# Lipid peroxidation and total antioxidant capacity in azoospermic semen

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**ABSTRACT**

**Objective:** This study was carried out to assess identify new biochemical markers of azoospermia based on dosage of malonyldialdehyde and of total antioxidant capacity.

**Methods:** The assay of MDA and TAC were carried out in the seminal plasma of 30 azoospermics and 30 normozoospermics. Normospermics served as the control group.

**Results:** The average concentration of MDA in the seminal plasma of azoospermics was 16.86 ± 1,347µM and for normospermics was 17.88 ± 1.615 µM. They showed no significant difference with $P > 0.05$. The TAC of azoospermic was 2091 ± 88.93 µM and for normozoospermics the concentration was 1410 ± 196.7 µM. The concentration of the TAC, demonstrated a significant difference with $P < 0.05$.

**Conclusion:** MDA in the seminal plasma presents no diagnostic value for azoospermia. However, the TAC is very high in azoospermics. Its evaluation may prove essential in the diagnosis of azoospermia.

**KEY WORDS:** Azoospermia, Malonyldialdehyde, Normozoospermia, Seminal plasma, Total antioxidant capacity

## Introduction

Reactive oxygen species (ROS) are produced naturally in the male reproductive system and play many roles in fertility. The presence of moderate concentrations and controlled thereof is essential to hyperactivation process, maturation, capacitation, acrosome reaction of spermatozoa. They also intervene in mobility, fertilization and adhesion of spermatozoa to the oocyte. Oxidative stress is result from the profound imbalance between ROS and antioxidants in favor of first. It is one of major causes of male infertility [1,2]. It induces changes in the plasma membrane and in the nucleus which lead to loss of mobility and which decreased fertilizing capacity of spermatozoa [3-5]. Different methods are used to measure the prooxidant and antioxidant status of semen.Indeed, the malonyldialdehyde (MDA) is reliable biomarker of lipid peroxidation. Its measurement allows the assessment of the prooxidant status [6-8].

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This marker is correlated to degree of alteration of spermatozoa function [9]. It results from the degradation of hydroperoxides formed during the peroxidation of polyunsaturated fatty acids. Lipid peroxidation is a process of bioactive chain reactions due to the oxidation of polyunsaturated lipids in the presence of oxygen [10]. It leads to the formation of primary products such as hydroperoxides or secondary products final such as MDA, 4-hydroxynonenal (4-HNE) and isoprostanes [6]. Moreover, semen contains many antioxidant substances to fight against oxidative stress [11]. Some are present in the spermatozoa, but the seminal plasma, is the best protection [12,13]. It contains

antioxidants of two types: enzymatic antioxidants (superoxide dismutase, glutathione, catalase) and those non-enzymatic, such as ascorbic acid, α-tocopherol, uric acid, albumin, carnitine, carotenoids, flavonoids and coenzyme Q10 [14, 15]. The determination of the total antioxidant activity (TAC) enables to explore the antioxidant status of semen. It determines the sperm's ability to neutralize ROS [16]. So the low level of the TAC and the high concentration of MDA in the seminal plasma have a key role in male infertility. In view of the action of oxidative stress markers in maintaining semen quality, assessment of oxidative stress status in seminal plasma of azoospermics could serve of tool azoospermia diagnostic. Azoospermia is a male infertility that affects less than 1% of men in the general population, being 5-15% of infertile men. It is characterized by the absence of spermatozoa in the ejaculate. Diagnosis is formal when no spermatozoon is found after centrifugation of the whole ejaculate to ensure that it no contains very few spermatozoa [17]. It is so important to develop reliable biochemical methods for the diagnosis of azoospermia.

The objective of this study is to determine firstly MDA and TAC concentration in azoospermics seminal plasma and secondly to establish the relationship between thereof and azoospermia.

