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Determination of Ceruloplasmin Oxidase Activity, Copper and Iron in Seminal Fluid of Fertile and Infertile Males

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Abstract

The copper transport protein ceruloplasmin (CP) is suggested to have a role in male infertility. It considers one of antioxidant enzymes by controlling the reaction of iron. Ceruloplasmin oxidase activity was measured by using the colorimetric method. The seminal fluids of patients and control were collected from Infertility Diagnosis & Assisted Reproductive Technologies-Baghdad/ Iraq. The study was included eighty one infertile male patients and forty four fertile male, copper concentration and iron concentration were estimated for all the samples by using atomic absorption spectrophotometer. The result of this study was shown no significant difference ($p>0.05$) between mean oxidase activity of ceruloplasmin in normozoospermic (5.116 ±2.681 U/L) and mean oxidase activity of cp in asthenozoospermic (3.586± 1.744 U/L), oligozoospermic (3.9472 ± 1.105 U/L), but there was showed highly significant decreased ($P<0.001$) between mean activity of ceruloplasmin in azoospermic (2.133 ± 0.940U/L) and mean activity of ceruloplasmin in normozoospermic (5.116 ±2.681 U/L). Also, the result of copper concentration was appeared no significant difference between normozoospermic and all infertile subgroups ($P =0.6270$), otherwise there is highly significant in iron concentration between normozoospermic and all infertile subgroups ($P <0.0001$).

Keywords: Ceruloplasmin; Infertile; Copper; Iron

1. Introduction

Low sperm production, poor sperm function, or obstructions that limit sperm delivery are all common causes of male infertility [1]. Male infertility can be caused by illnesses, injuries, chronic health problems, lifestyle choices, and other factors. [1]. It affects approximately 7% of all men [2]. The male factor is responsible for around half of all documented couple infertility cases. [3, 4]. The inability to conceive a child is the major common sign of male infertility. No additional evident signs or symptoms would exist. In certain situations, however, signs and symptoms are caused by an underlying problem such as a hereditary condition, hormonal imbalance, dilated veins around the testicle, or a condition that prevents sperm from passing through. [5]. Hormonal defect, physical problems, lifestyle problems, psychological concerns, sex problems, genetic disorders, and single-gene anomalies are some of the causes of infertility in men. Despite countless attempts by experts to pinpoint the causes of male infertility, around 70% of cases remain unclear. [6, 7]. Seminal plasma is a complex fluid made up of secretions from the seminal vesicles, the prostate, the bulbourethral glands, and the lumen / epididymides / vasa deferentia of the seminiferous tubules. Although it has been formed that seminal plasma serves as a functional modulator of sperm function as well as a medium for carrying, protecting, and nourishing sperm after ejaculation until fertilization, more research is needed to fully characterize the molecular make-up of seminal plasma in fertile men and to understand how this is changed in diverse causes of male infertility. [8].Ceruloplasmin is the major copper-carrying protein in the blood, and in addition plays a role in iron metabolism [9]. Ceruloplasmin (EC1.16.3.1) is a copper-containing enzyme (EC1.16.3.1) that is produced in the liver, [10] also it secreted by Sertoli

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cells (11). Ceruloplasmin carries more than 95% of total copper in healthy human plasma [12]. Ceruloplasmin has a copper-dependent oxidase activity, which is related to the oxidation of Fe2+ (ferrous iron) to Fe3+ (ferric iron) and so facilitates in its transport in the plasma through conjunction with transferrin, which can only carry iron in the ferric state [13].From this function, it is obtained an antioxidant property [14].Antioxidants, which help to eliminate ROS excess in the seminal ejaculate and facilitate in the transition of ROS to substances that are less harmful to cells [15], are a new emerging function in male infertility management [16]. When there is more ROS than the antioxidants can eliminate, oxidative stress builds up, affecting sperm protein, lipid and DNA breakage, and sperm malfunction. [14]. The aim of the present study is evaluated the oxidase activity of ceruloplasmin, copper and iron concentration in subgroups of infertile male and fertile subjects.

2. MATERIALS AND METHODS

2.1 Samples

The current study included infertile and fertile subjects collected from High Institute for Infertility Diagnosis & Assisted Reproductive Technologies-Baghdad/ Iraq. The period of collection was between November 2019 and March 2020.

