Section: Anatomy

Original Research Article

ASSOCIATION OF SEMINAL BIOCHEMICAL MARKERS, BODY MASS INDEX, SEMEN PARAMETERS, AND Y-CHROMOSOME MICRODELETIONS WITH MALE INFERTILITY IN THE POPULATION OF WEST BENGAL

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ABSTRACT

Background: Almost half of all cases of infertility are caused by male factors, making infertility a serious public health concern that affects millions of couples globally. Numerous physiological, metabolic, genetic, and lifestyle-related factors contribute to the complicated situation of male infertility. Of them, genetic disorders such as microdeletions in the Y chromosome and obesity are important contributors to poor spermatogenesis. A common measure of obesity, body mass index (BMI), has been connected to hormonal abnormalities that impair sperm production and testicular function. Furthermore, fructose and citric acid, two biochemical markers found in seminal plasma, are crucial for sperm motility, metabolism, and overall fertility. These markers also reflect the functional status of accessory reproductive glands such as the seminal vesicles and prostate. Furthermore, Y-chromosome microdeletions in the AZF (Azoospermia Factor) regions are among the most common genetic causes of male infertility, particularly in cases of azoospermia and severe oligozoospermia. The present study is designed to evaluate semen parameters, seminal fructose and citric acid levels, body mass index (BMI), and the presence of Y-chromosome microdeletions in infertile males from West Bengal. The study also aimed to analyse the interrelationship among these variables and their combined impact on male fertility.

Materials and Methods: This study was conducted in IQ City Medical College Durgapur West Bengal from July 2023 till December 2023 with a total of 150 male participants, including 100 infertile cases and 50 fertile controls, aged 23-30 years. BMI was calculated using the standard formula (weight in kg divided by height in meters squared). Molecular analysis for Y-chromosome microdeletions in the AZFa, AZFb, and AZFc regions was performed using multiplex polymerase chain reaction (PCR).

Results: The infertile group showed significantly lower sperm count (15.8 ± 2.85 million/ml) compared to the control group (56.57 ± 8.47 million/ml). Seminal fructose levels were significantly reduced in infertile men (92.68 ± 3.75 µmol/ejaculate) compared to fertile controls (108.29 ± 2.97 µmol/ejaculate). In contrast, seminal citric acid levels were significantly higher in infertile subjects (42.16 ± 1.22 µmol/ejaculate) than in controls (36.70 ± 1.65 µmol/ejaculate). BMI was significantly higher among infertile men (30.36 ± 4.45) compared to fertile controls (24.54 ± 2.19), indicating a strong association between obesity and male infertility. Y-chromosome microdeletions were detected in 5% of infertile men, with the majority involving the AZFc region, which is commonly associated with impaired spermatogenesis.

Conclusion: The findings of this study suggest that higher BMI is significantly associated with deteriorated semen quality and altered seminal biochemical markers, highlighting the negative impact of obesity on male reproductive health. Additionally, Y-chromosome microdeletions, particularly in the AZFc region, contribute to severe spermatogenic failure in a subset of infertile men. Therefore, a combined approach involving biochemical analysis, semen evaluation, and genetic screening can enhance diagnostic accuracy and improve management strategies for male infertility.
Keywords: Male infertility, Seminal biochemical markers, Semen analysis, Semen parameters, Y-chromosome microdeletions, AZF deletions, Azoospermia factor (AZF), Body mass index (BMI)

INTRODUCTION

Infertility is recognised as a major global health concern, affecting approximately 15–19% of couples worldwide. Among these, male infertility accounts for nearly 50% of cases either as a primary or contributing factor. In recent years, increasing trends in infertility have been observed due to changing lifestyles, environmental exposure, stress, and metabolic disorders. In the region of Jammu and Kashmir, a considerable proportion of the population faces infertility-related problems, which may be influenced by socioeconomic conditions, environmental pollutants, dietary habits, and genetic predispositions. [1-6]

Role of Seminal Plasma and Biochemical Markers

Semen is composed of spermatozoa suspended in seminal plasma, which constitutes nearly 90–95% of the total ejaculate volume. Seminal plasma is produced by accessory reproductive glands, mainly the seminal vesicles, prostate, and bulbourethral glands. It provides a protective and nutritive environment for sperm cells, facilitating their survival, motility, and fertilizing ability. [7-10]

Seminal vesicles contribute fructose, which serves as a primary energy source for sperm motility and metabolism. Low levels of seminal fructose may indicate impaired seminal vesicle function or obstruction of the ejaculatory ducts. The prostate gland secretes citric acid, which plays a role in semen liquefaction, buffering capacity, and sperm metabolism. Abnormal citric acid levels may reflect prostatic dysfunction or inflammation. Therefore, measuring these biochemical markers provides valuable insights into accessory gland function and sperm health. [11-15]

