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## Correlation of nerve growth factor with antioxidants and sperm parameters among Iraqi infertile males

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### ABSTRACT

The level of nerve growth factor (NGF) in the blood and seminal plasma of asthenozoospermia and oligoasthenozoospermia patients were compared to normozoospermic males and the relationship between NGF with sperm parameters and seminal antioxidant capacity were estimated to determine the role of NGF in the etiology of male infertility. Eighty-one infertile males and 40 normospermic control subjects were included in this stud. NGF levels were measured in the blood and seminal plasma, and its correlation with total antioxidant capacity (TAC), catalase (CAT) and glutathione (GSH) levels and sperm parameters were studied. There was a general trend of decreased in serum and seminal NGF concentrations of asthenozoospermic and oligoasthenozoospermic when compared to the normozoospermic samples. There was no significant association between NGF and seminal antioxidant status in asthenozoospermia and oligoasthenozoospermia males. Nevertheless, CAT positively correlated with sperm concentration, total sperm motility, and normal sperm morphology but only showed a statistically significant correlation to total sperm motility in asthenozoospermic males. A strong positive association was detected between seminal plasma TAC activity and total sperm motility in both asthenozoospermic and oligoasthenozoospermia males. A decrease NGF levels in serum and seminal plasma could have a significant role in the etiology of impaired sperm functions.



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### INTRODUCTION

Nerve growth factor (NGF) is a twenty- six kDa homodimer protein that belongs to a family of neurotrophins which is typically recognized for its involvement in neuronal survival as well as maintenance (Kolbeck *et al.*, 1994). It was discovered by Rita Levi-Montalcini and Stanley Cohen in the

1950s (Cowan *et al.*, 2001). Nevertheless, this particular protein has been involved in many biological actions outside of the central nervous system. NGF has been shown to have a crucial role in stimulating the growth, differentiation of the testis, as well as maturation, and motility of the spermatozoa (Lipps, 2000). *In vitro*, NGF could stimulate human sperm motility by cumulative the number of (A) grade spermatozoa and the movement distance. Additional, *in vitro* examination established that NGF stimulated the sperm motility in a dose-dependent manner. These results may simplify the advance studies on human fecundity and assisted reproduction techniques (Lin *et al.*, 2015).

Oxidative stress is related to inferior semen features and function. Reactive oxygen species (ROS) amounts have been discovered to be considerably higher in seminal ejaculates of healthy fertile males much older than forty years. The mechanism for damaging sperm arrives if the ROS go into the cell

nucleus, bind to the DNA, and consequence in its fragmentation. Defensive antioxidant enzyme concentrations are deficient in spermatozoa, and they reduce the ability to combat the unwanted effects of the reactive oxygen species, which might decrease the chances of pregnancy as males become gradually much less fertile with age (Desai *et al.*, 2010; Cocuzza *et al.*, 2008).

Deficits of enzymatic or non-enzymatic antioxidant systems in seminal plasma which together determine its total antioxidant capacity (TAC) are usually related with male sterility as the lack of any of these systems leads to the accumulation of unnecessary levels of ROS, causing a diminishing of both the structural and functional integrity of spermatozoa (Aitken *et al.*, 2006). The determination of TAC has confirmed to be applicable in humans for fertility valuation since low levels of SP-TAC are linked with sterility and irregular semen parameters (Eroglu, *et al.*, 2014). As a result, it is sensible to accept that quantitative variations in TAC among ejaculates would clarify modifications in sperm capability to tolerate defence actions and presentation best in vivo fertilizing capability.

Nerve growth factor is an antioxidant supplement that gained great attention in recent years. Due to the lack of previous studies assessing the relationship between NGF and seminal antioxidant status, in this study, we will attempt to elucidate the relationship between serum and seminal NGF as a biomarker with sperm parameter and seminal antioxidant capacity.

