ORIGINAL ARTICLE

Seminal Plasma Myeloperoxidase in Patients with Oligoasthenoteratozoospermia

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ABSTRACT

Keywords: MPO, OAT syndrome, seminal plasma, infertility

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Background: Infertility is defined according to WHO is inability of couple to conceive during one year of marriage despite of not using contraception methods and regular sexual intercourse. Oligoasthenoteratozoospermia (OAT) syndrome refers to a condition of abnormally low sperm count, decrease motility and abnormal sperm morphology in the ejaculate. **Objectives**: To determine the seminal plasma Myeloperoxidase (MPO) level in OAT patients and normal controls and to correlate MPO level with semen parameters. **Patients and Methods**: This study was designed as a case control study. This study included 30 OAT patients, and 15 healthy individuals as a control group (45 informed consent). MPO kit colorimetric method for in vitro was used to assess seminal level of MPO in OAT patients. **Results:** There was a relation between MPO level and semen microscopic features like count, motility and morphology. Correlation between seminal plasma MPO level and age. Relationship between smoking status and seminal plasma MPO which was a statistically significance. **Conclusions**: The seminal plasma level of MPO was a significantly higher in patients with OAT. Relations between MPO and semen parameters could be considered as an active biomarker of oxidative stress status.

INTRODUCTION

Infertility is defined by the World Health Organization (WHO) as the inability of a couple to achieve spontaneous pregnancy after one year or more of regular, unprotected sexual intercourse. [1]

Oligoasthenoteratozoospermia (OAT) is a condition of abnormal low sperm concentration, motility and normal sperm morphology in the ejaculate. OAT probably affects 30% of all infertile men. Many factors are responsible for the etiology of OAT like age, genetic factors, genital infections, environmental pollutants, smoking, hormonal abnormalities, varicoceles and drugs. [2]

The above mentioned causes are responsible for increasing reactive oxygen species (ROS) concentration and increasing oxidative stress (OS) which negatively affect spermatogenesis. ROS can affect sperms by many ways include lipid peroxidation, mitochondrial damage, apoptosis, and DNA damage. Many factors that negatively affect semen quality act through decreasing energy availability by mitochondrial dysfunction. [3]

Other cause that may damage the sperm is the presence of neutrophils in seminal leucocytes that may play a role in male infertility. Neutrophils, account for 50-60% of seminal leucocytes with macrophages 20-30%. Semen leucocytes count greater than $1x10^6$ WBC/ml is termed leukocytospermia and that arise from infection of secondary sexual glands, especially the prostate.[4] Neutrophils and macrophages generate superoxidase and hydrogen peroxidase as a part of the oxidative burst. Neutrophils contain large amounts of heme enzyme myeloperoxidase that utilizes the hydrogen peroxide generated, to produce hypochlorous acid and other highly reactive oxidants. Myeloperoxidase (MPO) is a major neutrophil protein with lower amount found in monocytes and possibly macrophages and it is excellent biomarker of neutrophil activation. Seminal plasma myeloperoxidase was also inversely correlated with the percentage of rapidly motile spermatozoa assessed by computer-assisted sperm analysis and the total number of spermatozoa per ejaculate. [5]

Our objective of this study is to determine the seminal plasma MPO level in OAT patients and normal controls and to correlate MPO level with semen parameters**.**

PATIENTS AND METHODS

This study was case-control study carried out between December 2018 and September 2019 in the Andrology Outpatient Clinic of Aswan University Hospital to evaluate seminal plasma MPO in patients with OAT. This study included 45 participants; they were divided to two groups. Group A; included 30 infertile patients, who had OAT, according to WHO 2010 (low sperm count ≤ 15 million/ ml, progressive motility ≤ 32 %, and abnormal morphology ≤ 4 %). Group B; included 15 healthy control subjects, who had normal semen parameters according to WHO 2010.

Inclusion criteria:

This study included infertile participants between 20-60 years with OAT due to varicoceles or smoking.