## Materials and methods

The study was conducted at the Pasteur Institute of Côte d'Ivoire (IPCI). The semen samples were collected with the patients consents in accordance to the standards established by the National Ethics and Research Commission of Côte d'Ivoire (NIRB-CI); Order No. 36 / MLS / NIRB / TB. Sixty samples of normozoospermic and azoospermic semen of men were selected for this study. Normozoospermics semen samples were considered the control group.

### Inclusion criteria

Only men with azoospermia and normozoospermia after realization of their spermogram and respecting conditions semen collections were included in this study

### Exclusion criteria

The exclusions criteria have permitted to reject all people who have not observe conditions for realization of spermogram and those with leucospermia and hyperviscosity.

### Sample analysis

### Realization of spermogram

The semen collection was done by masturbation after three days of abstinence. The semen analysis was performed according to the standards of the World Health Organization (WHO) [18]. After collection, semen samples were liquefied at a temperature of 37 °C in an incubator for one hour and then used for analysis. Macroscopic examination of the semen was performed to determine the color, volume, pH and viscosity of samples. Concentration, motility, morphology of spermatozoa was assessed by microscopic examination.

For the biochemical analysis of semen collected, samples were centrifuged at 3000 tr/mn for 10 minutes and seminal plasma was collected and stored at -8°C until the day of the analysis.

Thereafter samples were thawed at room temperature, and then 100 µl of seminal plasma was diluted with 900 µl of distilled water. This dilution was used immediately for the assay of MDA and TAC.

### MDA assay

It was evaluated using the method of determination of thio barbituric acid reactive substances (TBARS) described by Rao et *al*. [19]. To 1 ml of diluted seminal plasma was added 500 µl of thiobarbituric acid reagent, (consisting of 0.67 g of 2-thiobarbituric acid dissolved in 100 ml of distilled water with 0.5 g NaOH and 100 ml of glacial acetic acid) then the assembly was heated for 1 hour in a boiling water bath at 100° C. After cooling to room temperature, each tube was centrifuged for 10 minutes at 4000 trs / min, and the absorbance of the supernatant was read with a spectrophotometer at 534 nm. The calibration solution was prepared from of 1,1,3,3_tétraméthoxypropane (TMP) at 0.01%.

### TAC assay

It was performed using the method of iron reduction; ferric reducing antioxidant power (FRAP) described by Benzie and Strain [20]. The FRAP reagent was prepared in the proportions 10/1/1 by mixing buffer solution sodium acetate at 300 mM, pH 3.6; a solution of 2,4,6-Tripyridyl-s-Triazine (TPTZ) at 10 mM and a solution of 20 mM of FeCl3, 6H20. Then 1.5 mL of FRAP reagent was placed in glass tubes and then maintained at 37 ° C in water bath for 5 minutes. 50 µl of diluted seminal plasma was added to the FRAP

reagent and maintained at 37 ° C in water bath for 10 minutes. Then the absorbance is read at 593 nm. The calibration range was made from a stock solution of FeSO4,7 H20 at 100 µM.

**Statistical analysis**

The obtained data was analyzed using Graph Pad Prism 5.0 software. The results are summarized as arithmetic mean values and standard deviation (SD). The differences between the mean values of MDA and TAC in seminal plasma of azoospermics and normozoospermics were analyzed for statistical significance by student T-test (Nonparametric tests) and Mann–Whitney U test. Probability level values at $P < 0.05$ were regarded as significant.

## Results

The study included 30 normozoospermics and 30 azoospermics according to WHO criteria. The average age of azoospermics group was 39 (range 23 - 45) years. While the control group had an average age of 39 years with a 26 - 45-year interval. The data obtained from the spermogram analysis of azoospermics group and controls are shown in table 1.

