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Original Research Paper

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LEVEL OF ZINC, COPPER AND GOLD IN HUMAN SEMEN

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ABSTRACT In this study 24 semen samples were studied in detail. Seminal plasma was separated and the level of three metals zinc, copper and gold was measured. Gold was shown correlated to total sperm count.

KEYWORDS :

INTRODUCTION

World over male infertility is on rise[7]. Causes of male infertility is not fully known. When a man's fertility is in doubt he is advised by the clinician for semen evaluation, the major investigation for a male partner.

Details of semen is not fully understood. Our knowledge on semen study improved since the second half of last century, yet not acquired in full. Several group of workers are engaged working on different aspects of semen both organic and inorganic matter. Electrolytes and metals in semen secured large attention. The output of work of some improved our knowledge on physical and biochemical aspects of semen. Few electrolytes are important for action potential and others are for different other functions of sperm[3, 10, 12, 34]. Continued efforts of researchers improved our knowledge on understanding the level of Sodium, Potassium, Calcium, Magnesium, Iron, Zinc, Copper, Cadmium, Nickel, Chromium, Manganese, Lead, Aluminium, Cerium, Cobalt, Gallium, Molybdenum, Strontium, Barium and Titanium in semen[8, 9, 11, 24, 33, 34, 39-41, 44, 49, 50]. Some of these elements enter spermatozoa [17]. Some electrolytes and metals in seminal plasma are responsible for creating a favourable atmosphere for spermatozoa for its smooth functioning[26]. Employing instrumental neutron activation analysis we observed Gold in semen[26].

X-ray diffraction analysis showed that there existed a tri metal complex of zinc, copper and gold and throughout the length of human male reproductive system and semen[45]. In this study, these elements Zinc, Copper and Gold are further evaluated.

MATERIALS AND METHODS

The study was conducted in the Department of Physiology, Sree Devaraj Urs Medical College at Kolar, in the Province of Karnataka.

Semen samples were collected from Outpatient Department Laboratory of the Hospital. Samples were submitted by patients who reported with their partners to the hospital for evaluation of infertility. We have selected a total number of 24 semen suppliers. They were physically healthy. Their age was from 20-25 years. Sample was collected at a place close to laboratory. Collection was done after maintaining an abstinence of five days[29, 42, 56]. Collection was done at 8 am ± 30 minutes onto a clean sterile glass container supplied by our laboratory[30]. After receiving a sample which was given a number and was routinely examined[1, 57]. Following this, seminal plasma was separated from each sample by centrifugation and stored separately in glass containers.

A total number of 24 samples were used in this study.

Samples were sent to Sophisticated Test and Instrumentation Centre at Cochin for analysis of Zinc, Copper and Gold in it.

Each sample was digested with 5 ml HNO_3 and 2ml $HClO_4$, added 3ml Aqua-regia and made up into 50ml using HPLC grade water and analysed with ICP-AES system to find out the concentration of Zinc, Copper and Gold in it.

RESULTS

Distribution of total sperm count (mill/ml) (Table 1) and its percentage of motility (Table 2) observed in samples are shown. Level of Zinc (in ppm) (Table 3), Copper (in ppm) (Table 4) and Gold (in ppm) (Table 5) in samples are shown.

The mean value of Zinc detected in samples is 135.42 ppm (range, 55.3 to 253.36), Copper is 0.45 ppm (range, 0 to 3.4) and Gold is 0.38 ppm (range, 0.0 to 1.15). Table 6 shows the mean values of all parameters included in this study. Correlation of Zinc, Copper and Gold with total sperm count (mill/ml) (Table 7) and with percentage of sperm motility (Table 8) is presented.

DISCUSSION

Rise in rate of male infertility in different parts of world is alarming[7]. Clinicians and scientists are in search of probable causes for this. Considering semen, as the ideal material for investigation, researchers are concentrating on this to find out different substances present in it and their probable role on sperm production and sperm function. Groups of researchers are attending on this. Many of them explored the association between fertility and inorganic substances present in semen.

Employing spectroscopic analysis we observed different elements like sodium, potassium, calcium, magnesium, phosphorus, iron, manganese, zinc, copper, boron, silicon, thallium, vanadium, aluminium, mercury and gold in normal and pathological semen samples[32]. Some trace elements are essential for function of sperm motility[5, 39, 40].