Exclusion criteria:

- Elderly patients above 60 years old.
- Genital trauma
- Genital infections (mumps orchitis, sexually transmitted diseases, tuberculosis, bilharziasis)
- History of chronic diseases.
- History exposure to gonadotoxins as cytotoxins, androgenic steroids, alcohol, opiates, radiation, and thermal exposure.
- Patients with abnormal hormonal profile (FSH, LH, and testosterone).
- Patients with gynecomastia and hyperprolactinemia.

All the participants were subjected to the following:

Clinical evaluations: Detailed medical history taking, Smoking index= cigarettes per day (CPD) × years of tobacco use. That defined heavy smoker: a smoker who consumed ≥ 20 CPD, moderate smoker: a smoker who consumed between 11-19 CPD and mild smoker who consumed ≤ 10 CPD (Sulsky et al., 2014), Duration of marriage, Previous marriages for both, Number of children in patients with 2ry infertility, The ages of the older child and youngest child and If the wife taking any of contraception methods, Infertility history, Assessment of erection, ejaculation, desire, and orgasm , History of chronic diseases and Past history. All the participants were subjected to General examination included, Local examination included: Penis, Scrotum, Testis and Epididymis. Examination of the scrotal neck for varicocele was done in supine and upright positions using Valsalva maneuver and Varicocele grades. Investigations was included; Semen analysis; was

performed for all participants after 3-4 abstinence days, Scrotal ultrasonography; was done to assess the diagnosis of varicoceles and Seminal plasma MPO level assessment using MPO assay kits.

Ethical approval:

The study was approved from Scientific and Ethical Committees at Faculty of Medicine, Aswan University. 45 informed consents were obtained from all participants.

Statistical analysis:

Analysis of data were verified, coded and analyzed using IBM-SPSS (statistical program for social science version 21). Descriptive statistics: means, standard deviation, medians and ranges were calculated. Significant p value was considered when it is equal or less than 0.05.

RESULTS

Participants were classified as 15 normal control subjects and 30 infertile OAT patients upon 2 groups (15 OAT smoking patients with no other risk factor and 15 OAT patients with varicocele only as the etiological factor).

There was no significance difference between the patients and the control regarding age (table 1)

No significant correlation in MPO level between smoker group and varicocele group (P= 0.746) (table 2)

There are significant inverse correlation in MPO level and semen parameters (figure 1) (table 3) sperm concentration (P= 0.011), total sperm count (P = 0.017), progressive motility (P = 0.002), total motility (P < 0.001) (figure 2), and morphology (P < 0.001).

The study showed the correlation of seminal MPO and semen parameters between the smokers and varicocele group. (Table 4). There were inverse significant correlation of volume (P=0.030), sperm concentration (P=0.042), total sperm count (P=0.012) and morphology (P=0.044) at the group of smokers (figure 3).

The study showed inverse significant correlation at volume (P=0.033), sperm concentration (P=0.047) and morphology (P=0.041) at the group of varicocele patients (Figure 4)

Insignificant correlation for the control (P= 0.372) and significant correlation for the OAT patients (P= 0.012) according age. (Table 4) (Figure 5)

Relationship between smoking status and seminal plasma MPO among moderate and heavy smoking patients which was a statistically significance (P= 0.024) (table 5)

Insignificant correlation between semen MPO level and degree of varicocele (P= 0.773). (Table 6)

DISCUSSION

Infertility is defined according to WHO is the one year long failure of pregnancy in sexually active couples without using any contraceptives. Based on this definition, about 15% of couples do not achieve pregnancy within one year and look for medical treatment of infertility.[6] Impaired spermatogenesis leads to increased ROS concentration and to unbalanced germ cell apoptosis. The result of these processes is an affected sperm concentration, motility and morphology **el-Taieb et al., [3]**. Increased ROS concentration and reduced total antioxidant capacity (TAC) were found in the tubula and the seminal plasma of the majority of OAT patients which caused by lipid peroxides accumulation in the spermatozoa produce end-products such as malondialdehyde (MDA) which found to cause acrosomal anomalies, so that OS has negative effect on acrosome structure **el-Taieb et al., [7].** MPO is a peroxidase lysosomal enzyme, expressed in neutrophil granulocytes that produce hypochlorous acid and other highly reactive oxidants that became biomarker for leukocytes activation.[8] This study aimed to assess the seminal plasma MPO level by calorimetric

method in OAT patients and normal controls and correlate MPO level with semen parameters, also correlate MPO with smoking and varicocele as they considered the two subtypes of etiological factors of OAT.

