RESEARCH PAPER

Biochemical Markers In Seminal Plasma And Serum: A Comparative Assessment In Fertile And Infertile Men

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Abstract

Male infertility is primarily diagnosed through semen analysis; however, this method provides only a limited understanding of the underlying causes. Various factors, including infections, systemic diseases, and environmental exposures, can alter semen composition hence semen analysis is alone to diagnose of male infertility and often leads to inconclusive results and delayed diagnoses. To address these limitations, current research is focused on identifying biomarkers in seminal plasma, which may enhance diagnostic accuracy. Seminal plasma is a crucial biological fluid containing key reproductive gland secretions essential for sperm motility and its overall function. **Aim:** The present study aims to conduct a comparative analysis of biochemical markers in seminal plasma and serum among fertile and infertile men.

Methodology: this prospective study was carried out in the Department of Embryology, Chennai, India. A total of 76 male participants, categorized as either fertile or infertile, were assessed for serum and seminal plasma levels of biochemical markers. Glucose, urea, uric acid, copper, albumin, SGOT, SGOT, LDH, and total cholesterol were determined using spectrophotometric method. Statistical analysis revealed significant differences between the groups, highlighting the importance of biochemical factors in male fertility.

Result: no significant differences were observed in serum biochemical parameters. However, in seminal plasma, levels of lactate dehydrogenase (LDH), total cholesterol, aspartate aminotransferase (SGOT) were significantly different ($p<0.01$) between the groups.

Conclusion These findings underscore the potential of seminal plasma biochemical profiling as a valuable diagnostic tool for evaluating male fertility. The role of trace elements and biochemical markers in sperm function is critical, offering essential insights into the diagnosis, management, and prevention of male infertility.

Keywords: Seminal Plasma; Blood serum; Biochemical Markers; Male infertility

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1. Introduction

Global infertility prevalence is increasing and currently affects around 20% of all couples (Chandra *et al*., 2013; Rezaeiye*et al*., 2022). One crucial indicator of male reproductive health is the quality of semen. Numerous factors have been demonstrated to have an impact on semen quality, including age, lifestyle, socioeconomic status, environmental exposure, anatomical anomalies, endocrine disorders, or the use of specific drugs (Agha et al.,2022; Chen et al., 2024). Semen is a complex fluid aggregation that is composed of 5% cellular (mostly sperms) and 95% non-cellular components (seminal plasma) (Agha et al., 2022; Vashisht&Gahlay, 2024). Semen analysis (spermogram) after three days of abstinence is the main diagnostic method for male infertility to differentiate based on factors like sperm count, morphology, vitality, progressive motility, etc. (Mahdi, 2021; Placzkowska et al., 2024). Some of the common male fertility disorders are oligozoospermia featuring low sperm count of less than 15 million sperm/ml, asthenozoospermia characterized by low sperm motility, teratozoospermia where abnormal sperm

morphology is seen, azoospermia showing absence of sperm, sperm necrosis involving decreased vitality and other mixed disorders (Vashisht et al., 2021; Agha et al., 2022; Placzkowska et al., 2024).

The spermogram which concentrates on sperm count, motility, morphology, and percentage of viable cells does not offer complete information regarding the underlying causes of specific fertility disorders andconsidering spermogram alone as a diagnostic method of male fertility sometimes fail; few reported cases there where assome men with normal semen characteristics would be categorized as infertile (Allahkarami et al., 2017; Placzkowska et al., 2024). In 30-40% of infertile patients, physical examinationand hormone testing along with semen analysis have been unable to determine the cause of their infertility (Vashisht&Gahlay, 2024). Seminal plasma (SP), a heterogeneous complex fluid made up of the secretions of the testicle, epididymis, and dependent sex glands, is crucial for male fertility (Agha et al., 2022). Numerous biological components, including enzymes, proteins, hormones, peptides, cytokines, various cations, DNA, cholesterol, RNA, etc. as well as non-protein nitrogenous compounds (NPNs) like ammonia, urea, uric acid, creatinine and other substances, make up SP (Allahkarami et al., 2017; Placzkowska et al., 2024). trace elements like copper, zinc, sodium, calcium, magnesium, potassium and manganese essential for spermatogenesis, sperm maturation, sperm motility, and capacitation are also found in SP (Chen et al., 2024). Current research aims to understand infertility resulting from changes in levels of these molecules present in the seminal SP. SP thus as useful as a non-invasive diagnostic fluid and a source of information about the processes causing diminished male fertility the components or parameters of its rich and varied makeup can be used as biochemical markers to determine male fertility (Placzkowska et al., 2024; Vashisht&Gahlay, 2024). Blood serum is another biofluid that is routinely accessed for early disease detection and analysis of disorder progresses (Zhang et al., 2012). The levels of different parameters in serum may be translated into their corresponding levels in SP (Placzkowska et al., 2024). Serum study can complement SP analysis in analyzing male fertility.