Impact of BMI on Male Fertility: Body mass index (BMI) is widely used as a measure of obesity and overall body fat composition. Obesity has been associated with multiple adverse effects on male reproductive health, including hormonal imbalance, reduced testosterone levels, increased estrogen levels, and impaired spermatogenesis. Excess adipose tissue promotes the conversion of testosterone to estrogen through aromatization, leading to decreased sperm production and quality. [16]

Several studies have demonstrated that overweight and obese men exhibit lower sperm concentration, reduced motility, abnormal morphology, and increased DNA fragmentation. Obesity is also associated with increased oxidative stress, which can damage sperm cells and further compromise fertility. [17-19]

Genetic Factors: Y-Chromosome Microdeletions: Spermatogenesis is regulated by genes located in the AZF (Azoospermia Factor) regions of the Y chromosome, which are divided into AZFa, AZFb, and AZFc. Microdeletions in these regions are among the most common genetic causes of male infertility, particularly in men with azoospermia or severe oligozoospermia.

Detection of Y-chromosome microdeletions is important for clinical diagnosis, prognosis, and genetic counselling, especially for couples considering assisted reproductive techniques such as intracytoplasmic sperm injection (ICSI).

The present study integrates biochemical, physiological, and genetic aspects of male infertility in the population of Jammu and Kashmir to provide a comprehensive understanding of its underlying causes.

MATERIALS AND METHODS

Study Population: This Case-control prospective study was conducted in IQ City Medical College Durgapur West Bengal from July 2023 till December 2023 with a total of 150 male participants, including 100 infertile cases and 50 fertile controls, aged 23-30 years. The subjects were divided into two groups:

- Test group: 100 infertile males diagnosed based on semen analysis
- Control group: 50 fertile males with proven fertility

Samples were collected after obtaining informed consent from all participants.

Inclusion Criteria

- Men clinically diagnosed with infertility based on semen analysis
- Age between 23 and 30 years
- No history of recent fever or acute illness

Exclusion Criteria

- Individuals suffering from chronic diseases such as diabetes, hypertension, or kidney disease
- Men with known genital infections or history of testicular surgery
- Heavy smokers or alcohol abusers
- Men undergoing hormonal therapy

Semen Collection and Analysis

Sample Collection:

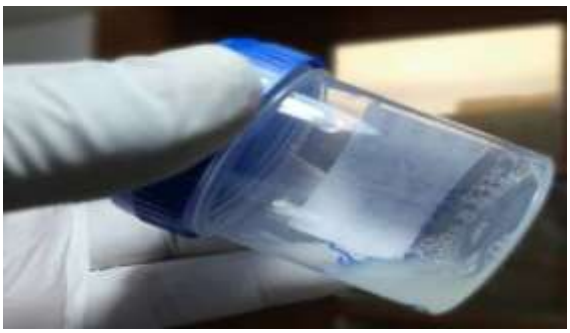
All the samples were collected and run in the institutional lab (NABL accredited). After ejaculatory abstinence of 3–5 days, semen samples from patients and controls were collected in a sterile plastic container and examined after 30 min according to World Health Organization -2010 criteria. [7] Infertile 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. The following parameters were assessed:

- Sperm count
- Sperm motility
- Semen volume
- Sperm viability
- Semen pH

Semen samples were analysed according to the WHO 2010 guidelines. Biochemical estimation of seminal fructose and citric acid was carried out using standard laboratory methods.

Estimation of Seminal 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 minute 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 Seminal 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.



BMI Calculation

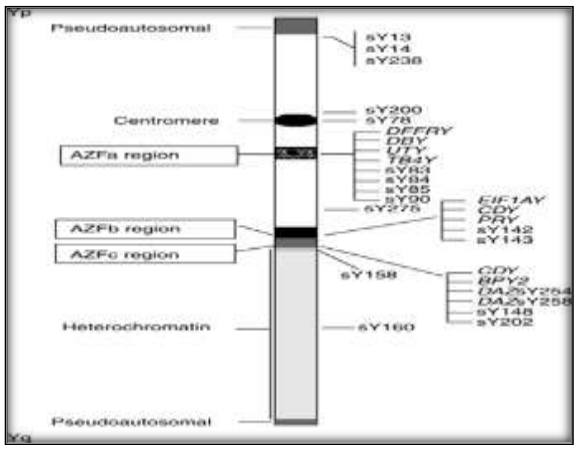
The height and weight were recorded using standard stadiometer and digital weigh machine**.**

BMI was calculated using the formula:

BMI = Weight (kg) / Height (m²)

Molecular Analysis for Y-Chromosome Microdeletions

DNA was extracted using a Nucleon BACC1 kit from peripheral blood samples taken from infertile guys. Genomic DNA was extracted using the phenol-chloroform technique from samples obtained from both the control group and test group. Multiplex PCR was performed using specific primers targeting AZFa (sY84, sY86), AZFb (sY127, sY134), and AZFc (sY254, sY255) regions. Amplified products were analyzed using gel electrophoresis.



