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Original Article

Effect of serum antioxidant levels on sperm function in infertile male

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ABSTRACT

Antioxidant and reactive oxygen species (ROS) has been implicated in male infertility. Antioxidants are compounds which scavenge formation or oppose of the actions ROS. Spermatozoa are particularly vulnerable to ROS because of inherent deficiencies in intracellular antioxidant enzyme protection, thus total body antioxidant capacity become more substantial to protect sperm. In this study to try to evaluate the role of serum total antioxidant capacity, uric acid, glutathione, total thiols, ferritin, albumin and malodialdehyde in male infertility. 66 men included in this study 40 were infertile and 26 were healthy fertile. Results showed a significant decrease in sperm progressive motility and a significant increase in sperm tail abnormality, also there were a significant decrease in serum total antioxidant capacity, uric acid and albumin in infertile group. Because of lack of the intracellular antioxidant enzymes sperm cannot protect plasma membrane that surrounds the tail, the protection depends on the antioxidant defense afforded by the seminal plasma and on the entire body antioxidant capacity. Decrease of serum antioxidant capacity, uric acid and albumin may cause destroy of sperm tail membrane and this may lowering of sperm progressive motility.

1. Introduction

Spermatozoal dysfunction is the single most common cause of infertility [1]. New researches focused on the investigation of the causes of spermatozoal dysfunction. In recent years, Oxidative stress (OS) has become the major interest in the pathophysiology of human sperm function and male infertility [2,3]. OS develops as a result of an imbalance between reactive oxygen species ROS (e.g. H_2O_2, $O^{\cdot 2-}$, $OH^{\cdot}$, etc.) generating and antioxidants defense [4]. The excessive generation of ROS has been identified as one of the most important defined etiologies for male infertility [5]. ROS have a potential to damage the sperm membrane particularly on membrane polyunsaturated fatty acid (PUFA) lipids, when ROS damage the unsaturated bonds of PUFA, a lipid peroxidation chain reaction begins which in turn reduces the sperm motility [2–6]. To prevent the damaging effect of excessive ROS, enzymatic and non-enzymatic antioxidant pathways scavenge excess ROS and allow a balance to be achieved between beneficial oxidant generation and damaging harmful ROS [7]. Antioxidants are the most important defense substances against free radical induced infertility [8].

Human body provides a complete antioxidant defense system composed of enzymatic and non-enzymatic antioxidants [9]. Enzymatic antioxidant, (e.g., superoxide dismutase, glutathione peroxidse, and glutathione reductase), which catalyze free radical quenching reactions [10]. A number of compounds can be considered as non-enzymatic antioxidants such as; ascorbate, urate, α-tocopherol, pyruvate, glutathione, albumins, and β-carotene and ubiquinol [11,12]. These molecules have the capability to take free electrons from the radicals without being as reactive as free oxygen radicals [13].

2. Materials and methods

A total of 66 serum samples were included in this study, 40 samples were obtained from patients who attended to clinical laboratories for infertility treatment and 26 samples were obtained from normal fertile men.

Semen samples have been collected in the laboratory or home by masturbation after 3–5 days of sexual abstinence and ejaculated in a wide mouth plastic container, delivered samples incubated at (37 °C). Blood samples were collected using a sterile syringe and serum were separated by centrifugation and frozen in (−45 °C) deep freeze for further analysis.

2.1. Semen analysis

Semen analysis was performed according to (WHO, 2010). Semen examinations include; sperm count, viability activity and morphology [14].

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Table 1
Semen analysis variables of infertile and fertile groups.

Semen analysis variables	Infertile men (n = 40)		Fertile men (n = 26)		*P* value
	Mean	±SEM	Mean	±SEM	
Count (million/ml)	60.66	±8.01	55.72	6.53	n.s
T. count (million/ejaculate)	215.23	32.32	257.89	38.51	n.s
Viability					
Live %	70.94	2.78	73.00	2.12	n.s
Dead %	29.06	2.78	27.00	2.12	n.s
Activity					
Active %	47.56	2.75	54.73	2.71	n.s
Progressive %	27.79	2.35	39.84	3.28	0.003
Non-progressive %	19.78	2.27	14.90	2.64	n.s
Sluggish	23.38	2.07	18.27	1.65	n.s
Morphology					
Normal %	53.24	2.34	58.29	2.65	n.s
Abnormal %	46.76	2.34	40.88	2.25	n.s
Head %	31.12	2.13	27.02	1.72	n.s
M.P %	4.60	0.52	5.53	1.05	n.s
Tail %	8.36	0.78	5.66	0.64	0.016
C.D %	1.47	0.30	1.03	0.36	n.s

Sperm count calculated using a Hematocytometer after 10 μl of semen diluted with 190 μl of sample diluent and sperm concentration described as millions per ml.

