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Research

The Impact of Leukocytospermia and Semen Hyperviscosity on Sperm Parameters Among Men with Suspected Infertility

İnfertilite Şüphesi Olan Erkeklerde Lökositopermi ve Semen Hiperviskozitesinin Sperm Parametreleri Üzerine Etkisi

Muhamet Afşin[1], Ayşe Feyda Nursal[2]

[1]University of Health Sciences Turkey, Gazi Yaşargil Training and Research Hospital, Clinic of Andrology, Diyarbakır, Turkey
[2]Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey

ABSTRACT

Objective: Leukocytospermia and semen hyperviscosity (SHV), impair sperm motility and can lead to male infertility. Therefore, this study aimed to evaluate the effect of leukocytospermia, and SHV on semen parameters in men with suspected infertility in Turkey.

Methods: A total of 1,316 semen analysis were included in the study. Sperm parameters were compared in the men with suspected infertility with and without leukocytospermia and SHV.

Results: Leukocytospermia was found in 335 (25.45%) men, and SHV was found in 195 (14.81%) men. Regarding leukocytospermia, there was a significant difference in the liquefaction period and progressive motility among groups. The men with leukocytospermia had increased liquefaction period and sperm concentration compared with men without leukocytospermia (p=0.008, p=0.000, respectively). The ejaculate volume and progressive motility decreased in men with leukocytospermia compared to men without leukocytospermia (p=0.01). Liquefaction period and immotility increased in men with SHV compared to those without SHV (p=0.000, p=0.000, respectively). Progressive motility, non-progressive motility, and total progressive motile sperm count decreased in men with SHV compared to without SHV (p=0.000, p=0.000, p=0.000, respectively).

Conclusion: This study showed that leukocytospermia and SHV frequently occur in men with suspected infertility. Although the total sperm count did not appear to be influenced by leukocytospermia and SHV in our study, and it deserves a detailed investigation in couples with unexplained infertility due to other effective semen parameters.

Keywords: Suspected infertility, leukocytospermia, semen hyperviscosity, semen parameters

ÖZ

Amaç: Lökositospermi ve semen hiperviskozitesi (SHV), sperm motilitesini bozar ve erkek infertilitesine yol açabilir. Bu nedenle, bu çalışmanın amacı Türkiye'de infertilite şüphesi olan erkeklerde lökositospermi ve SHV'nin semen parametreleri üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntem: Çalışmaya toplam 1.316 semen analizi dahil edildi. Lökositospermi ve SHV olan ve olmayan infertilite şüphesi olan erkeklerde sperm parametreleri karşılaştırıldı.

Bulgular: Üç yüz otuz beş (%25,45) erkekte lökositospermi, 195 (%14,81) erkekte SHV saptandı. Lökositospermi açısından gruplar arasında sıvılaşma periyodu ve progresif motilite açısından anlamlı fark vardı. Lökositospermisi olan erkeklerde, lökositospermisi olmayan erkeklere göre daha yüksek sıvılaşma süresi ve sperm konsantrasyonu vardı (sırasıyla p=0,008, p=0,000). Lökositospermisi olan erkeklerde ejakülat hacmi ve progresif motilitesi, lökositospermisi olmayan erkeklere göre azaldı (p=0,01). SHV'li erkeklerde SHV olmayanlara göre sıvılaşma süresi ve hareketsizlik arttı (p=0,000, p=0,000, sırasıyla). SHV'li erkeklerde, SHV olmayanlara kıyasla progresif motilite, ilerleyici olmayan motilite ve total progresif motil sperm sayısı azaldı (p=0,000, p=0,000, p=0,000, sırasıyla).

Address for Correspondence: Ayşe Feyda Nursal, Hitit University Faculty of Medicine, Department of Medical Genetics, Çorum, Turkey
Phone: +90 546 606 75 90 E-mail: feyda.nursal@gmail.com ORCID ID: orcid.org/0000-0001-7639-1122

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Sonuç: Bu çalışma, infertilite şüphesi olan erkeklerde lökositospermi ve SHV'nin sıklıkla görüldüğünü göstermiştir. Çalışmamızda toplam sperm sayısı lökositospermi ve SHV'den etkilenmemiş gibi görünse de, diğer etkili semen parametreleri nedeniyle açıklanamayan infertilitesi olan çiftlerde ayrıntılı bir araştırmayı hak etmektedir.

