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Evaluation of Glutathione Peroxidase Enzymatic Activity in Seminal Plasma of Patients Treated at the Institute Pasteur in Cote d'Ivoire

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Abstract

Glutathione peroxidase (GPx) is an antioxidant that plays an important role in the maintenance of male fertility. The aim of this study was to compare the profile of enzymatic activity of glutathione peroxidase in the seminal plasma of normozoosperm and those of pathological sperm. Thus, the activity of glutathione peroxidase was determined in the seminal plasma of 20 normozoosperms, 9 azoosperms and 31 oligoasthenoteratozoosperms. It was 37.58 ± 3.14 U/L in normozoosperms, 39.39 ± 2.27 U/L in oligoasthenoteratozoosperms, and 29.77 ± 2.62 U/L in azoosperms. The mean GPx enzyme activity of normozoosperms did not differ significantly from that of oligoasthenoteratozoosperms and azoosperms. In contrast, comparison of enzyme activity between abnormal sperms gave a significant difference. This study showed that glutathione peroxidase enzymatic activity is not related to sperm quality.

Keywords

Glutathione Peroxidase, Antioxidant, Oligoasthenoteratozoospermia, Azoospermia, Normozoospermia

1. Introduction

Seminal plasma provides nutrition, protection, mobility and survival of spermatozoa [1]. This medium has been the subject of numerous biochemical investigations due to its complex composition and its important role in male fertility [2] [3]. It is considered to be the biological fluid that contains the greatest amount and variety of antioxidants [4]. These are involved in the protection of spermatozoa. Reactive oxygen species (ROS) are also present in semen. They can have both beneficial and detrimental effects on sperm. They are essential for sperm survival, integrity and fertilization. The balance between ROS production and sperm antioxidants is a key determinant of sperm homeostasis [4]. Oxidative stress is characterized by an imbalance between ROS production and cellular antioxidant capacity. The ROS in excess in sperm are responsible for cellular damage leading to infertility [5]. In semen, antioxidants prevent or slow oxidative stress by neutralizing ROS [4]. Glutathione peroxidase (GPx) is a seleno-protein enzyme with an enzyme function. Several GPx are present in seminal plasma and on spermatozoa [6]. Some genes encoding GPx are differentially expressed in the male genital tract. Failure of expression of these genes is correlated with male infertility [7]. Several authors have shown the important role of GPx inhibitor in human sperm lipid peroxidation in vitro [8] [9]. In addition to its role as an antioxidant, it regulates the process of sperm DNA compaction [10].

Thus, GPx plays an important role in the maintenance of male fertility. Also, can characterization of GPx in seminal plasma improve the diagnosis and medical management of male infertility? It appears important to evaluate the activity of GPx in seminal plasma in order to better understand its action in the latter. Therefore, the aim of this study is to evaluate the enzymatic activity of glutathione peroxidase in the seminal plasma of men with different fertility potentials.

2. Material and Methods

2.1. Biological Materials

This study involved the analysis of human semen samples. These were collected from voluntary patients who came to Institute Pasteur in Cote d'Ivoire for a biological exploration of fertility and who respected the three-day abstinence period.

2.2. Methods

Thus, 60 semen samples were selected for this study: 20 normozoosperms, 9 azozoosperms and 31 oligoasthenoteratozoosperms.

2.2.1. Spermogram Realization

The spermogram was carried out in accordance with the instructions of the World Health Organization reference manual for semen analysis, sixth edition [11].

2.2.2. Seminal Plasma Collection

After cytological analysis of the semen, the remaining semen was centrifuged at 3000 rpm for 10 min. The seminal plasma was collected and stored at −20˚C until the day of analysis.

2.2.3. Determination of GPx Enzymatic Activity

The enzymatic activity of glutathione peroxidase was determined in seminal plasma by the spectrophotometric method described by Paglia and Valentine using the RANSEL® kit (RANDOX Laboratories, Ltd.) [12]. The absorbance is measured at 340 nm. This method is based on the following principle: GPx catalyzes the oxidation of reduced glutathione (GSH) to cumene hydroperoxide in the presence of glutathione reductase (GR) and NADPH. In the presence of glutathione reductase, oxidized glutathione (GSH) is immediately converted to the reduced form with the oxidation of NADPH to $NADP^+$. GPx activity was expressed as units per liter.

