THE EFFECT OF LEUKOCYTOSPERMIA ON SPERM QUALITY IN INFERTILE MEN VISITING NATIONAL IN-VITRO FERTILIZATION CENTER–MISURATA

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ABSTRACT

Leukocytospermia is highly prevalence condition among infertile males. However, the role of Leukocytospermia in the pathogenesis of male infertility remains controversial. Our study aimed to evaluate the prevalence of Leukocytospermia in study population and assessment the effect of seminal leukocytes on various semen parameters.

This is a prospective study conducted between June-October 2017 at National Infertility Center/ Misurata-Libya. 187 infertile men with no history of azoospermia, varicocele and any chronic underling diseases like diabetic, hypertensive and chronic viral infection were included. The demographic data that include age, infertility type, infertility period, and smoking status were collected from their medical records. Semen specimens were examined for ejaculation volume, acidity degree (pH), sperm concentration, sperm motility percent, sperm progressive motility percent, sperm morphology percent and leukocytes concentration according to the standard method of the WHO 2010 guidelines. Therefore, participants were grouped based on seminal leukocytes concentration into (Group A :< 2 WBC/HPF; Group B: 2-4 WBC/HPF; Group C: 4-6 WBC/HPF; Group D: <6WBC/HPF). All collected data were analyzed by Statistical Package for the Social Sciences (SPSS) version 20 using chi-squared X2 test, one way analysis of variance (ANOVA) and Fisher,s least significant difference LSD test. A P value $\leq$ 0.05 was defined as statistically significant.

The study found that, 42 (34.8%) of study population have Leukocytospermia ($\geq$3WBC/HPF). The sperm parameters, which have effected significantly by increase of seminal leukocytes were total motility (F 5.381; P 0.001<0.05), progressive motility (F 3.022; P 0.031<0.05) and normal morphology (F 3.518; P 0.02<0.05). The total motility was significantly higher in Group A (62.69±17.63; P<0.05) compare with other Groups, and was significantly lower in Group D (43.21±26.25; P<0.05) especially compare with Group A. The progressive motility was a significantly lower in Group D (11.57±7.6; P<0.05) compare to rest of leukocytes concentration Groups. The normal morphology was significantly higher in Group A (62.69±17.63; P<0.05) compare with Group B (7.92±8.22; P<0.01) and C (8.13±7.30; P<0.05), while, it was not any difference between Group D (10.11±11.03; P> 0.05) and other Groups.

The study concluded that seminal leukocytes have bi-effects of sperm quality. While, they caused of deterioration of different motility forms of sperm, they contributed of improvement of normal morphology percentage especially in high concentration level. However, Future studies are needed to examine bi-effects of seminal leukocytes on sperm quality.

Keywords: Leukocytospermia, Semen specimens, MGT, Spermatogenesis, testis, Lymphocytes.

1. INTRODUCTION

Leukocytes are considered as cellular component of human immune system, which are generated and functioned as body's guardian cells at early stage of developmental life. These cells characterized by supreme ability to protective the body from foreign without impair of body component through a property known as immune tolerance. Therefore, Leukocytes gain ability to recognize xenoantigen and ignored alloantigen, thus killing foreign without incidence of autoimmune responses [1].

Leukocytes are supported by immune tolerance in early development life. Therefore, self-body's components, which are generated at puberty as spermatozoa may deal as foreign by Leukocytes. However, Male Genital Tract MGT, where spermatozoa are produced and maturated, is not completely free of Leukocytes. Therefore, researchers of Male Factor Infertility interest a relationship between spermatozoa and Leukocytes and the disturbance of spermatozoa properties because of increase Leukocytes in the male genital tract **(**MGT) [2].

The Male Genital Tract (MGT) structures:

The MGT consists of number of external and internal organs and is located on the outside of the body and within the pelvis. All of its organs play together to produce spermatozoa and coordinate transport, maturation, storage and ejaculate them. The external organs are consisting of the penis,

which is the male organ for sexual intercourse and urination. The scrotum that is a loose, pouch-like sack of skin that hangs from the body at the front of the pelvis, containing the testes and epididymis. The testis is a location for Spermatogenesis and steroidogenesis process. Moreover, the epididymis is a located at the back of the testis and connects it to the vas deferens, functioning to maturate, store and carry sperm. Otherwise, the internal organs of the male MGT are consisting of the vas deferens that transports mature sperm to the urethra in preparation for ejaculation. Seminal vesicles, which produce molecules such as fructose that serve as energy sources for sperm and make up most of the seminal fluid of a man's ejaculate. Prostate gland makes up additional seminal fluid for sperm nourishment. In addition, bulbourethral (Cowper's) glands that produce a clear, slippery fluid that empties directly into the urethra to lubricate the urethra and neutralizes acidity associated with residual urine [3], [4].