Urea and uric acid levels in SP play an important role in male fertility as they have a significant negative correlation with sperm motility, morphology, and fertilization rate (Allahkarami et al., 2017). There are differences in the uric acid concentrations in the seminal plasma of infertile and normal people. Regulating the amounts of urea and uric acid in SP may enhance the rate of fertilization and the results of assisted reproductive technology (ART). Oxidative stress, caused by hyperuricemia in serum impacts several organs and systems including the male reproductive system (Ma et al., 2022). Copper (Cu), a microelement present in SP is an essential part of many metalloenzymes like ceruloplasmin, and superoxide dismutase which regulate vital energy metabolic pathways and redox-equilibrium-related processes (Vashisht&Gahlay, 2024). Oligozoospermic, asthenozoospermic, and azoospermic patients have substantially greater SP-Cu concentrations than normozoospermic people (Herman et al., 2020). Variations in serum Cu level is significant as they may result in smaller ejaculates and affect sperm concentrations, sperm motility and morphology. The metrics returned to normal after the Cu concentration was changed, making Cu another biomarker in male fertility (Yuyan et al., 2008).

Male infertility, like numerous other illnesses, has been linked to ROS (reactive oxygen species) and oxidative stress (Agha et al., 2022). Oxidative stress damages sperm axons, produces a rapid loss of sperm ATP, makes morphological abnormalities in sperm, and reduces sperm motility (Ma et al., 2022). Serum and SP albumin, an antioxidant providing thiol groups for "chain breaking" antioxidant actions, can be used as a biomarker (Palani, 2018). Men who are infertile had lower albumin levels than men who are fertile, which may impair serum and SP antioxidant capacity. Low antioxidant levels in SP cause ROS to destroy sperm tail membrane. Giving infertile men exogenous antioxidants like albumin in ART significantly increased sperm motility

Studies showed the association between male fertility and SP-cholesterol with lower levels in teratozoospermic men than those of normozoospermic fertile men. (Mehrparvar et al., 2020; Placzkowska et al., 2024). Serum cholesterol is the main building block for the synthesis of a number of steroids and hormones that have been shown to affect male steroidogenesis. Sperm lipid content has also been linked to increasing sperm motility. Patients with oligo-asthenozoospermia and teratozoospermia were shown to have greater sperm

concentrations of total HDL and LDL cholesterol as well as triglycerides than those with normozoospermia (Vashisht&Gahlay, 2024). Human sperm cholesterol levels vary greatly, even between ejaculates; on the other hand, sperm morphology and reproductive potential are strongly correlated with the percentage of cholesterol in sperm membranes (Pakpahan et al., 2022). Insulin resistance can result in hyperglycemia, which damages sperm DNA by increasing the production of ROS in the testes and epididymis (Zanko et al., 2024). Glucose metabolism is a key component of spermatogenesis, which is the process by which basic cell activities and their unique characteristics, like motility and the activity are maintained. Noticeably elevated glucose levels are seen in infertile patients (Placzkowska et al., 2024). This is corroborated by the fact that spermatozoa use glucose as an energy source for metabolic functions. Greater SP-glucose content in teratozoospermia patients could be due to the increased number of defective spermatozoa using less glucose. Serum and SP levels of cholesterol and glucose can therefore be used for male fertility analysis.

Enzymes like testis-specific lactate dehydrogenase (LDH-C4), leaked from sperms into SP is also a biomarker for male fertility because it maintains glycolysis and adenosine triphosphate (ATP) synthesis in sperm flagella during sperm capacitation. Fertile men showed a greater significant increase in LDH-C4 activity than did the oligospermia and azoospermia groups. Sperm motility and count may be adversely affected by decreased LDH-C4 activity caused by mitochondrial abnormalities in the area where LDH-C4 is situated (Saeed et al., 2021). There is a negative correlation between another enzyme, serum glutamate pyruvate transaminase (SGPT) and the percentage motility, which, in conjunction with low LDH levels, may account for the motility deficit in asthenozoospermia sperms (Vashisht et al., 2021). This, along with serum glutamate oxaloacetic transaminase (SGOT) in SP are linked to the secretory activity of male accessory sex glands. Serum and SP levels of LDH, SGPT, and SGOT can be employed as indicators for male fertility.