In the present study, the seminal plasma levels of MPO were significantly higher in OAT patients than control subjects that may be due to MPO generates oxidants that are highly reactive, that closely correlated with sperm damage and alter sperm functions. This finding was in agreement with **Pullar et al., [9]** who found that MPO if present in seminal fluid could be potentially damaging to spermatozoa. Also this finding was in agreement with **Omes et al., [10]** who found that protein expression in semen fluid seemed to be associated with a poor quality of spermatozoa. The current study reported a strong inverse significant correlation between MPO levels and sperm concentration of the OAT patients. This also was in agreement with **Pullar et al., [9]** who found that median MPO concentrations were 45-fold higher in semen samples with low sperm concentration. It may be that MPO as an excellent biomarker for oxidative stress that may be more closely correlated with sperm damage. This finding came with the agreement of **Omes et al., [10]** while this study demonstrated a significant negative correlation between MPO levels and low sperm count in OAT patients. It also was in agreement of **Pullar et al., [9] and Omes et al., [10]** it is possible that, rather than causing the decrease in sperm concentration, MPO may be present as a consequence of spermatozoa being of low quality and becoming apoptotic or damaged within the male reproductive tract, thereby attracting neutrophils, to remove defective cells. [11] This study found that seminal plasma MPO level was significantly negative correlated with sperm total motility in OAT patients. Also there was a significant negative correlation between progressive motility and MPO concentration, suggesting that oxidative stress affects the motility also inflammation and/or infections can cause production of cytokines (eg: IL-1 and IL-8), reduction of sperm motility and fertilizing power, as well as alterations in the composition of the seminal fluid **Vakhrusheva et al.[12]** This finding also come in agreement with **Pullar et al.,[9] and Omes et al.[10]** In the present study, there we a significant negative correlation between MPO levels and age of OAT patients; that could be explained by the majority of the sample was middle aged males whom more subjected to OS, also there is decline in mitochondrial level activity that affect the sperm and neutrophil function in the middle age group **Purandhar et al.[13]** This study reported a significant correlation between smoking status among patients and seminal MPO levels that come with agreement of **Lavi et al.,[14] Iho et al.,[15]** due to smoking causes a local inflammatory burst and that accompanied by the influx of polymorphonuclear leucocytes in particular neutrophils that significantly activated in smokers, suggesting a systematic inflammatory state **Lavi et al.[14]** Also nicotine leads to increase production of IL-8 by neutrophils that induce nicotinamide adenine dinucleotide phosphate reduced oxidase activity leading to an enhanced superoxide production and release of myeloperoxidase neutrophil-derived enzyme, which are significantly elevated in smokers compared with non-smokers **Iho et al.[15]**

CONCLUSION

The study concluded that the seminal plasma level of MPO was a significantly higher in patients with OAT versus healthy control subjects. There was significant correlation between MPO and smoking status. There was no significant correlation between MPO and varicoceles. MPO could be considered as an essential biomarker for the inflammatory process in the body and active biomarker of oxidative stress status.

Limitations: The study has some limitations. It has been conducted at small sample size. The short duration of follow up. The pre and post treatment of MPO in varicocele patients.

RECOMMENDATIONS

The use of MPO as a biological marker for oxidative stress that affects semen quality and leads to impairment of motility, concentration and morphology of the sperm; this issue needs further and

more detailed investigation. MPO can help in determination of therapeutic regimen and prognosis as it considered a marker of OS.

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Table 1: Baseline data comparisons between the study groups.

	Control (n=15)	**Case (n=30)**	**P-value**
Age/years			= 0.355*
• **Mean ± SD**	36.87 ± 7.2	34.53 ± 6.2	
• **Median (Range)**	32 (22 - 60)	33.5 (23 - 47)	
Smoking			**< 0.001****
• **Non-smoker**	15 (100%)	15 (50%)	
• **Moderate smoker**	0 (0%)	8 (26.7%)	
• **Heavy smoker**	0 (0%)	7 (23.3%)	
Varicocele			**= 0.001****
• **No**	15 (100%)	15 (50%)	
• **2nd Degree**	0 (0 %)	8 (26.7%)	
• **3rd Degree**	0 (0%)	7 (23.3%)	

Table 2: Semen MPO level comparison according to smoking and varicoceles.