Eighty one primary infertile men were included and forty four fertile male individuals were used in this investigation as a control group. The infertility of subjects was confirmed by specialist and sub groups according to lab assessments. Macroscopic and microscopic examination of semen was performed according to WHO recommendations (17). A portion of each semen sample was examined for sperm count, motility and morphologic features. Infertile male patients were then divided into the following three groups count /motility and/or morphology, WHO criteria (17). The subjects were then sorted into the four groups listed below: The first group normozoospermic (sperm motility (Progressive + non-progressive) ≥ 40%, n=44) of subjects (22 - 55) years. Second group Asthenozoosperic (sperm motility <40%, n=31) of patients (20 - 47) years. Third group Oligozoospermic (sperm motility <20%, n=28) of patients (26 - 44) years. Fourth group Azoospermic (sperm concentration - zero, n=22) of patients (17 - 45) years. The subjects who passed traumatic abnormality or any type of infertility which affected sperm analysis were excluded.

Laboratory measurements: Adequate amount of semen were collected in specific container after abstinence period 3 to 5 days. Seminal plasma was obtained for all participation subjects via centrifugation of semen sample at 3000 rpm for 15 minutes.

2.2 Method

Seminal plasma was aspirated and divided into plastic tubes. Copper and Iron concentration was checked in plasma seminal fluid using atomic absorption spectrophotometer (Shimadzu AA 7000, Germany).

For estimation the activity of ceruloplasmin oxidase, rice method was applied using p-phenylene diamine -2HCL as a substrate [18].

2.3 Statistical analysis

Statistical analysis for this research was done by using Graph Pad prism9 software. One way ANOVA statistics was used. The critical value ($P<0.05$) was considered as the level of significant. Pearson correlation coefficient was applied for testing the correlation between sperm analysis variables and metals.

3. Results and Discussion

There was no significant difference shown ($p>0.05$) between mean activity of ceruloplasmin in normozoospermic (5.116 ±2.681 U/L) and mean activity of cp in asthenozoospermic (3.586± 1.744 U/L), oligozoospermic (3.9472 ± 1.105 U/L), but there was showed highly significant decreased ($P<0.001$) between mean activity of ceruloplasmin in azoospermic (2.133 ± 0.940U/L) and mean activity of ceruloplasmin in normozoospermic (5.116 ±2.681 U/L).

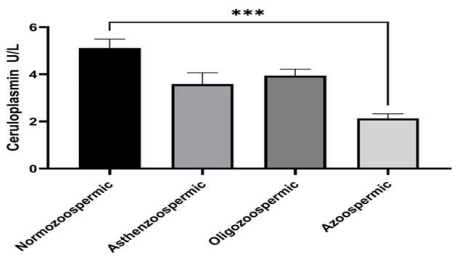

Figure1: Level of ceruloplasmin oxidase activity (U/L) in semen plasma from studied infertile subgroups versus control subjects. Significant reduction ($P<0.001$) observed in Azoospermic patients. Data are shown as mean and SE*** indicates high significant ($P<0.001$) differences.

There was no significant variation ($P >0.05$) between the mean concentration of copper in cases with normozoospermic (0.09562 ± 0.2918µg/ml) mean concentration of copper in patients with

asthenozoospermic (0.07924 ± 0.03868µg/ml), mean concentration of copper in patients with oligozoospermic (0.07450 ± 0.01953 µg/ml) and mean concentration of copper in patients with azoospermic (0.09377 ± 0.04922 µg/ml).

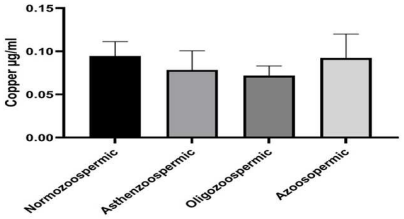

Figure 2: Copper concentration (µg/ml) in semen plasma from studied infertile subgroups and control subjects. No significant differences were seen among all studied groups. Data are shown as mean and SE.

There was high significant increased (P <0.0001) between mean concentration of iron in cases with normozoospermic (0.4234 ± 0.0821µg/ml) and asthenozoospermic (0.8163 ± 0.1097µg/ml), mean concentration of iron in patients with oligozoospermic (0.8809 ± 0.1105 µg/ml) and mean concentration of iron in patients with azoospermic (1.225 ± 0.2933 µg/ml).furthermore, There was high significant increased (P <0.0001) between mean concentration of iron in cases with azoospermic (1.225 ± 0.2933 µg/ml) and asthenozoospermic (0.8163 ± 0.1097µg/ml),and high significant difference (P <0.0001) between mean concentration of iron in cases with azoospermic (1.225 ± 0.2933 µg/ml) and oligozoospermic (0.8809 ± 0.1105 µg/ml) while there was no significant difference (P >0.05) between mean concentration of iron in cases with asthenozoospermic (0.8163 ± 0.1097µg/ml) and oligozoospermic (0.8809 ± 0.1105 µg/ml).

