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EVALUATION OF SEMINAL PLASMA TAC IN RELEVANCE TO SERUM DHEA, TESTOSTERONE LEVELS AS A DIAGNOSTIC VALUE IN INFERTILE MEN

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ABSTRACT

Oxidative stress (OS) plays a vital role in human reproduction. It rises as a consequence of excessive reactive oxygen species (ROS) production and/or reduced total antioxidant capacity (TAC) protection. Oxidative stress mediated damages to the plasma membrane of the spermatozoa may account for defective spermatozoa function parameters that are observed in a high percentage of infertile patients. This study is aimed to evaluate the relationship between level of seminal plasma TAC in relevance to serum dehydroepiandrosterone (DHEA) and testosterone in serum with the assessment of routine spermatozoa parameters in different groups of infertile men. Blood and semen samples were collected from the infertile and fertile subjects. The total numbers of samples were 80, the infertile men were divided into three groups (azoospermic, oligozoospermic and asthenozoospermic) each with 20 samples, and 20 fertile men. Then the levels of serum hormones and seminal plasma TAC were measured using Enzyme Linked Immunosorbent Assay (ELISA). The results of hormonal levels in serum showed that there were no significant differences ($p>0.05$) in the level of DHEA between control group and infertile groups, even though the level of DHEA were in the lower limit of normal among azoospermic group. There was no significant difference ($p>0.05$) in the level of testosterone among study groups but it was in the lower limit among azoospermic men. This study is reveals that there was a highly significant ($p<0.001$) difference in the level of TAC between study groups and control group which was increased among normozoospermic men and decreased among infertile groups. The lower limit of TAC was seen in asthenozoospermic men. The results of this study also showed that there were a relationship between TAC of seminal plasma and hormonal levels, and that TAC are positively correlated with testosterone and DHEA. From the results obtained it could be concluded that; DHEA and testosterone have an effect on male fertility via regulation of seminal plasma TAC. On the other hand seminal plasma TAC are negatively correlated with the number of immotile spermatozoa and positively correlated with concentration, progressively motile and morphologically normal spermatozoa.

KEYWORDS: *Total antioxidant capacity (TAC), dehydroepiandrosterone (DHEA), testosterone, seminal plasma, infertile, fertile subjects.*

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INTRODUCTION

According to world health organization (WHO) infertility can be defined as the inability of a sexually active and non-contracepting couple to achieve spontaneous pregnancy after one year of regular unprotected intercourses [1]. The control of spermatogenesis is by the integration between local and systemic factors. Healthy and mature spermatozoa reflects a convenient testicular tissue function in response to reproductive hormones [2]. The male caused infertility can be found in about 50% of infertile couples [3]. The etiology of declining male fertility can be associated with oxidative stress (OS), however low levels of reactive oxygen species (ROS) can be involved in important physiological processes of spermatozoa, such as capacitation and acrosome reactions [4]. OS can be defined as the loss of balance between the amount of total antioxidant capacity (TAC) and the amount of reactive oxygen species (ROS) that are generated in the spermatozoa, either through the ROS overproduction and/ or the decreased concentration of TAC which can cause a shortage in the scavenging processes of abnormal free radicals [5]. Some studies in relevance to male infertility have suggested that the infertile men may have an impaired seminal plasma TAC, showing that the decreased TAC levels may have a pathogenic effect on male fertility [6]. The balance between free radicals production and antioxidants defense in the seminal plasma are under the influence of different urogenital diseases and damaging factors, such as smoking, varicocele, long sexual abstinence, infections and inflammations [7]. A relation between Hormones and TAC are suggested, a correlation between plasmatic TAC and reproductive hormones level has been observed [8], levels of several other hormones (e.g., prolactin and growth hormone) have shown good correlation with several enzymatic antioxidant activities in different types of tissue [9]. Other studies reported that TAC is correlated with serum testosterone and estradiol in male[8]. However, it is not clearly understood if TAC levels are under systemic control, especially by the endocrine system. It has been observed that Dehydroepiandrosterone (DHEA) which is a derivative of C19 steroid that is mainly secreted by the adrenal cortex may play a role in the level of antioxidants. Exogenous DHEA can exert an antioxidant or prooxidant effect, depending on dose, schedule, and target tissue type [10].

