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TO ESTIMATE LEVEL OF ZINC AND FRUCTOSE IN NORMOSPERMICAND OLIGOSPERMIC MALES

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ABSTRACT

INTRODUCTION: Infertility has often been defined as failure to achieve pregnancy within one year of unprotected intercourse. Seminal plasma is very important for sperm metabolism, function, survival, and transport in the female genital tract. Zinc deficiency may lead to gonadal dysfunction, decrease in testicular weight and shrinkage of seminiferous tubules. Zinc may have a role in sperm production, viability and in the prevention of spermatozoa degradation. Fructose concentration is considered a good measure to evaluate seminal vesicular function and has been studied in great detail, and its concentration can depend on number of factors such as time since collection and the age of the donor.

MATERIAL AND METHODS: This is a cross-sectional study. Semen examination of the patients visiting the gynecology department of the Government Medical College, Aurangabad was carried out according to the standardized method of the World Health Organization from January 2016 to January 2017. A complete medical history of the patients was recorded. The collection and analysis of semen were done by properly standardized procedures as mentioned in WHO Laboratory Manual (2010). Males with primary and secondary infertility without treatment, of theages between 20-45 years males.

RESULTS: The mean of sperm count in oligospermic subjects is 10.84 ± 7.118 millions/ml of semen ranging 1-15 million/ml which is significantly lower than that of the normospermic subjects i.e; 92.88 ± 79.93 millions/ml, within a range of 32 — 170 million/ml ($p < 0.001$). In addition, the mean sperm motility in oligospermic men is 40.56 ±53.82%, ranged within 0-100% is significantly lower than that of the controlgroup 65.6 ± 41.50 %, which ranged from 30 - 98 % ($p < 0.001$). The mean of seminal plasma zinc in oligospermic men is1.65 ± 0.936µmol/ejaculate, ranged within 0.2- 2.9µmol/ejaculate is significantly lower than that of the control group4.074 ±1.199µmol/ejaculate which ranged from 2.66 — 4.9µmol/ejaculate ($p < 0.001$).

CONCLUSION: From our study we can conclude that biochemical parameters that is zinc and fructose level in seminal plasma have direct effect on physical parameters like sperm count and motility that‟s why we should incorporate these parameters in routine semen analysis. Also as dietary zinc affects seminal zinc level, zinc should be supplemented and dietary zinc should be adequate.

Keywords: Zinc, Fructose, Normospermic, Oligospermic Males

INTRODUCTION

Infertility has often been defined as failure to achieve pregnancy within one year of unprotected intercourse [1]. Infertility has multiple causes and consequences depending on the gender, sexual history, life style and cultural background of people. Infertility affects about 8-12% of the world‟s population and in about half of cases, men contribute the couple‟s infertility [2]. This problem has been worsening by as much as3% per year suggesting that male reproductive problems may be increasing over time[3].

Infertility is one of the most tragic of all marital problems. Unfortunately some married couples are infertile and infertility is a social stigma in our society. It can be said that the investigations of a childless couple may fail for the two main reasons namely ignorance and inadequate knowledge. Approximately15% of couples trying to conceive are infertile, in that about 30% cases are due to males only and in another 20% cases both partners have detectable abnormalities. Thus male factor plays an important role in 50% of infertile couples [4].

About 1 out of 15 couples of reproductive age experience difficulty conceiving. Both men and women are more or less equally affected. The prevalence of male infertility is similar to that of Type 1 and2 Diabetes combined. As per WHO study, incidence of infertility in India is 10 to 15% [5]. According to world Health Organization 60 to 80 million couples across the world presently suffer from infertility. Infertility varies across the world and it probably affects 8 to 12 per cent of couplesworldwide [5].

Semen is whitish milky fluid, slightly viscous, containing water and small amounts of salt, protein, fructose, citric acid and other substances [6]. Sperms make up only about 5 to 10% of the volume of semen. Fluid portion of semen is contributed by secretions of male accessory organs of reproduction. Seminal vesicles contribute 40 to 80% of semen. The secretions include fructose for sperm nutrition, bicarbonate to buffer the acidic vaginal vault, prostaglandins and coagulating substances. Most of the remainder is generated by the prostate (10 to 30%). Prostatic secretions in humans contain enzymes and proteases to liquefy the seminalcoagulum. It also contains high levels of citric acid, acid phosphatase, phospholipids and spermine [7].

