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Leukocytospermia is related to total motile sperm counts in infertile men at Sadewa Maternal and Child Hospital, Yogyakarta, Indonesia

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ABSTRACT

Background: Leukocytospermia is defined as the presence of leukocytes in semen at the concentration of ≥ 1 x 10^6/mL according to World Health Organization (WHO) manual procedure. Leukocytes are the source of reactive oxygen species (ROS), which may lead to oxidative stress conditions and sperm damage. This study aims to evaluate the association between leukocytospermia, poor semen quality, and infertility at Sadewa Maternal and Child Hospital, Yogyakarta, Indonesia.

Methods: This study was based on secondary data of semen analyses of males attending IVF clinics at Sadewa Maternal and Child Hospital, Yogyakarta, Indonesia, from May 2015 to November 2020. Variables assessed in this study were semen volume, sperm concentration, progressive motility, and Total Motile Sperm Count (TMSC). Data were analyzed using SPSS version 23 for Windows.

Results: The median sperm concentration of the leukocytospermia group was higher than that of the non-leukocytospermia group. There is a relationship between the number of leukocytes in semen and TMSC, with the higher TMSC median in the leukocytospermia group.

Conclusion: This is the first study to report a significant association between leukocytospermia and total motile sperm count (TMSC). The association is not expected with leukocytospermia patients who have better TMSC value.

Keywords: Leukocytospermia, total motile sperm count, infertile men, sperm analysis.
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INTRODUCTION

Leukocytospermia is defined as the presence of leukocytes in semen at the concentration of ≥ 1 x 10^6/mL according to World Health Organization (WHO) manual procedure.[1] The increase of leukocytes in semen is often found in infertile men, with the incidence varying between 22-30%. This condition is commonly associated with the infection in the epididymis, seminal vesicle, and/or prostate.[2]

Leukocytes are the source of Reactive Oxygen Species (ROS), leading to oxidative stress conditions and sperm damage.[3] Therefore, the association between leukocytospermia, poor semen quality, and infertility has been hypothesized, but it is still not clear to date. Lackner JE et al. reported a positive association between leukocytes concentration and sperm motility and morphology, and the association might be concentration-dependent.[4]

Similar results were found in the other studies by Fraczek et al. and Li et al., that leukocytes and bacterias affect the sperm parameters by enhancing sperm death through DNA fragmentation.[5] However, a recent meta-analysis of case-control studies concluded that leukocytospermia is not associated with reduced fertility after Assisted Reproductive Technology (ART) and altered semen concentration and motility.[6]

Total Motile Sperm Count (TMSC) has been introduced as consideration for choosing assisted reproduction treatment for male infertility.[7] TMSC value is obtained by multiplying semen volume with sperm concentration and progressive rate of sperm motility. Infertile men with TMSC 5-40 million could try intrauterine insemination for their treatment, while men with TMSC below 5 million should be considered for in vitro fertilization.[7] TMSC is also known as the better predictive value for intracytoplasmic sperm injection outcome compared to WHO 2010 semen parameters cut-off values based on a previous study.[8]

Considering previous reports, it is still unclear that leukocytes may affect semen parameters. The association between leukocytospermia and TMSC has rarely been studied. Based on those mentioned above, this study aims to evaluate the association between leukocytospermia and total motile sperm count in infertile men.

MATERIAL AND METHODS

Study Design

This study was based on 864 secondary semen analyses of males attending IVF clinics at Sadewa Maternal and Child Hospital, Yogyakarta, Indonesia, from May 2015 to November 2020. The hospital has approved access to medical record data by ensuring data security. The patient's identity is labeled with an initial code to maintain data confidentiality.

A semen sample was collected by masturbation after 2-7 days of abstinence. The inclusion criteria in this study were all infertile male patients examined for sperm analysis in the andrology laboratory of the Sadewa Maternal and Child Private Hospital, Yogyakarta, Indonesia, who were willing to be involved as research respondents. Meanwhile, the exclusion criteria for this study were infertile male patients with urogenital infections.

