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Original Research Article

Study of Prevalence of Leukocytospermia in GovernmentTeaching Hospital, JNKTMCH, Madhepura

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Abstract:

Background and Objectives: Infertility and childlessness is one of the major health and family problems throughout the world. The prevalence of infertility in the general population is estimated to be 15% to 20%. Of this, male factor alone is responsible for 20 to 40%. Human male infertility is normally assessed on the basis of semen analysis. Among the various parameters which are studied, the association between seminal Leukocytes and semen quality is still contradictory, but majority of the studies have proved the significant role of leukocytes in altering the semen quality by various mechanisms like ROS production, immune reactions etc., and causing deleterious effects on sperm count, motility, morphology, etc., resulting in infertility. Leukocytospermia is defined as the presence of more than 1 million leukocytes per milliliter of semen by the WHO and is also included in the WHO guidelines for semen analysis since long back.

To study Leukocytospermia in semen samples.

To find out the prevalence of leukocytospermia in the general (male) population of surrounding feeding areas with special reference to infertile men.

To study the relation/association between leukocytospermia and infertility status.

Methodology: A Total of 95 semen samples were received in the department of pathology with history of infertility, varicocoele, undescended testis, etc., One sample could not be processed because of urine contamination. Routine semen analysis including leukocyte count by Peroxidase staining test was done on all the remaining 94 samples. Out of these 94 samples, 6 were repeat samples of short duration, another 6 were with history other than infertility like varicocoele, undescended testes, etc., and 5 samples had symptoms of urinary tract infection. So,excluding these, the remaining 77 samples with history of infertility were taken up for leukocytospermia study. Conclusion: Leukocytospermia is not to be neglected as it is found to be associated with significant number of idiopathic male infertility cases, and in-turn, it is influenced bymany factors like smoking, etc. Detection of leukocytospermia by simple and cost- effective tests like Leukocyte Peroxidase staining should be included in the routine semen analyses especially in men under infertility investigation before putting them to higher, deeper and high-cost investigations, and appropriate and timely intervention may help the men with infertility due to leukocytospermia.

Keywords: Anti-Mullerian Hormone, Adenosine Tri-Phosphate; Human immunodeficiency virus, Immotile (Sperm), Luteinizing Hormone, Obstetrics and Gynaecology.

Introduction

Infertility and childlessness is a major health and family problem in the worldespecially in the third world countries. It is a distressing condition that adds to the psychological trauma of majority of infertile couples. The clinical definition of infertility used by the World Health Organization (WHO) is “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after12 months or more of regular unprotected sexual intercourse with the same partner”. [1] Infertility may be primary (inability to have any live birth) or secondary (inability to have additional live birth). Multiple factors—infectious, environmental, genetic, and even dietary in origin—can contribute to infertility. [2] These factors may affect the female, the male, or both partners in a union, resulting in an inability to become pregnant or carry a child to term. The prevalence of infertility in the generalpopulation is estimated to be 15% to 20%. Of this, male factor alone is responsible for20 to 40%. [3] In Indian couples seeking treatment for infertility, male factor is the causein approximately 23%. [4] The major causes for male infertility include varicocoele, genital tract obstruction, testicular failure, cryptorchidism, idiopathic or unexplained male infertility (UMI), gonadotoxin exposure, genetic conditions, infections, hormonal dysfunction,

immunological conditions, ejaculatory/sexual dysfunction, cancer and systemic diseases. [5] Despite advances in technologies and diagnostic methods in the field of andrology, there remains a significant subset of subfertile men who are classified as having 'unexplained male infertility' (UMI). Men are categorized as having UMI when they are infertile despite having normal semen analysis, normal history and physical examination and when female factor infertility has been ruled out. [6]

The average incidence of UMI is approximately 15% although reports of UMI instudy populations have ranged from 6% to 37%. Possible factors that might explain the difficulties to conceive in UMI include the presence of antisperm antibodies, sperm DNA damage, elevated levels of reactive oxygen species (ROS) and sperm dysfunction. [7,8,9] The presence and role of leukocytes in these factors are established in many studies and is still under further research. Human male infertility is normally assessed on the basis of Semen Profile with respect to Spermatozoa. Any process that affects sperm/semen production and quality is potentially harmful to male fertility. Leukocytes are found in virtually every ejaculate and function at multiple levels. [10] Among the various parameters which are studied under semen analysis, the association between seminal Leukocytes and semen quality is still contradictory and a subject of debate in literature. The concentration (rather than their absolute presence or absence) of leukocytes in the semen has more close association with the semen/sperm quality and quantity as revealed by many studies. [11] A leukocyte concentration of 1 million/mL of semen is taken as the standard and any value above this is termed Leukocytospermia by the WHO. Leukocytospermia is also regarded as one of the major semen parameters mentioned by WHO guidelines for semenanalysis. It has an incidence of 10 to 20% in the general population and is especiallycommon in infertile men. [12]

Objectives

To study Leukocytospermia in semen samples.

To find out the prevalence of leukocytospermia in the general (male) population of surrounding feeding areas with special reference to infertile men.

