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Original article

Cross-sectional study of zinc, cadmium levels and Zn/Cd ratio in seminal plasma and its relation with semen parameters

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Abstract:

Introduction:The aim of this study was to determine if there were any significant differences in Zinc (Zn) and cadmium (Cd) concentrations and Zn/Cd ratio in seminal plasma in men diagnosed with subnormal semen parameters compared to men diagnosed with normal semen profile group.

Materials and Methods: A total of 200 males investigated for infertility 100 men of normal semen parameters and 100 with subnormal semen parameters were evaluated. The seminal plasma Zn, Cd, concentrations were determined by atomic absorption spectrophotometer Perkin Elmer 5100), Semen analyses were performed using standard techniques as recommended by the World Health Organization.

Results: Study results showed the men with subnormal semen profile to be significantly older (Mann-Whitney U test: p= 0.014) and to have lower Zn levels (Mann-Whitney U test p= < .001) and Cd levels showed no significant difference in men with normal semen parameters and men had subnormal semen parameters (Mann-Whitney U test p= 0.007) and Zn/Cd ratios were significantly higher in men with normal semen profile. Further analysis men with subnormal semen parameters had lower zinc levels compared to men with normal semen parameters (Mann-Whitney U test: P<0.001), suggesting a connection between zinc and cadmium levels and Zn/Cd ratio in seminal plasma and male fertility.

Conclusion: Evaluation of seminal plasma, Zn, Cd levels and Zn/Cd ratio may be considered in a comprehensive investigation of the subfertile men.

Keywords: Seminal plasma, cadmium, zinc, zn/cd ratio, male infertility.

Introduction:

Human fertility is declining, and poor semen quality has been a contributing factor. [(1)(2)] Environmental exposure to toxic substances is increasingly being reported as a possible cause for decreased human fertility potential. Males are more likely to be affected by reproductive toxicants, and supposedly these toxicants have led to a decline in semen quality. [(1)(2)(3)]

The raised seminal cadmium levels were found despite the subjects being non-smokers and were not occupationally exposed to it. Contaminated food is a significant source of environmental exposure to cadmium among non-smokers. [(1)(4)(5)]

Zinc has beneficial effects on semen quality, and seminal zinc is significantly correlated with sperm concentration, volume, motility, viability, pH, and morphology. [(6)(7) (8)] The accumulation of heavy metals in the testis may decrease the zinc concentration in semen. [(9)(10)] The ratio of zinc and cadmium becomes an essential marker for reproductive health. Zn/Cd ratio reduction decreases the seminal and testicular immune response. [(11)]

India is among the fastest-growing industrial economies. However, the industrial effluents cause pollution of air, water and enter the food chain leading to exposure of toxic waste to the population in the region. [(12)(13)] There is increasing interest in

the contribution of lifestyle, smoking, occupational exposure, and toxic environmental exposure to declining sperm concentrations and human male infertility. [14][15] Identification of male infertility depends principally on semen analysis (sperm count and sperm motility sperm morphology). [16][17][18] With this perspective, the present study is done to assess the Zn/Cd ratio regarding the semen parameters in the subjects accessing infertility checkups.

Materials and Methods:

The study was carried out at the Government Medical College and Hospital (GMC), Aurangabad, located in the Marathwada Region of Maharashtra, India. This was a cross-sectional study with matched controls.

We decided to enroll 100 participants in each group to consider any loss due to protocol deviation. Approval was obtained before the collection of data from the Institutional Ethical Committee. In addition, written informed consent was obtained from all subjects before their participation. The study was carried out in 200 adult males aged 20-40 years during their attendance at infertility clinic GMC from September 2013 to October 2014.

Data on the age, medical and sexual history of the subjects were collected with the assistance of a physician.

Participants, Inclusion, and exclusion criteria.

Inclusion criteria:

Male subjects between age-group 20 to 40 years. Who are willing to participate in the study.