As a tri metal complex of zinc, copper and gold was reported in human male reproductive system and sperm[45]. A detailed analysis of these metals are done here in this study. A total number of 24 patients semen samples were included. Of which 17 (70.9%) had normal sperm count (Table 1) and 19 of them (79.2%) showed normal motility (Table 2).

Table 1: Distribution of samples according to total sperm count (million/ml)

Total Sperm Count (million/ml)	Number of patients	Percentage of sample %
1-20	2	8.3
21-40	5	20.8
41-80	10	41.7
>80	7	29.2
Total	24	100.0

Mean ± SD: 61.58±25.87

Table 2: Distribution of samples according to percentage of Motility (%)

Motility (%)	Number of patients	Percentage of sample%
<40	0	0.0
40-60	5	20.8
60-100	19	79.2
Total	24	100.0

Bondani et al. (1973)[6] believed seminal plasma is a source for through which excess electrolytes and metals are thrown out from the body. Contrarily Atig et al. (2012)[2] concluded their study that deficiency in trace elements is possibly related to poor sperm quality.

Table 3: Distribution of samples according to the level of Zinc (ppm)

Zinc (ppm)	Number of patients	%
<70.0	4	16.7
70.0-250.0	18	75.0
>250.0	2	8.3
Total	24	100.0

Table 4: Distribution of samples according to level of Copper (ppm)

Copper (ppm)	Number of patients	%
<0.20	9	37.5
0.20-1.0	14	58.3
>1.0	1	4.2
Total	24	100.0

Majority of patients (83.3%) showed the zinc above 70ppm (Table 3). Variation in zinc concentration is considered as normal[26, 27]. Among bivalent metals Zinc is the most commonly seen in seminal plasma[16, 27, 28] which in terms of its origin from prostate gland and different other parts of male genital system[27]. Along with zinc, two other bivalent ions magnesium and calcium are accumulated in the prostate gland and secreted in the prostatic fluid[15]. The level of zinc in semen differed in pathological conditions from normal[35, 36]. In daily ejaculates zinc concentration was 17.46 mg% on the first day. It differed on other days and on ninth day it was 13.16 mg %. Similarly copper level on the first day was 33.5 mg % and which fluctuated and 45 mg% on ninth day[42].

A strong positive correlation exist between total volume of ejaculate and the concentrations of zinc and copper in seminal plasma. Zinc and copper have a linear relationship[42]. The study on copper in male reproductive secretion started since 1856 when De Quatretages observed the effect of this metal on semen by adding from outside. Its toxicity to spermatozoa was repeatedly shown[23,38].

Copper is secreted throughout human genital tract[31, 55]. Copper was measured below 1ppm in 23 (95.8%) samples. All 24 patients showed presence of gold in their semen which was measuring below 1.55ppm (Table 5). The results of the present study is summarized and shown in Table 6.

Table 5: Distribution of samples according to the level of Gold (ppm)

Gold (ppm)	Number of patients	Percentage of sample %
<0.15	7	29.2
0.15-1.50	17	70.8
>1.50	0	0.0
Total	24	100.0

Table 6: Descriptive statistics

	Mean	SD ±
Count (Million/ml)	61.58	25.87
Motility (%)	72.08	10.41
Zinc (ppm)	135.41	62.34
Copper (ppm)	0.63	0.76
Gold (ppm)	0.51	0.32

We observed a negative correlation between total spermatozoa count and copper in seminal plasma[42].

Study of Battersby and Chandler (1977)[3] observed Copper on nucleus, acrosome and mid-piece of spermatozoa, further reported copper in mid-piece having a significant difference between fertile and non-fertile semen. Its presence in seminal plasma is important for sperm motility[32,37]. Reduction in its level may lead to infertility. Level of copper in seminal plasma was given as 153 µg/dL, 16.23 µg/dL [33], 52 µg/dL [53], 610 µg/dL [12], 33.5 µg/dL [42], 3.4 µg/dL [54] and 263 µg/dL [31,38].