Sperm analysis is carried out in a standardized andrology laboratory, with certified laboratory staff who have particular expertise in conducting sperm analysis examinations. Sperm analysis examination was repeated 3 times for each patient in order to minimize bias. All semen analyses were conventionally analyzed according to the WHO 2010 Laboratory Manual Procedure. Assessment of leukocyte concentration was performed after peroxidase staining. Patients with leukocyte concentration in semen ≥ 1 x 10^6/mL were classified into the leukocytospermia group. In comparison, the patients with leukocyte concentration in semen below 1 x 10^6/mL were classified into the non-leukocytospermia group. Semen volume was measured by aspirating the sample into the volumetric pipette. Sperm concentration was counted using an improved Neubauer chamber. Sperm motility was graded according to WHO criteria as progressive, non-progressive, and immotile, all as percentages. Total motile sperm count (TMSC) value was obtained by multiplying semen volume with sperm concentration and progressive rate of sperm motility.

Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 23.0 for Windows. Mann-Whitney test was conducted to assess the association between leukocyte concentration group with semen volume, sperm concentration, progressive rate of sperm motility, and TMSC. A p-value less than 0.05 was defined as statistically significant. Furthermore, we performed a chi-square test to analyze leukocytes level in three different TMSC groups.

RESULTS

A total of 864 semen analyses were included for this study. The median semen volume was 2.80 (0.80–9.50) mL, with an mL range. The median sperm concentration was 29.90 (3.50-251.50) x 10^6/mL. The progressive motility rate has a median value of 37.00 (1.00-84.00)%. The median of TMSC was 29.75 (0.11-264.08) x10^6 (Table 1). The median leukocyte concentration was 0.60 (0.03-16.50) 10^6/mL. A total of 220 samples were classified to the leukocytospermia group and 644 samples to the non-leukocytospermia group. The detailed analysis of semen parameters according to the leukocyte concentration group can be seen in Table 2.

The median value of semen volume was significantly lower in the leukocytospermia group compared to the non-leukocytospermia group (p=0.000). In addition, the median sperm concentration was significantly higher in the leukocytospermia group (p=0.000). Both groups' median progressive motility rate was not statistically different (p=0.182) (Table 1). The analysis of TMSC value according to the leukocyte concentration group can be seen in Table

Table 1. Sperm parameters according to leukocytospermia and non-leukocytospermia group.

Variables	Total (N=864)	Leukocytospermia (N=220)	Non-Leukocytospermia (N=644)	p
Semen Volume (mL)	2.80 (0.80-9.50)	2.60 (1.00-6.60)	2.9 (0.8-9.5)	0.000*
Sperm Concentration (10^6/mL)	29.90 (3.50-251.50)	50.87 (4.80-251.50)	25.87 (3.50-114.3)	0.000*
Progressive motility (%)	37.00 (1.00-84.00)	37.50 (3.00-84.00)	37.00 (1.00-74.00)	0.182

*Mann-Whitney test was statistically significant if p-value less than 0.05

Table 2. Analysis of TMSC according to leukocytospermia and non-leukocytospermia group.

Variables	TMSC	p
Leukocytospermia (10^6)	45.44 (0.95 - 264.08)	0.000*
Non-Leukocytospermia (10^6)	24.70 (0.11 - 212.42)	

*Mann-Whitney test was statistically significant if p-value less than 0.05; TMSC: Total Motile Sperm Count

Table 3. Analysis of leukocytes between TMSC groups.

Variables	Leukocytospermia (N=220)	Non-Leukocytospermia (N=644)	p
TMSC >40 x 10^6, n (%)	123 (36.20)	217 (63.80)	0.000*
TMSC 10-40 x10^6, n (%)	69 (21.60)	251 (78.40)	
TMSC <10 x 10^6, n (%)	28 (13.70)	176 (86.30)	

*Chi-Square test was statistically significant if p-value less than 0.05

2. There was a statistical difference in the median of TMSC between the two groups, where leukocytospermia had a statistically significant higher than the non-leukocytospermia group (p=0.000) (Table 2).

