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Seminal plasma levels of copper and its relationship with seminal parameters

Maryam Eidi[1] Ph.D., Akram Eidi[2] Ph.D., Omid Pouyan[3] M.D., Pouneh Shahmohammadi[1] M.Sc., Reza Fazaeli[4] Ph.D., Massih Bahar[5] M.Sc.

1 Department of Biology, Varamin Branch, Islamic Azad University, Varamin, Iran.
2 Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
3 Department of IVF, Shariati Hospital, Tehran, Iran.
4 Department of Chemistry, South Tehran Branch, Islamic Azad University, Tehran, Iran.
5 Department of Medical Genetics, Faculty of Medicine, Tarbiat Modares University, Tehran, Iran.

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Abstract

Background: The trace element copper has been identified as a highly toxic element for sperm. It is known to affect sperm motility in humans, and experimental implantation of copper in the epididymis, vas deferens, and scrotum of mammals has been demonstrated to affect fertility detrimentally.

Objective: Sperm concentration, motility, vitality and morphology are parameters used to evaluate potential male fertility. Since, copper is believed to be important for spermatogenesis; we conducted a study to investigate the correlation between seminal plasma copper concentration and human semen parameters in 232 males.

Materials and Methods: We selected 232 subfertile or infertile men who referred to Omid Fertility Clinic, randomly. Samples were categorized into normospermic (n=32), oligozospermic (n=73), asthenozospermic (n=111) and azospermic (n=16) groups according to their spermiogrames. Total seminal plasma copper concentration was determined by furnace atomic absorption spectrophotometer.

Results: The results showed that seminal plasma copper concentrations in oligozospermic, asthenozospermic and azospermic groups are significantly higher than normozospermic group ($p<0.01$). Also, negative correlations were found between seminal plasma copper concentration and sperm count ($p<0.05$), sperm motility ($p<0.01$), sperm vitality ($p<0.01$), normal morphology ($p<0.01$) and pH ($p<0.01$) in all groups.

Conclusion: It was suggested that excess copper in seminal plasma was detrimental for male reproductive capacity by reducing sperm count, motility, vitality and morphology.

***Key words*:** *Copper, Semen parameters, Male infertility.*

Introduction

"Exposure to environmental contaminants has been suggested to play a role in the pathophysiology of adverse reproductive health effects including decreased semen quality, sub-fertility, change in birth sex ratio, and an increase in the prevalence of developmental abnormalities of the male reproductive tract" (1-7).

Corresponding Author:
Maryam Eidi, Department of Biology, Varamin Branch, Islamic Azad University, Tehran, Iran.
Email: eidi@iauvaramin.ac.ir

Damage to human fertility, specifically a decline in male reproductive capacity has been suggested in many other reports, and the influence of environment factors including chemical substances and other pollutants in air, water, and soil have been examined (8-12). Copper products are used as the components of large systems, such as building, magnet, motor vehicle and telecom wire, copper tube, sheet and strip and many alloy products (13). "Copper in tailings and smelter slag is a potential environmental hazard (14) and high copper in drinking water transported through corroded copper tubes has been frequently observed" (15).

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The role of copper in male reproductive capacity appears to be largely unknown issue. Copper is a naturally occurring trace element that is essential for some metabolic processes. Copper depletion affects male reproduction in different species (16, 17).

Oster and Salgo (18) suggested that copper chelation was involved in suppression of spermatogenesis. On the other hand, it can be toxic at elevated concentrations (19,20). "Experimental implantation of copper in the epididymis, vas deferens, and scrotum of mammals has been demonstrated to affect fertility detrimentally. The trace element copper has been suggested as a highly toxic element for sperm and can affect sperm motility in humans" (21).

Since effect of copper on sperm quality is controversial, the present study was carried out to determine relation of seminal plasma copper concentration and human semen parameters.

Materials and methods

Subjects

Ejaculates were provided from a total of 232 males (mean age 32.15 ± 4.1), randomly. Subjects failed to have baby after 2 years of conception. Participants provided semen samples in polypropylene containers, via masturbation after an abstinence period of 2 to 3 days. Aliquots were taken after liquefaction at 37ºC. Exclusion criteria for subjects were cryptorchidism, vasectomy and varicocele.