Seminal plasma is very important for sperm metabolism, function, survival, and transport in the female genital tract [8]. Sperm count and sperm motility are prime parameters that determine the functional
ability of spermatozoa. [5]

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Zinc deficiency may lead to gonadal dysfunction, decrease in testicular weight and shrinkage of seminiferous tubules [9]. Zinc may have a role in sperm production, viability and in the prevention of spermatozoa degradation [10].

Fructose concentration is considered a good measure to evaluate seminal vesicular function and has been studied in great detail [11], and its concentration can depend on number of factors such as time since collection and the age of the donor [12]. Fructose is an important source of energy for the spermatozoa and primary source of lactic acid in semen. Fructose is also likely involved in protein complexes, particularly in coagulated semen [14]. Measurement of fructose concentration in whole semen will change over time as a result of fructolysis [15].

Fructose plays an important role in sperm motility and concentration, particularly with regard to energy metabolism [16]. A study conducted in 1997, shows that low levels of seminal fructose are positively correlated with low seminal volume, low sperm motility and high sperm chromatin stability. Seminal fructose is often routinely measured in the assessment of seminal vesicle function and male factor infertility [17].

Attempts to show a relationship between zinc and fructose concentrations in seminal plasma with andrological parameters have produced conflicting results. Semen analysis was performed with biochemical measurements of seminal zinc and fructose. Despite their often routine measurement in the evaluation of male fertility, attempts to correlate zinc and fructose concentrations with andrological parameters have been inconsistent [18].

Aim of our study is to estimate level of Zinc and fructose in study population and to correlate the same with semen parameters and thereby to assess the relationship of seminal zinc and fructose level and male reproductive parameters.

MATERIAL AND METHODS

This is a cross-sectional study.

Source of Study Population: Semen examination of the patients visiting the gynecology department of the Government Medical College, Aurangabad was carried out according to the standardized method of the World Health Organization from January 2016 to January 2017. A complete medical history of the patients was recorded. The collection and analysis of semen were done by properly standardized procedures as mentioned in WHO Laboratory Manual (2010) [19].

Sample Size: Total sample size = 100;
n1 = 50 Normospermic (Controls), n2 = 50 Oligospermic (Cases).

Study participants were grouped into:

Group 1: Cases –Oligozoospermic men- sperm count less than 15million/ ml

Group 2: Controls – Normozoospermic men- sperm count more than15 million/ ml

Inclusion Criteria:

1. Males with primary and secondary infertility without treatment, of the ages between 20-45 years males.
2. Subjects who are ready to give consent.

Exclusion Criteria:

1. Patients of pelvic surgery, Hernia repair, Diabetes mellitus, Thyroid disease.Patients on drugs like antipsychotics, antihypertensive, neuroleptics,alcohol and nicotine.
2. Patients having any other chronic medical/systemic illness other thaninfertility.
3. History of alcohol consumption, tobacco consumption and smoking.
4. Taking other drugs like corticosteroids, beta blockers, thiazide diuretics, fluoroquinolone antibiotics, etc.

Collection of semen sample:

1. The samples were collected after a minimum period of 48 hours but no longer than seven days of sexual abstinence. The name of the patient, the period of abstinence, the date and time of collection and interval between collection and analysis were recorded in the Performa.
2. Sample was collected in the morning during OPD hours.
3. The subjects were properly instructed and motivated to provide full cooperation.
4. The semen was obtained by masturbation into a clean, sterile wide mouthed plastic semen container.
5. The semen was passed in privacy of a room adjacent to the laboratory.
6. The semen containers were labeled with the patient"s name, registration number, date and time of collection.
7. The semen samples were protected from extremes of temperature during transport to the laboratory.
8. Sample is allowed to liquefy for 30 min at room temperature.
9. Semen volume, sperm concentration and motility were measured according to standard World Health Organization criteria.

Evaluation of sample:

Semen Analysis:

Semen analysis by conventional method and Computer Assisted Semen Analysis (CASA), Semen samples were analyzed according to WHO criteria.

Estimation of Zinc [20]:

After evaluation of physical parameters, whole semen sample was centrifuged at 3000 rpm for 10 minutes. Then, without disturbing the pellet at the bottom, supernatant seminal plasma was taken for zinc assay. Zinc was estimated by spectrophotometric method.