2.3. Ethical Consideration

Sperm samples were collected with written consent of the patients, and the study was approved by the National Ethics Committee for Life Sciences and Health (CNESVS), Order No. 036-13/CNESVS. The study was conducted in accordance with the legal and regulatory provisions of the Helsinki Law Statement.

2.4. Statistical Analysis

Graph Pad Prism 7 software and Excel (2013) were used for statistical analysis of the different data obtained. The results were reported as mean ± standard deviation. Comparisons between seminal plasma enzyme activities of azoosperms, teratozoosperm oligoasthenosperms, and normozoosperms were made using Mann-Whitney tests for unpaired samples. A 95% confidence interval was used. A P value < 0.05 was considered statistically significant.

3. Results

The distribution of the different categories of semen according to age groups showed a low number of azoospermia in all age groups (**Figure 1**). While the number of oligoasthenoteratozoosperms was high in all age groups except that for years. The average age of the different categories of sperm was 39.95 ± 1.29 years in normozoosperms. For oligoasthenoteratospermic and azoospermic sperm, the average age was 39 ± 1.23 and 37.78 ± 2.16 years, respectively (**Table 1**). There was no significant difference between the ages of the patients in the different sperm categories.

Table 1 shows the characteristics of the three types of semen. The mean volume was 2.90 ± 0.22 mL in normozoospermic patients, 2.869 ± 0.23 mL in oligoasthenoteratozoospermic patients, and that of azoosperms which was 2.4 ± 0.42 mL. The mean sperm concentration of normozoosperms was 66.1 ± 9.96 million, and 4.31 ± 0.73 million sperm in oligoasthenoteratozoospermia. The

Table 1. Profile of the different categories of semen.

Paramètres	Patients			P-value					
	NOR (n = 20)	OAT (n = 31)	AZO (n = 9)	NOR & OAT		NOR & AZO		OAT & AZO	
years	39.95 ± 1.29	39.13 ± 1.23	37.78 ± 2.16	0.65	NS	0.3742	NS	0.6005	NS
Vol (mL)	2.905 ± 0.22	2.869 ± 0.23	2.4 ± 0.42	0.91	NS	0.2498	NS	0.3344	NS
pH	7.75 ± 0.06	7.76 ± 0.05	7.81 ± 0.09	0.91	NS	0.5662	NS	0.6003	NS
Num. 10^6	66.1 ± 9.96	4.31 ± 0.73	0.00 ± 0.00	<0.0001	****	0.0001	***	0.0031	**
Morp	8.65 ± 0.84	1.1 ± 0.22	0.00 ± 0.00	<0.0001	****	<0.0001	****	0.0128	*
Vit (%)	74.4 ± 2.21	68 ± 2.22	0.00 ± 0.00	0.036	*	<0.0001	****	<0.0001	****
Mob (%) (a + b + c)									
1st hour	53 ± 1.72	27.9 ± 2.22	0.00 ± 0.00	<0.0001	****	<0.0001	****	<0.0001	****
4th hour	38.25 ± 1.63	11.45 ± 1.25	0.00 ± 0.00	<0.0001	****	<0.0001	****	<0.0001	****

P < 0.05: Significant difference. Num: numeration. Mob: Mobility. * weakly significant. Morp: Morphology. Vit: Vitality. ** moderately significant. *** very significant. **** highly significant. NS: not significant. N: number of samples. Nor: normozoosperms. OAT: oligoasthenoteratozoosperms. AZO: azoosperms.

