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Semen Biochemical Parameters in Infertile Men: A Comparison between Individuals with and without Leukocytospermia

Jiao Liang[1]*, Yi Feng Ge2, Jin Chun[2], Feng Lu[2], Hong Wang[1]

[1]The Reproductive Medical Center, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.
[2]Department of Laboratory Science, Nanjing Hospital, Jiangsu Corps, The Armed Police Force, PLA, Nanjing, Jiangsu, China.

Corresponding author:
Jiao Liang, The Reproductive Medical Center, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing, Jiangsu, China.
E-mail: jiao.liang@129.com

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Abstract

Objective: White Blood Cells (WBC) are a common presence in nearly every human semen sample, yet the clinical significance of leukocytospermia, defined as more than 1 × 10^6/mL of WBC in seminal plasma, remains unclear. This study aims to assess the correlation between leukocytopenia and sperm characteristics, as well as the biochemical markers of male accessory gland function in individuals experiencing infertility.

Methods: A total of 185 men with fertility concerns were examined, comprising two groups: those without leukocytospermia (n=115) and those with leukocytospermia (n=70). The infertile men underwent semen analysis along with measurements of fructose, acid-phosphatase, zinc, and γ-glutamyl transpeptidase in seminal plasma.

Results: The average age of the participants was 33.97 ± 6.45 years. Analysis of leukocyte concentration in semen revealed that 70 (37.8%) patients had leukocytospermia. Individuals with leukocytospermia exhibited significantly reduced sperm count and vitality, while other sperm parameters such as seminal volume, progressive motility, morphology of pathological forms, and seminal plasma pH remained unaffected. Levels of acid-phosphatase, fructose, and γ-glutamyl transpeptidase were significantly lower in infertile men with leukocytospermia compared to those without this condition. Seminal zinc levels did not differ between the two patient groups.

Conclusion: The findings suggest that leukocytospermia significantly impacts standard semen parameters and biochemical compounds reflecting the function of accessory glands, particularly the prostate and seminal vesicles.

Keywords: Sperm parameters; Seminal plasma; Male infertility; Leukocytospermia; Fructose; Zinc; Acid phosphatase; γ-glutamyl transpeptidase

Introduction

White Blood Cells (WBC) are typically present in small quantities in semen samples from healthy men [1]. Leukocytospermia (LCS), alternatively known as leukospermia, pyospermia, or pyosemia, refers to an unusually high concentration of WBC in semen [2]. The World Health Organization (WHO) defined LCS in 1992 as the presence of at least 1 × 106 WBC per mL of semen [3]. While it is generally believed that an increased number of leukocytes in bodily fluids suggests an infection, bacterial presence has only been confirmed in a minority of leukocytospermic samples [1,4,5]. The necessity of antibiotic therapy for LCS treatment remains a topic of controversy [6-10]. Additionally, it is crucial to note that, apart from infection, non-infectious factors such as autoimmunity, toxins, and medications can also contribute to LCS [11].

Polymorphonuclear neutrophils, the predominant type of WBC in semen, possess the ability to generate Reactive Oxygen Species (ROS) in response to various chemical and bacterial stimuli [12,13]. The potential role of oxidative stress in compromising the functional competence of human spermatozoa was recognized as

early as 1943 by McLeod's study, which demonstrated the negative impact of high oxygen tensions on sperm motility. Subsequent research has unequivocally shown that human spermatozoa can produce ROS [15-17], with increased ROS activity observed in infertile males [18-20].

The prevalence of LCS in male infertility patients ranges from 2% to 40% in published reports [21-23]. Despite the acknowledged public health burden of LCS, its clinical significance remains uncertain. Numerous studies have reported associations between LCS and abnormal standard semen parameters, sperm function, and male infertility [24,25]. However, conflicting findings and reports suggesting positive effects of leukocytes in semen, such as phagocytosis and elimination of morphologically abnormal spermatozoa, exist [26,27].

Therefore, this study aims to investigate the association of LCS with progressive sperm motility, sperm concentrations, and biochemical markers of sperm function. Evaluation of biochemical constituents of seminal plasma is essential for semen assessment, alongside physical characteristics, as these compounds appear to serve as valuable predictive markers of male infertility. This investigation focuses on examining the association of LCS with the seminal concentration of zinc, prostate-specific acid phosphatase, γ-glutamyltranspeptidase [GGT] from the prostate, and fructose from the seminal vesicles.

