

# ASSOCIATION OF LEUKOCYTOSPERMIA WITH SEMEN ANALYSIS PARAMETERS AMONG INFERTILE COUPLES

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## ABSTRACT

**Objective:** To evaluate various abnormalities of semen and their association with seminal fluid leukocytes among infertile couples.

**Material and Methods:** This descriptive cross-sectional study done at Department of Pathology in CMH Multan from 1<sup>st</sup> Sep 2019 to 30<sup>th</sup> May 2020. Total of 326 semen samples were received and analyzed. Sampling technique was non-probability consecutive sampling. These tests were advised by their clinicians/ consultants or out of their self-awareness of semen analysis importance. Sample of semen was collected by masturbation. The samples were kept at 37°C for 30 minutes for liquefaction and were examined within 1 hour.

**Results:** Subjects in study were between 20 to 50 years. Duration of their marriage was as; 213 individuals (65.3%) were between 1-5 years and 56 individuals (34.7%) were between 6 to 15 years. Out of 326 semen samples following results were revealed; Oligozoospermia 9 (2.8%), Asthenozoospermia 80 (24.5%), Oligoasthenozoospermia 76 (23.3%), Azoospermia 10 (4%), whereas 161 cases (49.4%) had semen essentially within normal limits. Hypospermia was seen in 24 (7.3 %) of patients & hyperspermia was seen in 5 (1.5%) of patients while 297 (91.2%) had normal semen volume. Pus cells were found in 64 (19.7 %) patients. Significant motility abnormality was seen in patient with >5 pus cells in semen (p <0.05).

**Conclusion:** Semen analysis among infertile couples revealed that 50% of individuals have abnormal semen parameters. Increased leukocytes count in semen is associated with sperm motility issues contributing to infertility.

**Key Words:** Semen analysis, Spermatozoa, Azoospermia.

This article can be cited as: Younas M, Nadeem A, Hussain M, Iqbal H, Sheikh S, Afzal K. Association of leukocytospermia with semen analysis parameters among infertile couples. Pak J Pathol. 2021; 31(1): 11-14.

## INTRODUCTION

In Pakistan, one of the main motives of marriage is to have children for continuation of inheritance to the descendants. Inability to conceive is extremely unpleasant experience [1]. Infertility brings negative influence on marital relation and satisfaction as it creates emotional disturbance, sexual dissatisfaction and impaired social adjustment and deterioration of marital relationships [2]. Married couples seek medical attention as soon as they experience infertility and approach for assessment of factors of infertility/ subfertility; infertility being unable to conceive beyond 12 months of unprotected intercourse [3]. It is estimated that 8-12% of couples are affected by infertility worldwide, with a male factor being a primary or contributing cause in approximately 50% of couples [4]. Worldwide 48.5 million couples are facing this issue [5]. Semen analysis plays fundamental role in distinguishing male causes of infertility with sensitivity of about 90% [6,7].

The common elements for male infertility are smoking, diabetes mellitus (DM), surgery, post

pubertal mumps, viral venereal diseases, exposure to pollution and history of fever [8]. Other possible factors may include exposure to chemical, radiation and thermal substances. Similarly, Alcohol/ drugs addiction and steroids also have adverse effects on sperm quantity and quality affecting fertility [9]. Presence of leukocytes in semen is commonly observed phenomenon and presence of > 1 X 10<sup>6</sup> leukocytes/ ml of semen has been used as a marker for significant leukocytospermia, as this number is associated with infection of the genital tract and there is an increased chance of significantly lowered semen quality [10].

Studies about association of increased leucocyte count in semen and their impact on male infertility in Pakistan are lacking. In view of above this study was conducted to find semen parameters that are disturbed in infertile males and to determine its association with raised leucocytes count in semen in our population.

## MATERIAL AND METHODS

This cross-sectional study was conducted at Department of Pathology in Combined Military Hospital Multan from 1<sup>st</sup> Sep 2019 to 30<sup>th</sup> May 2020. A sample size of 326 was calculated using WHO sample size calculator with prevalence of 15% and confidence interval of 95%. Non-probability

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Received: 11 Nov 2020; Revised: 18 Feb 2021; Accepted: 26 Mar 2021

consecutive sampling technique was used for sample collection. All patients reporting to department of Pathology for evaluation of male factor of infertility / subfertility through semen analysis with marital history of >12 months having unprotected intercourse for ≥12 months were included in the study irrespective of age. Subjects who did not give consent or those who were unable to give sample through masturbation were excluded.

After taking permission from Hospital Ethical Committee, written informed consent was taken from the patients. Patients' detailed clinical information was noted on Study Proforma including age, duration of marriage, previous children, inguinal/ scrotal surgery, history of Mumps and DM. Personal history of smoking, alcohol and tobacco chewing were noted. Patients were provided with labelled/ sealed sterile plastic jars for the collection of semen through masturbation. Samples were immediately transferred to incubator with temp of 37° C for liquefaction of semen for 30 minutes. Volume was measured with the help of a pipette and seminal fluid pH was carried out by pH paper. This was followed by microscopy of seminal fluid for sperm count, leucocytes count, sperm morphology and motility.