Exclusion criteria:

History of psychological dysfunction, with undescended testis, varicoceles, hydrocele, and history of surgery. With chronic illnesses like diabetes mellitus, hypertension, tuberculosis, and on zinc supplements or any long-term medication.

Participants above 40 years of age were excluded.

The age groups of subjects range from 20 to 40 years because even though sperm production starts at puberty and remains throughout life, there is an age-related decline in sperm count relatively after forty years of age. Cases below 20 years of age do not approach infertility centers, which might be due to the marriage age of 21 or the social stigma, or maybe waiting for terms. [19]

Collection of semen samples

Before sample collection, the subject had to abstain from any form of sexual activity for at least 3 days. Semen samples were collected by masturbation, and the entire ejaculate was passed into the sterile graduated centrifuged container for their volume measurement. The seminal plasma was parted from the semen sample by centrifugation at 3000 r. p.m. for 10 min at room temperature (22-25 C^{o}). An aliquot of the seminal plasma was carefully decanted into a metal-free plastic khan tube (washed thoroughly with 1% nitric acid and rinsed properly with de-ionized water) and stored at -20Co until required for analysis.

Microscopic semen analysis.

At least 200 scores were performed; 0.02 ml of the semen was diluted with 0.38 ml of sodium bicarbonate-formalin diluent. A new, improved Neubaur counting chamber was charged with diluted fluid using a Pasteur pipette, and preparation allowed settling for 3 min. The spermatozoa in 2 large squares were counted using 10x objectives, and the number of cells counted was multiplied by 10,000 to give the number of sperm cells per milliliters of sample. A drop of liquefied ample (1.5µL) was placed on a grease-free slide, and a coverslip was applied. The preparation was examined microscopically using 40x objectives. The motility of the spermatozoa was graded as:

- Motile
- Rapid progressive motility
- Slow or sluggish progressive motility
- Non-motile

Sperm cell morphology was evaluated according to the strict criteria of Kruger et al. (21), and 4.0% normal morphology was used as a cut-off value.

The contribution of morphologically normal sperm cells cannot be less than 4%.

Estimation of zinc and cadmium: This was done using the atomic absorption spectrophotometric method.

Quality control: All quality control standards are used to calibrate the equipment.

Zinc and Cadmium concentration was analyzed at the Govt Institute of science.

Cd was determined by the modified methods of Ediger and Coleman. Cd levels were measured in µg/dl, and zinc was determined by Smith et al., using AAS. zinc levels were measured in mg/dl. [2]

Categorical variables were articulated as absolute and relative frequencies. Numerical data were described using an arithmetic mean and standard deviation for normal data distribution and median and inter-quartile range for the remaining cases. If necessary, differences in categorical variables were tested using the $\chi 2$-test and the Fisher exact test. To test normal distribution of data the Kolmogorov-Smirnov test was used.Differences in normally distributed numerical variables between two independent groups were tested using Student's t-test and Mann-Whitney U test was used in occasion of deviation from the normal distribution. All p values were two-tailed. The significance of differences determined by statistical testing was expressed at the level $p<0.05$. The statistical software Jamovi (1.8.1) and XLSTAT free version were used for data analysis.

Results :

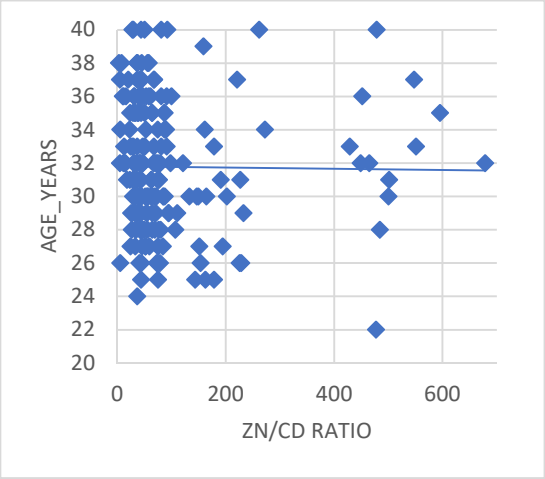
Table 1. Median age, sperm count, motility, morphology, and zinc/ cadmium level and zinc/cadmium ratio in seminal fluid of study subjects.

