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Impact of Leucocytospermia on Semen Parameters among Sudanese Sub-Fertile Men Port Sudan -2019

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ABSTRACT

Background: For a long time, bacterial infection of the male genital tract was thought to be one of the leading causes of male infertility. Various clinical studies have identified Leucocytospermia as a proxy marker for these infections, although other causes of inflammation may also play a role.

Objective: The study was conducted at the Dermatology Teaching Hospital in Port Sudan with the aim of determining the impact of leucocytospermia on semen parameters and defining the microbial etiology among infertile males.

Methods: Between September 2019 and February 2020, a descriptive, cross-sectional, hospital-based investigation was applied. After meeting the study requirements, 140 patients were randomly selected; patient information was collected via a closed-ended questionnaire after patients provided their authorization.

Results: A total of 140 male infertility patients were evaluated. The mean age of respondents was 43.5 + 2.6 years old, 61.4% of the patients had infertility for 1-5 years, 55.7% of the patients had secondary infertility, 32.1% of the patients demonstrated leucocytospermia on their semen analysis. Semen analysis results showed that 37.8 of the leucospermic patients' sperm count was <15 X 10^6. In 73.3% of the patients, the motile sperms were < 40%, and normal morphology was less than 4% in 46.7% of the patients. The analysis showed mixed infection by both gram positive and negative bacteria are common (42.2%).

Conclusion: According to the findings, there is an association between leucocytospermia and male infertility.

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Abbreviation

ICMART: International Committee for Monitoring Assisted Reproductive Technology
OS: Oxidative Stress
PDTH: Port Sudan Dermatology Teaching Hospital
ROS: Reactive Oxygen Species
WBCs: White Blood Cells
WHO: World Health Organization
DNA: Deoxyribo Nucleic Acid.

Introduction

The revised glossary exemplifies infertility as a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse, as defined by the international committee for monitoring assisted reproductive technology (ICMART) and the World Health Organization (WHO). If there have been no prior births, it is known as primary infertility; if there have been one

or two pregnancies, it is classified as secondary infertility [1,2].

Leucocytospermia is responsible for 10-20% of male factor infertility cases [3]. The remaining cases are caused by a host of other causes such as varicocele, hereditary and other systemic illness, lifestyle factors, gonadotoxin toxicity, hormonal dysfunction, chromosomal defects, testicular failure, ejaculatory disorders, and obstruction [4]. Leucocytospermia is described by the World Health Organization as the presence of one million or more leukocytes in one milliliter of semen [5]. Leucocytospermia is a disorder in which the number of leukocytes in the ejaculate increases. Leucocytes are white blood cells that play an essential function in the human immune system. They react to contamination and foreign materials by using an oxygen-based system to destroy bacteria and other pathogens [6].

Numerous experiments back up the idea that an increase in leucocytes in the sperm is harmful to sperm parameters like morphology and motility [7]. Leucocytospermia has been shown in recent years to have a negative impact on sperm parameters across a variety of mechanisms. An rise in leucocytes in the ejaculate has been linked to an increase in reactive oxygen species (ROS) and resulting oxidative stress (OS). By way of lipid peroxidation, both ROS and OS have long been linked to sperm dysfunction [6]. Lipid peroxidation alters the fluidity of the sperm cell membrane, causing DNA damage. Unfortunately, after maturation, adult sperm fail repair mechanisms. Reduced sperm motility, irregular sperm morphology, and total sperm fertilization capability and viability result from these combined effects [7,8].

The failure of a sexually active, non-contraceptive pair to reproduce after one year of regular intercourses is known as infertility. It affects about 15% of couples, with male causes accounting for 20% to 50% of the cases. Males' reproductive activity and reproduction are influenced by infectious processes in the genitourinary tract. Infectious etiologies accounted for up to 15% of male infertility cases [3].

Leucocytospermia is an abnormal laboratory finding identified by the World Health Organization (WHO) as the presence of one million leukocytes per milliliter of human ejaculate, which indicates the presence of genital tract infection [2]. Leucocytospermia has been shown to have a detrimental effect on sperm function and quality in previous research [9]. Leucocytospermia has been linked to high sperm deformity index scores, acrosomal injury, midpiece defects, and tail deformities in other studies. Infectious processes may also cause spermatogenesis to deteriorate, sperm function to be impaired, a leucocyte response to be triggered,and/or anatomical obstruction of the seminal tract. Leucocytospermia is thought to be caused by infection or inflammation of the male sex glands and urogenital tract [3]. As a result, broad-spectrum antibiotics with powerful antioxidants will minimize ROS formed within cellular mitochondria as a result of semen leucocyte inflammation, There is, still, no specific agreement about the outcomes of each medication or whether leucocytospermia should be treated or not. Furthermore, there is only one systematic analysis of leucocytospermia treatment provided, which was published in 2003, and there is insufficient information on this issue [9].