Anahtar Kelimeler: Şüpheli infertilite, lökositospermi, semen hiperviskozitesi, semen parametreleri

INTRODUCTION

Infertility is one of the important health problems that couples face worldwide. Infertility is defined as the inability of most couples to become pregnant after at least one year of frequent and unprotected sex and affects about 15% of couples. The main cause of infertility is among 20-50% in cases (1). Several factors impact sperm function, parameters, and then male fertility (2). Molecular mutations, genetic abnormalities, hormonal defects, impaired spermatogenesis, nutritional and some trace element deficiency, structural damages, environmental agents, life styles and obstructive problems are common factors affecting fertilization processes human sperm function (3).

Leukocytospermia is defined by the World Health Organization (WHO) as the presence of 1×10^6 leukocytes/mL in human ejaculate (4). Generally, leukocytospermia may indicate the male urogenital tract and sex glands' inflammation or infection (1). Based on prior studies, it was suggested that leukocytospermia negatively impacts sperm integrity and function (5). In a study found a significant correlation between leukocytospermia and defects in the tail function of sperm (6).

Semen hyperviscosity (SHV), with a coagulated and thick appearance, impacts the human seminal fluid's chemical and physical characteristics (7). Studies have shown the occurrence of SHV in 12%-29% ejaculates (8,9). Semen, which has normal viscose, contributes to sperm fertilization function and process, facilitating the spermatozoa entry into the cervical mucus (10). It maintains sperm swimming speed after mucus penetration (11), prevents the lipid peroxidation reaction (12), controls the surface charge distribution on the sperm membrane in the maturation process and keeps the spermatozoa's chromatin integrity (13).

Therefore, this study aimed to evaluate the effect of leukocytospermia, and SHV, on semen parameters in men with suspected infertility in Turkey.

METHODS

Study Population

This retrospective study was carried out from July 2019 to December 2021 at the University of Health Sciences Turkey, Gazi Yaşargil Training and Research Hospital, Andrology Laboratory. One thousand and three hundred and sixteen males with suspected infertility aged between 18 and 65 years were included in the study. We evaluated men with suspected infertility by dividing them into two groups, as with and without leukocytospermia and SHV after sperm analysis. The principles outlined in the Declaration of Helsinki were followed. The University of Health Sciences Turkey, Clinical Research Ethics Committee of Gazi Yaşargil Training and Research Hospital approved the study (decision no: 11, date: 28.01.2022).

Semen Analysis

Sperm samples were collected from the participants who were sexually abstinent for two-seven days by masturbating into sterilized disposable plastic cups without using any lubricant. Semen samples taken from the participants were examined in conformity with WHO criteria after liquefaction. The semen samples were first homogenized by pipetting with a Pasteur pipette. Approximately 10 µL of semen was pipetted and placed on the Makler camera (counting chamber) and sealed with a glass lid to determine the count and motility. Spermatozoa in 10 squares were counted through the x20 lens of the light microscope (Olympus CX31), and the results were expressed in millions. It was evaluated that sperm parameters including viscosity, leucocyte count, sperm concentration, total sperm count, motility, immotilty, and total progressive motile sperm count (TPMSC).

Leukocytospermia Analysis

A practical and reliable method for distinguishing leucocytes in the semen Papanicolaou dyeing method, which is a method, was used. A light microscope was used to distinguish spermatids and spermatocytes from polymorphonuclear leukocytes in a Papanicolaou-stained semen smear. The procedure is based mainly on the staining color, nuclear size, and shape differences. Polymorphonuclear leukocytes are stained a bluish color in contrast to the more pinkish color of spermatids.

Viscosity Analysis

After liquefaction, semen was aspirated into a disposable plastic pipette. The viscosity level is determined by the filament formed when it is left to drip (4). While a normal semen sample leaves small and separate drops from the pipette when the SHV drop forms a thread longer than 2 cm.