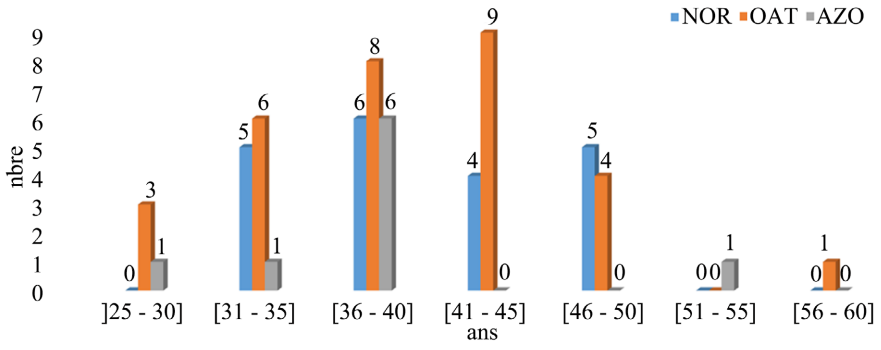

Figure 1. Distribution of the different categories of semen according to age groups.

mean pH measured in the samples was 7.75 ± 0.06 for normozoospermia, 7.76 ± 0.05 for oligoasthenoteratozoospermia, and 7.81 ± 0.09 for azoospermia, respectively. The average of typical forms of sperm was 8.65% ± 0.84% for normozoosperms. In contrast, it was 1.1 ± 0.22 in oligoasthenoteratozoosperms. The average vitality of normal spermatozoa observed was 74.40% ± 2.21% and 68% ± 2.22% for oligoasthenoteratozoospermic. The mean motility of normozoosperm observed at 1st hour was 53% ± 1.72% and 38.25% ± 1.63% at fourth hour. In oligoasthenoteratozoospermia, it was 27.9% ± 2.22% at first hour and 11.45% ± 1.25% at fourth hour.

Results from normozoosperms gave a mean GPx concentration of 37.58 ± 3.14 U/L. As for oligoasthenoteratozoospermic samples, the mean GPx concentration was 39.39 ± 2.27 U/L and that of the azospermic samples was 29.77 ± 2.62 U/L. The mean GPx enzyme activity in normozoosperms did not differ

greatly from that in oligoasthenoteratozoosperms, of which it was slightly lower (Figure 2). In azoosperms, GPx enzyme activity was lower than in normozoosperms but did not differ significantly (Figure 3). Comparison of enzyme activity between abnormal sperm yielded a significant P-value (Figure 4).

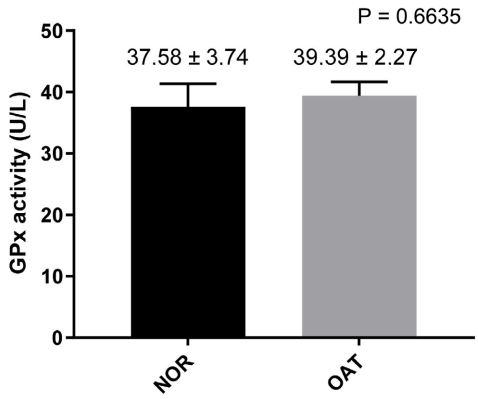

Figure 2. Comparison of average GPx activity values of normozoosperms and oligoasthenoteratozoosperms. *P < 0.05 significant difference. Nor: normozoosperms. OAT: oligoasthenoteratozoosperms.

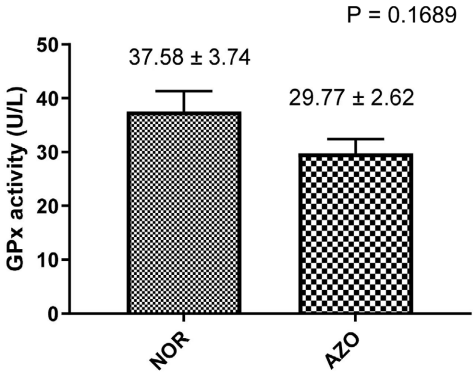

Figure 3. Comparison of mean values of GPx activity in normozoosperms and azoosperms. *P < 0.05 significant difference. Nor: normozoosperms. AZO: azoosperms.

