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Relevance of zinc concentration in seminal plasma with sperm morphology in infertile males

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Abstract: **Zinc is an essential micronutrient for spermatogenesis and normal sperm function. There have been conflicting reports on the relation of seminal zinc on various seminal parameters. This study aimed to evaluate zinc levels in seminal plasma of infertile men and its relation to infertility.**

Objective: To find out relation of zinc concentration in seminal plasma with sperm morphology in infertile subjects having normozoospermia, oligozoospermia and azoospermia.

Design: Cross sectional study

Setting: This study is carried out in the reproductive biology unit in department of Physiology &Central Research Lab, Jawaharlal Nehru Medical College Sawangi Wardha (MS) from June 2009 to Dec 2011.

Subjects and method: Study involved detail history taking; clinical examination followed by detailed semen analysis as per the WHO criteria. The seminal plasma was then classified in to six groups patients having normozoospermia (40), oligozoospermia (16) ,ashanospermia withnormal count (9),oligoasthenospermia (20),teratozoospermia(10) and azoospermia (10). They were used for zinc determination by zinc kit (supplied by Coral pharmaceuticals) using colorimeter. The morphology of the sperm is also evaluated. With WHO criteria, sample is normal if 30 % or more of the observed sperm have normal morphology.Slides for morphological smear were prepared and smear were fixed and stained with papanicolau stain, the procedure was done according to WHO manual.

Results: Zinc levels were lower among azoospermic & oligospermic as compared to normozoospermic patients. Seminal plasma zinc showed a positive correlation with normal spermatozoa morphology.

Conclusion: Zinc level in seminal plasma and its relationship to seminal parameters might give useful information regarding male reproductive dysfunction. It seems that the estimation of seminal plasma zinc may help in investigation and treatment of infertile subjects.

Keywords: **Normozospermia, oligospermia, oligoasthenospermia, taratozoospermia, asthenospermia, azoospermia.**

I. INTRODUCTION

What makes the earth a unique planet is entity called life. Reproduction is necessary for progress of species. With advance of civilization sterility has become more than eternal, a medical problem, a problem which closely affects the welfare of the society. Besides, the riddle of population explosion there are challenges to infertility as there is alarming increase in cases of infertility. Infertility is defined as failure of a couple to achieve pregnancy after 'a' year of unprotected intercourse with the same partner.

Semen is the fluid that a man ejaculates. The whole semen is the suspension of spermatozoa or sperms in the fluid medium called seminal plasma. The main portion of semen originates from three glandular organs. The constituents

contributed by gland are more or less specific for that gland e.g. spermatozoa from testes, fructose from seminal vesicle, and acid phosphates, citric acid zinc from prostrate. Semen analysis is the test most widely used to estimate the fertility potential of the male.

Zinc is involved in variety of cellular functions, including signal transduction, transcription and replication[1]. Bertrand and Vladesco were the first to notice the presence of zinc in semen[2]. Zinc concentration of seminal plasma is very high, over 30 times than that found in the blood [3]. Zinc itself is a characteristic constituent of the male reproductive organs and plays a definite, role in reproduction [4].

The association of zinc contents in seminal plasma and other spermatic parameters in both fertile and infertile men is still controversial. Because zinc plays a critical role in sperm function, and the relation of zinc in seminal plasma and spermatic function has not yet been concluded. This study attempts to assess a correlation between zinc contents in seminal plasma and in infertile men.

II. MATERIAL AND METHODS

The study has been carried out in the reproductive physiology unit in department of Physiology, Jawaharlal Nehru Medical College, Sawangi (Meghe) Wardha. The ethics committee of the institute cleared the study. The patients were referred from department of surgery, department of obstetrics and gynecology and sex and marriage counseling unit, JNMC Sawangi (Meghe) Wardha.

The ejaculates of 115 patients attending reproductive biology unit for fertility problems were collected after 2-7 days of sexual abstinence. At first clinical attendance, a detailed background history and physical examination were done on the subjects followed by detailed semen analysis and zinc estimation in seminal plasma.

Following inclusion and exclusion criteria is used for selection of subjects:

Inclusion criteria

Subjects from infertile couple.

- Mean age 21-45 years ,
- Couples having history of infertility, after an unprotected sexual intercourse more than one year.

Exclusion criteria

- Wives of the infertile subjects having obvious causes of infertility like tubal blockage or ovulation disorders.
- Individuals who had undergone pelvic surgery of hernia repair.
- Having diabetes or thyroid disease.
- Patients having history of mumps, tuberculosis, sexually transmitted diseases.
- Patients who were on antipsychotic or antihypertensive drugs, or taking vitamin and mineral supplementation were excluded from the study.

Study set up : Reproductive Physiology unit, department of Physiology, JNMC & Central research Laboratory, JNMC, Sawangi (Meghe) Wardha.