	Smoker(n=15)	***Varicocele (n=15)***	***P Value***
MPO level			
• **Mean ± SD**	0.78 ± 0.2	0.88 ± 0.2	= 0.746**
• **Median (Range)**	0.7 (0.1 – 3.5)	0.55 (0.1 – 3.5)	

Table 3: Correlation between seminal MPO and semen parameters.

Parameter	Seminal MPO	
	Control (n=15)	Case (n=30)
	rho* (P-value)	
Semen parameter		
• **Volume (ml)**	-0.194 (= 0.144)	-0.065 (= 0.366)
• **Concentration**	-0.042 (= 0.207)	**-0.459 (= 0.011)**
• **Total sperm count**	-0.144 (= 0.304)	**-0.390 (= 0.017)**
• **Progressive motility %**	-0.112 (= 0.280)	**-0.515 (= 0.002)**
• **Total motility %**	-0.136 (= 0.145)	**-0.702 (< 0.001)**
• **Morphology %**	-0.008 (= 0.489)	**-0.622 (< 0.001)**

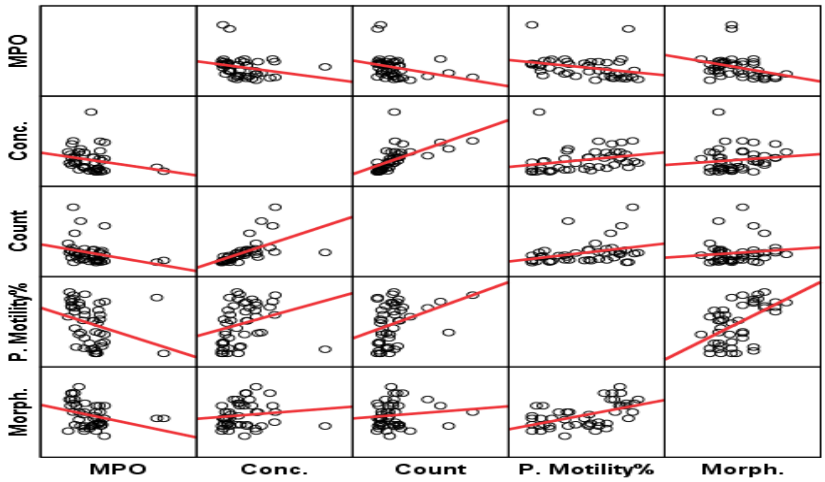

Figure 1: correlation between MPO and semen parameters.

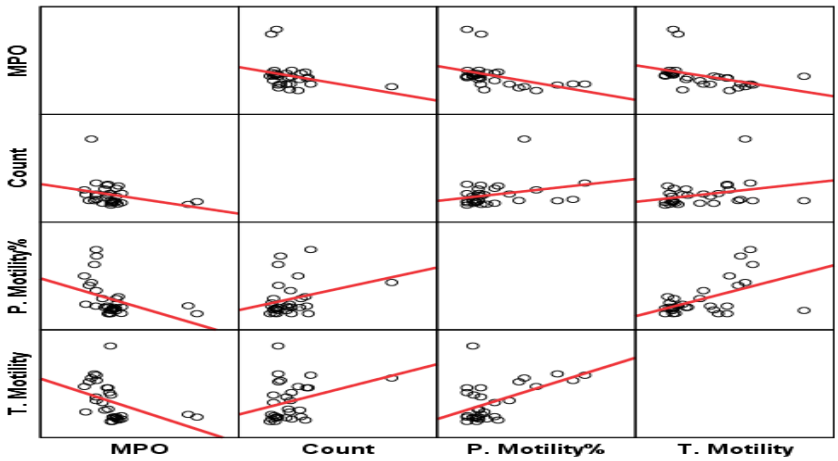

Figure 2: Correlation between MPO, total sperm count and motility (Case).

Table 4: Correlation between seminal MPO and semen parameters comparison between groups of smokers and varicoceles.