		Zn/Cd ratio	Age (Years)	Sperm count (Millions /ml)	Sperm motility (%)	Sperm normal Morphology (%)	Zn (mg/dl)	Cd µg/ dl)
Zn/Cd ratio	Pearson's r							
	p-value							
Age (Years)	Pearson's r	-0.01						
	p-value	0.849						
Sperm count (Millions/ml	Pearson's r	0.26***	-0.08					
	p-value	<.001	0.286					
Sperm motility (%)	Pearson's r	0.33***	-0.04	0.80***				
	p-value	<.001	0.591	<.001				
Sperm normal Morphology (%)	Pearson's r	0.19**	0.02	0.52***	0.59***			
	p-value	0.006	0.829	<.001	< .001			
Zinc (mg/dl)	Pearson's r	0.32***	-0.06	0.34***	0.35***	0.18**		
	p-value	<.001	0.379	<.001	< .001	0.009		
Cadmium (microgram/dl)	Pearson's r	-0.72***	0.10	0.21**	-0.20**	-0.19**	-0.12	
	p-value	<.001	0.154	0.003	0.005	0.008	0.091	

Table II: Correlation matrix of seminal plasma zinc/cadmium ratio and zinc, cadmium levels, and semen parameters in the study participants

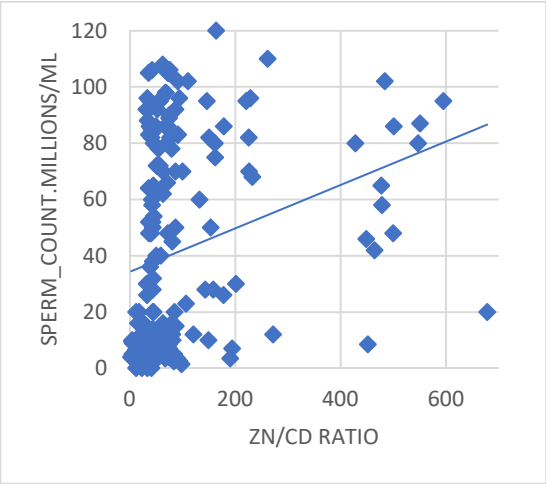
	Median [Q1, Q3]		
	Group A Normal n=100	Group B Sub-normal n=100	p*
Age (Years)	30 (28-34)	32 (30-36)	0.014
Sperm count >15x10^6/mL	81 (57-92)	9.25 (5.0-12)	< .001
Motility >40%	60 (50-66.25)	25 (15-30)	< .001
Morphology >4%	55 (52-60)	36 (32-54)	< .001
Zinc.mg/dl	100.25 (95.25- 110.25)	76.37(45.12-112.50)	< .001
Cadmium µg/dl	1.5 (0.6-2.2)	2.0 (0.6-2.4)	0.007
Zn/Cd ratio	69.7 (43.8-151.6)	44.3 (43.8-67.0)	< .001

Median [Q1, Q3] IQR; *Mann Whitney U tes [*p <.05, ** p < .01, ***p < .001]



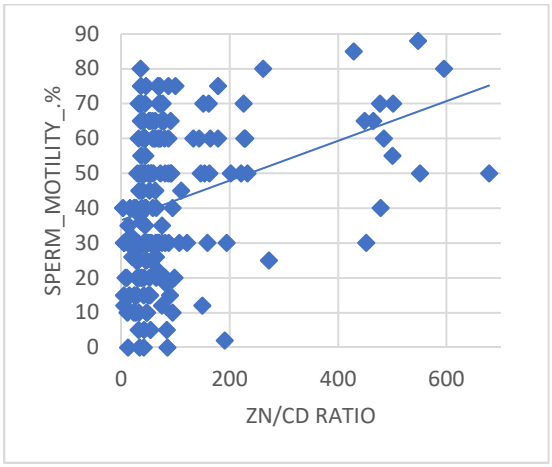
(r = -0.01 p = 0.849)

Fig.1.Scatter plots showing correlation between seminal plasma zinc/cadmium ratio and Age in the study participants.



