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RESEARCH ARTICLE

EVALUATION OF SEMINAL FRUCTOSE AND CITRIC ACID LEVELS IN INFERTILE MALES AND ITS CORRELATION WITH BODY MASS INDEX (BMI) IN POPULATION OF JAMMU AND KASHMIR

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Abstract

Introduction:30-35% of total population in Jammu and Kashmir reportedly has infertility problem which can be related to many factors. Overweight and obesity is one such factor which have a negative effect on fertility. It is generally assumed that obese patients have higher fat content in semen samples and that higher BMI value alters the level of reproductive hormones.

Materials and methods: 150subjects (100 infertile as test and 50 fertile subjects as controls) were taken after taking proper consent. Sample was collected by the criteria laid down by WHO 2010.

Results: In this study, the sperm count was found as 56.57 ± 8.47 in fertile and 15.8 ± 2.85 in infertile subjects. The fructose levels for controls and test were found out to be 108.29±2.97 and 92.68±3.75. And the citric acid levels are 36.70 ±1.65 and 42.16±1.22 in control and test respectively.

Conclusion: In our study conducted in the population of Jammu and Kashmir, it has been found that increase BMI value has a significant relation with infertility.

Introduction:-

Several biochemical markers are traced in the seminal fluid that is secreted at thetime of ejaculation. Like blood, semen also consists of cellular and non cellularcomponents namely spermatozoa and seminal plasma respectively. 5 to 10% of thesemen is constituted by spermatozoans while the major bulk is contributed by theseminal plasma. Seminal plasma constitutes the secretions from accessory reproductiveglands of the male (1). These constituents are incompletely mixedduring ejaculation leading to heterogeneity in the fractions of ejaculate (2). Much of the seminal plasma is secreted by the seminal vesicle which accounts for70-85% of semen. These include fructose, prostaglandins, coagulation factors,bicarbonate etc. Secretions of prostate form the second major component (10-30%) of theseminal plasma (3). Prostatic secretions in humans contain enzymesand proteases to liquify the semen, citric acid, acid phosphatase, phospholipids andspermine. Among this individual fructose occupies a vital position as it serves as theenergy donor for the spermatozoa.

Fructose is the major carbohydrate secreted by the seminal vesicle and occupies asignificant portion of the seminal plasma. Estimation of fructose is significant as thelevels of fructose in seminal plasma has been directly correlated to normal spermmotility, concentration and testosterone secretion of the interstitial cells of Leydig(4).The secretions of the accessory glands may not be absolutely essential forfertilization but they optimize condition for sperm motility, transport and survival in boththe semen and female reproductive tract. Thus the accessory glands produce

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substancesspecific for each gland which can be used as markers to determine glandular function (2). Accordingly, fructose is used as a marker for seminal vesicle andcitric acid/acid phosphatase is used as a marker for prostate.

Citric acid is an essential and a vital biochemical constituent of seminal plasma which not only rebounds the condition of the prostate, but also allied with coagulation and liquefaction of semen in humans. The importance of citric acid in altering sperm attributes during abstinence has been underrated, inspecting the levels of citric acid in seminal plasma thus may benefit to find the probable causes of male infertility (5).

Body mass index (BMI) has been demonstrated to affect female fertility; however, little information is available on the impact of BMI on male fertility or semen parameters. Overweight appears to be one of the major and neglected causes of infertility. Men with low BMI (<20 kg/m^2) may present with an abnormal semen analysis. Overweight and obesity are expected to be associated with changes to the male reproductive hormone profile. It has already been found that a high BMI is associated with alterations in the levels of testosterone and estrogens, as well as sex-hormone binding globulin (6).

Materials And Methods:-

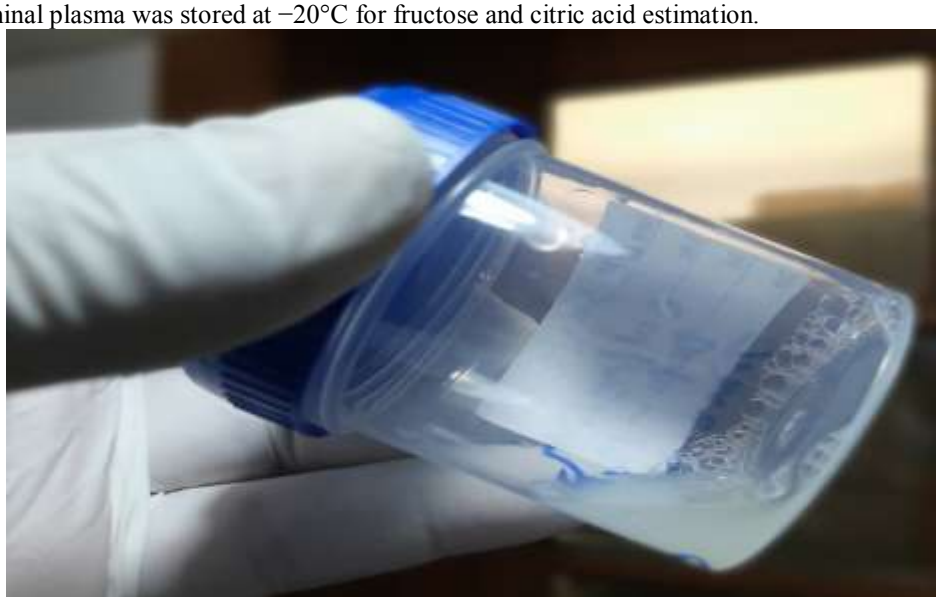
150 subjects (100 test and 50 controls) aged between 23-30 years were included in the study. All the samples were collected and tested in GK labs, Srinagar Jammu and Kashmir. Semen sample was collected after 3 days of ejaculatory abstinence according to World Health Organization criteria 2010 after taking proper consent from the subject.