## MATERIALS AND METHODS

**Subjects:** The study population was composed of 121 male partners of infertile couples (between 19 and 49 years of age) who presented for semen analysis at Kamal Al- Samurai Specialist Hospital (Baghdad) as a part of their fertility evaluation. Ethical approval was required from the Research and Ethics committee of the Hospital and it was given. Patient's age, height, weight, body mass index (BMI), alcohol status, smoking status, fertility history, and occupation was evaluated. All contributing males were interviewed by professional andrology doctor. After the initial screening, data were collected from all participants. According to the 5<sup>th</sup> edition of the World Health Organization (WHO) guidelines (Organization, 2010), the patients were divided into the following groups based on their semen parameters: Normozoospermic (40 cases) this study group represent controls, Asthenozoospermic (40 patients) and oligoasthenozoospermic (41 patients). Subjects of these groups involve of males whose infertility is distinct by less than normal level for sperm quality.

**Collecting and Analyzing Samples: Semen analysis:** The semen sample collection was achieved following the references of the fifth edition of the manual for the examination and processing of human semen. Semen samples were collected by masturbation after 3-5 days of sexual abstinence into a dry, clean, and sterile container labeled with the name and age of the patient, a period of abstinence and time of collection. The specimens were placed in the incubator at 37°C to allow the semen liquefaction. After liquefaction, manual semen examination was performed according to the 2010 guidelines by the WHO.

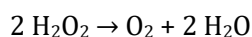
Aliquots of liquefied semen were centrifuge at 3,000 r.p.m for minutes; their supernatants were collected quickly and frozen at -20°C in tubes to study their NGF, total antioxidant capacity, catalase, and reduced glutathione levels.

**Blood samples and analysis:** Five ml of peripheral venous blood was collected from patients performed by using a disposable syringe. Samples were safely transferred into labeled gel tubes and allowed to clot at room temperature. The serum has been collected by centrifuge at 3,000 r.p.m per minute for 15 minutes; the sera were transferred by micropipette and frozen at -20 C° in tubes until further examination.

**Measurement of NGF:** NGF calculation was assessed using an enzyme-linked immunosorbent assay (ELISA) kit (E-EL-H1205, USA). This analyzes uses an antibody particular for human NGF coated on a 96-well plate. Reagents, samples and standards were all prepared as instructed. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of NGF in the samples was accounted by comparing the OD of the samples to the standard curve (ranging from 15.5 to 1000 pg/ml).

**Measurement of total antioxidant capacity:** Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999) (Benzie *et al.*, 1999). FRAP test that uses antioxidants as reductants in a redox-linked colorimetric method which depends on the reduction of ferric tripyridyltriazine complex into a coloured ferrous form that can be checked by measuring the change in absorption at 593nm.

**Measurement of catalase:** The activity of catalase was assayed by the method of Sinha (Sinha *et al.*, 1972).



**Measurement of glutathione:** The GSH concentration in seminal plasma was measured according to the method of Beutler (Beutler *et al.*, 1963; Sinha,

1972). DTNB is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen was measured at 412 nm and is directly proportional to the GSH concentration.

**Statistical analysis:** Statistical analysis was accomplished with Statistical Package for Social Sciences (SPSS) (version 23.0, SPSS Inc., Chicago, IL, USA). For comparison between two groups, one-way ANOVA was used. The independent correlation of NGF and (TAC, CTA, GSH) with semen parameters was tested by linear regression analysis with Pearson's correlation test. The significance level was set as two-tailed, and  $p < 0.05$  was considered statistically significant.

## RESULTS AND DISCUSSION

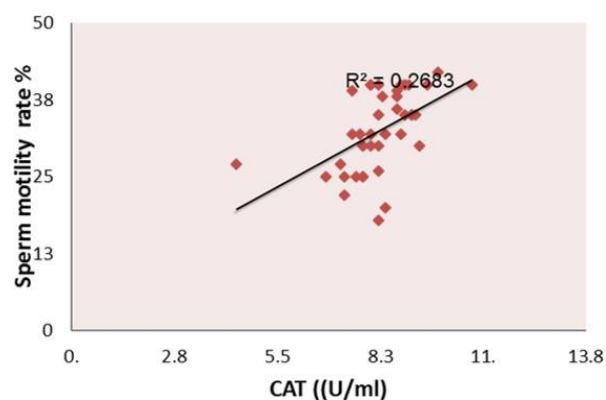
A total of 121 men completed this component of the study and were included in the determinations of correlation coefficients between NGF, antioxidants status, and semen quality measures. The standard characteristics of men with normal and abnormal semen parameters are listed in table 1. Mean values for age and Body mass index did not significantly different between studied groups.