Although it's easy to diagnose and treat, leucocytospermia accounts for a significant portion of male factor infertility. Antibiotics and antioxidants tend to reduce the number of leucocytes in the semen and improve fertility rates in such patients. In our neighboring countries, there is a scarcity of study, and Sudan has just a few studies.

Patients and Methods

Study Design, Area, Population, and Period

The study was implemented in a dermatology and venereology teaching hospital in Port Sudan and was retrospective, cross-sectional, and hospital-based. 140 Sudanese patients with primary or secondary infertility were recruited between September 2019 and February 2020 for the study.

Inclusion criteria

Sudanese males of reproductive age who have been married for more than a year, have primary or secondary infertility, are sexually normal, and live in the study field.

Exclusion criteria

Patients who live outside the research field, have been married for less than a year, have been diagnosed with other sexual problems (e.g. erectile dysfunction), or have been diagnosed with other causes of male infertility (e.g. trauma, varicocele, smoking, alcohol), and who follow the eligibility criterion but refuse to participate in the study.

Sampling

Sample size

The following formula will be used:

N= ((P x Q x Z2) / d2) + 10% (non- respondent rate)

Where n is the sample size, p is the sample proportion, q is equal to p-1, z is the appropriate cut-off point on the standard normal distribution at 95% confidence (standard value of 1.96) and d is the degree of precision.

Margin of error at 5% (standard value of 0.05)

The sample size calculation based on a study conducted in Khartoum on the assumption that the sero-prevelance of leucocytospermia was 16%:

0.16 x (1 – 0.16) x (1.96)2 \ (0.05)2 = 204

Non respondent rate = n x 10 \100

204 x10 \100 = 20.4

So the sample size is 20 +100 = 120 suspected cases.

Sample Selection Technique

Randomized cluster design was used to sample participants. The suspected cases were numbered in regular manner.

Data Collection

Data was gathered using pre-designed closed-ended questionnaires, direct personal interviews with patients, and completed questionnaires. For all patients, seminal analysis was performed in the same lab, from the same batch, and by the same lab technician. The same microbiologist performed Gram staining on leucocytospermic patients. Sub-fertile patients with leucocytospermia and sub-fertile patients without leucocytospermia were categorized into two classes.

Variables of the Study

The variables in the study were grouped into two types

Dependent variable

Association between leucocytospermia and male infertility.

Independent variable

Age, place of residence, levels of education, occupation, socioeconomic status, illness period, onset (primary or secondary), lifestyles, chronic diseases, long-term medications, among others are all factors to take into account.

Study Protocol

After dry masturbation, seminal fluid samples were extracted a septically from each participant, analyzed under a microscope, and cultured using specific antibiotic disks. The seminal fluid of 140 subfertile men with primary or secondary infertility was examined. After at least 48 hours of abstinence, seminal fluid was gathered in a clean, wide-mouth bottle through dry masturbation and taken to the laboratory straight away. Seminal fluid analysis was performed for 140 sub-fertile men. Color, viscosity, PH and volume were examined macroscopically, and motility was examined under the microscope within 20 minutes to one hour of collection time and stained by Giemsa stain10%,fixed by spirit 10-15min. Morphology and pathological sperms are individually examined to see whether there are any defects in the sperm's head, midpiece, or tail. Using the Peroxidase test, this manual procedure will differentiate immature sperm from white blood cells (WBCs).

WBCs in seminal fluid were counted using two different methods. On a direct smear of the semen stained using Couture et al's process, the amount of WBCs per 100 sperm was counted. By multiplying sperm concentration by the number of WBCs per 100 sperm and dividing by 100, the number of WBCs per milliliter of fluid was determined.

Counting the number of WBCs and sperm in 100 consecutive 400X high power fields or before at least 10 polymorphonuclear leukocytes is counted was often used to calculate the number of leukocytes. The number of WBCs per milliliter of semen was calculated by dividing the number of WBCs by the number of sperm. The number of sperm per milliliter was then multiplied by the number of leukocytes per milliliter of semen.
Microbiological studies:

Day 1: 2ml of semen added to 70 ml of Brain Heart infusion (nutrient media).
Day 2: Subculture overnight in blood, MacConkey and Choclate agars at 37 C.
Day 3: Gram staining and other biochemical testing to identify the micro-organisms. Add the antibiotic sensitivity disc.
Day 4: Read the antibiotic sensitivity infusion disc according to diameter of clear area around the antibiotic disc.

