernatant of seminal plasma was carefully removed, transferred to Eppendorf tubes and frozen at -80°C until examination for antioxidant activities.

Hormonal Dosage

The hormonal profile of each patient has been studied, including FSH, LH, and testosterone. Blood samples were collected in the hospital and samples were sent to laboratory for measurement of serum free testosterone, LH and FSH by Radioimmunoassay technique. Values considered normal for FSH, LH and testosterone are between 10 mU/ml; 1.1- 10 mU/ml; 3 -12 ng/ml, respectively.

Measurement of Antioxidant Activities

Seminal plasma SOD activity was measured using a Ransod kit (Randox Laboratories Ltd) with xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4 - iodophenyl) - 3 - (4- nitrophenol)-5-phenyltetrazolium chloride (I.N.T) to form a red formazan dye. Seminal plasma was diluted 31 fold with 10 mM phosphate buffer, pH 7. One unit of SOD was the amount that caused a 50% inhibition in the rate of I.N.T. reduction. The SOD activity was detected at 505 nm and expressed as U/ml seminal plasma.

Seminal plasma GPX was determined by a Ransel kit (Randox Laboratories Ltd., London, U.K.). GPX catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured. The GPX activity was expressed as U/mL seminal plasma.

Catalase activity was measured by monitoring the initial rate of disappearance of hydrogen peroxide (initial concentration 10 mM) at 240 nm. The catalase activity was expressed as U/mL seminal plasma [11].

Zinc activity was measured by Randox Zn kit (Randox Laboratories Ltd., London, U.K.). The zinc present in the sample is chelated by 5-Br-PAPS-2- (5-bromo-2-pyridylazo) -5- (N-sulfopropylamino) phenol in the reagent. The complex is measured then at 560 nm. The Zinc activity was expressed as $\mu\text{mol/l}$ seminal plasma.

Statistical Analysis

Data analysis was performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Data are represented as Mean \pm SD (standard deviation). The comparisons between fertile group and infertile group were calculated using t-test. Values were considered statistically significant when $p < 0.05$ and highly significant when $p < 0.001$.

Results

Conventional semen analysis

The conventional semen parameters of both fertile and infertile group are reported in Table 1. According to the World Health Organization (2010), sperm concentration, motility and morphology were significantly lower in infertile group in comparison with fertile group ($p < 0.005$).

Parameters	Fertile group (n=30)	Infertile group (n=119)	p-value
Volume (ml)	2.11 \pm 0.66	1.24 \pm 0.49	>0.05
Concentration (106 /ml)	229.70 \pm 8.31	4.76 \pm 1.31**	<0.001
Motility grade(1) (a+b) (%)	36.11 \pm 5.46	27.7 \pm 7.51**	<0.001
Leucocyte concentration (106 /ml)	0.60 \pm 0.09	0.79 \pm 0.08	>0.05
Normal sperm form (%)	60.67 \pm 2.25	9.2 \pm 1.12**	<0.001

(1)=Grade of sperm motility according to World Health Organization guideline (2010); a=rapid progressive; b=slow progressive; ** $p < 0.001$ highly significant; * $p < 0.05$ significant.

Table 1: Standard semen parameters in fertile and infertile men.

The main chromosomal abnormalities identified

The mean age of our study participants was 38.8 \pm 5.97 years. As shown in Table 2, we found 79 patients with numerical chromosomal abnormalities (klinefelter karyotype is the most common), 24 patients with structural chromosomal abnormalities, 19 patients with chromosomal mosaicism and one patient with complete sex reversal of XX (46,XX).

Karyotype	Cases number
47,XXY	71
46,XY/47,XXY	9
47,XYY/48, XY,+ mar [4]	1
45, X/46, XY	3
46, X, del Y	2
46, XX	1

47, XY,+mar[2]/46,XY	4
46, XY,t(7;9)	1
46, XY,del(7q11,2)	1
48, XYY	3
45, X/46,XY/47,XYY	2
45, XY,rob(13;14)	1
46, XY,t(10;20)	1
46, XY,t(8;10)	1
46, XY,t(4;17)	2
45, XY,der(13;14)	2
46, XY,t(3;14)	1
46, XY,ishY(p11.3)	1
46, XY,add(22p)	1
46, XY,t(9;13)(q33;q22)	1
46, XY,t(11;21)(p14;p11)	1
46, XY,t(7;20)(p12;p13)	1
46, XY,t(7;20)(p12;p13)	1
46, XY,t(3;7)(p24;p15)	1
45, XY,der(14;21)(q10;q10)	1
46, XY,t(11;14)(q22;q32)	1
46, XY,t(9;14)(q12;q10)	1
46, XY,t(4;10)(q34;q11)	1
46, XY,t(11;13)(q2;p11)	1
46, XY,t(4;21)(p11;p11)t(8;21)(q22;q21)	1

Table 2: Summary table of karyotype results of 119 patients.

