Relation between Level of Total Antioxidant Capacity and Semen Quality in Male Infertility

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Abstract:

Background: Male infertility continues to be a clinical challenge of increasing significance. Male factors such as decreased semen quality are responsible for more than 25% of all infertility issues. Oxidative stress is induced by reactive oxygen species (ROS) or free radicals. Although ROS are required for critical aspects of sperm function, excessive levels of ROS can negatively impact sperm quality. The seminal plasma includes many enzymatic and non-enzymatic antioxidants which protect the spermatozoa against oxidative stress.

Objective: Assessing the relation between the level of total antioxidant capacity (TAC) and semen quality in male infertility and to establish the cutoff value, sensitivity and specificity of TAC in seminal plasma from healthy donors (controls) and infertile patients.

Subjects and Methods: Semen samples were obtained from 50 male infertile partners who were attended to Damietta University Hospital seeking medical advices. Another 40 fertile males matched for age was included in the study as a control group. All subjects participating in this study were subjected to medical history, clinical examination, laboratory investigations including semen analysis by conventional method, computer assisted semen analysis (CASA), and determination of TAC in seminal plasma by spectrophotometric method.

Results: The total antioxidant capacity (TAC) was found to be significantly lower in the infertile patients than in the fertile donors. A statistically significant positive correlation was observed between TAC and all semen parameters such as the sperm concentration, sperm motility and sperm morphology. The best cutoff to distinguish between fertile controls and infertile men was ≤1.51 mmol/L. At this level, specificity was 87.5% and sensitivity 78%.

Conclusions: The reduced seminal TAC levels were associated with impairment of the sperm concentration, motility and morphology. So, the TAC may be used as a biomarker for assessing the oxidative stress in sperms.

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1. Introduction

Infertility is defined as no conception after at least one year of regular unprotected intercourse (Agarwal et al., 2014).

For healthy young couples, the probability of achieving pregnancy within the first year of fertility-focused sexual activity is 84% (Esteves et al., 2011).

Approximately 15% of couples do not achieve pregnancy within one year of unprotected sexual intercourse. A male infertility factor is identified in about 50% of these cases and is solely responsible in 20% of couples (American Urological Association, 2010).

According to a study conducted by the Egyptian Fertility Care Society, infertility in Egypt affects 12 % of Egyptian couples, 64 % of infertility causes lie with the female partner, while 20.5 % are due to problems with the male partner. The remaining 12.2 % ensue from factors in both partners and 3.3 % remain unexplained (Egyptian Fertility Care Society, 2013).

Reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism. Unstable free radical species attack cellular components causing damage to lipids, proteins, and DNA which can initiate a chain of events resulting in the onset of a variety of diseases (Koracevic et al., 2001).

Antioxidants are a family of vitamins, minerals and other nutrients that help to protect the body from the damage caused by free radicals. Some of such antioxidants are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. In males, antioxidants are found in the testis, epididymis, secretions of the male accessory organs and seminal plasma (Tremellen, 2008).

Antioxidants are one of the most important components to having healthy fertility in males. Everything in the body is made of cells that need to be protected from free radicals. If these cells are less than optimal, the organs will not be functioning at their best. These antioxidant systems include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, macromolecules such as albumin, ceruloplasmin, and ferritin, small molecules including ascorbic acid, α-tocopherol, β-carotene, and reduced glutathione. The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the system **(Agarwal et al., 2014)**.

The cooperation among different antioxidants provides greater protection against attack by reactive oxygen or nitrogen species, than any single compound alone. Thus, the overall antioxidant capacity may provide more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids (Koracevic et al., 2001). **The aim of this study** was to assess the relation between the level of total antioxidant capacity and semen quality in male infertility and to establish the cutoff value, sensitivity and specificity of TAC in seminal plasma from healthy donors (controls) and infertile patients.

2. Subjects and Methods

This is a case control study done on 50 infertile men who were attended to Damietta University Hospital seeking for infertility medical advice, during the period from February to July 2016. Another 40 fertile males matched for age was included in the study as a control group.

Inclusion criteria:

Include infertile males with no conception for at least one year with unprotected intercourse, and their semen analysis presented by abnormalities in the form of oligozoospermia, asthenozoospermia, oligoasthenozoospermia, and oligoasthenoteratozoospermia.