Semen analysis

Semen analysis was performed according to the World Health Organization (WHO) guidelines to obtain volume, pH, vitality, sperm concentration, motility and morphology (World Health Organization, 2000). Sperm concentration was determined by a Neubauer® counting chamber. Motility was expressed as the percentage of progressive motile spermatozoa. Morphology was determined according to the WHO criteria using the papanicolaou's staining procedure. At least 300 cells were examined at a final magnification of ×1000. The samples were divided into 4 groups of; normospermic, azospermic (no sperm in semen), oligozoospermic (sperm concentration fewer than 20×10^6/ml) and asthenozoospermic (fewer than 50% spermatozoa with forward progression) groups.

Analysis of seminal plasma copper concentration

Total seminal plasma copper concentration was measured by furnace atomic absorption spectrometry. Samples were digested by adding nitric acid and diluted in high purity water (1:2). Wavelength was 324.8 nm. Calibration copper was delineated using suitable standard concentrations (10, 50 and 100 μg/L) by diluting standard $CuCl_2$, H_2O solution (Merck, Darmstadt) (22).

Statistical analysis

Statistical analyses were performed with the SPSS program. Correlation between semen parameters and copper concentration were considered significant at $p<0.05$.

Results

Table I shows population characteristics, sperm concentration, sperm vitality, sperm motility, normal morphology and seminal plasma concentrations of copper. Semen parameters are given as Mean ± SD.

Table I. Population characteristics, sperm concentration, sperm vitality, sperm motility, normal morphology and seminal plasma copper concentrations.

Character	Mean ± S.D.
Age (year)	31.22 ± 5.3
Sperm concentration (* 10^6)	60.15 ± 12.1
Sperm vitality (%)	60.48 ± 11.08
Sperm motility (%)	42.92 ± 13.4
Normal morphology (%)	63.65 ± 18.2
Seminal plasma copper concentration (ppb)	82.04 ± 18.3
pH	7.72 ± 1.02

In the present study, seminal plasma copper concentration was compared among 4 groups. Figure 1 shows that seminal plasma copper concentration is higher in azospermic ($p<0.001$), oligozospermic ($p<0.01$) and asthenozospermic ($p<0.01$) groups compare to normospermic males. When studying the correlations between the seminal plasma copper concentration and semen parameters, significant negative correlations were found between seminal plasma copper concentration and pH ($r_s=-0.173$, $p<0.01$) (Figure 2), vitality ($r_s=-0.391$, $p<0.01$) (Figure 3), sperm concentration ($r_s=-0.114$, $p<0.05$), motility ($r_s=-0.399$, $p<0.01$) (Figure 4) and normal morphology ($r_s=-0.317$, $p<0.01$) (Figure 5).

Significant positive correlations were found between seminal plasma copper concentration and fructose concentration ($r_s=0.116$, $p<0.05$), tail defects ($r_s=0.121$, $p<0.05$) and number of short tail sperms ($r_s=0.127$, $p<0.05$).

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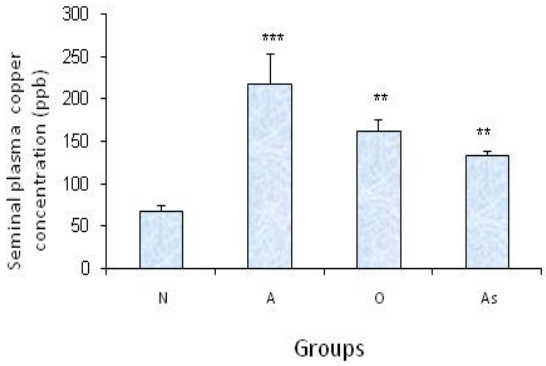

Figure 1. Seminal plasma copper concentrations in normospermic (N), azospermic (A), oligozospermic (O) and asthenozospermic (As) groups. **p<0.01, ***p<0.001 different from normospermic group.