Hormonal analysis

The hormonal levels of both fertile and infertile men involved in this study were reported in Table 3. FSH level was significantly higher in infertile group compared to the fertile group (18.98 ± 0.14 versus 6.55 mU/ml; $p < 0.001$). In addition, a significant increase of LH level was shown in infertile group compared to fertile group (8.63 ± 1.05 versus 4.50 mU/ml; $p < 0.05$).

Parameters	Fertile group (n=30)	Infertile group (n=119)	p-value
FSH (mU/ml)	6.55	$18.98 \pm 0.14^{**}$	<0.001
LH (mU/ml)	4.5	$8.63 \pm 1.05^*$	<0.05
Testosterone (ng/ml)	7.5	$2.915 \pm 2.01^*$	<0.05

** p<0.001 highly significant; *p<0.05 significant.

Table 3: Hormonal levels of both fertile and infertile group.

However, the average of testosterone level in infertile men was lower than that of fertile men (2.915 ± 2.01 versus 7.5 mU/ml; $p < 0.05$).

Seminal antioxidant measurements

The mean values of the various antioxidant enzymes and zinc level in seminal plasma of control and infertile group were given in Table 4.

Parameters	Fertile group (n=30)	Infertile group (n=119)	p-value
SOD (U/ml)	0.536 ± 0.06	$0.211 \pm 0.05^*$	<0.05
GPx (U/ml)	0.550 ± 0.04	$0.180 \pm 0.03^*$	<0.05
CAT (U/ml)	9.050 ± 2.5	$2.730 \pm 1.55^*$	<0.05
Zinc (μ mol/l)	14.37 ± 5.94	$3.83 \pm 0.99^{**}$	<0.001

*p<0.05 ; **p<0.001

Table 4: Values of seminal SOD, GPx, CAT and zinc concentration in both fertile and infertile group.

Compared to control group, a significant decrease of SOD level was shown in infertile group (0.211 ± 0.05 versus 0.536 ± 0.06 U/ml; $p < 0.05$). In addition, GPx level was significantly reduced in infertile group compared to controls (0.180 ± 0.03 versus 0.550 ± 0.04 U/ml; $p < 0.05$). We observed a significant decrease of CAT level in infertile group compared to the fertile group (2.730 ± 1.55 versus 9.050 ± 2.5 U/ml; $p < 0.05$). Zinc concentration in semen plasma of fertile men was significantly higher compared to infertile group (14.37 ± 5.94 versus 3.83 ± 0.99 μ mol/l; $p < 0.005$).

Discussion

Infertility affects approximately 15% of couples, and genetic abnormalities are thought to account for 15%-30% of male factor infertility. Genetic disorders contribute to infertility by influencing a variety of physiological processes including hormonal homeostasis, spermatogenesis, and sperm quality and biochemical reactions [12].

Chromosome analysis is a valuable tool for the diagnosis of male infertility, and chromosomal defects are the most common genetic abnormalities in infertile men [13]. These abnormalities may be numerical or structural and may involve sex chromosomes or autosomes [14]. The frequency of chromosomal aberrations in the general population is approximately 0.6%. However, karyotype abnormalities are reported in 2%-14% of males presenting with infertility. Increases in chromosomal aberrations have been clearly demonstrated to increase proportionally with increasing severity of the infertility [12].

Numerical sex chromosomal abnormalities in males are relatively common with Klinefelter syndrome (47,XXY) and 47,XYY syndrome [15]. In the present study, Klinefelter syndrome was found in 80 patients (71 patients had the homogeneous chromosomal formula (47 XXY) and 9 patients had the mosaic karyotype (46 XY/47 XXY). In accordance with our results, previous study reported that Klinefelter syndrome accounts for 66.7% of cytogenetic defects [16].

The X aneuploidy could indirectly alter gene expression genome wide through epigenetic effects; for example, the presence of an extra heterochromatic chromosome might act as a sink for specific protein complexes [17] and may be responsible for the phenotypic features of Klinefelter's syndrome [18].