In general, the testis and epididymis are considered as important organs of the MGT, whereas they performed major physiological functions of it. Interestingly, both the testis and epididymis have the same basic structural organization, which are comprised of tubules lined by a highly heterogeneous epithelium surrounded by aperitubular cellular layer and an interstitial tissue containing the vasculature and lymphatics. Although, the similarities are largely superficial, but the epithelium of the testis (so-called seminoferous epithelium) is comprised of a single somatic cell type (so-called Sertoli) cell supporting a population of rapidly differentiating and proliferating spermatogenic cells, whereas the epididymal epithelium is comprised of a number of relatively stable epithelial cell types, including principal cells, clear cells, basal cells, and halo cells, apical cells and narrow cells. [4], [5].

In view of the above, testes and epididymis are organized functionally into three compartments. The tubular compartment, which is a highly heterogeneous epithelium. The intertubular compartment, that is aperitubular cellular layer. And the vascular compartment, which is an interstitial tissue. Therefore, the intertubular compartment is a chief location of numerous of leukocytes, include resident macrophages, lymphocytes, mast cells, dendritic cells, eosinophils and polymorphonuclear leukocytes [3], [4].

Physiological Distribution of Leukocytes in the MGT

The spermatogenesis site of the MGT is immune privilege, which is supported by Cellular Components preserve the spermatogenic cells from negative effects of the autoimmune response. Leukocytes distribution are mainly rolled by four of the MGT components, which include: Blood-Epithelial Barriers (BEB), that are formed of Sertoli cells tight junctions or epididymis epithelial cells tight junctions, segregate spermatogenic cells in epithelial region away from leukocytes in interstitial regions of the MGT and protect germ cells from autoimmune attack and foreign tissue grafts. Sertoli cells, which are somatic cells formed seminiferous epithelium, secrete molecules capable of inhibiting proliferation of B and T lymphocytes. Leydig cells, which are androgen production cell, play role in immunoregulatory of macrophages and lymphocytes, as well as active suppression of antigen-specific immunity by regulatory cytokines, androgenic steroids, and other anti-inflammatory and immunosuppressive factors. The seminal plasma, which is nutrition media of spermatozoa, has immunosuppressive activities mediated by several factors as prostaglandins,

suppresses lymphocyte proliferation and Natural Killer cells activity and modifies cytokines release from antigen presenting cells [6], [7], [8], [9].

Disturbance of Leukocytes in the MGT:

Cellular Components underlying immune privilege of the MGT are susceptible to disruption by pathological alteration, that induce upset of Leukocytes distribution in MGT. Inflammatory reactions are the most common expected cause of seminal Leukocytes upset, which are correlate with cytokines overproduction and Leukocytes infiltration into spermatogenic cells site. Therefore, they interference with Sertoli cells and BEB function, result in spermatozoa deteriorations and post-testicular male infertility. Moreover, they contribute of cytokines production up to capacity of immunosuppressive activities of seminal plasma, induce defects of semen properties and weakness of sperm fertilization ability [10].

Infiltration high numbers of Leukocytes into semen is a sign of leukocytes disturbance in the MGT [11]. Therefore, World Health Organization WHO 1999 guidelineused Leukocytospermia term to describe elevation of Leukocytes in semen up to $1x10^6$ /ml and consider it one of routine semen analysis parameters that essential in Male Infertility screening.

Leukocytospermia has been frequently noted between infertile males. Whereas, clinical studies have reported the prevalence of it ranged from 16.1% to 60.7% of infertile male and indicated to it one of the expected causes of male infertility [2]. Previously, Leukocytospermia was considered as a heterogeneous etiology condition, consisting of infection, inflammation and autoimmunity [12]. As well, lifestyle factors like consuming cigarettes, alcohol, or marihuana are supposed as causes of Leukocytospermia [13].

Moreover, Infection has gotten highly importance of seminal leukocytes elevation [14].

Leukocytospermia has been strongly correlated with inflammation, autoimmunity and lifestyle factors as smoking and obesity more than infection. Where, Pasqualotto [16], Linked between chronic abacterial autoimmune prostatitis and elevated levels of Reactive Oxygen Species (ROS) that induced by increased levels of leukocytes. Lackner [15], demonstrated that Leukocytospermia has little diagnostic value in the detection of Bacteriospermia. Pasqualotto [16], showed a positive correlation between smoking and the presence of Leukocytospermia. Tunc [17], supposed that, oxidative stress increase with an increase in Body Mass Index (BMI), primarily due to an increase in seminal macrophage activation.