Seminal parameters of the normospermic males (control group) and the case of asthenozoospermic and oligoasthenozoospermic infertile males are reported in table 2. In asthenozoospermic and oligoasthenozoospermic males the mean sperm count total sperm motility and percentage of sperm with normal morphology were significantly ( $P < 0.05$ ) lower than those of normospermic males. Leukocyte concentration in infertile males showed profiles similar to that of normal group. A significant ( $P < 0.05$ ) difference was absorbed in the mean semen volume of oligoasthenozoospermic infertile males compared with Normozoospermic males.

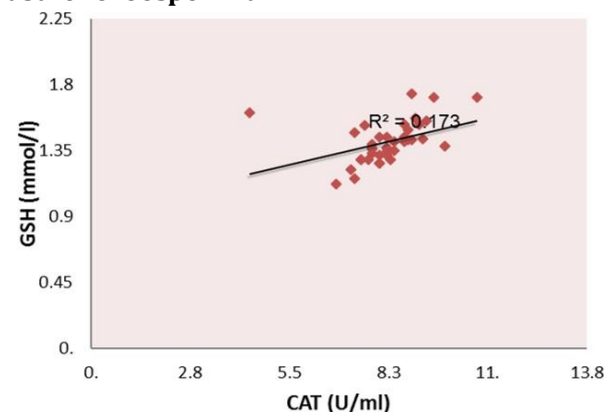
In this study, we found there was a general trend of a significant decreased in serum and seminal NGF concentrations of the abnormal semen samples (asthenozoospermia and oligoasthenozoospermia) when compared to the normozoospermia samples. NGF levels in serum and seminal plasma samples from oligoasthenozoospermic men were lower than those in seminal plasma from asthenozoospermic men, although these differences were not statistically significant ( $P > 0.05$ ). No significant correlations among sperm concentration, total sperm motility, normal sperm morphology rate and semen volume and serum levels of NGF.

Seminal plasma antioxidant statuses of the specimens are shown in table 3. Total antioxidant capacity (TAC), catalase (CAT) and glutathione (GSH) were significantly higher ( $P < 0.05$ ) in normozoospermic than in infertile groups.

The correlations between semen parameters, NGF and seminal plasma TAC, catalase, GSH levels in the infertile group were examined using Spearman correlation analysis. There was no significant correlation between NGF and seminal antioxidant status in asthenozoospermia and oligoasthenozoospermia groups. However, CAT positively correlated with sperm concentration, total sperm motility and normal sperm morphology but only showed a statistically significant correlation ( $r = 0.518$ ,  $p < 0.001$ ) to total sperm motility in asthenozoospermic males (Figure 1). Conversely, CAT, GSH and TAC were well correlated ( $r = 0.416$ ,  $p < 0.008$ ;  $r = 0.573$ ,  $p < 0.000$  respectively) to each other (Figures 2 and 3). In oligoasthenozoospermic males, CAT positively correlated with GSH ( $r = 0.892$ ,  $p < 0.000$ ) (Figure 4), but not significantly with TAC ( $p < 0.940$ ).



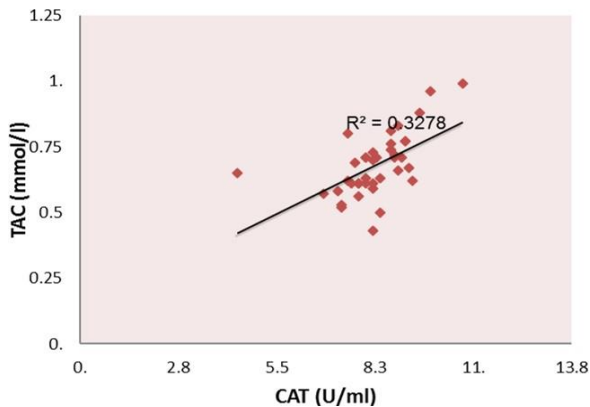
**Figure 1: The correlation between CAT and total sperm motility in patients with asthenozoospermia**