Like serum, seminal plasma consists of rich biochemical components. It has been shown recently that seminal plasma proteins could serve as important biomarkers for male infertility (Macanovic*et al*., 2015). In addition, functional proteomic analysis revealed that proteins are over or under expressed in the seminal plasma of men with poor semen quality (Sharma *et al*., 2013; Parvin *et al*., 2024). However, the origins of these components and their correlations with those in serum are unclear. It is also unknown whether these components could be used to evaluate male fertility. Several studies have been done to compare the levels of biochemical markers in seminal plasma and serum, most of these studies focused on animal reproduction, and investigations on biochemical markers were limited. Moreover, the origins and potential physiological effects of biochemical components in seminal plasma have been still poorly understood; thus, we designed this study to detect the levels of various kinds of biochemical markers in seminal plasma and serum on the basis of quality control for each marker, and all the data were compared and analyzed.

By assessing and contrasting biochemical indicators and heavy metal concentrations in seminal plasma among four groups— Oligoasthenoteratozoospermia (OAT), Severe oligoasthenoteratozoospermia (SOAT), Teratozoospermia (TERATO) and a healthy control group (Normozoospermia)—the present investigation fills this knowledge gap. It is anticipated that the results would shed light on the pathophysiology of infertility and promote the creation of focused treatment plans to reduce environmental risk factors. In order to support more precise diagnosis and individualized treatment choices, this current study aims to provide a fundamental understanding of the biochemical and heavy metal profiles that distinguish various infertility etiologies.

2. Materials and Methods

2.1 Study design and participants

This prospective study included a total of 76 subjects who attended the fertility clinic at Chennai Fertility Centre and Research Institute, Chennai, India. Subjects were grouped into four categories including three diseased conditions and one healthy control group. Each group is designated with terminology based on WHO criteria and listed in Table 1. Among 76 subjects, 26 men were identified as fertile and were assigned to the control group (NORMO) whereas 17 were diagnosed with teratospermia (TERATO), 19 with oligoasthenoteratozoospermia (OAT), and 14 with severe oligoasthenoteratozoospermia (SOAT). All men presenting for infertility evaluations were included in the study group (inclusive criteria). Individuals in the

NORMO group consisted of healthy volunteers with no history of fertility problems and whose partners conceived spontaneously within one year of regular, unprotected intercourse or were currently pregnant in the 1st trimester or recently delivered a child less than 6 months from the date of enrollment in the study, provided these are natural pregnancies without any medical interventions. However, men aged below 22 years and those who had undergone radiotherapy and chemotherapy related to malignancy were excluded from the study.

Table 1. Terminologies and characteristics of four groups in the study.

Group / Sl. No.	Term	Characteristics
1	Normozoospermia	Semen sample with normal characteristics including sperm concentration of 16 million/mL or more, Sperm motility (progressive+ non progressive) being < 42%, normal sperm morphology
2	Oligoasthenoteratozoospermia (OAT)	Decreased concentration (<16 million/mL), Decreased motility percentage (< 42%), decreased normal sperm morphology (< 4%)
3	Severe oligoasthenoteratozoospermia (SOAT)	low sperm count, poor motility, poor morphology
4	Teratospermia	Decreased normal sperm morphology (<4%)

2.2 Ethical consideration

The study was conducted after getting approval from Research Committee of the Institutional Ethical Committee of Chennai Fertility Centre and Research Institute (CFCRI/IHEC/2023/001). Informed consent was obtained from all participants before sample collection.

2.3 Collection of semen sample and evaluations of physical and morphological characteristics

Semen samples were collected from four groups of men and were subjected to physical and morphological evaluations following the protocol described by Franken *et al*. (2011). At first, samples were collected by masturbation into a sterile container after 2-7 days of abstinence. The specimens were then allowed to liquefy at room temperature for 30 minutes before analysis. Sperm count, percentage of motile sperm, and sperm with normal morphology were objectively evaluated through microscopic examination. These parameters were assessed according to the WHO Laboratory Manual for the Examination and Processing of Human Semen (WHO, 2021) (Scheiber*et al*., 2017).

2.4 Preparation of serum sample for biochemical analysis

Blood samples were collected from the study participants,5 mL of blood was drawn from a hand vein. Following collection, the blood was placed in gel tubes and allowed to clot. After clotting, the samples were centrifuged at 2000 rpm for 10 minutes to separate the serum (Agha *et al*., 2022). The serum was then collected in gel tubes and was immediately used for measuring the levels of biochemical markers specified in this study. All samples were sent to the Biochemistry Department at Chennai Fertility Centre for analysis.