Data Management

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 22. If the P. value was less than 0.05, the relationship between various study variables was evaluated for statistical significance.

Ethical Consideration

The Sudan Medical Specialization Board's Dermatology and Venereology Council, the Educational Development Center's (EDC) ethical committee, and the administrator of the Port Sudan dermatology and venereology hospital all issued their authorization. As an eligibility criterion, the patient's informed consent was obtained.

Results

The current study investigated 140 Sudanese male patients presented to Dermatology and Venereal disease clinic at Port Sudan Hospital complaining of infertility. Socio-demographic characteristics: The current study found 83 out of 140 patients were in the age group 20-40 years, followed by 51 patients were in the age group 41-60 years, 6 patients were in age group more than 60 years. The mean age was 40 years.

Infertility types: Sixty-two patients (44.3%) had primary infertility, while 78 patients (55.7%) had secondary infertility. Infertility duration: Eighty-six patients (61.4%) of the patients had infertility for 1-5 years, while 54 patients (38.6%) had infertility for more than 5 years. (Table1)

Table 1: Distribution of study participants according to age, infertility types and duration

Variable		Frequency	Percent %
Age	20 - 40	83	59.3
	20 - 40	51	36.4
	60 and More	6	4.3
Infertility type	Primary	78	55.7
	Secondary	62	44.3
Duration of infertility	< 5.0 Years	86	61.4
	> 5.0 Years	54	38.6

Presence of more than 10 leucocytes per high power field in semen was detected in 45 patients (32.1%) and not detected in 95 patients of infertility (67.9%). (Table 2) Semen analysis results: The sperm count was <15 X 10^6 in 37.8% of leucocytospermic patients, and 30.5% in non leucocytospermic group, while it was >15 X 10^6 in 62.2% of leucocytospermic patient and 69.5% in non leucocytospermic group (P value was >0.05) (Table.3).

Table 2: Distribution of Leucocytospermia among study group

Presence of Leucocytes in semen	Frequency	Percent
≥10 Leucocytes/HPF	45	32.1 %
< 10 Leucocytes/HPF	95	67.9 %
Total	140	100 %

Table 3: Leucocytospermia in relation to sperm count among study group

Variable	sperm count				Total		
	L ess than 15 million		more than 15 million				p-value
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Leucocytospermic	17	37.8 %	28	62.2 %	45	100%	> 0.05
Non- leucocytospermic	29	30.5%	66	69.5%	95	100%	

The abnormal sperm morphology was detected in 46.7% of leucocytospermic group and in 40% in non leucocytospermic patients, while the normal sperm morphology was detected in 53.3% of leucocytospermic patients and 60% in non leucocytospermic semen (P value >0.05). (Table 4)

Table 4: Leucocytospermia in relation to sperm morphology among study group

Variable	sperm count				Total		
	less than 4%		equal or more than 4%				p-value
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Leucocytospermic	24	53.3%	21	46.7%	45	100%	> 0.05
Non- leucocytospermic	57	60%	38	40%	95	100%	

The low sperm motility (<40%) was detected in 73.3% of leucocytospermic patients and it was 46.3% in non leucocytospermic in contrast to normal sperm motility (>40%) in about 26.7% of leucocytospermic patients and 53.7% in non leucocytospermic group (P value <0.05) (Table 5).

Table 5: Leucocytospermia in relation to sperm motility among study group

Variable	sperm count				Total		
	less than 40%		more than 40%				p-value
	Frequency	Percent	Frequency	Percent	Frequency	Percent	
Leucocytospermic	33	73.3%	12	26.7%	45	100%	> 0.05
Non- leucocytospermic	44	46.3%	51	53.7%	95	100%	

Gram staining of semen sample: Micro-organisms were detected in all leucocytospermic patients, 12 patients showed Gram positive micro-organisms, 14 showed Gram negative micro-organism, 19 of them showed mixed infections by both Gram positive and negative (Table 6).

Table 6: Detection of different micro-organisms using Gram stain study group

Micro-organisms	Frequency	Percent
gram positive	12	26.7 %
gram negative	14	31.1 %
Mixed	19	42.2 %
Total	45	100 %

Discussion

Infertility is a major worldwide issue that affects 15% of couples. In 2010, 48.5 million couples were infertile, and almost 50% of infertility is affected by male influences [7]. In tropical countries, leucocytospermia was reported in 10% of the community [8]. As a consequence, the incidence in Sudan is estimated to be higher, necessitating further investigation. During the study time, infertile Sudanese males attending Port Sudan Dermatology Teaching Hospital were examined to determine the prevalence of leucocytospermia and its impact on semen parameters. Gram stain was used in this analysis to identify the various microorganisms colonizing the semen of sub-fertile Sudanese males in order to ensure proper antibiotic coverage. The present study reported leucocytospermia in 45 of 140 subfertile males, indicating that the incidence of leucocytospermia is 31.2 %. These are troubling results, since they demonstrate that leucocytospermia has a clear impact on overall male subfertility,which greater than that [10] documented 12% prevalence rate.