Furthermore, it could be seen in Table 3 that there was also a significant association between leukocytes levels in each TMSC group (p=0.000). Leukocytospermia could be found mostly in TMSC >40 million group (36.20%), followed by TMSC 10-40 million group (21.60%), and TMSC <10 million group (13.70%) (Table 3).

DISCUSSION

Based on the recent findings, it is known that the median sperm concentration of the leukocytospermia group was higher than that of the non-leukocytospermia group. This is in line with the result of a study by Lackner JE et al., where the lowest leukocyte group has a lower sperm concentration than the other groups.[4] Based on statistical analysis, it is known that there is a relationship between the number of leukocytes in semen and TMSC, with the higher TMSC median in the leukocytospermia group.

Total Motile Sperm Count (TMSC) can be used as a reference to decide on the treatment of infertility through assisted reproductive technology. TMSC value can be obtained by: semen volume x (progressive motility/100) x sperm concentration.[7,8] The value can be calculated before and after sperm preparation. A post-preparation TMSC between 0.8 to 5 million spermatozoa has a positive predictive value in couples who took intrauterine insemination.[9] TMSC components, which are obtained from semen parameters, are possible to be affected by leukocytes.

High seminal leukocyte concentration or leukocytospermia is known as a parameter of the presence of inflammation in the male reproductive tract. This condition is more often found in infertile men compared to the normal population.[10] Granulocytes are the predominant type of leukocyte in human semen with the percentage of 50-60%, followed by macrophages (20-30%) and T-lymphocytes (2-5%).[11] Leukocytospermia is also known as the possible factor contributing to sperm damage and male infertility subsequently. The pathophysiology may be initiated by an increase in reactive oxygen species (ROS).[3]

At a higher level, ROS may lead to oxidative stress and disrupt sperm physiology. Lipid peroxidation of the plasma membrane has been recognized as the mechanism of sperm damage in oxidative stress conditions. An altered membrane may enable ROS to attack sperm nuclear DNA and cause DNA fragmentation.[11-14] Oxidative stress also has a role in impaired spermiogenesis that results in increased production of defective sperm and may alter the sperm parameters.[13,14] The higher median sperm concentration and TMSC in the leukocyte group seem contradictory to the earlier theory.

The previous study stated that leukocytes might have a positive and negative dual effect on the sperm parameters, concentration-dependent effect.[4] Semen parameters and leukocytes have a complex relationship and involve other pro-inflammatory cytokines and ROS components.[5] Besides the adverse effects caused by ROS, there are also physiological functions involved in the cell signaling and the fertilization process. The presence of ROS in the male reproductive system in physiological amounts will induce spermatozoa capacitation, hyperactivation, and acrosomal reactions.[15,16]

This dual effect of ROS produced by leukocytes on spermatozoa may be due to its short half-life.[17] The negative impact of ROS will appear clinically depending on the balance of the number of oxidants and antioxidants. This negative impact will appear if the amount of antioxidants exceeds a certain threshold. Seminal plasma is known to have a large number of antioxidants to reduce the negative impact caused by ROS in the leukocytospermia group.[17-19] Further observational studies are needed to include these contributing factors in the analysis.

CONCLUSIONS

This is the first study to report a significant association between leukocytospermia and total motile sperm count (TMSC). The association is not expected with leukocytospermia patients who have better TMSC value. Further studies are needed to explore more contributing factors regarding this topic.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

ETHICS CONSIDERATION

Ethical clearance was obtained from the Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada-DR. Sardjito General Hospital with the protocol number of the clearance is KE/FK/0434/EC/2021.

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AUTHORS CONTRIBUTION

DMR conceptualized the presented idea. DMR and MCH conducted data collection. MCH and NS analyzed the data. DMR verified the analytical methods. MCH and NS wrote the manuscript with support from DMR. All authors discussed the results and contributed to the final manuscript.

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