Normal semen analysis parameters as defined by WHO included; volume 1.5 ml (1.4-1.7), total sperm count 39 million/ ejaculate (33-46); sperm concentration 15 million/ ml (12-16); sperm vitality 58% live (55-63); total (progressive and non-progressive) motility 40% (38-42), progressive motility 32% (31-34); and morphologically normal forms >4.0% [11].

Data was analyzed by SPSS version 24.0. Descriptive statistics like frequency, percentages, mean and Standard Deviation (SD) was calculated. Chi square test was applied to see association between seminal fluid leukocyte count and final result of report. P-value of ≤ 0.05 was considered significant.

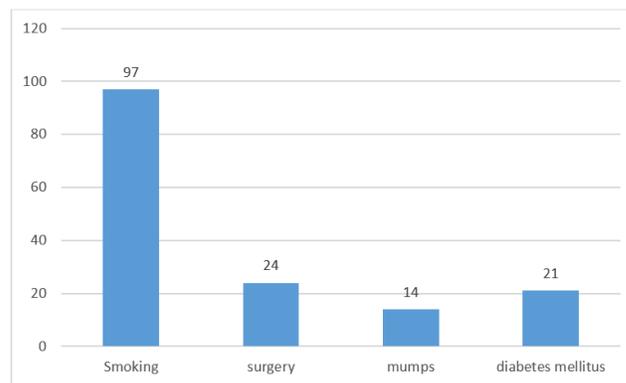
**RESULTS**

Most of the patients who reported to us for semen analysis were younger with quite short duration of marriage and most of them had primary infertility (Table-I).

**Table-I: Demographic Data (n = 326)**

Parameter	No. of patient	Percentages
<b>Patient age groups</b>		
20 -35 years	270	82.5 %
36- 50 years	56	17.2 %
<b>Duration of Marriage</b>		
1-5 years	213	65.3%
6-15 years	56	34.7%

Cigarette smoking was identified as most common risk factor in history in these infertile males, followed by less common findings including history of DM, inguinal surgery, mumps and infections (Figure- I)



**Figure-I: Risk factors among infertile males (n=326).**

Around 50 % of patients had normal semen analysis. Asthenozoospermia was seen in 80 (24.5%) subjects and Oligoasthenozoospermia in 76 (23%) subjects. Hypospermia was seen in 24 (7.3%) of subjects & hyperspermia was seen in 1.5% of subjects while 297 (91%) had normal semen volume. Pus cells were found in 64 (19.7 %) patients. When compared seminal pus cells to final result of the report, there was significant motility abnormality in patients with pus cells >5 in semen (p value <0.05). (Table-II & III)

**Table-II- Semen analysis parameters and result of report (n=326).**

Parameters	Number of patients	Percentage
<b>Volume</b>		
< 1.5 ml	24	7.3%
1.5 ml to 6 ml	297	91.1 %
>3 ml	5	1.5%
<b>Sperm Count</b>		
<20 million	9	2.8%
>20 million	315	97.2%
<b>Motility</b>		
Abnormal	80	24.5%
Normal	246	75.5%
<b>Morphology</b>		
Abnormal	19	5.8 %
Normal	307	94.2 %
<b>Pus cells</b>		
No pus cells	262	80.4%
1-5	39	12%
>5	25	7.7%

**Table-III: Association of pus cells with final result of report (n = 326).**

	1-5 pus cells	>5 pus cells	No pus cells	p value
Oligozoospermia	0	0	9	0.01
Asthenozoospermia	19	19	42	
Oligoasthenozoospermia	18	2	56	
Essentially within normal limits	2	4	155	

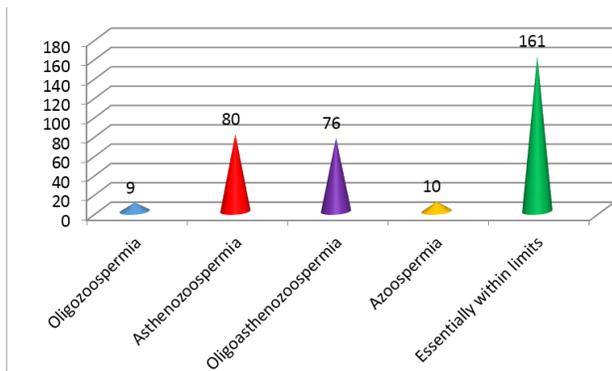


Figure-II: Final Result of semen analysis report (n=326).