Statistical Analysis

The data obtained in the study were expressed as the arithmetic mean ± standard deviation. The Statistical Package Program for the Social Sciences (version 21) was used for statistical analysis. The conformity of the data to the normal distribution was analyzed using the Shapiro-Wilk test, and the homogeneity was analyzed by Levene's test. Student' t-test was used for statistical analysis. The significance was taken as $p \leq 0.05$.

RESULTS

A total of 1,316 semen analyses were included in the study. Leukocytospermia was found in 335 (25.45%) men, and SHV was found in 195 (14.81%) men. Sperm parameters were compared in the men with suspected infertility with and without leukocytospermia and SHV. Regarding leukocytospermia, there was a significant difference in the liquefaction period and progressive motility among groups. The men with leukocytospermia had increased liquefaction period and sperm concentration compared with men without leukocytospermia (p=0.008, p=0.000, respectively). The ejaculate volume and progressive motility decreased in men with leukocytospermia compared to men without leukocytospermia (p=0.01). The sperm parameters of men with and without leukocytospermia are presented in Table 1.

Then, we evaluated the sperm parameters in men with infertility who were suspected of SHV. For SHV, the liquefaction period, progressive motility, nonprogressive motility, immotility, and TPMSC were statistically different between the groups. Liquefaction period and immotility increased in men with SHV compared to those without SHV (p=0.000, p=0.000, respectively). Progressive motility, non-progressive motility, and TPMSC decreased in men with SHV compared to without SHV (p=0.000, p=0.000, p=0.000, respectively). The results are shown in Table 2.

DISCUSSION

The genitourinary tract's infectious process may contribute to males' fertility and reproductive function. Generally, leukocytospermia is assumed to indicate underlying genitourinary infection, and its incidence vary between 22 and 30%. This is commonly related to the seminal vesicle, epididymis, or prostate infection (14). Due to the common leukocytospermia in infertile men, whether the seminal leukocyte presence is correlated with the quality of semen may be questioned. Some studies' results show a the negative impact of leukocytes on the quality of semen due to the reactive oxygen species (ROS) presence. Activated leukocytes secrete protease, cytokines, and ROS, leading to damage sperm through deoxyribonucleic acid (DNA) fragments and lipid peroxidation (15). According to Agarwal et al. (16), patients presenting with low-level leukocytospermia have seminal oxidative stress. Although the present WHO guidelines do not categorize these patients as leukocytospermic, these men benefit from antibiotic treatment, testing for antioxidant supplements, or bacterial cultures to decrease the sperm DNA fragmentation induced by ROS and improve their fertility chances.

Leukocytospermia have been reported to hinder the spermatozoa's fertilization potential by interfering with sperm and egg fusion and the acrosome reaction (7,17). For this reason, the leucocytes presence in seminal plasma is

Table 1. Semen analysis of groups according to leukocytospermia

Semen quality parameters	Without leukocytospermia (n=981)	With leukocytospermia (n=335)	p
Abstinence period (day), mean ± SD	3.27±0.49	2.23±0.5	0.27
Age (year), mean ± SD	30.75±6.68	29.87±7.48	0.05
Liquefaction period (minute), mean ± SD	34.79±10.54	36.59±10.75	0.008
Ejaculate volume (mL), mean ± SD	3.02±1.77	2.77±1.38	0.01
Sperm concentration (million/mL), mean ± SD	35.8±30.46	43.28±32.69	0.00
Total sperm count (million), mean ± SD	101.33±95.57	109.23±87.54	0.18
Motility (%)			
Progressive (%), mean ± SD	47.72±20.8	44.97±17.90	0.02
Non-progressive (%), mean ± SD	10.28±6.64	9.96±5.97	0.41
Immotility (n, %), mean ± SD	41.77±20.13	45.24±18.23	0.05
TPMSC (million), mean ± SD	54.45±62.93	51.53±47.04	0.37