The volume, count, morphology, and motility of semen were investigated in this study. The non-leucocytospermic semen had average values for semen parameters. Leucocytospermic sperm had a lower proportion of semen volume and motility. Other study showed that individuals with leucocytospermia had reduced sperm volume, though this was not statistically significant [11].

Male fertility can be influenced by sperm count, since a lower sperm count reduces the chances of having a partner pregnant. In the current study, 62.2% of the leucocytospermic patients (69.5% in non leucocytospermic patients) sperm count was >15 X 10^6 while 37.8% of the leucocytospermic patients (30.5% of non leucocytospermic patients) showed the sperms count was < 15 X 10^6. Despite the reality that patients with leucocytospermia have a poor sperm count, the study found no statistically significant correlation between leucocytospermia and sperm count, because p value of chi square was > 0.05. Other study reported that total sperm count showed lower values in men with leucocytospermia [11]. when compared to men with non leucospermic semen (P<0.05). Findings by [10] agreed with the current study because they found this relation was attenuated and became not significant after correction for confounding factors.

Sperm morphology means the size and shape of sperms. It is one factor that's examined as part of a semen analysis to evaluate male infertility. Sperm morphology results are reported as the percentage of sperm that appear normal when semen is viewed under a microscope. The results of this study found no interaction between leucocytospermia and sperm morphology. There was 46.7% of leucocytospermic (40% of non leucocytospermic patients) has egual or more than 4% of abnormal sperm morphology, while 53.3% of leucocytospermic patients (60% of non leucocytospermic patients) has less than 4% of abnormal sperm morphology,[12] noticed a lower proportion of sperm with normal morphology associated with leucocytospermia.

Sperm motility refers to sperms' capacity to travel quickly and in a forward-progressive direction. Our findings showed that, among 73.3% of the leucocytospermic patients (46.3% of non leucocytospermic patients), the motile sperms were < 40% while in 26.7% of the leucocytospermic patients (53.7% of non leucocytospermic); the motile sperms were>40%. This indicates that the majority of leucocytospermic patients

have sperm motility concerns. By the other hand analysis of sperm motility with leucocytospermia showed inverse relation between leucocytospermia and sperm motility (*p value* <0.05). Similarly with other study [11] noticed that leucocytospermia was correlated with reduced sperm motility, the previous results and the main suggestion that a possible inverted U shaped' relation of leucocytospermia to sperm parameters. Correspondingly, [12] showed that the leucocytospermia has significant negative effect on semen parameters.

Bacterial microorganisms: The Gram stain was used to identify various bacterial microorganisms in leucocytospermic patients; 26.7 % were Gram positive, 31.1 % were Gram negative, and 42.2 percent were infected with both Gram positive and Gram negative bacteria.

Seminal fluid culture and antibiotic susceptibility testing should be performed to isolate different colonizing microbes from infertile sperm and to ensure appropriate antibiotic therapy before leucocytospermia resolution, regardless of the number of leucocytes in the semen. The limited number of participants could have a negative impact on the probability of reporting significant findings in our study; Nevertheless, statistical significance was achieved after accounting for confounding factors, so that doesn't seem to have an influence on the results. As a result, the authors suggest that the same study be conducted in other andrology clinics in order to generate evidence and take action depending on the outcomes.

Conclusion

Leucocytospermia was found in about a third of infertile Sudanese patients who visited a dermatology teaching hospital in Port Sudan. There was a statistically significant difference in sperm motility between patients with and without leucocytospermia. In individuals with and without leucocytospermia, there was no statistically important difference in sperm count and morphology. Gram staining of leucocytospermic sperm revealed a combination of gram positive and negative microbes.

Recommendations

Physicians should be aware about the association of leucocytospermia and male infertility especially with low sperm motility. The same study would be conducted in other hospitals with large sample size to generalize the findings and to elucidate definite management strategies for infertile males with leucocytospermia as well as to eliminate infection and reduce reactive oxygen free radicals. To evaluate the effect of treatment of leucocytospermia on semen parameters, rate of resolution of leucocytospermia, the bacteriological cure rates and on the chances of pregnancy in furthers large studies.

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