2.5 Preparation of seminal plasma sample for biochemical analysis

After the evaluation of the physical and morphological parameters, the seminal plasma samples were centrifuged at 1500 rpm for 10 minutes to remove the cellular components. The supernatant was then carefully

extracted for the assay, ensuring the pellet at the bottom remained undisturbed (Kumar *et al*., 2020).

2.6 Estimation of biochemical parameters in serum and SP samples

The biochemical levels of specified markers were quantified using the ErbaChem 5X Semi-Automated Biochemistry Analyzer® spectrophotometric analyzer.

2.6.1 Estimation of uric acid

Uric acid, which absorbs light at 293 nm, is converted by uricase to allantoin, that is non-absorbing at 293 nm. The change in absorbance at 293 nm due to the disappearance of uric acid is directly proportional to the concentration of uric acid in the sample (serum / SP) and is measured using a dichromatic (293,700 nm) endpoint technique (Mohammed Jumaah, 2013; Allahkarami*et al*., 2017).

2.6.2 Estimation of urea

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia is used by the enzyme glutamate dehydrogenase (GLDH) to reductively aminate α-ketoglutarate (α-KG), with simultaneous oxidation of reduced nicotinamide-adenine dinucleotide (NADH). The change in absorbance at 340 nm due to the disappearance of NADH is directly proportional to the urea concentration in the sample (serum / SP) and is measured using a dichromatic (340, 383 nm) rate technique (Zou *et al*., 2016).

2.6.3 Estimation of copper (Cu)

The estimation of Cu was done using kits from Diaspertz. The test principle can be outlined as follows. At pH 4.7, Cu is released from the carrier protein and forms a chelate complex with 4-(3,5-Dibromo-2-Pyridylazo)-N-ethyl-N-sulfopropylanaline. The increase of absorbance of this complex is proportional to the concentration of total Cu in the sample (serum / SP) (Hussain, 2020; Mayasula*et al*., 2020). The concentration of copper present in sample can be calculated using the formula: Copper [µg/dL] = Sample / Standard X Concentration of Standard [µg/dL]

2.6.4 Estimation of albumin

In the presence of a solubilizing agent, BCP binds to albumin at pH 4.9. The amount of albumin-BCP complex is directly proportional to the albumin concentration in the sample. The complex absorbs at 600 nm and is measured using a polychromatic (600, 540, 700 nm) endpoint technique (Sharma *et al*., 2013; Dan *et al*., 2013).

2.6.5 Estimation of total cholesterol

Serum and SP total cholesterol was determined by enzymatic method (Allain *et al*., 1974; Pesce and Bodourian, 2022). Total cholesterol is analyzed by mixing 5 µL of sample with a reagent consisting of cholesterol esterase, cholesterol oxidase, catalase, acetylacetone, methanol, and hydroxy poly ethoxy dodecane in an ammonium phosphate buffer at pH 7.0. The rate of increase in absorbance of the dihydro lutidine product is measured at 37^0C and 405 nm. The change in absorbance between 4 and 10 min is used to calculate the cholesterol concentrations by using simultaneously determined free cholesterol standards.

2.6.6 Estimation of glucose

The method for glucose estimation relies on the enzymatic reaction of the sample where glucose is oxidized by the enzyme glucose oxidase to produce gluconic acid and hydrogen peroxide (Mahmoud, *et al*., 1998). The hydrogen peroxide reacts with a chromogenic oxygen acceptor (e.g., phenol and 4-aminoantipyrine) in the presence of peroxidase, forming a colored complex. The intensity of the color is directly proportional to the glucose concentration and is measured spectrophotometrically at 407 nm

2.6.7 Estimation of lactate dehydrogenase (LDH)

The LDI method for estimation of Lactate dehydrogenase (LDH) uses L-lactate buffered at a pH of 9.4 as substrate. LDH oxidizes the substrate in the presence of NAD+ to yield pyruvate and NADH which absorbs at 340 nm. LDH activity is measured as a rate reaction at 340/700 nm, proportional to the amount of lactate dehydrogenase in the sample (Hashemi *et al*., 2016; Kumar *et al*., 2018).

2.6.8 Estimation of serum glutamate-pyruvic transaminase (SGPT)

The SGPT enzyme catalyzes the reversible transfer of an amino group from L-alanine to α-ketoglutarate, forming pyruvate and L-glutamate. In the presence of lactate dehydrogenase (LDH), pyruvate is reduced to L-lactate using NADH (Nicotinamide Adenine Dinucleotide, reduced form), which is oxidized to NAD+ in the process. The rate of decrease in NADH, measured at 340 nm, is directly proportional to the SGPT activity in the sample (Rosecrans *et al*., 1987; Vashisht*et al*., 2021).