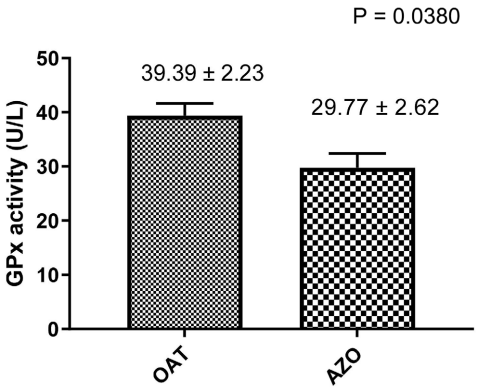

Figure 4. Comparison of average GPx activity values of oligoasthenoteratozoosperms and azoosperms. *P < 0.05 significant difference. OAT: oligoasthenoteratozoosperms. AZO: azoosperms.

4. Discussion

Age influences semen quality in men. Endocrine changes, morphological and functional alternations of the aging testis, lead to a decrease in testosterone production [13]. Increasing age leads to a decrease in sperm function and furthermore male fertility [14]. It affects semen parameters such as volume, motility and sperm morphology [15]. Also our study showed that the mean age of normozoospermes was approximately equal to those of semens azoospermic and oligoasthenoteratozoospermic. The P-value showed no significant difference between the subjects of the study. Therefore, the characteristics of the different sperms analyzed were not related to age. Batou *et al.* showed that the average age of men consulting for infertility in Libreville (GABON) was 39.5 ± 7.9 years; which is roughly equal to the average age of the patients selected for this study. This age range corresponds to the period when the desire for procreation becomes a concern for couples [16].

Most studies suggest that increasing paternal age has negative effects on fertility and presents some genetic risk to offspring. It is also important to note that advanced paternal age appears to be associated with an increased risk of spontaneous abortion in women and an increased frequency of certain autosomal dominant conditions, autism spectrum disorders and schizophrenia in children [17]. But the age at which this decline in male fertility appears and the features of it are poorly defined [14].

Volume, vitality, concentration, motility, and morphology are the essential criteria for semen characterization [11]. Semen volume is a function of annexing gland secretions, permeability of the reproductive tract and smooth muscle contraction [18]. Volumes of the different categories of semen did not show any significant difference. Indeed, all sperm selected for the study showed normal volume.

Sperm count is an important parameter of sperm. On the way to the egg, many sperm are lost or die naturally. Thus, the passage through the vagina, the cervix and the arrival at the egg through the fallopian tubes constitute a real obstacle course in which many sperm do not reach their destination [19]. Also, the WHO has set the sperm count of normal semen at fifteen million [11]. Male infertility secondary to oligozoospermia is surprisingly common. Although some cases are idiopathic, oligozoospermia can be caused by endocrine dysfunction, anatomical abnormalities, medications, or environmental exposures [20]. Azoospermia, which is an absence of sperm in the semen, has several etiologies. These fall into three general categories: pre-testicular, testicular and post-testicular. Pre-testicular causes of azoospermia are endocrine abnormalities that adversely affect spermatogenesis. Testicular etiologies involve intrinsic disorders of spermatogenesis within the testes. Post-testicular causes of azoospermia include obstruction of the ductal system at any location in the male reproductive system [21].

Sperm vitality was normal for normozoospermic and oligoasthenoteratozoos-

permic sperm selected for this study. The causes of necrozoospermia are numerous. They can be infectious, or linked to seminal plasma abnormalities (problem of viscosity, collection, prostatovesicular markers, prolonged abstinence, etc.), epididymal abnormalities (disturbance of epididymal markers), testicular pathologies, the presence of anti-sperm antibodies (cytotoxic) and the existence of general or local hyperthermia [22].

Sperm motility is reduced in oligoasthenoteratozoospermia. Asthenozoospermia corresponds to reduced or absent mobility sperm in the ejaculate. It is one of the main causes of male infertility. This condition can result from structural defects of the sperm flagellum related to genetic factors or the presence of sperm antibodies, excessive use of alcohol, tobacco, marijuana and other drugs. Other factors include age, fever, exposure to toxic agents such as fertilizers and chemical solvents, infections, poor diet, prolonged exposure to heat, oncological treatments such as chemotherapy and radiation, and varicoceles, which can lead to decreased sperm motility [23].