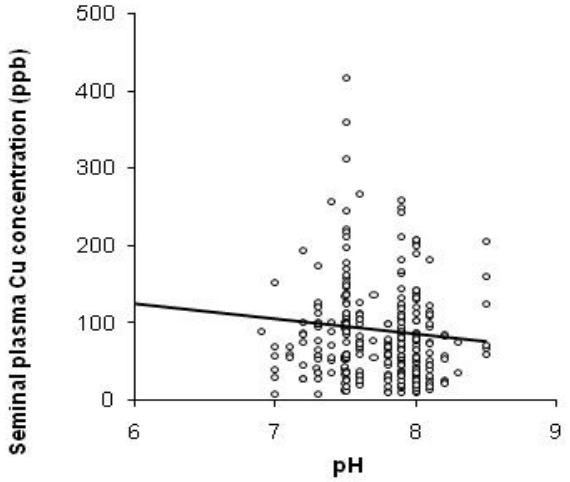

Figure 2. Correlation between seminal plasma copper concentration and pH (r_s=-0.173, p<0.01).

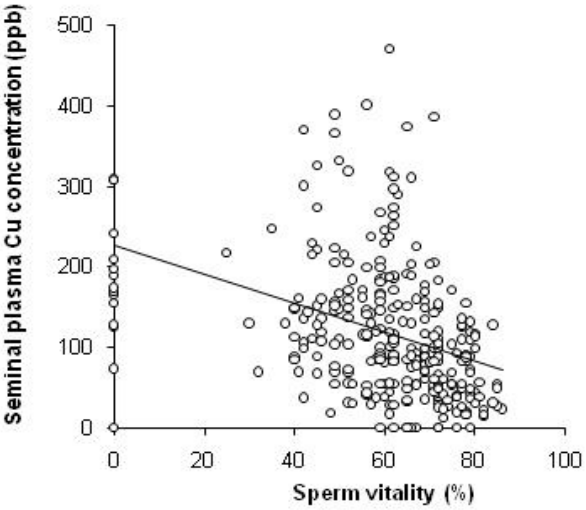

Figure 3. Correlation between seminal plasma copper concentration and vitality (r_s=-0.391, p<0.01).

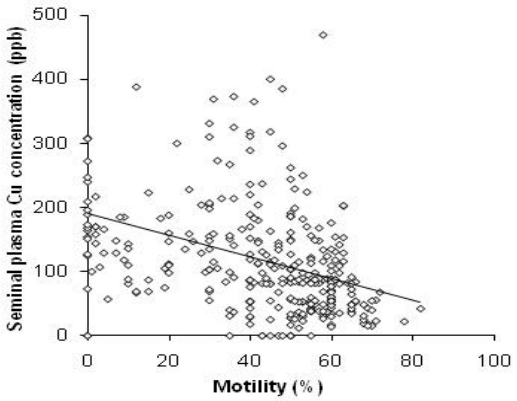

Figure 4. Correlation between seminal plasma copper concentration and total motility (r_s=-0.399, p<0.01).

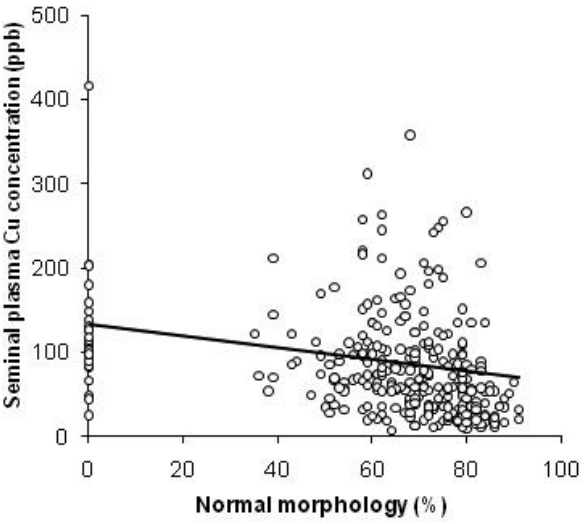

Figure 5. Correlation between seminal plasma copper concentration and normal morphology (r_s=-0.317, p<0.01).

Discussion

In recent years, it has been suggested that environmental factors may adversely affect the reproductive organs (1). Many metals are discharged as environmental pollutants from the combustion of fossil fuels, such as diesel fuels. While certain trace amounts of metals are essential for physiological homeostasis, it is well known that excessive or insufficient concentrations of these elements will induce toxicity and deficiency symptoms.