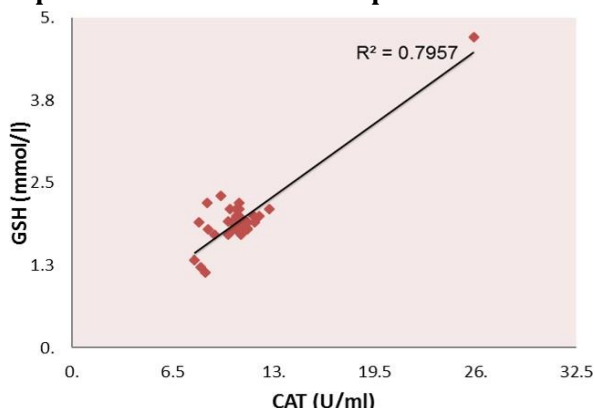
**Figure 2: The correlation between CAT and GSH in patients with asthenozoospermia**

In both infertile groups, sperm concentration, total sperm motility and normal sperm morphology rate were all positively correlated with GSH, but none showed statistical significance. Highly positive relationship was observed between seminal plasma TAC activity and total sperm motility in both asthenozoospermic ( $r = 0.864$ ,  $p < 0.001$ ) and oligoasthenozoospermic ( $r = 0.831$ ,  $p < 0.001$ ) males (Figures 5 and 6). A positive correlation ( $r = 0.536$ ,  $P < 0.001$ )

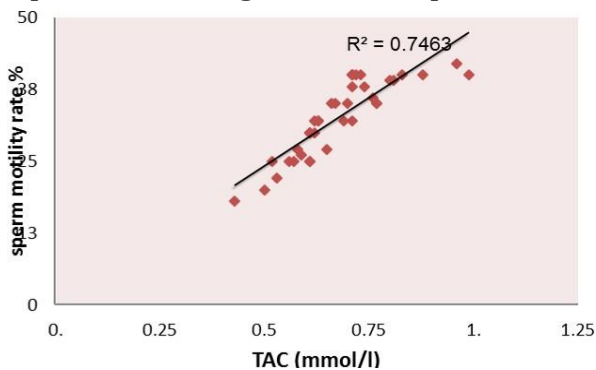
between spermatozoa count per mL and TAC in asthenozoospermic group (Figure 7).



**Figure 3: The correlation between CAT and TAC in patients with asthenozoospermia**



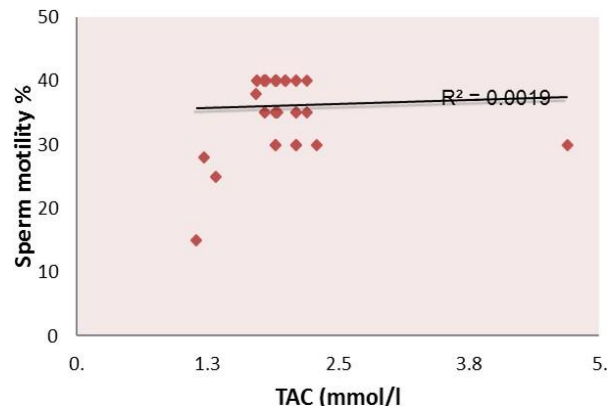
**Figure 4: The correlation between CAT and GSH in patients with oligoasthenozoospermia**



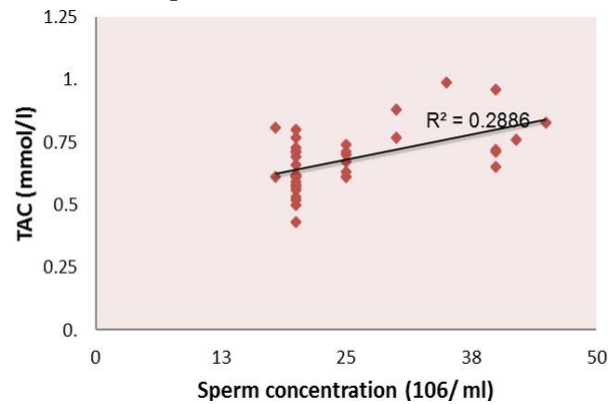
**Figure 5: The correlation between TAC and total sperm motility in patients with asthenozoospermia**