Exclusion criteria:

Patients with one or more of the following criteria were excluded from the study: 1) History of chronic diseases e.g. chronic liver and kidney diseases. 2) Patients with genital infection e.g. Pyospermia, prostatic infection. 3) Patients on antioxidants supplementation. 4) Patients having history of hormonal therapy for any reasons. 5) Patients with history of smoking or alcohol abuse.

All subjects participating in this study were analyzed for: 1) Full history taking including personal history, complaint, present and past history. 2) Clinical examination including general and local examination. 3) Laboratory investigations including: a) Semen analysis by conventional method and computer assisted semen analysis (CASA).b) Determination of total antioxidant capacity (TAC) in seminal plasma by spectrophotometric method.

An informed consent was taken from all subjects participating in this study.

Statistical analysis of the present study was conducted using the mean, standard deviation, student's t- test, and linear correlation coefficient tests by statistical program for social science V17 (SPSS). Description of quantitative variables by mean ± SD, range and description of qualitative variables by number and percentage. A value of $P \leq 0.05$ was considered significant.

3. Results

As regard to parameters of seminal fluid examination, there were high statistical significant differences between infertile and control groups ($p<0.001$). The control group has higher level of seminal TAC than infertile group with high statistical significant differences between them (P value < 0.001)-(Table 1).

There were a significant positive correlation between seminal TAC level and volume of seminal fluid, count, % of total motility and % of normal morphology of sperms among studied groups (Table 2).

The cutoff that maximized the sum of the sensitivity and specificity when using TAC as a predictor of fertility was ≤ 1.51 mmol/L seminal plasma TAC level. The receiver operating characteristic curve (ROC) showed 78% sensitivity and 87.5% specificity for this optimal cutoff (Figure 1).

Table (1): Comparison (Mean ±SD) between infertile and control groups as regard to age, seminal fluid examination parameters and seminal TAC levels.

Parameters	Groups						P-value
	Infertile group			Control group			
Age (Years)	33.4	±	4.3	32.1	±	5.1	0.19
Volume of seminal fluid (ml)	2.8	±	0.9	3.2	±	0.8	0.03*
Count of sperms (million/ml)	14.2	±	6.8	70.3	±	33.2	<0.001*
Total motility (PR+NP) %	30.8	±	14.4	78.5	±	12.8	<0.001*
Normal morphology of sperms (%)	10.1	±	4.8	28.3	±	10.7	<0.001*
Seminal TAC (mmol/L)	1.34	±	0.20	1.86	±	0.24	<0.001*

Table (2): Correlation between seminal TAC and other parameters.

Parameters	Seminal TAC (mmol/L)	
	r	**P-value**
Age (Years)	-0.258	0.114
Volume of seminal fluid(ml)	0.209	0.048*
Count of sperms (million/ml)	0.705	<0.001*
Total motility (PR+NP)	0.619	<0.001*
Normal morphology of sperms	0.543	<0.001*

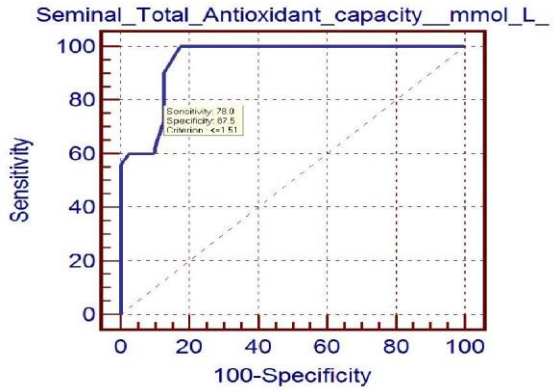

Figure (1): ROC curve showing the sensitivity and specificity of seminal TAC between the infertile patients versus the control.

4. Discussion

Infertility is a major clinical problem, affecting people medically and psychologically. Approximately 15% of couples trying to conceive are infertile, in that about 30% of cases are due to male factor only and in another 20% of cases both partners have detectable abnormalities. Thus male factor plays an important role in 50% of infertile couples. Causes of infertility are anatomic defects, endocrinopathies, immunologic problems, gene mutation, radiation, chemotherapy, ejaculatory failures and environmental exposures (Badade et al., 2012).

A defective sperm function has been recognized as one of the most important causes of the male infertility. The seminal plasma possesses a rich source of different enzymatic and non-enzymatic antioxidants such as vitamin C (ascorbic acid), that protect the spermatozoa against oxidative stress which is one of the mediators of infertility which causes sperm dysfunction and a low sperm quality. Growing evidences have indicated that an imbalance between the oxidative and anti-oxidative substances in the semen leads to metabolic and functional disorders of the male germ cells and that these may be a primary cause of some types of infertility (Fraczek and Kurpisz, 2007).