MATERIALS AND METHODS

This prospective study was carried out in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University during the period from August 2017 to March 2018. Written informed consent was obtained from all patients. The method involved collection of serum for the assessment of DHEA and testosterone and seminal plasma for the assessment of TAC of 80 subjects who attended the male infertility clinic of the High institute for infertility diagnosis and ART's. The total number of infertile patients involved in this study was 60 as study group (SG) which classified respectively into three subgroups as: Azoospermia (SG1=20), Oligozoospermia (SG2=20) and Asthenozoospermia (SG3=20). Twenty healthy fertile men were considered as control group (CG=20). Serum and seminal plasma were stored at -36°C until hormonal and TAC analysis. After liquefaction and seminal fluid analysis for all seminal fluid samples were done, the seminal plasma was separated by centrifugation of seminal fluid at 3000 rotation per minute(Rpm) for 15 minutes and stored at -36°C until TAC analysis is done. Semen samples were obtained from the subjected males by masturbation, and the ejaculated semen were deposited immediately in sterile petri dishes, the samples within each subject were in acquaintance, the patients and normal individuals were asked to keep away from sexual intercourse for three days in order to enhance ideal quality and quantity of seminal fluid. The newly ejaculated samples were allowed to liquefy at 37^{o}C in the incubator before evaluation of spermatozoa characteristics according to standard criteria of WHO (2010) [11]. Serum levels of DHEA (ng/ml), testosterone (ng/L) and seminal plasma TAC (U/ml) were measured by using enzyme-linked immunosorbent assay (ELISA).

STATISTICAL ANALYSIS

All statistical analysis was done with version 23.0 SPSS (Statistical Package for the Social Sciences) statistical Program, USA. Microsoft Office Excel software program was used to present data in the form of figures. Numeric variables, such as parameters of SFA, hormonal levels in serum and TAC levels in seminal plasma, were presented in the form of mean and standard error of mean(SE), however nominal data were presented as numbers and percentages[12].