The pathological products of Leukocytospermia:

The high production of ROS have been associated with impaired male fertility [18].

Whereas, ROS have ability to induced lipid peroxidation and weakening of polyunsaturated fatty acids of the sperm cell membrane, and eventually decrease sperm motility [19]. In fact, leukocytes (especially polymorphonucler (PMN) leukocytes) are primary source of seminal ROS, which are produced as a consequence of inflammatory processes [20], [21], [22], [23]. Besides, Leukocytes enhance the ROS production by human spermatozoa via direct cell-cell contact or by soluble products released by leukocytes, therefore elevation of seminal leukocytes may contribute in irreversible oxidation damage of mature sperm, which lacks repair mechanisms of oxidative stress damage [24], [25].

The ROS have been noted and correlated with sperm deterioration in semen samples with Leukocytes concentration less than $1x10^6$ cell/ml, means not Grouped as Leukocytospermic according WHO guidelines. Therefore, some reporters indicated that the WHO guidelines for Leukocytospermia may need to be revised [26], [27].

The Effect of Leukocytospermia on Sperm Quality:

Elevated seminal leukocytes have been correlated with a positive as well as negative effect on sperm quality. Tomlinson [28], noted three types of seminal phagocytic cell swim with spermatozoa: small PMN leukocytes, monocytes and macrophages capable of engulfing multiple sperm heads and enhance normal morphological sperm percent in semen sample. Tomlinson [29], insured that Leukocytes concentration was not associated with either reduced semen quality or conception rates. Bouvet [30], concluded that macrophages are present in human semen and participate in immunovigilance contributing to improve the seminal quality. In contrast, Aziz [31], observed a significant positive correlation between leukocytospermia and sperm tail defects, acrosomal damage, and high Sperm Deformity Index scores. Besides, Moskovtsev [32], indicated that leukocytes concentration was significantly negative correlated with standard semen parameters such as sperm concentration, motility, and normal morphology. Zorn [33], Concluded that slightly increased leukocytes and elastase are associated with slightly poorer sperm characteristics and increased sperm necrosis, DNA denaturation and intracellular ROS and decreased mitochondrial membrane potential. Domes [34], found that elevated seminal leukocytes was the dominant factor associated with deterioration in sperm concentration, motility, normal morphology and

DNA fragmentation index (DFI). Ismail [35], demonstrated that, the presence of Leukocytospermia concomitant with a reduction of sperm motility and increase percentages of abnormal sperms. Eldamnhoury [36], illustrated a significant association between increase levels of leukocytes and decrease levels of total sperm count, sperm concentration and sperm progressive motile sperm percentage.

The purpose of this study was to evaluate seminal leukocyte concentration in infertile men, and the central hypothesis is to assess the effect of seminal leukocytes on various semen parameters in infertile men.

2. MATERIALS AND METHODS

The review committee of Life Science Department in Libyan Academy-Misurata, approved this study. Moreover, all participants were given informed consent for the use of a fraction of their sperm for research purposes.

The study was conducted between June-October 2017 National Infertility Center/ Misurata-Libya. The study was rolled on 187 infertile men. All participants were given informed consent for the use of a fraction of their sperm for research purposes. The participants weren't diagnostic as azoospermia or varicocele. In addition, they did not complain of any chronic underling diseases like diabetic, hypertensive and viral infection. The demographic data and clinical information about patients include age, infertility type, infertility period, and smoking status and were collected from their medical records. Semen specimen collection and standard semen analysis for all participants carried out according to the standard method of the WHO [37], guidelines. The participants were Grouped based on seminal leukocytes concentration into:

Group A: includes samples without Leukocytospermia (< 2WBC /HPF).

Group B: includes samples with mild Leukocytospermia (2-4WBC /HPF).

Group C: includes samples with moderate Leukocytospermia (4-6 WBC /HPF).

Group D: includes samples with sever Leukocytospermia in semen (<6WBC/HPF).

Semen collection:

All the semen samples were collected in private room near the Andrology Laboratory in Infertility Center after 48-72 hours' period of sexual abstinence by means of masturbation into a sterile wide mouth plastic container, then placed in an incubator (37°C) for liquefaction within 30 to 60 minutes. Thereby, liquefaction time and appearance of the semen were assessed, as well, semen volume was measured in a graded tube with 0.1ml accuracy and semen pH were measured by indicator paper strips (working range pH 6.5–10.0).