Table 7: Correlation of Zinc, Copper and Gold with Total Sperm Count (million/ml)

	Count (million/ml)				p value
	<20 million/ml) (n=2)		>20 million/ml) (n=22)		
	No	%	No	%	
Zinc (ppm)					
• <70.0	0	0.0	4	18.2	1.000
• 70.0-250.0	2	100.0	16	72.7	
• >250.0	0	0.0	2	9.1	
Copper (ppm)					
• <0.20	1	50.0	8	36.4	1.000
• 0.20-1.0	1	50.0	13	59.1	
• >1.0	0	0.0	1	4.5	
Gold (ppm)					
• <0.15	2	100.0	5	22.7	0.076*
• 0.15-1.50	0	0.0	17	77.3	
• >1.50	0	0.0	0	0.0	

***Significant (0.05<p<0.10)**

There was no difference in copper concentration in seminal plasma between normal and pathological conditions [33,38].

Table 8: Correlation of Zinc, Copper and Gold with Motility (%)

	Motility (%)				P value
	<60 % (n=5)		>60% (n=19)		
	No	%	No	%	
Zinc (ppm)					
• <70.0	0	0.0	4	21.1	0.712
• 70.0-250.0	5	100.0	13	68.4	
• >250.0	0	0.0	2	10.5	
Copper (ppm)					
• <0.20	2	40.0	7	36.8	0.300
• 0.20-1.0	2	40.0	12	63.2	
• >1.0	1	20.0	0	0.0	
Gold (ppm)					
• <0.15	0	0.0	7	36.8	0.272
• 0.15-1.50	5	100.0	12	63.2	
• >1.50	0	0.0	0	0.0	

Zinc and copper in semen have a linear relationship [42].A negative correlation existed between total spermatozoa count and copper in seminal plasma [42].

Gold was not considered as an important trace metal for human body function. The total amount of gold present in human body was measured as less than 1 mg [18]. Oser in (1965)[18] reported its presence as accidental. Gold was discovered in human semen in 1981[26]. Its presence in semen was repeatedly shown[32]. Gold reported in semen is the highest level ever reported in biological material[26,32].

Gold is present both inside and outside spermatozoa[47, 48]. No significant difference was seen between the level of gold in seminal plasma and spermatozoa.

The metal is present in whole length of male genital system except seminal vesicle[47, 48]. Gold in semen is mainly reaching

from caput epididymis[32,47,48]. The metal is also detected in male reproductive tract of small animals[43]. It was also detected on the rim of seminiferous tubule of frog[43].

Gold was shown in seminal plasma and spermatozoa of all normal samples[47, 48]. Gold is present both in normal and pathological samples. It was not present in few samples where motility was below detectable level. Possibly the lack of gold is responsible for low motility. Chronobiological study observed the highest amount of gold in semen was present at 0800 hours and lowest at 0000 hours of the day[51].After 3 hours of collection gold was less in seminal plasma showing the entry of metal to spermatozoa [25].

Human semen study conducted at gold mine and non-gold mine area showed fertility was higher at gold mine area [19]. Gold was present in all normal samples studied in gold mine area[19,21] and gold deposit area[22]. Above studies show that gold in semen as important for fertility.

Suvarna bhasma (SB) is a drug prescribed by Ayurvedic physicians to some male infertile patients. In a study we observed after administration of SB to infertile patients their sperm count and sperm motility increased and which was sustained for longer period of time in normal and pathological conditions[46]. SB is prepared from pure gold. The level of gold in SB was measured by Prasad et al.[20] (2011b). SB improved spermatogenesis and increased sperm motility of epididymal spermatozoa in rats [13,14]. In vitro studies showed SB increases human sperm motility[52].Similarly Biswas et al. (2004)[4] observed improved testicular steroidogenic and gametogenic function in immature male albino rats. In one study infertile patients were prescribed SB and results showed their semen parameters improved and sustained for longer period of time[13].

Our statistical study to find out a correlation of 3 metals with total sperm count showed significant correlation between gold and total sperm count. No correlation was observed when three metals was studied with percentage of motility (Table 8).

In conclusion, this study showed the level of three metals, zinc, copper and gold in seminal plasma of patients where gold showed a significant result when studied in terms of total sperm count.

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