**Table 1.** Azoospermics and normozoospermics semen characteristics.

| Semen characteristics | Normozoospermic | Azoospermic |
|---|---|---|
| **n** | 30 | 30 |
| **Volume (ml)** | 3.42 ± 0.24 | 2.96 ± 0.25 |
| **pH** | 7.58 ± 0.05 | 7.61± 0.04 |
| **Concentration ($10^6$/ml)** | **68.88 ± 6.74** | **00 ± 00** |
| **Mobility 1H (a+b) (%)** | 47.20 ± 1.53 | 00 ± 00 |
| **Mobility 4H (a+b) (%)** | 31.20 ± 1.48 | 00 ± 00 |
| Morphology (%) | 23.52 ± 1.82 | 00 ± 00 |

Comparison of the MDA levels in the seminal plasma of the two groups is shown in figure 1. The mean concentration of MDA among two groups was not significantly different; with a *P*-value of 0.684 ($P > 0.05$) (figure 1).

The mean value of MDA in seminal plasma of normozoospermics was 17.88 ± 1.615µM and that of azoospermics was 16.86 ± 1.347 µM. TAC of azoospermics was 1.5 times higher than that of normozoospermics. The mean concentration of normozoospermics TAC (1410 ± 196.7 µM) was significantly lower than that of azoospermics (2091 ± 88.93 µM) with a *P*-value of 0.0002 (figure 2).

**Figure 1.** Comparison of mean (SD) value of seminal plasma MDA level among groups.

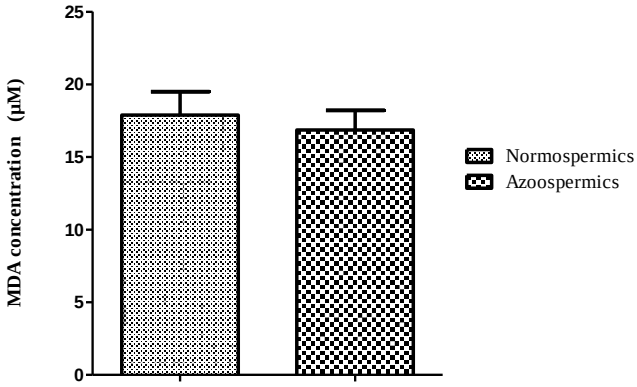
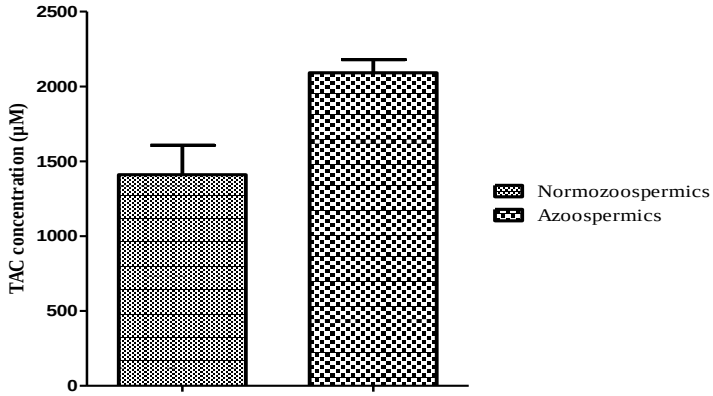



**Figure 2.** Comparison of mean (SD) value of seminal plasma TAC level among groups.




## Discussion

MDA assay in semen is used in biochemical tests to monitor the degree of peroxydative alterations of spermatozoa [21]. The high levels of MDA concentration in seminal plasma represent increased levels of lipid peroxidation which lead to decrease fertility [5,21,7]. This is a characteristic of oxidative stress and it shows an imbalance of the balance between ROS and antioxidants [1-3]. Lipid peroxidation was highlighted in the plasma membrane of spermatozoa as reported by several authors [8,22]. It is responsible for the reduction in membrane fluidity, aggregation and rearrangement of the phospholipid bilayer [10]. Shiva et *al.*, as well as Abdul-Rasheed, showed a significant increase of MDA concentration in azoospermic semen com-