RESULTS

The mean and standard error of normozoospermic men TAC were (15.58 ± 2.63), azoospermic men (10.64 ± 3.56), oligozoospermic men (11.03 ± 2.92) and asthenozoospermic men (8.75 ± 3.58) with highly ($p>0.005$) significant difference among theme as shown in table (1) and figure (1).The mean and standard error of DHEA for normozoospermicmen were (5.69 ± 4.17), azoospermic men were (3.58 ± 2.14), oligozoospermic men were (4.23 ± 1.95) and asthenozoospermic men were (5.06 ± 3.43) there were no significant ($P>0.05$) differences among them, Even though there were no significant difference in the levels of DHEA, the results showed a decreased level among azoospermic men and the highest level were seen in normozoospermic men, as shown in table (1) and figure (1).The mean and standard error of testosterone in normozoospermic men were (4.12 ± 1.11), azoospermic men were (3.27 ± 1.25), oligozoospermic men were (3.46 ± 1.14) and in asthenozoospermic men were (3.95 ± 1.39) with no significant difference among them, Even though there were no significant difference in the levels of testosterone, it appeared that the lower level were seen among azoospermic men and the highest level were seen in normozoospermic men as shown in table (1) and figure (1). The results of seminal plasma TAC and serum hormonal levels revealed that, there were significant relationships between seminal plasma TAC and serum hormones. The highest level of TAC was seen in normozoospermic men with mean and standard error (15.58 ±2.63) that are positively correlated with DHEA (5.69 ± 4.17) and testosterone (4.12 ± 1.11). While the level of TAC are decreased in other groups that have low levels of DHEA and testosterone, the lowest levels of DHEA and testosterone can be seen in azoospermic men with mean and standard error (3.58 ± 2.14) and (3.27 ± 1.25) respectively correlated with decreased TAC level (10.54 ± 3.56) as shown in table(1) and figure (1).The results of seminal plasma TAC and spermatozoa function parameters revealed that, there were significant relationships between TAC and spermatozoa function parameters. The highest level of spermatozoa concentration (million/ml) were seen in normozoospermin men (75 ± 27.3) that were positively correlated with the highest level of TAC (15.58 ± 2.63). On the other hand the lowest level of concentration were seen in oligozoospermic men (10.95 ± 3.95) that are correlated with decreasing in TAC level (11.03 ± 2.92) as shown in table (2) and figure (2). The highest level of progressively motile (PM) were seen in normozoospermic men (47.3 ± 4.8) that are correlated with highest level of TAC and the lowest level of PM were seen in asthenozoospermic men with mean and standard error (12.55 ± 7.83) that are correlated with the lowest level of TAC (8.75 ± 3.58) as shown in table (2) and figure (2). The highest values of non-progressively motile (non-PM) were seen in asthenozoospermic men (31.3 ± 9.92) that are negatively correlated with TAC (8.75 ± 3.58). The level of non-progressively motile seen in normozoospermin men were (29.2 ± 5.80) that are correlated with increase in the level of TAC as compared with other study groups. The highest level of immotile spermatozoa appeared in asthenozoospermic men (56.1 ± 15.2) that were correlated with the lowest level of TAC while the lowest level of immotile spermatozoa were seen in normozoospermic men (23.5 ± 4.29) that are correlated with the highest level of TAC among other study groups as shown in table (2) and figure (2). On the other hand the highest number of round cells (R-cells) (cell/HPF) were seen in asthenozoospermic men (8.43 ± 5.45) that are negatively correlated with TAC among other groups of study, meanwhile the lowest level of R-cells were seen in azoospermic men that are associated with increasing in TAC level as compared with asthenozoospermic men group as shown in table (2) and figure (2).The highest level of morphologically normal spermatozoa (MNS) % were seen in normozoospermic men (44.15 ± 4.39) that are correlated with the highest level of TAC (15.58 ± 2.63), while the MNS were (28.4 ± 8.33) positively correlated with TAC level (11.03 ± 2.92) as shown in table (2) and figure (2).

Table 1

Comparison of hormonal levels in different study groups with TAC

Study groups	M ± SE		
	DHEA	Testosterone	TAC
Normozoospermia	5.69± 4.17	4.12± 1.11	15.58±2.63
Azoospermia	3.58± 2.14	3.27± 1.25	10.64±3.56
Oligozoospermia	4.23± 1.95	3.46± 1.14	11.03±2.92
Asthenozoospermia	5.09± 3.43	3.95± 1.39	8.75±3.58
f-test	1.82^{NS}	2.12^{NS}	16.42**
p-value	0.15	0.103	0.00001
R-coefficient	0.024	0.024	0.024
R^2-coefficient	0.0006	0.0006	0.0006

M±SE=Mean±Standard Error

**** (P<0.05), ** (P<0.001), NS=Non significant***

Table 2

Comparison of seminal fluid parameters among different study groups

Study groups	M ± SE							
	Concentration		Concentration		Concentration			
Normozoospermia	75.0± 27.3	Normozoospermia	75.0± 27.3	Normozoospermia	75.0± 27.3	Normozoospermia		
Azoospermia	0	Azoospermia	0	Azoospermia	0	Azoospermia		
Oligozoospermia	10.95± 3.95	Oligozoospermia	10.95± 3.95	Oligozoospermia	10.95± 3.95	Oligozoospermia		
Asthenozoospermia	45.9± 24.6	Asthenozoospermia	45.9± 24.6	Asthenozoospermia	45.9± 24.6	Asthenozoospermia		
f-test	45.22**	f-test	45.22**	f-test	45.22**	f-test		
p-value	<0.00001	p-value	<0.00001	p-value	<0.00001	p-value		
R-coefficient	0.33	R-coefficient	0.33	R-coefficient	0.33	R-coefficient		
R^2-coefficient	0.114	R^2-coefficient	0.114	R^2-coefficient	0.114	R^2-coefficient		