Materials and Methods

Patients

This cross-sectional clinical study included 185 consecutive men with fertility issues, referred to the Uromedica Polyclinic in Belgrade between June and November 2017, meeting the specified inclusion criteria. Inclusion criteria comprised an age range of 20 to 39 years and providing written informed consent. Exclusion criteria involved the presence of urogenital infection, azoospermia, or recent antibiotic therapy within the last month. Ethical approval for this study was obtained from the Ethical Committee of the Association of Serbian Private Healthcare Providers in Belgrade, Serbia.

Semen Analysis

All semen analyses were conducted within 2 hours after ejaculation, adhering to the criteria outlined in the WHO Laboratory Manual 2010 [28].

Leukocytospermia Determination

Leukocytes in semen were quantified using a peroxidase enzyme identification procedure specific to polymorphonuclear granulocytes (PMN), the predominant type of WBC in semen. Peroxidase staining utilized ortho-toluidine, following the guidelines of WHO 2010 [28]. Peroxidase-positive cells, mainly granulocytes, stained brown, while peroxidase-negative cells remained unstained. The counting was performed in an improved Neubauer chamber under 400x magnification (Nikon SE, Japan).

Biochemical Assays

Zinc Determination: Zinc levels in semen were assessed using a commercially available kit (Randox, ZN2341, United Kingdom), originally designed for serum zinc measurement but adapted for semen. The assay relied on the compound 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropyl amino)-phenol (5-Br-PAPS) binding to zinc, inducing a color change measured at a wavelength of 560 nm.

Fructose Determination: Fructose levels were determined with a commercially available kit (Roche-glucose HK cobas, Gluk2, Switzerland). The enzymatic determination of fructose in semen utilized Hexokinase, with the increase in absorbance at 340 nm being directly proportional to fructose concentration.

Acid Phosphatase Determination: Acid phosphatase levels were determined using the Konetic colorimetric method (modified Hillman method) with a kit from Randox (AC1011, United Kingdom).

γ-Glutamyltranspeptidase (GGT) Activity Determination: GGT activity was measured using an enzymatic rate method with a kit from Clinischem Ltd (GGT-47261, Budapest, Hungary). The reaction involved the transfer of a gamma-glutamyl group from the substrate, γ-glutamyl-p-nitroaniline, catalyzed by GGT, resulting in the production of a colored product, p-nitroaniline.

Statistical Analysis

Statistical analysis included calculating descriptive measures (mean value, standard deviation, minimum, and maximum value) using SPSS software. Differences between groups with normal and elevated levels of leukocytes were explored using t-tests or Mann-Whitney tests based on data distribution characteristics, considering $p<0.05$ as statistically significant.

Results

The average age of the study participants was 33.97 ± 6.45 years. Analysis of leukocyte concentration in semen revealed that 70 (37.8%) patients had leukocytospermia, defined as a leukocyte count in semen exceeding 1.0 × 106. There was no statistically significant difference in age between patients with or without leukocytospermia ($p=0.394$).

Sperm parameters in patients with and without leukocytospermia are detailed in Table 1. The study findings demonstrated a statistically significant higher sperm concentration in the group of men without leukocytospermia compared to those with leukocytospermia ($p=0.038$). Additionally, there was a notable difference in the percentage of vital spermatozoa between these two groups ($p=0.009$), indicating that individuals with leukocytospermia exhibited lower sperm vitality. No statistically

significant differences were observed between the study groups concerning other investigated sperm-related characteristics (Table 1).

Seminal biochemical markers in males with and without leukocytospermia are presented in Table 2. The analysis of group-specific differences related to seminal biochemical markers demonstrated that seminal vesicle secretion (fructose) and various prostatic markers (acid phosphatase, GGT) were statistically significantly lower in patients with leukocytospermia compared to those with a normal number of white blood cells in semen. The level of zinc in semen did not differ between patients with or without leukocytospermia (Table 2).

Discussion

The significance of genital inflammation in male infertility remains uncertain in many aspects. Leukocytospermia has been considered an indicator of male genital tract inflammation and has been associated with poor semen parameters [22,29-32]. The objective of our study was to investigate the relationship between leukocytopenia and seminal quality, as well as biochemical markers of sperm function, in 185 men with fertility problems. Leukocytospermia was present in 37.8% of infertile men in this study, aligning with the reported incidence of this condition (2-40%) [2].