Statistical Analysis:

The data was analyzed using a statistical software- SPSS version 20.0. The level of significance was set at 95% confidence interval, where p-value less than 0.05 ($p<0.05$) was considered as statistically significant. First, Unpaired t- test for statistical significance was applied between cases and controls for all the parameters like count, motility, zinc and fructose. Pearson‟s correlation test was used to correlate count, motility with zinc and fructose in normospermic and oligospermic men in SPSS. (Pearson‟s correlation coefficient (r) values are between -1 to +1; Positive correlation means that as one parameter increases the other alsoincreases.

When r = 0 to 0.29, it shows weak correlation, When r = 0.30 to 0.49, it shows moderate correlation andWhen r = 0.50 to 1, there is strong correlation)

RESULTS

Table 1 shows the mean of sperm count in oligospermic subjects is 10.84 ± 7.118 millions/ml of semen ranging 1-15 million/ml which is significantly lower than that of the normospermic subjects i.e; 92.88 ± 79.93 millions/ml, within a range of 32 — 170 million/ml ($p < 0.001$). In addition, the mean sperm motility in oligospermic men is 40.56 ± 53.82%, ranged within 0-100% is significantly lower than that of the control group 65.6 ± 41.50 %, which ranged from 30 - 98 % ($p < 0.001$). The mean of seminal plasma zinc in oligospermic men is1.65 ± 0.936μmol/ejaculate, ranged within 0.2- 2.9μmol/ejaculate is significantly lower than that of the control group4.074 ±1.199μmol/ejaculate which ranged from 2.66 — 4.9μmol/ejaculate ($p < 0.001$).

Table I - Mean value of comparison of count, motility, zinc andfructose between normal and oligospermic men.

Parameter	**Normal (Mean± SD) n= 50**	**0ligo (Mean± SD) n= 50**	**p value**
Count (millions/ml)	92.88 ± 79.93	10.84 ± 7.118	< **0.001****
Motility (%)	65.60 ± 41.50	40.56 ± 53.82	< **0.001****
Zinc (μmol/ejaculate)	4.074 ± 1.199	1.65 ± 0.936	< **0.001****

Fructose (μmol/ejaculate)	14.042 ± 8.64	17.29 ± 10.56	**0.001****

In table 1, the mean of seminal plasma fructose in oligospermic men Which is 17.29±10.56μmol/ejaculate, ranged within 9.18- 26.68 μmol/ejaculate is significantly higher (p < 0.001) than, control group14.042±8.64μmol/ejaculate, which ranged from 3.98 – 22.78 μmol/ejaculate.

Table II – Correlation between sperm count and zinc concentration.

Variable	Number of patients	Correlation coefficient((r)	P value
Oligospermic	50	0.715	0.000

Table II, shows correlation between sperm count and seminal plasma zinc level in the oligospermic men which is statistically significant with a strong positive correlation. (r = 0.715, p = 0.000).

Table III – Correlation between sperm count and fructoseconcentration.

Variable	Number of patients	Correlation coefficient((r)	P value
Oligospermic	50	-0.113	0.433

Table III, shows the correlation between sperm count and seminal plasma fructose level in the oligospermic men which is statistically notsignificant. (r = -0.113, p = 0.433).

Table IV – Correlation between sperm motility and zinc concentration.

Variable	Number of patients	Correlation coefficient((r)	P value
Oligospermic	50	0.171	0.234

Table IV, 15 shows the correlation between sperm motility and seminal plasma zinc level in the oligospermic men which is statistically not significant. (r = 0.171, p = 0.234).

Table V – Correlation between sperm motility with fructoseconcentration.

Variable	Number of patients	Correlation coefficient((r)	P value
Oligospermic	50	-0.477	0.000

Table V, 16 shows correlation between sperm motility and seminal plasma fructose level in the oligospermic men which is statistically significant with a moderate negative correlation. (r = -0.477, p = 0.000).

DISCUSSION

In the present study, the mean levels of sperm count, motility, zinc and fructose concentration were estimated and correlated between oligospermic and normospermic subjects.

The mean of seminal plasma zinc in oligospermic menis1.65 ± 0.936μmol/ejaculate, ranged within 0.2- 2.9μmol/ejaculate is significantly lower than that of the control group 4.074 ± 1.199 μmol/ ejaculate which ranged 2.66 — 4.9μmol/ ejaculate (p < 0.001).

The correlation between sperm count and zinc concentration in oligospermic men is statistically significant with a strong positive correlation (r = 0.715, p = 0.000). The correlation between sperm motility and seminal plasma zinc concentration in μmol/ejaculate in oligospermic men is statistically not significant (r = 0.171, p = 0.234).