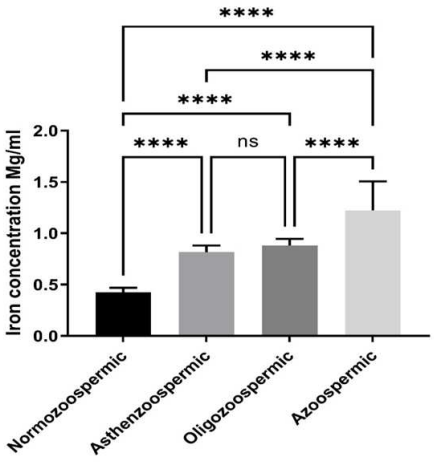

Figure 3: Iron concentration (µg/ml) in semen plasma from studied infertile subgroups and control subjects. Significant elevation (P<0.0001) of iron concentration were recorded in all infertile subgroups patients compared to control group. Data are shown as mean and SE**** indicates high significant (P<0.0001) differences.

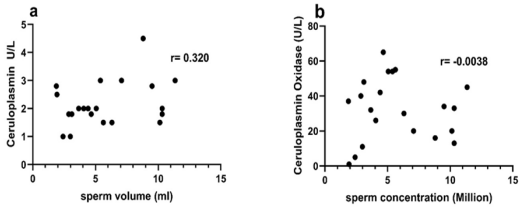

Figure 4: Correlation between activity of ceruloplasmin oxidase enzyme and sperm analysis variables in infertile studied subgroups. (a) Sperm volume and (b) Sperm concentration. The correlation coefficients showed no statistical correlation at (P<0.05) using Pearson's correlation coefficients.

Ceruloplasmin is a serum ferroxidase responsible for 90% of copper transport [19]. Ceruloplasmin is a positive acute-phase reactant [20]. Liver hepatocytes mostly synthesize ceruloplasmin, also some somatic cells can produce it, like sertoli cells [11], mammalian cells [21] and brain cells [22]. Cp oxidase activity was shown no significant difference between the normospermic males and asthenozoospermic, oligozoospermic groups while there was a highly significant decrease in the azoospermic group which is agreed with Suha H. H. et al. [23]. Likewise, the level of Cu was shown no significant difference between the normospermic males and infertile male groups which is agreed with Suha H. H. et.al. [23] and Mohammad M. H.et.al.[24] and disagreed with Eidi, M. et.al. which found highly significant elevated of copper in infertile groups [25]. Fe was significantly increased in all infertile male groups comparing with normospermic group which is disagreed with Mohammed M. H.et al. [24]. Many causes of male infertility, the overall results of this study which is decreasing cp activity and increasing Fe concentration support that oxidative stress (OS) is one factor that impacts fertility status. Like any other aerobic cell, spermatozoa are continually confronted with the "oxygen-paradox" [26]. Oxygen is required for life to exist because physiological levels of reactive oxygen species (ROS) are required for normal cell activity. On the other hand, oxygen breakdown products such as ROS can be harmful to cell function and survival [27]. Oxidative stress occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds the body's own natural antioxidant defenses, resulting in cellular damage [28]. Reactive oxygen species are produced by sperm and seminal leukocytes within the sperm

and cause infertility via two distinct mechanisms. First, they cause damage to the sperm membrane, reducing sperm motility and ability to fuse with the oocyte. Second, reactive oxygen species can change sperm DNA, resulting in the transmission of defective paternal DNA to concepts. [29]. Till now, the role of ceruloplasmin in the seminal fluid is unclear, while cp synthesizes and secretes by Sertoli cells that indicate cp is one of the essential proteins in the maintenance and control of spermatogenesis [11]. The function of cp in the male infertility spout of two ways: cp is a carrier of copper which considers an important element for the production of male gametes and in the processes of cell division - mitotic and meiotic, Both copper increase and deficiency leads to a significant reduction in male fertility [30].The other role of cp emerges from ferroxidase activity of it which grant it the antioxidant property by prevent the production of free radical that come from iron (Fenton reaction) [31].

4. Conclusion and Recommendation

In current study, there was no significant difference in ceruloplasmin oxidase activity and copper concentration between azoospermic, oligozoospermic and asthenozoospermic when compared with fertile individuals; otherwise, there was a highly significant difference of iron concentration in all types of infertile when compared with the fertile group. Further studies can occur for ceruloplasmin instance of measuring ferroxidase activity and the concentration of it in different types of infertile male. Measure transferrin and ferritin in the seminal fluid which are related with iron metabolism.

5. Conflicts of interest

There are no conflicts to declare.

6. Formatting of funding sources

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