pared to normozoospermic [8,14]. Abdul-Rasheed concluded that azoospermia, oligozoospermia and asthenozoospermia are associated with an increase of MDA concentration in the seminal plasma [8]. Similarly, Huang et *al.* indicated that the concentration of MDA in normozoospermics semen is significantly lower than that of asthenozoospermic and oligoasthenozoospermic semen [22]. Li et *al.*, reported a significant difference in MDA concentration in seminal plasma only between non-obstructive azoospermia and normozoospermia [23]. For Pasqualotto and *al.*, MDA concentration in semen is negatively associated to ROS except idiopathic infertility [24]. However, the concentration of MDA in seminal plasma of normozoospermics and azoospermics evaluated in this study show no significant difference. Also according to our study, MDA cannot be used as biochemical diagnostic marker of azoospermia. Haiba et *al.* have shown that the concentration level of MDA in seminal plasma is not correlated to the concentration and motility of spermatozoa [25]. The presence of MDA concentration in seminal plasma of azoospermic semen cannot to express the lipid peroxidation of spermatozoa, owing to the absence of spermatozoa. The results of Huang et *al.* suggested that the MDA concentration in seminal plasma is not all derived from the spermatozoa, but it may also in part have its origin in lipid peroxidation occurring in the prostate, seminal vesicles, and other accessory organs [22]. MDA formation is not specific of lipids. It may be formed upon activation of the cyclooxygenase pathways, and appear during the interaction of the hydroxyl radical with vitamin C or with deoxyglucose [26].

TAC of seminal plasma enables to appreciate its ability to neutralize free radicals. It assesses the cumulative effect of all antioxidants present in seminal plasma, but without individualizing them [16,27]. Seminal plasma contains many antioxidants that act as scavengers of free radicals. They ensure the protection of spermatozoa against oxidative stress [12,13,15]. The low TAC level has a key role in male infertility [27]. It is generally related to a deficiency in antioxidant influenced by several factors such as nutrition, age, infection, hormones [28]. This study showed that the value of the total antioxidant capacity in azoospermic semen was higher relative to that of normozoospermic semen and it had a significant difference. However, it was noted in general that, the seminal plasma of fertile men has a higher total antioxidant capacity than that of infertile men [11,27,29]. Shiva et *al.* have found an average concentration of superoxide dismutase (SOD) significantly lowered in the seminal plasma of azoospermic semen [14]. For catalase and ascorbic acid, they found no significant difference between the concentrations in normozoospermic and azoospermic semen [14]. Mahfouz et *al.* have described a level of TAC of 1420 µM from which we can define fertile semen [27]. Whereas the average concentration of the TAC in our study was 1410 µM. The results of this study show that the antioxidant activity is not deficit in azoospermics and TAC can serve as a selective biochemical marker of azoospermia. It distinctly shows that azoospermia is associated with increase in seminal plasma antioxidant level. These results can be explained by the fact that in semen, the antioxidants production is expressed in several male reproductive tract tissues, including testis, epididymis, prostate, and seminal vesicles; it is possible that antioxydant expression in these tissues is upregulated in response to defective spermatogenesis. This assumption is similar to that emitted by Kumar et *al.* and Zini et *al.* [30,31]. But also the antioxidants present in seminal plasma are essential for maintaining male fertility but the excess of these may also cause the reverse effect [32]. These results support the idea that high levels of ROS detected in some cases of male infertility are mainly due to their excessive production and not to a failure of the antioxidant activity of seminal plasma [33].

The study of evaluation of oxidative stress in seminal plasma of azoospermics has considered lipid peroxidation and total antioxidant activity. The concentration of MDA in the seminal plasma of azoospermics and that of normozoospermics did not show a significant difference, this parameter cannot be used as a biochemical marker of azoospermia. Therefore, lipid peroxidation is not the only source of production of MDA in the seminal plasma. On the other hand, total antioxidant activity of azoospermics has showed a significant difference compared to normospermics. Therefore, TAC could serve as a biochemical marker for diagnosis of azoospermia.

## Conflict of Interest

We declare that we have no conflict of interest.

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