Sperm viability examined by (Hypo-osmotic swelling test) test, semen sample mixed with hypo-osmotic solution in a ratio (1:9) and incubated at 37 °C for 30 min slides prepared and seen under phase contrast microscopy, the live and dead spermatozoa were calculated and expressed as percent.

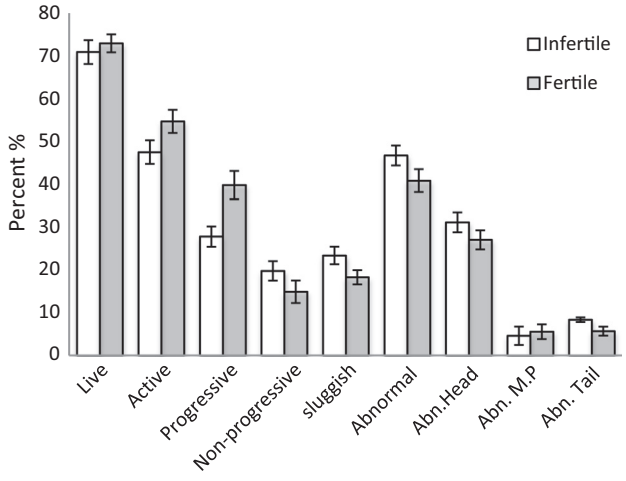

Fig. 1. Mean ± SEM of semen analysis variables of infertile and fertile groups.

Sperm activity has been examined by taking 10 μL of semen sample directly after liquefaction on a pre-warmed slide, the result described as progressive motility (PR), Non progressive motility (NP) and immotility (IM). Morphology examined by staining with India ink, the slides has been seen by oil emersion lens. The abnormality recorded as abnormal head, midpiece and tail.

2.2. Serum biochemical parameters

Frozen serum samples thawed and allowed to reach room temperature for biochemical analysis. TAC estimated using a kit of (CUPRAC) method [15]. The uric acid/uricase assay based kit is used for measuring uric acid concentrations [16]. Glutathione measured using colorimetric method [17], based on the reaction of 5,5-dithiobis(2-nitro-benzoic acid) [DTNB] with the aliphatic thiols at pH 8.0. The estimation of total thiols has carried out as the same as glutathione method except in the step of the addition of TCA which have not been added in the total thiols assay. Total protein is determined using sensitive lowery method [18]. Albumin estimated using commercially available kit demanding on bromocresol green (BCG) reagent. Ferritin determined by enzyme-linked immunosorbent assay (ELISA) [19], and MDA measured using a colorimetric method depends on the reaction of thiobarbituric acid (TBA) with MDA [20].

2.3. Statistical analysis

Results presented as Mean ± SE, and the differences between groups determined by ANOVA table using SPSS computer software program, $P \leq 0.05$ has been considered statistically significant.

3. Results

Results of semen analysis are showed in Table 1 and Fig. 1. Data showed both groups have no difference in sperm count and viability. The groups have high significant difference in progressive motility, Mean ± SEM (27.79 ± 2.35% and 39.84 ± 3.28%, $P \leq 0.003$) for infertile and fertile groups respectively.

The following evidences suggest that the cause of infertility in this study is the lack of sufficient motility as shown in Table 1. Total motility was lower but not significant in infertile group ($P \leq 0.08$). This results also reported by Roya Rozati et al. [21]. Also results showed high significant difference in the morphology of sperm tail Mean ± SEM (8.36 ± 0.78 and 5.66 ± 0.64, $P \leq 0.01$).

Table 2 and Fig. 2 show serum biochemical variables. The groups have high significant difference in TAC levels, Mean ± SEM (665.55 ± 32.25 and 813.70 ± 36.72 μM, $p \leq 0.015$) and significant difference in uric acid (261.80 ± 20.99 and 451.20 ± 24.97 μmol/l, $p \leq 0.03$) for infertile and fertile groups respectively. Also albumin was significantly decreased in infertile men as compared to fertile men (41.5 ± 2.2 and 49.2 ± 1.4 g/l, $p \leq 0.05$) respectively.

Table 2
Serum biochemical variables of infertile and fertile groups.

Serum biochemical variables	Infertile men (n = 40)		Fertile men (n = 26)		*P* value
	Mean	±SEM	Mean	±SEM	
TAC (μM)	665.55	32.25	813.70	36.72	0.015
Uric acid (μmol/l)	361.80	20.99	451.20	24.97	0.030
Glutathione (μmol/l)	197.10	9.32	177.50	13.50	n.s
Thiols (μmol/l)	188.82	9.29	181.67	10.64	n.s
Ferritin (ng/ml)	126.44	13.82	143.82	15.16	n.s
T. Protein (g/l)	64.94	2.77	63.04	10.78	n.s
Albumin (g/l)	41.5	2.2	49.2	1.4	0.050
MDA (nmol/ml)	198.10	15.54	247.06	13.45	n.s

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