SD: Standard deviation, TPMSC: Total progressive motile sperm count

Table 2. Semen analysis of groups according to SHV

Semen quality parameters	Without SHV (n=1,121)	With SHV (n=195)	p
Abstinence period (day), mean ± SD	3.26±0.49	3.27±0.49	0.8
Age (year), mean ± SD	30.52±6.85	30.55±7.19	0.95
Likefaction period (minute), mean ± SD	32.27±6.98	52.30±11.82	0.00
Ejaculate volume (mL), mean ± SD	2.94±1.73	3.06±1.41	0.33
Sperm concentration (million/mL), mean ± SD	38.21±31.51	34.75±29.32	0.15
Total sperm count (million), mean ± SD	103.78±94.13	100.00±90.19	0.60
Motility (%)			
Progressive (%), mean ± SD	49.00±19.56	35.66±19.97	0.00
Non-progressive (%), mean ± SD	10.37±6.41	9.24±6.80	0.03
Immotility (%), mean ± SD	40.52±18.8	54.9±20.46	0.00
TPMSC (million), mean ± SD	56.34±61.24	38.31±43.5	0.00

SD: Standard deviation, SHV: Semen hyperviscosity, TPMSC: Total progressive motile sperm count

an important prognostic factor for failed embryo transfer and *in vitro* fertilization (18). A study by Ziyyat et al. (19) paradoxically decreased sperm motility in semen samples with a higher threshold of 1×10^6 leukocytes/mL and found that sperm motility increased among the semen samples with moderate leukocytospermia. Lackner et al. (20) found that the concentration-dependent association of leukocyte concentration was positively associated with sperm motility and morphology.

The chemical and physical characteristics of seminal fluid can be seriously impaired by SHV. Recent studies show that 12%-29% of ejaculates had SHV as the main cause of male infertility (21). In this regard, more research attention has been paid to it because of its serious effect on sperm function. The probable causes of SHV are infection, inflammation, and dysfunction of the immune system or the male accessory glands (8). More importantly, it seems that SHV is related to decreased sperm motility, probably because the trapping effect prevents the progression of normal sperm through the female genital tract (21). Gonzales (22) and Andrade-Rocha (8) found that fructose levels in SHV were reduced and hypothesized that the seminal vesicles inadequately functioned as the explanation. A study by Lampiao and Chisaka (23) found significantly higher progressive motility, normal morphology, sperm concentration, viability, and total motility in the normal viscosity group than in the abnormal viscosity group. A study found significantly reduced sperm and vitality sperm motility while SHV significantly increased polymorphonuclear granulocyte elastase levels (24).

In this study, we evaluated the men with suspected infertility in leukocytospermia and SHV. Our rates of leukocytospermia and SHV were consistent with the literature. There was a significant difference among the groups under study regarding leukocytospermia and SHV. This study demonstrated that leukocytospermia had a negative effect on the liquefaction period, sperm concentration, ejaculate volume, and progressive motility. Men with leukocytospermia had increased liquefaction period and sperm concentration compared with men without leukocytospermia, whereas the ejaculate volume and progressive motility decreased in men with leukocytospermia compared with those without leukocytospermia (Table 1). Also, this study clearly showed a significant negative correlation between SHV and liquefaction period, immotility, progressive motility, non-progressive motility, and TPMSC. The liquefaction period and immotility increased in men with SHV compared to those without SHV, whereas progressive motility, non-progressive motility, and TPMSC decreased in men with SHV compared to those without SHV (Table 2).

One limitation of this study was that it was retrospective. However, the high number of samples will help us understand the factors affecting suspected infertility.

CONCLUSION

This study showed that leukocytospermia and SHV frequently occur in men with suspected infertility. Although total sperm count did not appear to be influenced by leukocytospermia and SHV in our study, deserves a detailed investigation in couples with unexplained infertility due to other effective semen parameters.

ETHICS

Ethics Committee Approval: The University of Health Sciences Turkey, Clinical Research Ethics Committee of Gazi Yaşargil Training and Research Hospital approved the study (decision no: 11, date: 28.01.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: M.A., Design: M.A., A.F.N., Data Collection or Processing: M.A., Analysis or Interpretation: M.A., Literature Search: M.A., Writing: A.F.N.

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