The result of present study showed significance differences of seminal plasma copper concentration between normospermic, azospermic, oligozospermic and asthenozospermic males. Also, our study demonstrated significant correlations between seminal plasma copper concentration and

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sperm concentration, pH, vitality, motility and normal morphology.

The role of copper in male reproductive capacity appears to be largely unknown, but this heavy metal appears to be involved in spermatozoa motility and it may also act at the pituitary receptors which control the release of LH (23).

Wong *et al* (2001) demonstrated a weak but significant positive correlation between blood copper concentrations and sperm motility (24). In a similar study, Jockenhövel *et al* (25) showed significant correlation between seminal plasma copper concentrations and sperm count, motility and normal morphology. It is known that copper is an essential trace element that plays an important role in several enzymes such as superoxide dismutase. Human spermatozoa are particularly susceptible to peroxidative damage because they contain high concentrations of polyunsaturated fatty acids and also possess a significant ability to generate reactive oxygen species (ROS), mainly superoxide anion and hydrogen peroxide. Superoxide dismutase protects human spermatozoa from this peroxidative damage. Oxidative stress caused by accumulated ROS is closely involved in a variety of pathological processes. Germ cells are as vulnerable as other cells to the potential detrimental effects of ROS and may thus require antioxidant protection at sites of gamete production, maturation and storage and embryo implantation (26). It is reported that "copper acts as a catalyst in the formation of ROS that can lead to oxidative stress and destructive lipid peroxidative damage" (27).

It has been shown that copper "in vitro" increased lipoprotein oxidation (28, 29). "Spermatozoa are highly susceptible to damage by excess concentrations of ROS due to the high content of polyunsaturated fatty acids within their plasma membrane and, although conventional basic semen characteristics other than motility are not obviously influenced by the oxidative state of semen" (30), such damage may underlie several aspects of male infertility. Increased lipid peroxidation and altered membrane function can render sperm dysfunctional through impaired metabolism, motility, acrosome reaction reactivity and fusogenic capacity as well as oxidative damage to sperm DNA (31).

High concentration of copper in seminal plasma is correlated with reduced sperm motility. Excess levels of monovalent and divalent copper ions in solution should result in lipid peroxidation in sperm plasma membrane, an effect that may render sperm immotile (24, 32).

Katayose *et al* (33) demonstrated that higher concentrations of copper had significant adverse effects on sperm motility. Salsabili *et al* carried out a study with spinal cord-injured men. They showed that seminal plasma copper had a relationship with sperm motility (34).

Also, Aydemir *et al* showed that copper levels in serum and seminal plasma in the subfertile male group were significantly higher than those in the fertile male group. Copper might be mediator of the effect of oxidative damage and play an essential role in spermatogenesis and male infertility (35). Shinohara *et al* found significant correlations between copper concentration in semen and sperm concentration, semen volume and abnormal morphology (36).

Ackerman *et al* (37, 38) carried out a study on impala living in the Kruger National Park, South Africa and demonstrated an adverse effect of high concentrations of copper on sperm morphology. This report and previous studies found that a large variety of sperm abnormalities are in impala, both in control and in animals exposed to copper. The frequency of occurrence abnormalities in elevated copper levels in the animals compared the normal, as presented by the liver copper concentrations, revealed a statistically significant correlation between the occurrence of sperm neck vacuoles and copper levels.

On the other hand, our study demonstrated significant and negative correlation between seminal plasma copper concentration and pH in seminal plasma. The present data showed that high concentration of copper is related to lowering pH of seminal plasma, acidic pH, with changing condition of seminal plasma due to decrease motile or alive percent of spermatozoa. Controversially, Yuyan *et al* did not show significant effect of high or low serum copper levels on sperm quality (39). The excessive copper intake has a negative effect on the organs of reproduction of males and females (25, 33).

It has been reported that copper has a toxic effect on the seminiferous epithelium (32). The toxic effects of copper on seminal plasma are manifested in the decrease percentage of motile spermatozoa and decrease number of malformed sperm cells (34).

Accordingly, it is plausible to consider seminal plasma copper concentration as a good marker for evaluating reactive oxygen radicals, sperm metabolism, vitality, motility and relevant semen parameters. Therefore determination of copper in seminal plasma during infertility is recommended.

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