Standard Semen Analysis:

Makler counting chamber (SEFI-Medical Instruments LTD) using were to assessment the sperm concentration, sperm motility and leukocyte concentration. A 10µl well mixed undiluted semen was put in the center of the disc area of the chamber. Then covered by round coverslip, which was grasped gently by finger to insure the drop spread on the entire area of the disc into a thickness of 10 microns. The chamber was placed on the microscope stage, and the sperm concentration, motility and seminal leukocyte concentration were investigating under the 20x-40x objective and 10x eyepiece lenses [37].

Sperm concentration was calculated by count sperms in a strip of 10 squares. Therefore, a number of sperms counted were representing in millions per ml. If very few sperms, the concentration was a number of sperms counted in a 100 squares representing in hundreds of thousands per milliliter. The total sperm count per ejaculate was the volume of the ejaculate times the concentration per milliliter [37].

Sperm motility was determined by counting all motile and immotile spermatozoa within 10 squares. By the same way, progressive motility that represented spermatozoa moving actively, either linearly or in a large circle, and non-progressive motility that represented all other patterns of motility with an absence of progression, e.g. swimming in small circles were counted. Each motility Group calculated as following:

1. $\text{Total motility}(\%) = \frac{\text{Total Motile Sperm}}{\text{Total Sperm Count}} \times 100$
2. $\text{Progressive motility } (\%) = \frac{\text{Progressive Sperm}}{\text{Total Sperm Count}} \times 100$
3. $\text{Non-progressive motility } (\%) = \frac{\text{Non Progressive Sperm}}{\text{Total Sperm Count}} \times 100$
4. $\text{Immotile } (\%) = \frac{\text{Total Immotile Sperm}}{\text{Total Sperm Count}} \times 100$

Furthermore, Seminal leukocytes were counted in 400X of High Power Filed HPF [37].

Normal morphology was calculated by using smear slides, which were prepared by placed 20µl well mixed undiluted semen on slide, smeared by using edge of another slide, then dried by heat and stained by Leishman stain. The smear was examined under 100x oil immersion lens of microscope, and normal forms will be calculated.

However, Total Normal Sperm Concentration TNSC was calculated as following:

TNSC = sperm concentration (%) x total motility (%) x normal morphology (%) [37].

Statistical Analysis:

The Statistical Package for the Social Sciences (SPSS 20.0.0) computer software program was used for all statistical analysis. The data were expressed as mean ± SD (range) or number (%). Differences in demographic characteristics (patients' age, infertility type, infertility period and smoking status) among the Leukocytospermia Groups were checked using the chi-squared χ^2 test. The effect of leukocyte concentrations on semen parameters were tested by one way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test. A P value ≤ 0.05 was defined as statistically significant.

3. RESULTS AND DISCUSSION

General Characteristics of Study Population:

A total of 187 infertile male patients attending the National Infertility Center/ Misurata-Libya for infertility screening, who satisfied the inclusion criteria, were involved in this study to estimate seminal parameters with different leukocytes concentration. Therefore, the mean age of participants was 40.15±7.06 years with range from 26 to 65 years. 128 (68.4%) of total participants had got primary infertility and 59 (31.6%) of them got secondary infertility. However, the mean of infertile period was 6.21±4.76 years with range from 1 to 27 years. 80 (42.8 %) of participants were smoker and 107 (57.2%) were non-smoker. The mean ejaculate volume of

semen samples was 2.70±1.43 ml in range of 0.2-7 ml, semen pH was 7.76±0.42 in range of 7-9, the mean of sperm concentration was 71.49±64.74 $x10^6$/ml in range of 0.200-400 $x10^6$/ml, the mean of sperm count per ejaculate was 164.70±214.14 $x10^6$ in range of 0.200-1200 $x10^6$, the mean of total motility was 56.60±21.62% in range of 0.0-90%, the mean of progressive motility was 16.30 ± 8.62% in range of 0.0-54%, the mean of normal morphology was 10.27±10.15% in range of 0.0-44%, the mean of Total Normal Sperm Concentration (TNSC) was 28.12±48.89$x10^6$/ml in range of 0.0-268.8$x10^6$/ml and the mean of seminal leukocytes was 3.15±3.11cell/HPF in range of 0.5-25 cell/HPF (Table 3.1.)

Table 3.1 General Characteristics of 187 infertile men included in the study. The values are presented either mean± SD (range) or number (%) TNSC = Total normal sperm concentration; HPF= high power filed. * Rolled on 163 infertile male of the population study.