2.6.9 Estimation of serum glutamate oxaloacetic transaminase (SGOT)

The SGOT enzyme catalyzes the reversible transfer of an amino group between L-aspartate and α-ketoglutarate, forming oxaloacetate and L-glutamate. The oxaloacetate produced reacts with NADH (Nicotinamide Adenine Dinucleotide, reduced form) in the presence of malate dehydrogenase (MDH), which is subsequently converted into NAD+. The decrease in absorbance due to the oxidation of NADH to NAD+ is measured at 340 nm and is proportional to the SGOT/AST activity in the sample.

2.7 Statistical analyses

Experiments for the estimation of various biochemical parameters were performed thrice. In each experiment, mean values were recorded along with their standard deviation values and at the end, average of three values and standard errors were taken to plot the corresponding graphs. Statistical analysis was done using SPSS software version 23 (Sun *et al*., 2017). Values with p-value <0.01 were considered significance.

3. RESULTS In the present study, semen and serum samples were collected from fertile and infertile men and were subjected to physical, morphological and biochemical evaluations. Semen samples from fertile men were considered as control group (NORMO), while those from infertile men were categorized as OAT, SOAT and TERATO groups based on sperm characteristics.

3.1. Evaluation of physical and morphological parameters of semen samples

The physical and morphological parameters of sperms, assessed through microscopic examination of semen samples, are presented in Table 2.Except for non-progressiveness, all other physical parameters, including semen volume, sperm concentration, motility, rapid progressiveness and slow motility, were significantly reduced in all three infertile groups compared to the control group. Among the infertile groups, semen volume was lowest in TERATO, while sperm concentration, motility and rapid progressiveness were lowest in SOAT. In contrast, slow motility was lowest in the OAT group. The sperms of the study groups also showed marked abnormalities in morphology. Table 2 shows that, semen samples from control group(fertile men) and study groups (infertile men) and the observed variations after post Hoc Tukey test ($p<0.005$)

Table 2. Evaluation of physical and morphological parameters

Physical parameters	Fertile (Mean ±SD)	Infertile (Mean±SD)			Significance
	NORMO	SOAT	OAT	**TERATO**	
Semen volume	3.20±1.26	2.35±0.82	2.4±0.78	1.96±0.74	**
Sperm concentration	31.90±11.78	4.28±3.99	8.3±2.84	21.88±4.38	**
Sperm motility %	61.5±7.47	34.28±5.34	34.33±9.04	53.53±4.92	**
Rapid progressive %	23.5±4.74	6.43±3.78	8±5.28	18.23±5.28	**
Slow motility %	26.5±5.79	17.14±6.98	15±5.67	24.12±5.07	**
Non progressive %	11.5±3.37	10.71±4.49	15.33±12.88	11.18±3.32	
Normal sperm Morphology %	6.1±1.73	1.00	1.00	1.41±0.51	**

** **NORMO –** Normozoospermia; **SOAT –** Severe Oligo Astheno Teratozoospermia; **OAT -** Oligo AsthenoTeratozoospermia; **TERATO** – Teratospermia.

3.2. Evaluation of biochemical parameters in Seminal plasma (SP) and serum samples3.2.1. Biochemical evaluation of SP samples The levels of various biochemical parameters estimated in the SP samples are illustrated in Figure 1A. Significant variations were observed in the levels of two key enzymes, serum glutamic oxaloacetic transaminase (SGOT) and lactate dehydrogenase (LDH), which are essential for cellular energy production, sperm quality and motility (Table 3). Among the study groups, the TERATO group exhibited the highest levels of these enzymes (Table 3). Similarly, significant differences were observed in the level of copper (Cu) and total

cholesterol among the control and infertile groups. The Cu level in the SP of OAT group was significantly higher than in the control group, whereas the SOAT group registered the lowest Cu level. Total cholesterol was highest in the TERATO group, followed by OAT and SOAT groups.

Variations were also observed in the levels of glucose, serum glutamic pyruvic transaminase (SGPT), albumin, urea and uric acid in SP samples across the four study groups. However, these differences were not statistically significant (Figure 1A). The mean uric acid level was highest in the control group and lowest in the TERATO group. Seminal plasma glucose levels were highest in the SOAT group while the control group had the lowest value. Albumin was absent in the SP of both the control and SOAT groups, with only negligible amounts detected in the other two groups. Additionally, urea levels in SP samples were higher in the control group compared to the infertile groups.