## DISCUSSION

Infertility is one of most distressing challenge to public health and is associated with negative effect on marital/ family relationship as well as a risk to dissatisfaction and separation of couples [12]. Our study revealed that most patients reporting for semen analysis were younger (<35 years), they had primary infertility and 65% of them reported within 5 years of marriage. Age range was 20-50 years. Our data is comparable with Sheikh AH *et al* who reported the similar results with 82% patient between 20-35 age groups and 56% reported fertility clinic within 1-5 years of marriage. In the same study author found DM in 2%, impotency in 2%, varicocele in 4%, mumps in 6% and premature ejaculation in 2% of subjects [13]. Duration of marriage in present study participants was quite short as; 213 individuals (65.3%) were between 1-5 years and 56 individuals (34.7%) 6-15 years which is in accordance with earlier studies [9].

Out of 326 semen samples following was revealed; Oligozoospermia 9 (2.8%), Asthenozoospermia 80 (24.5%), Oligoasthenozoospermia 76 (23.3%), Azoospermia 10 (4%), whereas 161 cases (49.4%) had semen essentially within normal limits which is in accordance with Khan MS *et al*. Hypospermia was seen in 12 % of patient and hyperspermia was seen in 7.3% of patients while 85% had normal semen volume. Pus cells were found in 19.2 % patients. When comparing pus cells to result of report, there was significant motility abnormality in patient with >5 pus cells in semen ( $p < 0.05$ ) which is in accordance with Khan MS *et al* (n= 1521) who found 13 % had azoospermia, 23% oligozoospermia, 1% polyzoospermia, 14% normozoospermia, 35% asthenozoospermia, 11% oligoasthenozoospermia and 2.4% teratozoospermia [14].

In present study increased leucocytes were detected in different subclasses of infertile males and fewer leucocytes were noticed among subjects with normal parameters of semen in present study. Pus

cells showed an inverse relationship to sperm motility and count. Similarly, fewest leucocytes were observed among subjects where normal forms were more frequent. More leucocytes were observed in cases where motility, concentration or morphology was disturbed. Similarly, Ajai AB *et al* have found that more seminal fluid leucocytes were observed among obese men having azoospermia, reduced sperm motility and lower count of sperms with oval head morphology. They concluded that pyospermia should be analyzed in accordance with body mass index (BMI) and mean progressive motility in their management of male infertility but this aspect was not included in present study [15].

Male factors of infertility should be considered precisely to manage the patients properly and earlier. In the present study 19.7% of patients exhibited pyospermia which is in accordance with previous studies where pyospermia was found in up to 30% semen samples of infertile men [16]. Similarly, Guzick DS *et al* have concluded that sperm concentration, motility, and morphology are extremely important in workup of infertility [17].

In our study abnormal semen parameters were seen in 50% of subjects. The most common semen abnormality seen was asthenozoospermia and oligoasthenozoospermia which is 24.5% and 23% respectively where as azoospermia and teratozoospermia constituted only minority of cases. This is in accordance with Butt F *et al* (n= 396) who revealed that semen evaluation is the basis for assessment of male infertility. Sperm concentration, motility and morphology are interrelated; factors that disturb one of them usually also have negative impact on the other two as well. Out of them 293 (74%) had normospermia, 59 (15%) had azoospermia and 44 (11%) had oligospermia. The subjects with oligospermia had lower volume and significantly higher percentage of non-motile sperms and abnormal morphology  $55\% \pm 16\%$  in comparison to normal samples ( $p$ -value 0.0001) [18, 19]. In another study Khan MS *et al* (n=396) found that 35% subjects had isolated asthenozoospermia and 11% had oligoasthenozoospermia [20]. Similarly Memon BA *et al* (n=500) have concluded that 17% of tested individuals had azoospermia which supports present study too [21].

Cigarette smoking was identified in 97 (29.7%) cases as most common risk factor in these infertile males, followed by DM 21(6%), inguinal surgery 24 (7%), mumps and infections which is in accordance with earlier studies [22,23].

WHO recommends that motility of sperms and their concentration is better forecaster of fertility potentials than sperm morphology, which is an agreement to our study as well, as motility is significantly lower in the infertile subjects. Recently a number of computer-assisted sperm analysis (CASA) systems have been developed, but traditional methods have a greater clinical utility and significance [24]. Sperm motility is an important indicator of quality of semen and its fertility potentials, because it is required for penetration of cervical mucus, transport across the female genital tract and penetration through the corona radiata zona pellucida before oocyte fertilization [25].

## CONCLUSIONS

Semen analysis among infertile couples revealed 50% of subjects had abnormalities. Increased leukocytes in semen are associated with sperm motility issues contributing to infertility.

## RECOMMENDATIONS

Further research is recommended to assess correlation between the presences of leukocytes in semen with semen culture results.

## AUTHOR CONTRIBUTION

**Muhammad Younas:** Draft preparation

**Aamir Nadeem:** Proof reading

**Mohsin Hussain:** Literature review, statistical analysis

**Hamid Iqbal:** Idea conception, data collection

**Shagufta Sheikh:** Data analysis

**Kamran Afzal:** Study design

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