M±SE=Mean±Standard Error

**** (P<0.05), ** (P<0.001), NS=Non significant***

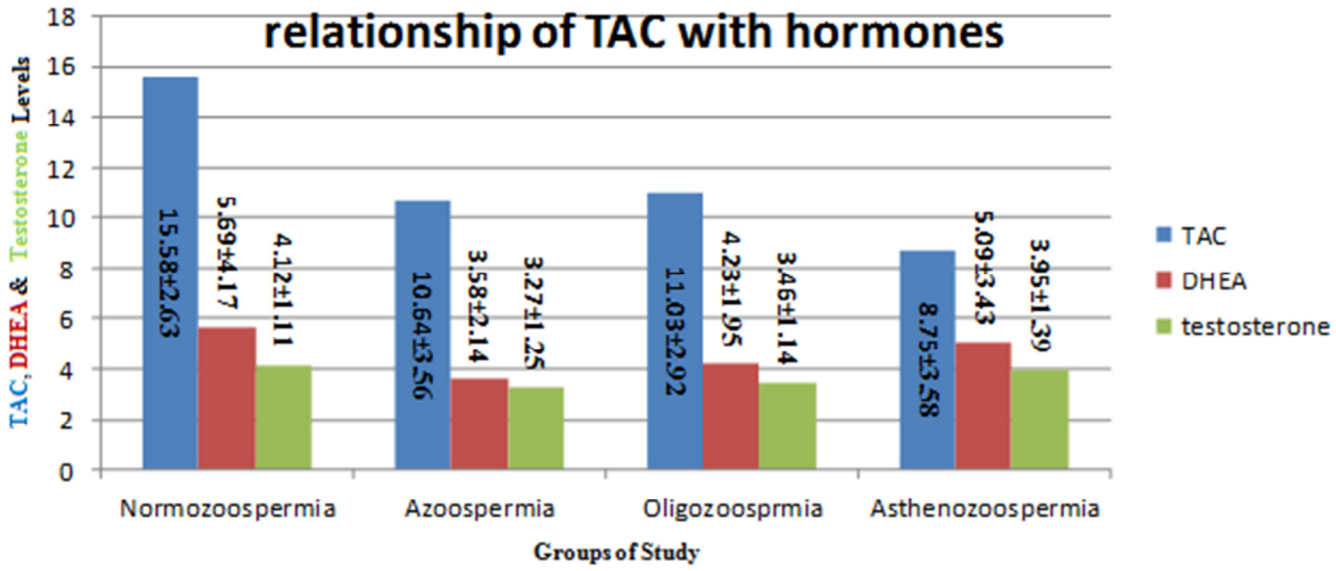

Figure 1

Relationship of seminal plasma TAC with serum hormones levels

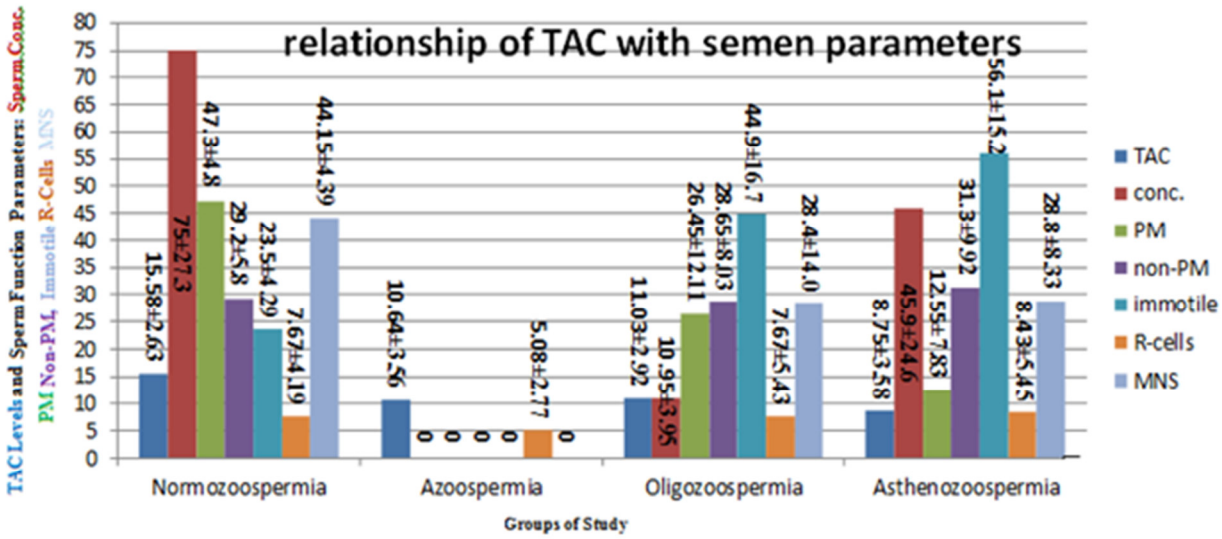

Figure 2
Relationship of seminal plasma TAC with seminal fluid parameters.

DISCUSSION

Spermatogenesis is controlled through the integration of local and systemic factors. Healthy mature spermatozoa reflect appropriate testicular tissue function in response to androgens[13]. Sperm chromatin structure plays a major role in the fertility potential of a male. Its status is reflective of both maturation and integrity and susceptibility to modification throughout spermatogenesis. Oxidative stress and serum hormones may affect final sperm chromatin status[14]. In the present study, there were positive correlation between TAC, testosterone and DHEA. This correlation could be due to the normal function of the cells that are in charge of hormones production and enzymatic antioxidants and in case of damage the production of hormones may be reduced as well as the normal level of TAC. These findings seems to be in line with a previous observations by Mancini *et al.,* (2007) who find a significant inverse correlation between FSH, LH levels and seminal plasma TAC in varicocele patients[15]. Mancini *et al*., (2008) show that male hypogonadism is accompanied by low TAC levels, which are corrected by testosterone administration [16]. Bednarek-Tupikowska *et al*., (2000) revealed that DHEA exhibits a potent mediator property for ROS scavenger production and have a positive correlation with TAC[17]. Demirbag *et al.,* (2005) represents that TAC are significantly correlated with total testosterone in male subjects and also with estradiol in a group of pre- and postmenopausal women[18]. In the present study, there was a positive correlation between concentration, progressively motile and morphologically normal spermatozoa with TAC level. On the other hand the immotile spermatozoa were negatively correlated with TAC which may be caused by high level of ROS produced by immotile