NGF is a polypeptide member of the neurotrophin protein family that plays an essential role in the differentiation and regulation of neuronal survival, mediated through tyrosine kinase receptors (Barbacid, 1994). Evidence added over the past few years displays the existence of NGF and its receptors outside the nervous system, mainly in the male reproductive organs (Artico *et al.*, 2009; Perard *et al.*, 2007; Li *et al.*, 2010), which directs the possible role of NGF in the reproductive system. The first indication of a measured NGF content in human exposed that the level of NGF protein in

seminal plasma gained from oligoasthenozoospermic and asthenozoospermic was lower in comparison to fertile semen although these differences were not statistically significant (Li *et al.*, 2010). Parallel to the study above, a work is done by Saeednia *et al.* in 2016 showed that the seminal NGF concentration was significantly higher ( $P$ -value  $<0.05$ ) in normozoospermic compared with asthenozoospermic men (Saeednia *et al.*, 2016). The decrease in serum and seminal NGF levels in asthenozoospermic and oligoasthenozoospermic men may have a substantial part in the aetiology of sperm dysfunction.



**Figure 6: The correlation between TAC and total sperm motility in patients with oligoasthenozoospermia**



**Figure 7: The correlation between TAC and sperm concentration in patients with asthenozoospermia**

In the present study there was no significant correlation between NGF and semen parameters, a study by Li *et al.*, (Li *et al.*, 2010) shows that the adding of exogenous NGF to bovine sperm can escalate the viability and motility of spermatozoa. Furthermore, *in vitro* NGF supplementation, defeats human endothelial cell apoptosis and increase cell proliferation (Graiani *et al.*, 2004). Recent results demonstrated that NGF affected viability and apoptosis of sperm, and the motion pattern of the sperm was influenced in a dose- and time-dependent manner (Sanchez-Rodriguez *et al.*, 2018).

**Table 1: Average characteristics of each group**

Characteristics	Normospermia	Asthenozoospermia	Oligoasthenozoospermia	p-value summary
Age (years)	32.9±1.005	32.4±1.0971	31.122±1.005	NS
Body weight (kg)	84.5745±1.858	83.1429±2.1486	80.4167±1.9506	NS
Height (cm)	175.4681±1.3656	170.4107±1.8894	173.4167±1.2765	NS
Body mass index	27.1876±4.5825	27.9202±4.9985	26.544±4.6809	NS

Values are presented as mean ± SE; N.S: Non-significant

**Table 2: Comparison of seminal parameters, including NGF levels between normozoospermic, asthenozoospermic and oligoasthenozoospermic males**

Parameters	Normozoospermic males	asthenozoospermic infertile males	oligoasthenozoospermic infertile males
Sperm concentration (10 <sup>6</sup> / ml)	<sup>a</sup> 32.425±1.3872	<sup>b</sup> 24.95±1.2305	<sup>c</sup> 11.6111±0.4981
Sperm motility (PR+NP) %	<sup>a</sup> 75±1.2142	<sup>b</sup> 32.55 ± 1.0196	<sup>c</sup> 36.122±0.8577
Normal morphology %	<sup>a</sup> 67.9787±1.0388	<sup>b</sup> 45.0893±1.909	<sup>b</sup> 34.9167±1.31
Leukocyte concentration	<sup>a</sup> 1.175±0.1181	<sup>a</sup> 1.1±0.123	<sup>a</sup> 1.3902±0.1301
Volume (ml)	<sup>a</sup> 2.134 ± 0.06828	<sup>ab</sup> 2.4554 ± 0.1482	<sup>b</sup> 2.75±0.1679
NGF (pg/mL)			
Serum	<sup>a</sup> 657.8535+56.4869	<sup>b</sup> 481.2885+44.0855	<sup>b</sup> 370.1398+34.3548
Seminal plasma	<sup>a</sup> 631.5508+ 38.8221	<sup>b</sup> 459.595+37.5279	<sup>b</sup> 370.1398+34.3548