Similar studies on seminal plasma zinc concentration in oligospermic subjects were conducted. The studies are as follows. Carreras and Mendoza (1997) have reported that zinc was significantly correlated with sperm count [21]. Lewis-Jones et al (1996) reported a mean seminal plasma zinc concentration of 117.6 mg/L (range between 75.2 –176.5). Seminal plasma zinc concentration were poorly correlated (r = 0.039) with motile sperm concentration [22].

Fuse et al (1999) observed significantly low levels of zinc in oligospermic patients (p<0.05) and showed significantly positive correlation with sperm count (r=0.33, p<0.05) and sperm motility (r=0.22, p<0.05) [23]. Sin-Eng Chia et al (2000) The means of the seminal plasma zinc concentration which were significantly lower in the oligospermic group 183.6 mg/L (range, 63–499) compared with those in the normal group 274.6 mg/L (range, 55–420). Seminal plasma zinc concentration was significantly correlated with sperm count (r=0.341, P=0.0001), motility (r=0.253, P=0.0001) [24].

Wai Yee Wong et al (2001) when studied the correlations between the elements zinc and the semen analysis parameters, weak correlations were found between plasma zinc concentrations and sperm concentration (r=0.18; P, 0.01), sperm motility (r= 0.15; P, 0.05).

Zinc concentrations in seminal plasma correlated with sperm count ($r = 0.17$; p, 0.05) [25]. Mankad et al (2006) found positive correlation between zinc level and sperm count ($r = 0.29$, $p < 0.05$) but no significant correlation was observed between zinc and sperm motility [26].

Hasan ali et al (2007) observed significant positive correlation of seminal plasma zinc concentration with sperm count in oligospermic ($r = 0.49$), significant low level of serum and seminal plasma zinc levels in oligospermic males [27].

Basil et al (2008) found a significant positive correlation between seminal plasma Zn concentrations and sperm motility in oligospermic group ($r = 0.68$; $p < 0.022$). There was significant low concentration of seminal plasma Zn in oligozoospermic males when compared with that of fertile controls [28].

The mean of seminal plasma fructose in oligospermic men is 17.29 ± 10.56 µmol/ejaculate, ranged within 9.18- 26.68 µmol/ejaculate is significantly higher ($p < 0.001$) than, control group14.042 ± 8.64 µmol/ejaculate, which ranged from 3.98 – 22.78µmol/ejaculate. In our study the correlation between sperm count and seminal plasma fructose level in the oligospermic men is statistically not significant. ($r = -0.113$, $p = 0.433$). The correlation between sperm motility and seminal plasma fructose level in the oligospermic men is statistically significant with a moderate correlation. ($r = -0.477$, $p = 0.00$).

Jather et al (1977) observed the mean values for fructose in normal subjects (381±17.4) was significantly lower than the corresponding mean value observed in the oligospermic group (467±19.4). Analysis of fructose values in normal volunteers revealed that, seminal plasma fructose levels decreased as the sperm count increased 59. [29] In study conducted by Shibabrata Biswas et al (1978) fructose concentration showed a significant negative correlation with sperm motility ($r = -0.2315$; $p = 0.02$). The semen with the highest sperm count showed the lowest amounts of fructose 60. [30]

Lewis et al (1996) proved that there was a significant negative correlation between the number of motile spermatozoa and fructose concentrations ($r = 0.062$, $p = 0.0048$) lack of correlation between increasing numbers of motile spermatozoa in the ejaculate and the concentration of zinc in the seminal plasma ($r = 0.0392$, $p = 0.0713$). [31]

CONCLUSION

According to the results in our study, there is a positive, significant and strong correlation seen between sperm count and zinc concentration ($r=0.715$, $p < 0.05$) and there was significant negative correlation between sperm motility and fructose concentration ($r = -0.477$; $p < 0.05$). This study also showed that, zinc concentration wassignificantly lower ($p < 0.001$) in oligospermic men (1.65 ± 0.93µmol/ejaculate) as compared to normal healthy controls (4.07 ± 1.19µmol/ejaculate). And fructose was higher in oligospermic men (17.29 ± 10.56 µmol/ejaculate) as compared to normal (14.04 ± 8.64µmol/ejaculate).

From our study we can conclude that biochemical parameters that is zinc and fructose level in seminal plasma have direct effect on physical parameters like sperm count and motility that‟s why we should incorporate these parameters in routine semen analysis. Also as dietary zinc affects seminal zinc level, zinc should be supplemented and dietary zinc should be adequate.

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