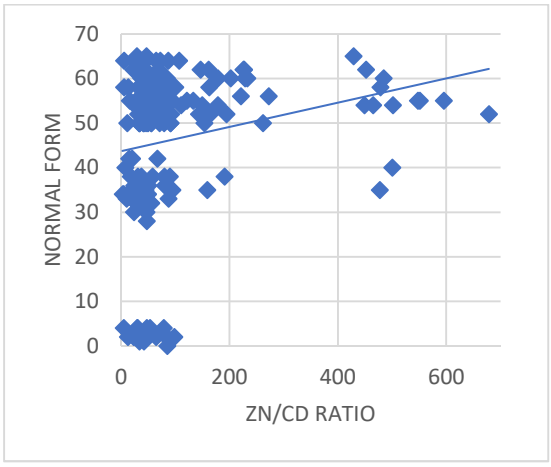
(r = 0.26 *** p = < .001)

Fig.2.Scatter plots showing correlation between seminal plasma zinc/cadmium ratio and sperm count in the study participants



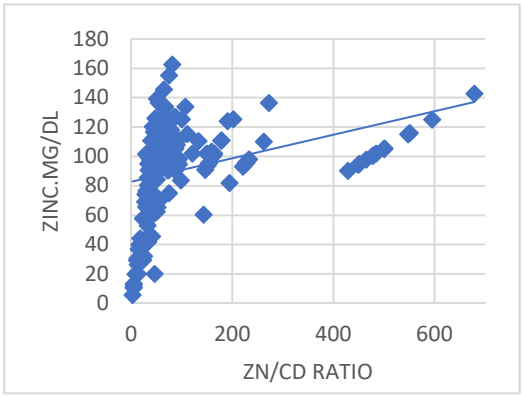
(r = 0.33 *** p = < .001)

Fig.3.Scatter plots showing correlation between seminal plasma zinc/cadmium ratio and sperm total motility in the study participants.



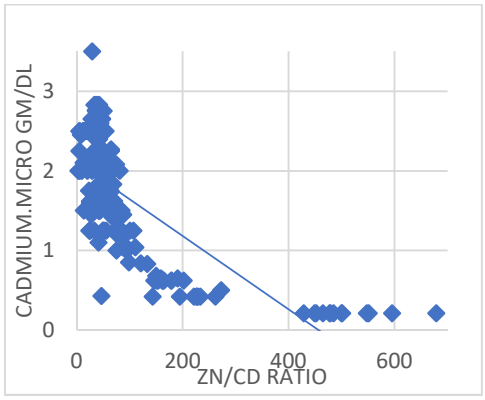
(r = 0.19 ** p = 0.006)

Fig.4.Scatter plots showing correlation between seminal plasma zinc/cadmium ratio and sperm morphology in thestudyparticipants



(r= 0.32 *** p = < .001)

Fig.5.Scatter plots showing correlation between seminal plasma



(r= -0.72 *** p= < .001)

Fig.6.Scatter plots showing correlation between seminal plasma

The research was carried out in 200 men, out of which 100 had normal semen parameters, and 100 had been with subnormal sperm parameters.

The subjects with sub-normal semen parameters were significantly older (median 32, interquartile range 30-36) than subjects with normal semen parameters (Mann Whitney U test; p=0.014). In the former, median sperm count was 9.25 (interquartile range 5.0-9.25), motility 25 % (interquartile range15-30), morphology 36 % (interquartile range 32-54), all significantly lower compared to men with normal semen parameters analysis (Mann Whitney U test; p<0.001). Zinc and cadmium values in the seminal plasma of men having sub-normal semen parameter had a significantly lower median of 76.37 (interquartile range 45.12-112.50) (Mann Whitney U test; p=< .001) cadmium values median of 2.0 (interquartile range 0.6-2.4) (Mann Whitney U test; p= 0.007) (Table.1)

Correlation between zinc/cadmium ratio and zinc cadmium levels on semen profile.