Variable are reported as mean + SE; PR: Progressive motility; NP: Non-progressive motility; Different letters mean significant at (P<0.05)

**Table 3: Seminal plasma TAC, CAT and GSH activities in normozoospermic, asthenozoospermic and oligoasthenozoospermic males**

Variables	TAC (mmol/l)	CAT(U/ml)	GSH(mmol/l)
Normozoospermic males	<sup>a</sup> 1.6468±0.02194	<sup>a</sup> 21.9350±0.32827	<sup>a</sup> 5.1580±0.10366
Asthenozoospermic infertile males	<sup>b</sup> 0.6780±0.01850	<sup>b</sup> 8.2450±0.15779	<sup>b</sup> 1.4110±0.02220
Oligoasthenozoospermic infertile males	<sup>c</sup> 0.8641±0.01666	<sup>c</sup> 10.8976±0.41127	<sup>c</sup> 1.9469±0.49820

Variable are reported as mean +SE; Different letters mean significant at (P<0.05)

The male issue is revealed a principal related purpose to infertility. Apart from the predictable explanations is oxidative stress (Makker *et al.*, 2009). Unbalance between antioxidant status and reactive oxygen species in the male reproductive system have a substantial impact on spermatozoa fertilization ratio. Evaluating and maintenance these factors would be a decent contribution in order to accelerate the natural conception. Increased ROS producing and reduced antioxidant capacity in infertile men is inversely correlated with sperm motility leading to a reduced percentage of motile spermatozoa in those patients (El-Taieb *et al.*, 2009).

In 2017, a study was done by Donatus *et al.*, showed that the mean serum level of TAC was significantly higher infertile male partners with normal semen parameters when compared to male partners of an infertile couple with abnormal semen parameters (Donatus *et al.*, 2017). Moreover, researches demonstrate a decreased activity of antioxidant parameters such as GSH in the seminal plasma of asthenozoospermic men in comparison

with normospermic males (Atig *et al.*, 2012; Tomar *et al.*, 2017). Insufficiencies of enzymatic or non-enzymatic antioxidant systems in seminal plasma are extensively linked with male infertility as the deficiency of any of these systems leads to the accumulation of ROS, causing a potential cellular and DNA damage leading to increased male infertility (Aitken *et al.*, 2006; Venkatesh *et al.*, 2011).

Seminal plasma antioxidant defense activity and its association with sperm quality have been examined so far, but the outcomes achieved are debatable. Our results are in accordance with the study of Koca *et al.*, reported that antioxidant capacity is positively correlated to sperm motility (Koca *et al.*, 2003) Likewise, different studies detected a statistically significant positive correlation between seminal plasma TAC sperm concentration, sperm motility, and normal sperm morphology (Pahune *et al.*, 2013; Faramarz *et al.*, 2016; Colagar *et al.*, 2013). Shi *et al.* , connected the level of TAC concentration with semen parameters of 225 infertile men, the seminal plasma TAC level had a significantly positive association with sperm with grade

a and sperm density (Shi *et al.*, 2005). In contrast, Appasamy *et al.* detected a negative association between antioxidant activity and sperm concentration, which proposes that oxidative stress could affect sperm concentration (Appasamy *et al.*, 2007). Yet, there was no correlation between sperm count and TAC levels in either fertile men or men with idiopathic infertility (Faramarz *et al.*, 2016). In 2007, a study by Khosrowbeygi & Zarghami indicates a straight association between catalase activity and sperm motility, concentration morphology in seminal plasma of asthenozoospermic, oligoasthenoteratozoospermia and asthenoteratozoospermia males (Khosrowbeygi *et al.*, 2007).

The main limitation of our study is the low sample number which might be an issue affecting the results.

## CONCLUSION

Our study revealed that there were significant differences in the levels of NGF, TAC, CAT and GSH in the seminal plasma of infertile and normospermic participants which may support the application of NGF and seminal antioxidants statuses as a specific biomarker for assessing the semen quality.

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