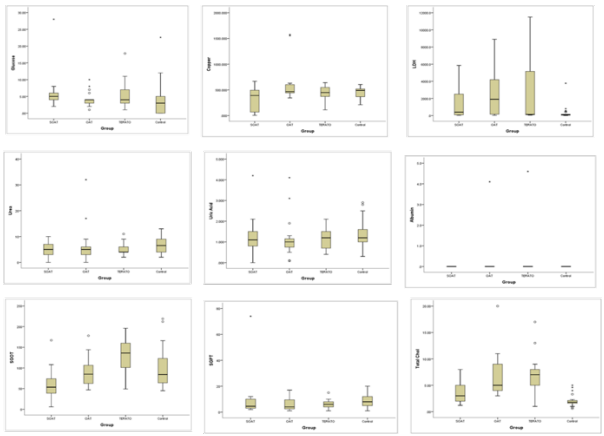

Figure 1: A Graphical Representation of biomedical analyses in SP samples of four groups under study

Figure 1 shows that, the graphs of the amounts of glucose, copper, LDH, albumin, urea, uric acid, SGOT, SGPT, and total cholesterol present in each out of four groups are depicted separately in the figure.Table 3 Shows that, The results of estimation of all biochemical markers in SP samples collected from all the four groups were analyzed, recorded and tabulatedafter post Hoc Tukey test, ($p<0.05$), significant values are given in bold.

Table 3. Biochemical marker analysis of SP samples

SP Parameters	SOAT	OAT	TERATO	Control	p value
Glucose	6.55±6.37 (2.87-10.23)	4.21±2.15 (3.17-5.25)	5.4±4.34 (3.17-7.63)	3.98±4.94 (1.99-5.98)	0.337
SGOT	**61.14±43.61 (35.96-86.32)**	**90.05±35.33 (73.02-107.08)**	**128.59±44.36 (105.78-151.39)**	**99±46.6 (80.18-117.82)**	**0.001**
SGPT	10.5±18.62 (-0.25-21.25)	6.11±4.69 (3.85-8.37)	6.41±3.34 (4.7-8.13)	8.69±5.24 (6.58-10.81)	0.459
LDH	**1478.79±1963.63 (345.02-2612.55)**	**2398.37±2662.95 (1114.87-3681.87)**	**2760.29±4135.61 (372.46-5148.12)**	**316.81±748.41 (7.88-625.74)**	**0.002**
Copper	**324.32±235.62 (188.28-460.37)**	**593.5±353.14 (423.3-763.71)**	**432.01±150.73 (354.51-509.51)**	**444.35±112.64 (398.85-489.85)**	**0.01**
Albumin	0±0 (0.0-0.0)	0.22±0.94 (-0.24-0.67)	0.27±1.12 (-0.3-0.84)	0±0 (0.0-0.0)	0.521
Urea	4.93±3 (3.2-6.66)	6.42±7.14 (2.98-9.86)	5.06±2.33 (3.86-6.26)	6.46±3.43 (5.08-7.85)	0.585
Uric Acid	1.26±1 (0.68-1.84)	1.17±0.96 (0.71-1.64)	1.14±0.54 (0.86-1.42)	1.36±0.67 (1.09-1.63)	0.808

Total Cholesterol	**3.51± 2.05 (2.33-4.69)**	**6.83±4.11 (4.79-8.87)**	**7.24±3.67 (5.35-9.12)**	**2.11± 1.08 (1.65-2.56)**	**0.000**

Control – Normozoospermia; **SOAT** – Severe OligoAsthenoTeratozoospermia; **OAT** - OligoAsthenoTeratozoospermia; **TERATO** – Teratospermia

3.2.2. Biochemical evaluation of serum samples

The level of the various biochemical parameters estimated in the serum samples are presented in Figure 1B. Serum glucose levels were significantly higher in all three infertile groups compared to the control group, with the highest value observed in the OAT group (Table 4). While no other biochemical markers showed statistically significant differences among the study groups, certain trends were noted. The OAT group exhibited the highest mean levels of uric acid, albumin, Cu, SGOT and LDH. In contrast, serum SGPT and urea levels were highest in the TERATO group. Similarly, total cholesterol levels were highest in the OAT group and lowest in the control group.