Correlation studies showed seminal plasma zn/cd ratio positively correlated with sperm count (r=0.26, p< .001) Although zn/cd ratio was positively correlated with sperm motility (r=0.33, p< .001) and moderately correlated with sperm morphology (r= 0.19, p=0.003) while seminal

plasma zn/cd ratio correlated with seminal plasma zn levels (r= 0.32, p< .001) showed significantly positive correlation. While on other hand seminal plasma zn/cd ratio inversely correlated with seminal plasma cd (r= -0.72, p =1.000) and age (r= -0.01, p= 0.576) (Table.2)

Discussion:

The diagnosis of male factor infertility has a tremendous impact on affected couples' physical and emotional health and quality of life. The deterioration in semen quality over the past 60 years has become a public health challenge worldwide. [22] During meta-analysis done by Sun et al., the interaction between men's fertility changes and trace elements concentration in seminal fluid, including Zn and Cd, was studied. It has been found that even a slight elevation of cadmium level significantly reduces the semen quality, but not to such a high extent as a decrease of zinc concentration. ([23] This study evaluated that the seminal plasma Zn/Cd ratio levels were significantly higher in seminal plasma of subjects with normal semen profiles than subjects with sub-normal semen parameters. This is in agreement with the previous study ([22]. In this study, the interactions of trace elements in seminal plasma with the spermiogram demonstrate very complex mechanisms which may determine the sperm quality of the participants. Similarly, in their study,(24) found that a high zinc/cadmium (Zn/Cd) ratio of more than 200 was associated with a normal sperm count and motility, suggesting an inverse relationship between the Zn/Cd ratio and impairment of spermatozoa motility. [24] Therefore, it may be reasonable to suggest that Zn/Cd ratio may be a better index of assessing sperm quality than seminal zinc and cadmium independently. [11]

This study showed that semen parameters below the reference range decrease seminal plasma zinc levels. Also, seminal plasma zinc levels were higher among subjects with normozoospermia. Subjects with low sperm count, motility, and abnormal morphology had significantly reduced levels of seminal plasma zinc when compared with normozoospermic men, and these subjects with normal sperm count showed a weak correlation between their seminal plasma cd levels and age. This observation is inconclusive with previous studies [25][26][27][28]. Endocrine-disrupting chemicals such as Cd and Pb are toxic to the testis and adversely affect wildlife reproduction. [29], disrupting steroidogenesis and spermiogenesis in laboratory animals. ([30] Changes in human and animal sperm morphology and motility have been associated with toxic exposures and may relate to damage to differentiating cells or, over time to stem cells. [31] Cadmium in seminal plasma has been associated with low semen volume and sperm motility. [32] Increasing seminal plasma cadmium was significantly predicted to cause abnormal sperm morphology. [33] This may explain the significant increase in abnormal morphology in infertile normospermics compared to the fertile normospermics. [33] In this study, no significant difference was found between seminal plasma cd concentration among the study groups and consider them . [34]

In contrast to previous reports. [1][9], cadmium levels provide no clear correlation with semen parameters in this study. However, Zn/Cd ratio and zinc levels provided clear correlations with the semen parameters. –as assessed by t-tests and Mann Whitney U test. To verify the accuracy of this analytical approach for use in clinical examinations of male infertility, it is essential to accumulate a large number of specimens and examine as many other sperm parameters as possible.

Conclusion:

From this study , we conclude that , valuation of seminal plasma, Zn, Cd levels and Zn/Cd ratio may be considered in a comprehensive investigation of the subfertile men. Correlation analysis of whole specimens revealed that trace elements in seminal human plasma were found to be correlated positively or negatively with various sperm properties. It was revealed that Zn/Cd ratio and zinc and cadmium levels were key metals for the diagnosis of male infertility by AAS, and this method could be used to complement the diagnosis of male infertility.This study highlight one of important issue underling need of knowledge at primary care level.

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