Study design : Cross sectional study

Study duration: About 2 years

Collection of semen sample[5].

The following instructions based on WHO recommendations are used for semen analysis. The subject was provided with clearly written or oral instructions concerning the collection of sample. The sample was collected after a minimum of 48 hours and no longer than 7 days of sexual abstinence. Two semen samples were collected for initial evaluation. The sample was collected in the privacy of a room near the laboratory.

The samples obtained by masturbation and ejaculated into a clean, wide-mouthed glass or plastic container and protected from extremes of temperature (not less than 20°c and not more than 40°c) in the laboratory. The sample was examined immediately after liquefaction and certainly within 1h of ejaculation. Semen analysis was carried out by conventional analysis to determine the volume, pH, sperm count, sperm motility, and sperm morphology according to WHO criteria.

Examination under microscope

The semen sample was mixed well in the beaker for few minutes. One drop of sample, of approximately 2cms was placed on clean glass slide with the help of dropper the covered with cover slip. The glass slide and cover slip were warmed to 37 degree C before use. Several fields were examined under low power of microscope to see homogenous distribution or any spontaneous agglutination. Then the lens was turned to high power objective and then parameters were assessed as concentration, motility ,viability and morphology .

The morphology of the sperm is evaluated. With WHO criteria, sample is normal if 30 % or more of the observed sperm have normal morphology[6].Slides for morphological smear were prepared and smear were fixed and stained with papanicolau stain, the procedure was done according to WHO manual[6].

Papanicolau staining done accourding to WHO manual

Estimation of zinc in seminal plasma

There is an increasing interest in the significance of zinc in the physiology and pathology of the male genitourinary apparatus, in particular with regard to fertility[7]. Thus, adequate technique is necessary for determining zinc in the appropriate matrices: seminal fluid, seminal plasma, seminal plasma, spermatozoa and prostatic tissue.

The technique most widely used for determining zinc in seminal plasma is atomic absorption spectroscopy which has several inconveniences as follows:

- Indispensable background correction in the ultraviolet region.
- Heavy matrix effect due to high protein content and high viscosity of samples, so large dilutions are required with consequent manipulation, which entails risk of contamination or of analytic loss.
- Difficulty in assessment of adequate standards.
- The need for frequent preparation of calibration curves.
- High cost of instrument.

Zinc estimation in seminal plasma is done by colorimetric method6. It can be used to determine the zinc “whole” semen, seminal plasma, or isolated spermatozoa[8, 9]. The accuracy of this method is proved by statistical comparison with atomic absorption[8].

Evaluation of a commercially available kit for the colorimetric determination of zinc in seminal plasma is done by comparing with the atomic absorption spectrophotometery as a reference method[8].this method can be recommended for use in semen analysis laboratories[10].

For the estimation of zinc in seminal plasma following method is used1[11, 12.]

Principle : Zinc in alkaline medium reacts with Nitro-PAPS to form a purple colored complex. Intensity of the complex formed is directly proportional to the amount of zinc present in the sample.

Zinc + Nitro – PAPS ⟶ Purple colored complex

III. OBSERVATION AND RESULT

Table 1: Division of subsets into six groups on the basis of sperm count, % motility and percent morphology

Group	Name of the group	No. of patients
Group 1: Control	Normozoospermia	40
Group 2	Oligozoospermia	16
Group 3	Asthenospermia with normal count	9
Group 4	Oligoasthenospermia	20

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Group 5	Teratozoospermia	10
Group 6	Azoospermia	20
Total		110

Table 1 shows division of 115 subjects on the basis of sperm count, % motility and % morphology.

Table 2: Descriptive Statistics for % normal Morphology in all the groups

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	40	71.87	9.85	1.55	68.72	75.02	40.00	95.00
Group 2	16	55.31	18.92	4.73	45.22	65.39	30.00	80.00
Group 3	9	47.77	17.15	5.71	34.58	60.96	30.00	70.00
Group 4	20	48.00	26.67	5.96	35.51	60.48	20.00	100.00
Group 5	10	20.00	4.08	1.29	17.07	22.92	10.00	25.00
Group 6	20	0.00	0.00	0.00	0.00	0.000	0.00	0.00

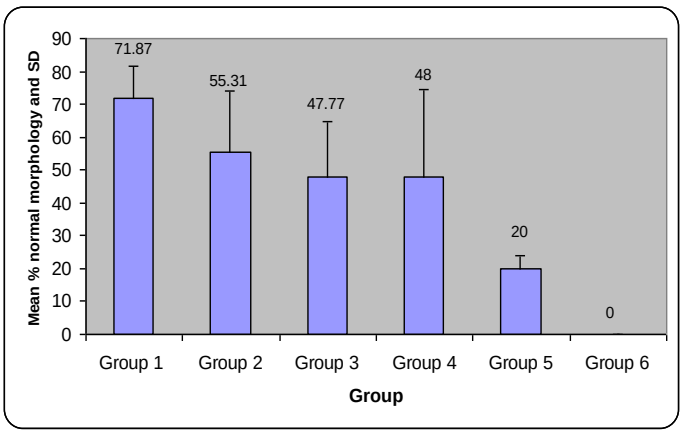
Spermatozoa having normal morphologies are maximum in normozoospermic patients (group 1) and minimum in teratozoospermic group.