Characteristics		**Value**
Age (y)		40.15±7.055 (26-65)
Infertility type	Primary	128 (68.4)
	Secondary	59 (31.6)
Infertile period (y)		6.21±4.76 (1-27)
Smoking status	Smoking	80 (42.8)
Sperm parameters	Non smoking	107 (57.2)
Ejaculate volume (ml)		2.70±1.43 (0.20-7.00)
*Semen pH		7.67±0.42 (7.00-9.00)
Sperm Concentration ($x10^6$/ml)		64.74±71.49 (0.200-400)
Total count ($x10^6$)		164.70±214.14 (0.200-1,600)
Total motility (%)		56.61±21.62 (0.00-90)
Progressive motility(%)		16.30±8.62 (0.00-54)
Normal Morphology (%)		10.27±10.15 (0.00-44)
TNSC ($x10^6$/ml)		28.12±48.89 (0.00-268.80)
Leukocytes /HPF		3.15±3.11 (0.50-25.00)

The seminal leukocytes concentrations of study population:

The levels of seminal leukocytes concentration of study population are represented in Table 3.2 and Figure 3.1, which show 27 (14.4%), 48 (25.7%), 47 (25.1%), 23 (12.3%), 13 (7%), 10 (5.3%), 2 (1.1%), 4 (2.1%), 7 (3.7%) and 6 (3.2%) of our study population have Seminal leukocyte in concentration of 0-1 WBC/HPF, 1-2 WBC/HPF, 2-3 WBC/HPF, 3-4 WBC/HPF, 4-5 WBC/HPF, 5-6 WBC/HPF, 6-7 WBC/HPF, 7-8 WBC/HPF, 8-9 WBC/HPF and ≥ 10 WBC/HPF, respectively. So, 122 (65.2%) of our study population have <3 WBC/HPF in their semen samples. While, 42 (34.8%) of them have Leukocytospermia (≥3 WBC/HPF) in their semen samples.

According to our results, the prevalence rate of Leukocytospermia (≥3 WBC/HPF) is higher than 23% and 7.4% reported by Ajayi [38], Therefore, the prevalence rate of Leukocytospermia of our study population is relatively high.

Table 3.2 Frequency of seminal leukocytes concentrations of study population.
WBC= white blood cell; HPF= high power filed

Seminal leukocyte concentration (WBC/HPF)	Frequency	Percentage	Cumulative Percentage
0-1	**27**	**14.4**	**14.4**
1-2	**48**	**25.7**	**40.1**
2-3	**47**	**25.1**	**65.2**
3-4	**23**	**12.3**	**77.5**
4-5	**13**	**7.0**	**84.5**
5-6	**10**	**5.3**	**89.8**

6-7	**2**	**1.1**	**90.9**
7-8	**4**	**2.1**	**93.0**
8-9	**7**	**3.7**	**96.8**
≥10	**6**	**3.2**	**100.0**

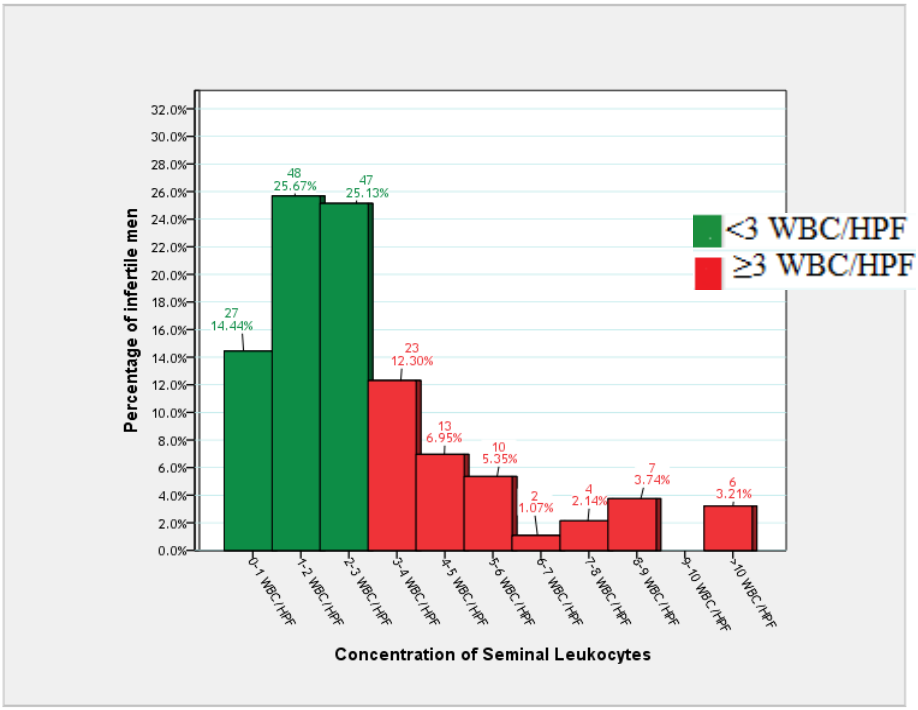

Figure 3.1 Frequency of seminal leukocytes concentration of study population

Grouped study population according to seminal Leukocyte concentrations:

To study the effect of seminal leukocytes on sperm parameters, we have Grouped our population study based on seminal leukocyte concentration into four Groups represent in Table 3.3 and Figure 3.2. Therefore, 78 (41.7 %) of our study population assigned to Group A (<2 WBC/ HPF), 68

(36.4%) to Group B (2-4 WBC/ HPF), 22 (11.8%) to Group C (4-6 WBC/ HPF) and 19 (10.2%) to Group D (>6 WBC/ HPF).

Table 3.3 Frequency of Leukocytospermia Groups of study population

Leukocytospermia Groups	Frequency	Percentage	Cumulative Percentage
< 2 WBC/ HPF	78	41.7	41.7
2-4 WBC/ HPF	68	36.4	78.1
4-6 WBC/ HPF	22	11.8	89.8
>6 WBC/ HPF	19	10.2	100.0

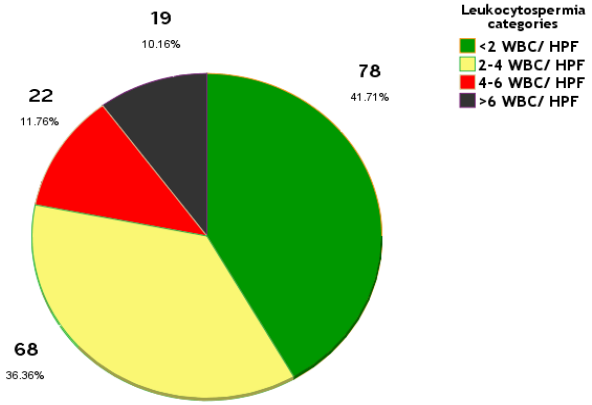

Figure 3.2 Leukocytospermia Groups of study population

Comparison of demographic characteristics between Leukocytospermia Groups

Comparison of demographic characteristics between Leukocytospermia Groups (Table 3.4) observes similarity of age groups, infertility period and smoking status between Leukocytospermia Groups (P>0.05). In contrast, it shows a significantly difference (P=0.05) of percentage of primary infertility and secondary infertility between Leukocytospermia Groups, while primary infertility percent dropped dramatically with increase of

seminal leukocytes, secondary infertility percent increased with increase of seminal leukocytes between Leukocytospermia Groups.

Regarding to our study, aging, infertility period and smoking do not play role in elevated seminal leukocytes. On other hand, secondary infertility has a relationship with elevated seminal leukocytes more than primary infertility. These results are first evidence that reported the relationship between Leukocytospermia and infertility type. However, we suggest that Leukocytospermia contributed of secondary infertility more than primary infertility. In fact, more studies are needed to clarify the relation between Leukocytospermia and infertility types.

Table 3.4 Comparison of demographic characteristics between Leukocytospermia Groups
The values are presented as number (%), WBC= white blood cell; HPF= high power filed; X^2=chi squares.
*statistically significant differences ($P \leq 0.05$)

Parameter			Leukocytospermia Groups				χ^2	*p*
		Total N=187(%)	< 2 WBC/ HPF N=78	2-4 WBC/ HPF N=68	4-6 WBC/ HPF N=22	>6 WBC/ HPF N=19		
Age group (years)								
	20-30	17 (9.1)	12 (15.4)	5 (7.4)	0 (0.0)	0 (0.0)	12.38	0.19
	31-40	89(47.6)	38 (48.7)	32 (47.1)	11 (50)	8 (42.1)		
	41-50	65(34.8)	24 (30.8)	23 (33.8)	10 (45.5)	8(42.1)		
	>50	16(8.5)	4 (5.1)	8 (11.8)	1 (4.5)	3(15.8)		
Infertility type								
	1ry	128 (68.4)	61 (78.2)	45 (66.2)	12 (54.5)	10 (52.6)	7.77	0.05*
	2ry	59 (31.6)	17 (21.8)	23 (33.8)	10 (45.5)	9 (47.4)		
Infertility period (years)								
	<5	82 (43.9)	36 (46.2)	29 (42.6)	10 (45.5)	7 (36.8)	3.38	0.95
	5-9.9	68 (36.4)	29 (37.2)	24 (35.3)	9 (40.9)	6(31.6)		
	10-14.9	25 (13.4)	8 (10.3)	11 (16.2)	2 (9.1)	4 (21.1)		
	≥ 15	12 (6.4)	5 (6.4)	4 (5.9)	1 (4.5)	2 (10.5)		
Smoking status								
	Smoking	80 (42.8)	33 (42.3)	28 (41.2)	10 (45.5)	9 (47.4)	0.306	0.959
	non-smoking	107(57.2)	45 (57.7)	40 (58.8)	12 (54.5)	10 (52.6)		