Exclusions:

1. Patients suffering from chronic diseases such as diabetes, kidney disease, atherosclerosis, vascular disease and hypertension
2. Subjects with genital diseases, heavy smoking.
3. Patients having any previous surgery (e.g., vasectomy reversal or varicocele removal).

Sample Collection:

After ejaculatory abstinence of 3–5 days, semen samples from patients and controls were collected in a sterile plastic container and examined after 30 min according to World Health Organization -2010 criteria(7). Infertile groups were classified based on sperm concentration, motility, and morphology. Later, samples were centrifuged at 3000 rpm for 10 min and seminal plasma was stored at −20°C for fructose and citric acid estimation.



Estimation of fructose:

20 µL of plasma from a seminal fluid sample was thoroughly combined with 220µL of distilled water. The solution was then deprotonated using 50 µL of ZnSO4 and 50 µL of NaOH. A sample was spun at 2500 rpm for 15 minutes,

and the clear liquid that resulted was combined with 200 µL of Indole reagent and 32% hydrochloric acid. The absorbance at 470 nanometers was measured after cooling the mixture that had been heated for 20 minutes at 60°C.

Estimation of Citric acid:
100 µl seminal plasma was mixed with 100 µl of 50% trichloro acetic acid (TCA) and stored in an ice bath for cooling. The supernatant was centrifuged at 2000 rpm for 15 min. 800 µl of anhydrous acetic anhydride was added to 100 µl of supernatant and incubated at 60°C for 10 min in a water bath. Later, dry reagent grade pyridine was added and incubated at 60°C for 40 min. Cooled on ice bath for 5 min and absorbance was measured at 400 nm.

Body mass index (BMI):
It is a measure of body fat based on height and weight that applies to adult men and women.We used the following table as a reference and calculated the BMI using the formula:

Weight(kg) divided by height (m^2).

Evaluation	**Measure**
Underweight	<18.5
Normal	18.5-24.9
Overweight	25-29.9
Obese I	30-34.9
Obese II	35-39.9
Morbidly obese	≥40

Results:-
The observed values of sperm count, fructose levels, citric acid levels and BMI in semen sample of fertile (control) and infertile (test)subjects

Parameters	**Control (n=50)**	**Test (n=100)**	**Significance**
Sperm count (mill/ml)	56.57 ± 8.47	15.8 ± 2.85	<0.0001
Fructose (µmole/ejaculate)	108.29±2.97	92.68±3.75	< 0.0001
Citric acid (µmole/ejaculate)	36.70 ±1.65	42.16±1.22	< 0.0001
Body mass index	24.54 ±2.19	30.36 ± 4.45	< 0.0001

Discussion:-
In this study, BMI was found to be significantly related to the semen parameters like sperm count, fructose levels and citric acid levels which suggest that higher BMI value was associated with infertility.

Lewis Jones, et al., (1996) found that fructose concentrations were inversely ratio to sperm motility with R=-, 062 (p <0.05(9). However, Andrade Rocha FT (2001) confirmed that seminal fructose concentration was related to sperm concentration, survival, motility and morphology, but the results were not statistically significant(10). In the study of Ndovi (2006), seminal fructose concentration was negatively correlated with sperm motility (R=-0.04) but not statistically significant (11). Jansen et al. in 2004found out that a higher BMI value was related to lower sperm concentration and sperm count inDanish military conscripts (12).MacDonald et al.,investigated the association between BMI and semen characteristics or sperm concentration and total sperm count but were not able to show any association [13], but the analysis was limited by a low number of included studies. Sermondadeset al included a larger number of studies and more than 13000 men [14]. No association was found between BMI and sperm concentration or total sperm count when comparing means across BMI groups. However, a significantly increased risk of azoospermia and oligozoospermiawas found in overweight and obese men, indicating that high BMI may affect sperm production.

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