spermatozoa. And there were a correlation between round cells and non-progressively motile spermatozoa with TAC, this correlation could appear because of the small number of study groups. These finding were in agreement with Pahune *et al.,* (2013) and revealed that higher seminal plasma levels of TAC have been positively correlated with sperm concentration, motility and morphology[19]. Benedetti *et al.,* (2012) reported a positive correlation between blood TAC concentrations and sperm concentration, motility and morphology[20]. Keskes-Ammar *et al.,* (2003) observed that higher level of ROS was correlated with a decreased number of motile spermatozoa; conversely greater sperm motility was observed in samples with lesser amount of ROS[21].

CONCLUSIONS

The results of this study may lead us to draw the following conclusions

1. There were no significant difference in testosterone and DHEA serum levels, Even though it showed a decreased level among infertile men when compared with control group.
2. A significant differences between the level of TAC between study groups and control groups. The highest level of TAC was seen in normozoospermic men and the lowest level of TAC was seen in asthenozoospermic men

3. Total antioxidant capacity was significantly correlated with hormonal levels, it was positively correlated with DHEA and testosterone.
4. A significant differences in spermatozoa function parameters between study groups and control group.
5. Total antioxidant capacity was significantly correlated with spermatozoa function parameters. It was positively correlated with concentration, progressively motile spermatozoa and morphologically normal spermatozoa and negatively correlated with immotile spermatozoa.

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AUTHORS CONTRIBUTION STATEMENT

This research was done by M.Sc. student Fahad D. Olewei as a part of his thesis under the supervision of Dr.Hayder (corresponding author) and Dr. Mohammad Oda and all of authors were in team work.

CONFLICT OF INTEREST

Conflict of interest declared none.

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