The Effect of Seminal Leukocytes on Semen parameters:

The effect of leukocytes concentration on sperm parameters (Table 3.5) shows no significant effect of leukocytes concentration on ejaculate volume (F 1.126; *P* 0.34> 0.05), semen pH (F 0.141; *P* 0.94> 0.05), sperm concentration (F 1.721; *P* 0.16>0.05), total count (F 1.894; *P* 0.13> 0.05) and TNSC (F 0.606; *P* 0.61>0.05). While, it shows a significant effect on total motility (F 5.381; *P* 0.001<0.05), progressive motility (F 3.022; *P* 0.031<0.05) and normal morphology (F 3.518; *P* 0.02<0.05).

Fisher's LSD test (Table 3.6) determines which of Leukocytospermia Groups is significantly effect on total motility, progressive motility and normal morphology. It illustrates that mean of total motility is significantly higher in Group A (62.69±17.63; *P*<0.05) compare with other Groups, while mean of total motility is significantly lower in Group D (43.21±26.25; *P*<0.05) especially in compare with Group A (Table 3.6 Fig 3.3). Additionally, mean of progressive motility is a significantly lower in Group D (11.57±7.6; *P*<0.05) compare to a rest of leukocytes concentration Groups (Table 3.6 Fig 3.4). Furthermore, the total morphology mean was significantly higher in Group A (62.69±17.63; *P*<0.05) compare with Group B (7.92±8.22; *P*<0.01) and C (8.13±7.30; *P*<0.05), while, it was not any difference between Group D (10.11±11.03; *P*> 0.05) and other Groups (Table 3.6 Fig 3.5).

According to our results elevation seminal leukocyte appear a significant effect on sperm quality. Where, the sperm motility is gradually decreased with increase of seminal leukocytes concentration, which are consistent with Lackner [39], who suggest that the association between leukocytes and semen quality might be concentration dependent. Domes [34], and Lobascio

[40], who suggested that the elevated seminal leukocytes was associated with a statistically significant deterioration in sperm motility. Therefore, the data from this study insured that total motility one of sperm parameter that highly sensitive to presence of leukocytes in seminal fluid even in low concentration. Moreover, this study demonstrates a significant effect of high concentration of seminal leukocytes on progressive motility of sperm. These data align with Chan [41], who indicated that progressive motility of sperm effected significantly by high concentration of seminal leukocytes. Similarly, previous study [42], illustrated a significantly negative effect of seminal leukocytes on progressive motility. These data are consistent with the hypothesis that the progressive motility of sperm induces just by high concentration of seminal leukocytes (>6 WBC/HPF). A closer examination of the effects of seminal leukocytes on normal morphology of sperm suggests dual effects, means that they have positive and negative effect based on their concentration. These finding in agreed with Zorn [33], observation, that the best sperm normal morphology at low and high seminal leukocyte concentration, while observed the lowest sperm normal morphology at moderate seminal leukocyte concentration. We suppose that dropped of normal morphology of sperm with slightly increase of seminal leukocyte (2-6 WBC/HPF) it may due to production of ROS [31]. On other hand, re-elevated of normal morphology of sperm at >6 WBC/HPF may due to phagocytosis activity of seminal leukocyte to word abnormal sperm morphology [30].

Table 3.5 The effect of leukocytes concentration on sperm parameters
[a] ANOVA test * Significant P <0.05

Semen parameters	F [a]	*P*
Ejaculate volume (ml)	1.126	0.34
Semen pH	0.141	0.94
Sperm concentration (x10^6/ml)	1.721	0.16
Total count (x10^6)	1.894	0.13
Total motility (%)	5.381	0.001*
Progressive motility (%)	3.022	0.03 *
Normal morphology (%)	3.518	0.02 *
TNSC (x10^6/ml)	0.606	0.61

Table 3.6 Comparison of total motility, progressive motility and normal morphology between Leukocytospermia Groups
Values are presented as mean ± SD.
[a-h] Same letters indicate statistically significant differences ($P \leq 0.05$).