RESULTS

Sperm count: Based on the present study, 4 different infertile groups were demonstrated as depicted in [Table 1].

Table 1

S.no	Infertile subgroups	n = 100	Percentage
1	Aspermia	12	12%
2	Azoospermia	49	49%
3	Oligozoospermia	16	16%
4	Teratozoospermia	26	26%

Table 2: Comparison of semen parameters between fertile and infertile subjects.

Semen parameters	Test (n=100)	Control (n=50)	P value
Sperm count	15.8 ± 2.85	56.57 ± 8.47	<0.0001
Motility	37.6±1.8	54.4 ±1.8	<0.0001
Volume	2±0.8	2.6±0.1	<0.0001
Viability	53.7±1.4	66±1.2	<0.0001

pH: WHO criteria for normal pH ranges from 7.2 -7.8.

Table 3: Shows the observed value of semen pH in 100 patients.

pH	Total subjects=100
>7.8 (alkaline)	69 %
7.2-7.8 (normal)	21%
<7.2 (acidic)	10%

Infertile men exhibited significantly lower sperm count, motility, volume, and viability compared to fertile controls ($p < 0.0001$). These findings indicate compromised spermatogenesis and reduced semen quality in the infertile group.

Seminal fructose levels were significantly lower in infertile men, suggesting impaired seminal vesicle function or reduced sperm metabolic activity. Conversely, elevated citric acid levels in infertile subjects may indicate altered prostatic secretion or inflammatory changes.

BMI was significantly higher in infertile men, supporting the hypothesis that obesity negatively affects male reproductive function.

Y-chromosome microdeletions were detected in 5% of infertile men, with AZFc being the most commonly affected region. These deletions were associated with azoospermia or severe oligozoospermia, confirming their role in impaired spermatogenesis.

Table 4: ?

S.no	Deleted region	Spermatozoa in spermogram	Clinical diagnosis	Karyotype	Phenotypic data
1	AZFc	0	Azoospermia	46, XY	Android adiposity, gynaecomastia, brachydactyly
2	AZFc	5 (motionless spermatozoa in spermogram	Severe oligozoospermia	46, XY	Epidemic parotitis in childhood, in testis biopsy only spermatids found
3	AZFc	1-2 immobile spermatozoa in spermogramm	Severe oligozoospermia	46, XY	Epidemic parotitis in childhood
4	AZFa, AZFb, AZFc	0	Azoospermia	46, X del (Y)(q)	Android adiposity. Gynaecomastia, small testis in right side
5	AZFa, AZFb, AZFc	0	Azoospermia	46, X del (Y)(q)	No data

DISCUSSION

The present study highlights a strong association between elevated BMI and impaired semen quality, reinforcing the role of obesity as a major risk factor for male infertility. Higher BMI is linked to hormonal disturbances, oxidative stress, and metabolic abnormalities that negatively impact sperm production and function.

Reduced seminal fructose levels in infertile men suggest compromised seminal vesicle activity, which may affect sperm motility and energy supply. Increased citric acid levels may reflect altered prostatic function or inflammation, further contributing to reduced fertility.

Genetically, Y-chromosome microdeletions were present in 5% of infertile men, consistent with global prevalence rates. AZFc deletions were the most common, aligning with previous research that identifies this region as crucial for spermatogenesis.

Overall, the study emphasizes the importance of a multidisciplinary approach combining semen analysis, biochemical testing, and genetic screening for accurate diagnosis and management of male infertility.

CONCLUSION

Higher BMI is significantly associated with male infertility and poor semen quality.

Infertile men exhibit reduced seminal fructose levels and altered citric acid concentrations, indicating accessory gland dysfunction.

Y-chromosome microdeletions, particularly in the AZFc region, are a significant genetic cause of infertility.

A comprehensive diagnostic approach integrating semen analysis, biochemical assessment, and molecular testing should be adopted for effective evaluation and management of male infertility.

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