Several studies have demonstrated a negative correlation between WBC count in semen and seminal parameters [22,33-35]. Our findings affirm that leukocytospermia is linked to lower sperm concentration and vitality, while other sperm parameters, such as motility, pathological forms, and semen volume, did not differ between patients with and without leukocytospermia. The plausible explanation for these findings may be that inflammation of the genital tract could induce partial obstructions of the

Table 1: Sperm-related characteristics in study participants.

Sperm-related characteristics	Patients without leukocytospermia (N=115)	Patients with leukocytospermia (N=70)	P-value
Seminal volume (mL)	3.14 ± 1.59	3.22 ± 1.52	0.725
Sperm concentration (x 10^6/mL)	45.87 ± 56.26	20.93 ± 30.71	0.038
Progressive motility	32.26 ± 12.68	31.15 ± 12.94	0.531
Vitality	64.33 ± 11.55	59.28 ± 13.07	0.009
Morphology of pathological forms (%)	56.76 ± 10.28	50.59 ± 10.84	0.092
pH	7.94 ± 0.42	7.89 ± 0.34	0.452

Table 2: Seminal biochemical markers in study participants.

Seminal biochemical markers	Patients without Leukocytospermia (N=115)	Patients with Leukocytospermia (N=70)	P-value
Acid phosphatase (U/L)	1.21 ± 0.72	0.83 ± 0.53	<0.001
Fructose (mmol/L)	13.71 ± 6.80	11.80 ± 6.10	0.049
GGT (U/L)	13.29 ± 6.93	10.49 ± 4.97	0.004
Zinc (mmol/L)	2.35 ± 1.41	2.03 ± 1.41	0.08

seminal tract, resulting in severe oligoasthenospermia [36]. The first indication that gland infection could impair excretory function was observed in the late 1960s. Approximately 50% of leukocytes in seminal fluid originate from the prostate and seminal vesicles during prostatitis or seminal vesicles. Thus, infection of the accessory glands leads to an increased number of leukocytes in the ejaculate.

In this study, biochemical compounds in semen derived from accessory glands, including zinc, γ-glutamyl transpeptidase (GGT), and acid phosphatase for the prostate and fructose for the seminal vesicles, were measured to assess gland function. The results show that men with fertility problems and leukocytospermia exhibit significantly decreased levels of acid phosphatase, GGT, and fructose compared to those without leukocytospermia. Although the zinc levels were also decreased in patients with an abnormally high polymorphonuclear neutrophil (PMN) count in semen, it did not reach statistical significance, consistent with reported data.

Inflammatory conditions significantly impact the secretory function of male accessory organs. Reduced levels of citric acid, acid phosphatase, fructose, zinc, and alpha-glutamyl transferase activity have been associated with decreased prostatic and seminal vesicle secretory function [39-44]. Our study's results align with these findings, showing significantly lower seminal fructose levels in infertile men with leukocytospermia. While Marconi et al. found a decrease in seminal GGT levels in patients with inflammatory chronic prostatitis/chronic pelvic pain syndrome, they concluded that GGT may not be a reliable diagnostic marker for inflammation in this context.

Zinc has been suggested to contribute to the bactericidal activity of human seminal plasma. Moreover, prostatic secretions contain the highest physiologic concentrations of zinc in men, with zinc identified decades ago as the "prostatic antibacterial factor".

Conclusion

The clinical significance of an elevated number of WBC in male ejaculation remains controversial. Evidence suggests that silent inflammation of the genital tract could compromise male fertility potential. The origin of infertility in men with leukocytopenia may be the presence of subclinical genital inflammation leading to spermatogenesis deterioration, impaired sperm function, and seminal tract obstruction. Further investigations are necessary to confirm these hypotheses.

The data from this study unequivocally indicate that leukocytopenia in infertile men is associated with the impairment of the secretory function of the prostate and seminal vesicles, reflected in decreased levels of biochemical compounds derived from these glands in seminal plasma. Additionally, leukocytopenia in infertile men is linked to abnormalities in certain program parameters, such as sperm concentration and vitality.

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