Parameter	**Seminal MPO**	
	Smoker (n=15)	**Varicocele (n=15)**
	rho* (P-value)	
Semen parameter		
• **Volume (ml)**	**- 0.495 (= 0.030)**	**- 0.488 (= 0.033)**
• **Concentration**	**- 0.384 (= 0.042)**	**- 0.336 (= 0.047)**
• **Total sperm count**	**- 0.579 (= 0.012)**	- 0.243 (=0.201)
• **Progressive motility %**	- 0.109 (= 0.350)	- 0.181 (= 0.259)
• **Total motility %**	- 0.175 (= 0.288)	- 0.074 (= 0.397)
• **Morphology %**	**- 0.397 (= 0.044)**	**- 0.441 (= 0.041)**

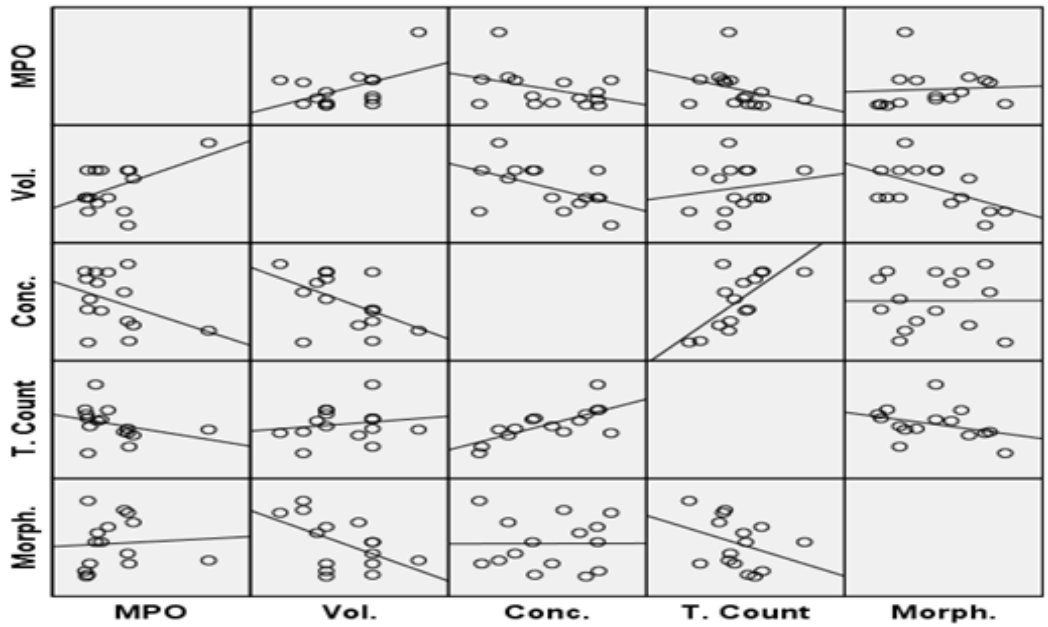

Figure 3: Correlation between MPO and semen parameters (Smokers).