Semen parameters	Leukocytospermia Groups			
	< 2 WBC/ HPF N=78	2-4 WBC/ HPF N=68	4-6 WBC/ HPF N=22	> 6 WBC/ HPF N=19
Total motility (%)	62.69±17.63[a,b,c]	55.04 ±22.91[a,c]	51.45±19.98[b]	43.21±26.25[c]
Progressive motility (%)	16.22±8.72[d]	16.71±8.44[e]	19.43±8.40[f]	11.57±7.64[d,e,f]
Normal morphology (%)	12.97±11.55[g,h]	7.92±8.22[g]	8.14±7.30 [h]	10.11±11.03

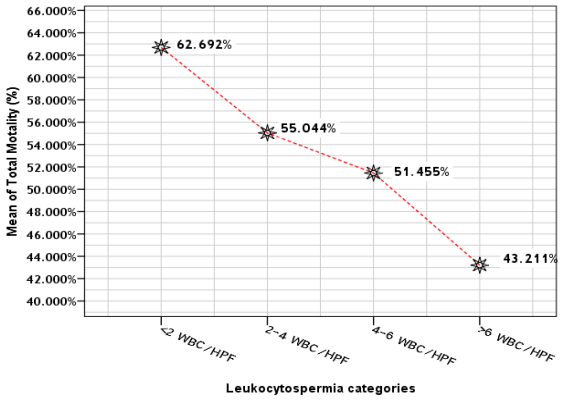

Figure 3.3 Comparison of mean sperm total motility between Leukocytospermia Groups.

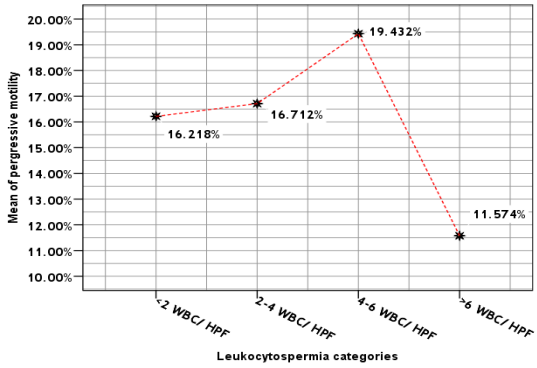

Figure 3.4 Comparison of mean sperm progressive motility between Leukocytospermia Groups.

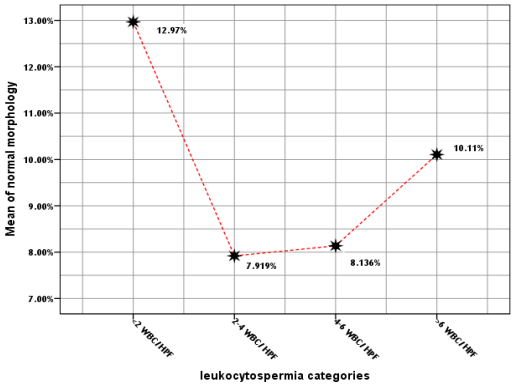

Figure 3.5 Comparison of mean sperm normal morphology between Leukocytospermia Groups.

4. CONCLUSION

Leukocytospermia is a pathological condition has been frequently noted between infertile males. Multifactor as infection, inflammation, autoimmunity, smoking and obesity, may contribute of Leukocytospermia appearance. The effects of Leukocytospermia on sperm quality and fertilization ability of sperm are controversial subject. The current study aimed mainly to evaluate the prevalence of Leukocytospermia in study population and assessment the effect of seminal leukocytes on various semen parameters. Therefore, it has gotten out many findings that reflect the significant effects of Leukocytospermia on sperm quality. However, the prevalence rate of Leukocytospermia in study population is relatively high compare to previous studies. Leukocytospermia is a risk factor of secondary infertility, whereas most of secondary infertility men have a high concentration of seminal leukocyte. Total motility of sperm is most sensitive seminal parameter to seminal leukocytes elevation, where is gradually decreased with increase of seminal leukocytes concentration. Progressive motility of sperm effected significantly by high concentration of seminal leukocytes (>6 WBC/HPF). Lastly, normal morphology of sperm has improved with high level of seminal leukocytes (>6 WBC/HPF).

In light of the above, we concluded that seminal leukocytes have dual effect of sperm quality. While, they caused of deterioration of different motility forms of sperm, they contributed of improvement of normal morphology percentage especially in high concentration level. However, Future studies are needed to examine the dual effect of seminal leukocytes on sperm quality. Although, our data has demonstrated that the prevalence of Leukocytospermia in study population and the effects of seminal leukocytes

on sperm quality, but it limits to identify the pathogenesis of these effects. Thus, future studies are necessary for extending the scope to determine which of seminal leukocytes products role Leukocytospermia effects on sperm quality and what extend of pathological changes due to seminal leukocytes on DNA, membranous organelles and other cellular structure of sperm.

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