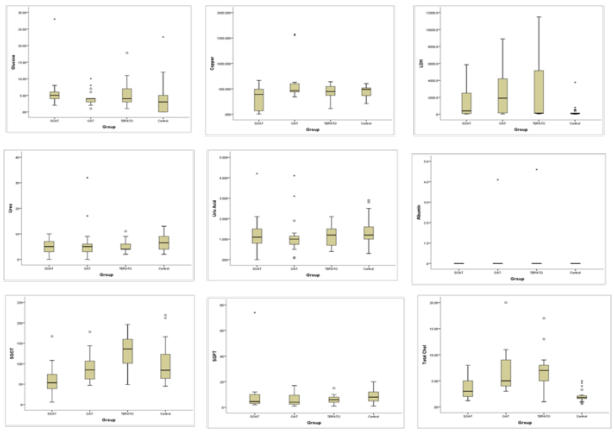

Figure 2: Graphical representation of biochemical analyses in serum samples of four groups under study

Figure 2 shows that, the graphs of the amounts of glucose, copper, LDH, albumin urea, uric acid, SGOT,SGPT, and total cholesterol present in each out of four groups are depicted separately in the figure.Table 4 shows that, The results of estimation of all biochemical markers in serum samples collected from all the four groups were analyzed, recorded and tabulated after post Hoc Tukey test ($p<0.01$).

Table 4. Biochemical marker analysis of serum samples

** **Control** – Normozoospermia; **SOAT** – Severe OligoAsthenoTeratozoospermia; **OAT** - OligoAsthenoTeratozoospermia; **TERATO** – Teratospermia.**4. DISCUSSION**

Diagnosis of male infertility is routinely done by assessing sperm motility, sperm counts and morphology (Mahdi, 2021). In the present study, reduction in sperm counts, % motility and abnormalities in morphology were observed in the OAT, SOAT and TERATO groups. These variations in the physical and morphological parameters of semen in these groups signify impairments in sperm production and function, indicating compromised fertility. Though these parameters are commonly used still today to evaluate male fertility status (Wang &Swerdloff, 2014), they have increasingly been proved to be inadequate and inaccurate over the years, requiring repeated sperm analyses (Vashisht&Gahlay, 2024). This is especially due to inconsistencies in results, as well as the prevalence of idiopathic infertile individuals. Therefore, it is crucial to find additional targets to diagnose male infertility. Seminal plasma (SP), constituting 95% of semen regulates sperm capacitation and aids sperm maturation. It can thus serve as a useful non-invasive biological tool having biomarkers linked to illnesses of the male reproductive system.

Uric acid (UA), a main anti-oxidant in SP can be used as a biomarker for male infertility (Ma et al., 2022). It removes hydroxyl free radicals and singlet oxygen species in association with Cu ions. So, male reproductive health, benefits from a higher level of SP-UA. This is in converse to serum-UA levels, where hyperuricemia results in oxidative stress. Though the present study showed the lowest SP-UA level in Terato group and the highest serum-UA concentration in the OAT group, these differences in UA levels between the control and study groups were not significant and so not conclusive. The amount of the trace element copper (Cu) in the SP of the control and study groups was estimated in this study as Cu is a cofactor for several enzymes involved in spermatogenesis and is crucial for the protection of sperms from oxidative damage, development of blood-testis barrier, synthesis of seminal fluid and also sperm motility (Elkhidir*et al*., 2022). Optimal levels of Cu are therefore essential for the functioning of male reproductive system-proteins like ceruloplasmin and superoxide dismutase (Herman et al., 2020; Manouchehri et al., 2022; Chen et al., 2024).

Both, Cu deficiency and abundance can result in male infertility. The infertility of this study's SOAT group could be due to higher total oxidant value of SP and the resulting oxidative damage which can be justified by its significantly elevated SP-Cu levels as known from the studies of Herman et al. (2020) and Manouchehri et al. (2022). The serum-Cu levels of this study were similar to the findings of Yuyan et al. (2008), who reported no significant differences between serum Cu levels of fertile and infertile men.

SP-glucose levels are higher in infertile individuals, malformed sperms utilize lesser glucose leading to its retention in SP (Placzkowska et al., 2024). Our results did not support this and SP-glucose levels of all the study groups did not significantly differ from the control group. However, its significantly higher serum levels in all of the study groups reestablish previous reports which link infertility and diabetes (Zhu et al., 2017; Panpalia et al., 2019; Mohammad & Ameen, 2021). Impaired glucose metabolism and insulin resistance thus led to reduced secretion of male reproductive hormones resulting in sperm defects (Zanko et al., 2024). These results therefore, emphasize the importance of estimating serum glucose levels in male fertility analysis.

Urea levels in SP and serum were evaluated in this study as urea helps with sperm motility, acrosome reaction and capacitation (Zhang et al.,2024). But its excess concentrations can be counteractive, leading to lesser mobility due to production of reactive oxygen species. There are differences in metabolic activity, renal function and nitrogenous waste processing between the fertile and the sub-fertile males which can be determined by differences in urea levels in SP and serum, emphasizing urea's importance as a potential male fertility biomarker (Allahkarami et al., 2017). However, SP- and serum urea levels of the study groups showed only slight variations that were not statistically significant.