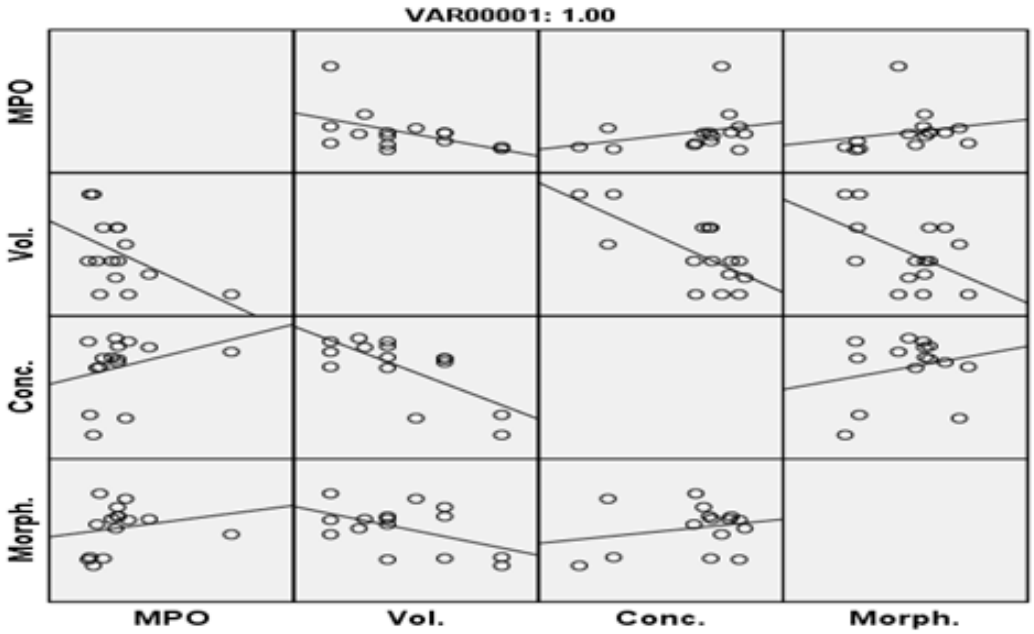

Figure 4: Correlation between MPO and semen parameters (Varicocele).

Table 5: Correlation between seminal MPO and age in OAT patients.

Parameter	**Seminal MPO**	
	Control (n=15)	**Case (n=30)**
	rho* (P-value)	
• **Age/years**	-0.092 (=0.372)	**-0.413 (=0.012)**

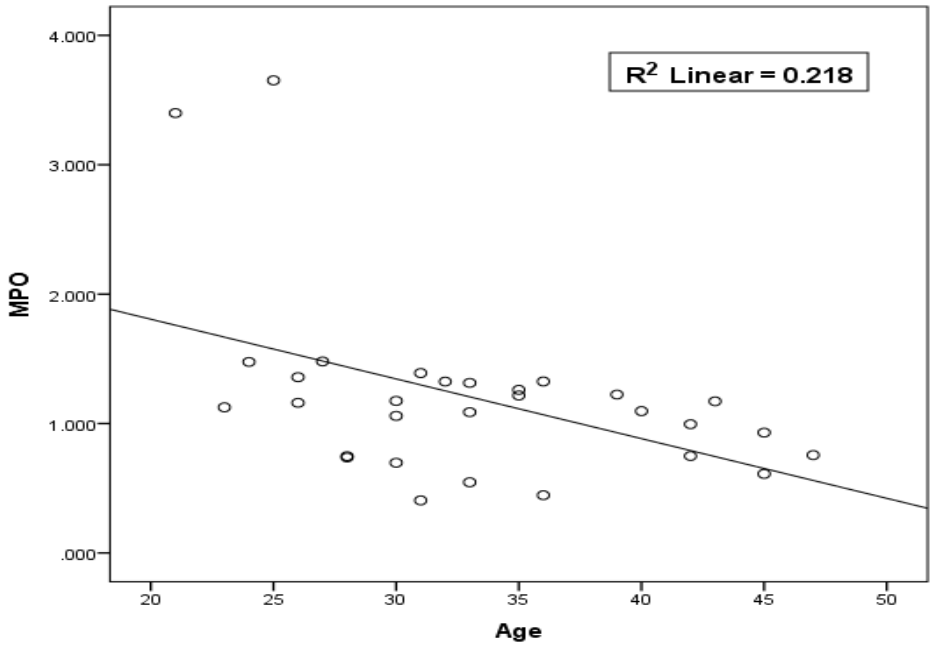

Figure 5: Correlation between MPO and age (Case).

Table 5: Relationship between smoking and seminal MPO level among smoker cases (n=15).

	Mean ± SD	**Median (Range)**	**P-value***
Smoking Status			**= 0.024**
• **Moderate**	1.23 ± 0.2	1.3 (1.1 – 1.5)	
• **Heavy**	1.87 ± 0.7	1.9 (1.1 – 3.7)	

Table 6: Relationship between varicocele and seminal MPO level (n=15).

	Mean ± SD	**Median (Range)**	**P-value***
Varicocele degree			
• **2nd Degree**	1.57 ± 0.8	1.30 (1.12 – 3.65)	= 0.773
• **3rd Degree**	1.61 ± 0.8	1.33 (1.24 – 3.39)	