Sperm formation is the culmination of a process that involves complex cell division and maturation in the nucleus, cytoplasm and plasma membrane during spermiogenesis. Dysfunctions in spermiogenesis lead to the production of morphologically or functionally abnormal spermatozoa. Teratozoospermia is a condition characterized by the presence of a large percentage of abnormally shaped sperm in the semen [24]. Human spermatozoa have a high percentage of morphological abnormalities [11]. Sperm morphology is important in the assessment of male fertility. Abnormal sperm morphology can be accompanied by sperm DNA damage [25]. The combination of many sperm abnormalities significantly impairs its fertilizing power. This is the case of oligoasthenoteratozoospermia. Frikh *et al.* during their study of the prevalence of infertility in Rabat noted that oligoasthenoteratozoospermia is the most representative associated sperm pathology with 26.2% [26].

Our results showed that semen pathologies such as azoospermia, oligoasthenoteratozoospermia are not associated with a variation in the enzymatic activity of glutathione peroxidase in seminal plasma. Indeed, the antioxidant activity of GPx was identical regardless of the values of the semen parameters. Our results are in agreement with those of Yeung *et al.* who showed that seminal plasma GPx enzymatic activity does not vary with semen quality [27]. Variation in GPx enzyme activity usually occurs when there is oxidative stress that results in an imbalance between pro-oxidants and antioxidants [28]. It is characterized by decreased motility, apoptosis, lipid peroxidation or DNA fragmentation of sperm [29]. Ammar *et al.* also showed that GPx enzymatic activity was reduced in sperm with varicocele-related oxidative stress [30]. This oxidative stress was evidenced by other parameters such as sperm DNA fragmentation, decreased enzymatic activity of superoxide dismutase and catalase, markers of lipid peroxidation [30]. Azoospermia may be associated with oxidative damage. Indeed, Le *et al.*, showed that azoospermia induced by estradiol benzoate treatment in male

mice was associated with oxidative stress [31]. Also subjects carrying heterozygous defects in the gene SECISBP2 have synthesis reduced of most of the 25 known human selenoproteins. They then present azoospermia, characterized by the failure of the last steps of spermatogenesis with a GPx deficiency [32]. Furthermore, the work of Sharma and Agarwal has shown that oxidative stress does not involve one compound or chemical reaction but a set of production and protection systems [33]. Currently, there is no recognized index or marker of oxidative stress. This is why a large number of indicators are used in order to obtain a set of presumptions concerning the importance of oxidative stress or the capacities organism's defense. Therefore, this study does not allow us to establish a relationship between GPx enzymatic activity in seminal plasma and the sperm pathologies azoospermia and oligoasthenoteratozoospermia. In addition, numerous studies have suggested that decreased antioxidant levels in seminal plasma may be a potential cause of infertility [34].

In oligoasthenosperms, Atig *et al.* showed that GPx enzyme activity was low compared to normozoosperms in contrast to our study which showed no significant difference between these two categories of semen [34]. Chyra-Jach *et al.* report GPx activities higher in seminal plasma of men with oligospermia and oligoasthenospermia [35]. While Garrido *et al.* indicate in their work that the enzymatic activity of GPx4 and GSH are correlated with the percentage of normal forms of spermatozoa in the semen. They conclude that GPx4 can be a biochemical marker of sperm maturation [36]. These results agree with Crisol *et al.* who showed a significant association of motility and morphological sperm parameters with GPx activity [37]. In contrast, Yeung *et al.* indicate that there was no correlation between GPx enzyme activity with morphology and the percentage of motile sperm in semen [27]. Nevertheless, it is important to note that GPx supplementation of semen improves sperm parameters when the latter has been affected by oxidative stress [38].

5. Conclusion

This study demonstrated the presence of glutathione peroxidase in the seminal plasma of normozoospermic, oligoasthenoteratozoospermic and azoospermic semen. It showed that the enzymatic activity of glutathione peroxidase is not related to the quality of the sperm. Enzymatic activity of glutathione peroxidase in seminal plasma is only related to oxidative stress. In order to better appreciate the activity of glutathione peroxidase in seminal plasma, it would be interesting to associate the enzyme activity with those of glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT), which are the main enzymes involved in the metabolism of glutathione peroxidase.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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