The extra Y chromosome in case of 47,XYY, may be responsible for abnormal germ cells which may reside at the primary and secondary spermatocyte or spermatid stages of development leading to a continuous elimination of these cells during spermatogenesis. This may cause varying degrees of maturation arrest as well as heterogeneous sperm concentrations seen in men with genetic abnormalities [19]. These observations are in accordance with our results that shows low semen parameters in the patients with 47,XYY syndrome compared with fertile group.

In the other hand, structural chromosomal abnormalities are an important cause of male infertility. In fact, chromosomal rearrangement may interrupt an important gene or alternatively may exert a position effect [20]. These rearrangements may alter genes functionality at specific breakpoints such as those with a specific role in spermatogenesis. This may cause defective spermatogenesis, resulting on semen parameters abnormalities [21,22].

In the current study, elevated serum levels of FSH and LH were observed in patients when compared with fertile men. Raised gonadotropin levels may indicate germ cell damage, hypogonadism, and increased pituitary drive and might be a cause of Leydig cell hyperplasia. These results are in accordance with other study that also showed a higher rate of FSH and LH in men with severe spermic insufficiency [23]. Moreover, a significant decrease of total testosterone level was found in infertile group in comparison with fertile group. Our results were in discordance with previous results those published who showed no significant difference of serum testosterone level between fertile and infertile men [24,25].

In order to assess seminal antioxidant activities, we have evaluated zinc level and three antioxidants enzymes (SOD, GPx and CAT) concentrations. The main results of our study showed a significant decrease of seminal antioxidant status in infertile group compared to fertile group.

We have stated significant higher level of SOD activity in fertile group than in group of patients.

This outcome confirms previously published observations of other authors [8,26]. It must be emphasized that SOD seminal plasma provides protection against lipid peroxidation of phospholipid and phospholipid-bound fatty acids in normozoospermic samples [27]. However, our results are in discordance with others who found no significant difference of seminal plasma SOD activity between men with normozoospermia and those with pathological spermogram [28,29].

Seminal plasma zinc level was found to be higher in fertile group compared to infertile group ($p < 0.05$). These results are in accordance with other studies who found a significant difference of seminal plasma zinc concentration among azoospermic, oligozoospermic and normozoospermic men [30,31].

Such depletion of seminal zinc concentration may be explained by an increase of sperm ROS level leading to abnormal sperm parameters [32]. A recent study has shown that *in vitro* supplementation of an appropriate dose of zinc increases significantly the total antioxidant status in infertile men with asthenospermia and asthenoteratospermia [33]. However, there was considerable contradictory evidence that there is no significant difference between seminal zinc content in fertile and infertile men [6,34,35].

Moreover, our findings indicated that seminal GPx activity was lower in infertile group compared to control group. Accordingly, other

showed that seminal GPx activity in healthy subjects was 10 times greater than that in infertile men [36]. Efficiently, increased GPx activity in seminal plasma of fertile men catalyzes seminal ROS, which might protect sperm against per-oxidative damage [37]. Seminal plasma GPx reduction may lead to defective sperm quality and reduced fertilizing capacity [38].

Our results showed either, that catalase activity was significantly lower in infertile patients compared to controls. In agreement with our findings, a previous study has shown that catalase activity was significantly decreased in seminal plasma of normozoospermic compared to infertile groups [39].

We believe that our work being the first one which point to combine the influence of chromosomal derangement on certain parameters such as antioxidants in semen plasma and gonadotropins hormones in men with numerical or structural karyotype abnormalities.

Conclusion

Our study shows that patients with unbalanced chromosomal abnormalities had significant reduction of seminal antioxidant SOD, GPx and catalase activities. Seminal zinc concentration was also significantly decreased in our patients. A significant increase of Gonadotropin hormones and a significant decrease of testosterone level were also showed in the present study. These alterations may be due to a loss or derangement of chromosomal segments containing genes involved in the regulation of these systems. Our results open interesting perspectives through the study of antioxidant genes which are involved in male infertility that has remained idiopathic until now.

Conflict of Interest

We have no conflict of interest to declare.

Acknowledgements

The authors would like to thank all the staff of cytogenetic, molecular genetics and human Reproductive biology Laboratory, CHU Farhat Hached, Sousse, Tunisia for excellent technical help. This study was supported by Ministry of Higher Education and Scientific Research, Tunisia.

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