It was reported that increased ROS in the semen of infertile males could cause abnormal and immature spermatozoal morphologies, motilities and concentrations (Song et al., 2006).

However, due to the high density of the mitochondria which may leak oxygen radicals in the cytoplasm, the ability of the spermatozoa in scavenging oxidants is limited. Therefore, the antioxidant capacity has to be present in the seminal fluid as well. That is why, the protection against ROS and the prevention of other damages are of critical importance and they can be provided by both enzymatic and non-enzymatic antioxidants (Pahune et al., 2013).

The antioxidant power of biological fluids can be evaluated either by quantification of individual antioxidants or by assessing their aggregate, cumulative action and synergic effect. This latter concept is known as the Total Antioxidant Capacity (TAC). The quantification of individual antioxidants (most often superoxide dismutase and catalase) is a complicated, expensive and time consuming task. Therefore the idea of a single measurement of TAC is very attractive (Fingerova et al., 2007).

The current study was performed to assess the relation between level of TAC and semen quality in male infertility and to establish the cutoff value, sensitivity and specificity of TAC in seminal plasma from healthy donors (controls) and infertile patients.

As regard to parameters of seminal fluid examination in the form of count, % of total motility and % of normal morphology of sperms, there were high statistical significant differences between infertile and control groups (P<0.001).

This result reported that the control group has higher level of seminal TAC than infertile group with high statistical significant differences between them (P value < 0.001).

There were a significant positive correlation between seminal TAC level and volume of seminal fluid, count, % of total motility and % of normal morphology of sperms among studied groups.

These results were in agreement with Amiri et al. (2006) study. They found that the mean of sperm concentration, motility and morphology of fertile males were significantly higher than that of infertile males.

These results were in agreement with Fingerova et al. (2007) study. They measured the seminal plasma TAC levels and found significantly lower values in the infertile patients compared with the control.

These results also were in agreement with Pahune et al. (2013) study who found that TAC was decreased in the infertile group as compared to that in the control group and there was a significant positive correlation between semen parameters and TAC among studied groups.

The study of Pahune et al. (2013) also reported that the TAC levels were significantly lower in the infertile groups versus the control group. Thus, low levels of the TAC indicate that the antioxidants are utilized to detoxify the excessive amount of reactive oxygen species. A positive correlation of the TAC with the seminal parameters, which was found in this study, supported the fact that the reduced antioxidant power was mainly responsible for the impaired sperm quality, which could lead to infertility.

Donnelly et al. (1999) reported that single or combined supplementation of ascorbate and tocopherol is not beneficial for sperm motility improvement. Their findings suggest the importance of the TAC assay instead of measuring individual antioxidants that is a complex and inaccurate. It may therefore be important to use TAC assay for monitoring and follow up for infertile patients.

The difference between this study and the study of Donnelly et al. (1999) was because of the type of antioxidants included in his study were ascorbate and α-tocopherol only, but in this study total antioxidants capacity were included.

These results were in agreement with Mahfouz et al. (2009) study that establish a cutoff value for TAC and found that all infertile men showed TAC levels below this cutoff value, whereas proven fertile men showed TAC levels higher than this cutoff value. Receiver operating characteristic curve showed high sensitivity and specificity.

These results indicated that the TAC levels in the seminal plasma of infertile group were significantly lower than those in control group (P value < 0.001).

Also, we found in this study that the reduced TAC levels were associated with impairment of the sperm concentration, motility and morphology.

So, there were a significant positive correlation between seminal TAC level and count, % of total motility and % of normal morphology of sperms among studied groups.

Evidence was suggested that a low seminal TAC was related to the male infertility (Sika, 2001).

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Therefore, the results indicate that the diagnostic capability of the seminal TAC test is beyond those of conventional tests of sperm quality and function. The TAC test can identify patients with a clinical diagnosis of male factor infertility.

5. Conclusion

This study suggested that the reduced seminal TAC levels were associated with impairment of the sperm concentration, motility and morphology and were related to the male infertility. Decreased TAC levels were associated with impaired sperm function as a result of either increased ROS production or insufficient antioxidant capacity. The positive correlation of the seminal TAC level with the sperm parameters indicated that oxidative stress adversely affects the semen parameters. So, the TAC may be used as a biomarker for assessing the oxidative stress in sperms.

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