The current study aimed to investigate the presence of globular vertebrate protein, 'serum albumin' in human SP and serum, and its connection to the motility and viability of sperm, as albumin is known to be used by the male reproductive system (Belinskaia*et al*., 2021). However, no differences in albumin levels were seen between the control and study groups here. SP-albumin, which serves as an antioxidant decreased in oligospermia as reported by Agha et al. (2022), and a similar decrease though not significant was observed in SOAT group. Though it was reported earlier that a weak significant association existed between SP-albumin levels and seminal volume (Belinskaia, 2020; Palani and Alahmar, 2020), no association between SP-albumin levels and sperm motility was found in the present study. Serum albumin is a non-enzymatic antioxidant that is responsible for the protection and maintenance of the sperm's tail membrane and therefore the sperm's motility (Palani, 2018). Infertile men had lower levels of serum albumin. But the present study results did not support this.

Serum glutamic pyruvic transaminase (SGPT) and Serum glutamic oxaloacetic transaminase (SGOT) are liver enzymes that are also responsible for proper sperm metabolism and the functioning of male accessory sex glands (Vashisht&Gahlay, 2024). They maintain semen quality by preventing acrosomal damage and disintegration of sperms. Significantly increased SGOT levels were seen in the SP of infertile individuals in the present study, making this a biomarker of male infertility. LDH, the glycolytic enzyme is another potent biomarker in distinguishing fertile and infertile SP. It influences sperm motility by providing energy through ATP production by LDH-mediated glycolysis (Vashisht et al., 2021; Vashisht&Gahlay, 2024) In the present study, LDH levels were found to be significantly higher among all the study groups compared to the control, just like the SGOT levels, indicating more energy requirement than usual in infertility (Saeed et al., 2021).

Cholesterol, a crucial lipid in male fertility present in SP, modifies the lipid bilayer of the sperm plasma membrane and prevents its fusion with the acrosomal membrane, thereby helping fertilization (Pushpendra and Jain, 2012; Naseer et al., 2014; Pakpahan et al., 2022). Its efflux is also essential for processes like sperm capacitation and maturation and it is a substrate for the synthesis of steroid sex hormones. Cholesterol levels are seen to be higher in infertile conditions as reported by Vashisht&Gahlay, 2024. The results of the present study are in line with this report, showing increased levels of total cholesterol among all the study groups. However, there are conflicting reports with Mehrparvar et al. (2020) observing lower cholesterol levels in infertile men and Placzkowska et al. (2024) not finding any correlation between cholesterol levels in SP as well as serum and infertility, and so further investigation regarding this is required.

In a systematic review, van den Berg et al (2024) reported 88 possible biomarkers in SP, which had the potential to be used in assisted reproductive technology (ART) programs. However, several of these have been reported only once and there is a huge dearth of complementary studies. Studies also differed in sample size, methodology, demographics and analysis, making interpretations complicated. Clinical studies correlating SP biomarkers and ART are also limited. So, high-precision biomarkers should be found using broad spectrum analysis, such as omics analyses (e.g., genomes, transcriptomics, proteomics, and metabolomics), with consistent techniques and sufficiently powered sample numbers. A reliable SP biomarker database that can potentially benefit ART programs can only then be established (van den Berg et al., 2024).

The findings of the present study suggest the potential utility of Cu, enzymes SGOT and LDH and cholesterol in SP as well as serum glucose as significant biomarkers for male fertility. However, variability across studies and between groups indicates a need for further research to elucidate their precise roles and clinical significance. For example, it is well-known that parameters like UA and urea negatively affected male fertility (Allahkarami et al., 2017), but such effects were not seen in this study. And Palani (2018) reported a high significant reduction of serum albumin in infertile men compared to fertile individuals, which was not confirmed by this study. Therefore, to fully understand and unleash the diagnostic potential of SP biomarkers, more research and development are necessary. The smooth integration of SP-based tests into standard clinical procedures will surely be facilitated by the discovery of new biomarkers as well as proper confirmation of the known ones, through the execution of thorough validation studies.

4. CONCLUSION

The current study found that, in comparison to blood serum, seminal plasma can serve as a more effective biomarker tool for determining male infertility. It is essential to create a non-invasive technique for evaluating, classifying, and identifying male infertility. Large, longitudinal cohort studies could be carried out by researchers to monitor how seminal plasma biomarkers evolve over time and in response to different drugs. Standardized protocols for biomarker evaluation, including sample collection, processing, and analysis, are necessary to convert research findings into clinical practice. While it is evident that the potential of seminal plasma (SP) in biomarker research is underutilized, a definitive judgment cannot be made.

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COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

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