Table 3: Correlation of zinc concentration with normal morphology

	Zinc concentration	Morphology	Correlation 'r'	p-value
Group 1	2.19±0.12	71.87±9.85	-0.07	0.66 NS,p>0.05
Group 2	1.61±0.31	55.31±18.92	0.14	0.58 NS,p>0.05
Group 3	1.56±0.41	47.77±17.15	0.95	0.000 S,p<0.05
Group 4	1.23±0.25	48.00±26.67	0.01	0.96 S,p<0.05
Group 5	1.36±0.24	20.00±4.08	0.18	0.60 NS,p>0.05
Group 6	1.28±0.27	-	-	-

When mean zinc concentration is correlated with % normal morphology positive correlation is observed between mean zinc concentration and normal morphology in all the groups except in group 1

Graph 1: Descriptive Statistics for % normal Morphology in all groups



The above graph shows percentage of normal spermatozoa is more as in normozoospermia patients as compared to other groups.

IV. DISCUSSION

An assessment of sperm morphology is a pre-requisite of semen analysis, allowing evaluation of the status of testicular germ cell production and providing indicators to factors contributing to infertility. Zinc in seminal plasma stabilizes the cell membrane and nuclear chromatin of spermatozoa [7]. Zinc is responsible for maintaining sperm integrity and modulates testis fatty acid composition by interrupting fatty acid composition[8]. Zinc is an essential cofactor for metalloenzymes and plays an important role in polymeric organization of macromolecules such as nucleic acids, protein synthesis cell division and stability of biomembrane[9]. The outer dense fiber extends along 60 percent length of principal piece, consist of few structural proteins, and comprise medulla and cortex. This outer dense fiber protein contain high amount of amino acid cysteine, which stabilizes and stiffens the structure during Epididymal maturation[10]. Zinc plays a regulatory role in process of capacitation and acrosome reaction[11].

However, evaluation of sperm morphology remains subjective, and interpretation of standard definitions can differ between technicians and laboratories[12]. Similarly, there are a number of staining procedures available, "assessment of sperm morphology from vitally-stained preparations may represent a useful innovation". In this study the Papanicolaoue (PAP) staining is used to study the morphology of spermatozoa.

Mean of percent normal morphology in normozoospermic patients (group1) is 71.87percent, in oligozoospermic patients (group 2) is 55.31percent, in asthenospermic patients with normal count (group3) is 47.77percent, in oligoasthenospermic patients (group 4) is 48percent and in teratozoospermic patients (group 5) is 20percent . Numbers of spermatozoa having normal morphologies are maximum in normozoospermic patients (group 1) and minimum in teratozoospermic group.

When mean zinc concentration is correlated with percent normal morphology positive correlation is observed between mean zinc concentration and normal morphology in all the groups. Correlation with zinc concentration and percent normal morphology is statistically significant in asthenospermic with normal count (group 3) and oligoasthenospermic patients (group 4).

Thus in the present study, mean seminal zinc concentration was found to be more in patients with good sperm morphology compared to patients with poor morphology.

MarmarJ et al (1975)[13] observed a trend towards increasing normal morphology in the groups with increasing zinc concentration. Tikkiwal M et al (1987)[14] observed the significant improvement in number of normal spermatozoa in oligospermic patients when treated with oral zinc sulphate. He also showed, increase in seminal plasma zinc levels.

Hunt C. D. et al (1992) observed slight increases in sperm head roundness when seminal plasma zinc level was low. Zinc in combination with other seminal factors affects the structural stability of spermatozoa. Kvist U (1980) observed that the stability of nuclear chromatin in human spermatozoa against decondensation by sodium dodecyl sulphate was significantly lower in the semen sample with subnormal zinc concentration [7].

On the other hand, Colleen S et al (1975)[15] observed the no correlation between zinc concentration in seminal fluid and percent of abnormal spermatozoa. Behne D et al (1988) found no correlation between the contents of zinc in seminal plasma and normal morphology[15].

V. SUMMARY AND CONCLUSION

The role of zinc in relation to male infertility is inconclusive Positive correlation is observed between zinc concentration and percent normal morphology of sperm It seems that the estimation of seminal plasma zinc may help in investigation and treatment of infertile men .So estimation of seminal zinc concentration an be done in cases of unexplained infertility .

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