To study the relation/association between leukocytospermia and infertility status.

To study the effect of leukocytospermia on various semen/sperm parameters.

Material and Methods

The Cross sectional study. was carried out in the department of Pathology, JNKTMCH, Madhepura. Semen samples of subjects were referred from department of OBG, and department of Surgery, JNKTMCH Madhepura. Observation method of primary source of information was used on these semen samples.

Total Study Time: 18 months, Statistical Methods Used: 1. Proportion, 2. Chi-Square/Z Test for proportion, 3.Contingency tables, 4. Bar graph, 5. Pie graph, 6. Phi-Coefficient. 7. Mean Deviation, 8. t-value, etc. Sample Size: Calculated by using the datas, 1. Level of Significance, 2. Error and Sample size calculated by statistical technique was 71 subjects. However, Enumeration technique adopted for the duration of 1 year was considered for the study and 95 samples were received out of which, 77 samples were taken for study after applying the exclusion criterias.

Inclusion Criteria

All Male patients sent for semen analysis for infertility investigation of the couples, for their follow up and also for assessing the quality and quantity of semen in various pathological conditions of a patient.

Exclusion Criteria

Subjects not willing for study.

Samples of men with symptoms of genital tract infection.

Samples of unmarried men.

Samples of men not under infertility investigation.

Repeat (Follow-up) samples and

The present study was done on all semen samples sent for analysis to detect the cause for infertility, or follow up of that couple and also for assessing the quality and quantity of semen of patients with various pathological conditions. The WHO-2010 guidelines for semen analysis were followed for analysing variousparameters of each semen sample.

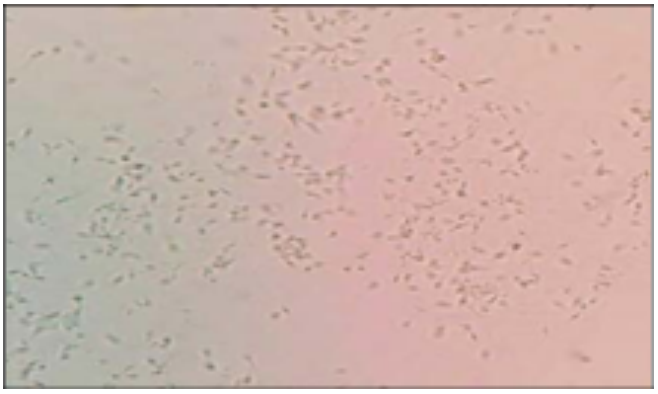

Figure 1: A wet-mount view 40x

Results

A total of 95 semen samples were received. Out of these, 1 sample could not be processed due to urine contamination. Routine semen analyses including Peroxidase staining test for leukocyte count for the remaining 94 samples were done and reported. Of these 94 cases, 6 were repeat samples after short durations (2-6 weeks). So, 88 cases were left. Out of these 88 cases, 67 (76.1%) cases were with history of primary infertility, 15 (17.1%) cases with secondary infertility and the remaining 6 (6.8%) cases were miscellaneous (Cases for investigation other than infertility: 2 cases were unmarried, one with bilateral undescended testes and the other with bilateral varicocoele, 1 case for follow up examination for vasectomy, 1 case wanting for recanalization and the remaining 2 cases were fertile with bilateral varicocoele). Out of these 88 samples studied, 24 (27.3%) cases were found to be positive for leukocytospermia. The 88 case-samples reported included 6 cases not under infertility investigation and 5 cases with symptoms of uro-genital tract infection. The 24 leukocytospermia cases included all the 5 cases with symptoms of genital tract infection and 3 cases not under infertility investigation. Thus, excluding the 5 cases with symptoms of uro-genital tract infection, 19 (22.8%) out of 83 cases were positive for leukocytospermia, i.e., the prevalence of leukocytospermia in the general population of men is 22.8%. Finally, excluding the cases which were not under infertility investigation, we got 16 leukocytospermia cases from 77 cases under infertility investigation with a percentage prevalence of 20.8 for the present study.

Table 1: Age-range of presentation

Age-range(years)	Number of cases(77)
25-29	19
30-34	31
35-39	22
40-43	05

Table 2: Risky habits compared with leukocytospermia

Habits	Total number of cases (Out of 77)	No. of men with leukocytospermia (Out of 16)	Percentage (%)
Smokers	47 (61%)	14	29.8
Non-smokers	30	2	6.7
Alcoholics	39 (50.6%)	10	25.6
Non-alcoholics	38	06	15.8
Risky sexual activity (Anal intercourse etc.,)	14 (18.8%)	06	42.9
Non-risky sexual behavior	63	10	15.9

As can be seen, Leukocytospermia is found in 14 (29.8%) out of47 smokers as against just 2 (6.7%) out of 30 non-smokers. Also, of the 16 leukocytospermic men, 14 (87.5%) are smokers and only 2 (12.5%) are non-smokers.

Table 3: Smoking * remarks cross tabulation

			Leukocytospermia		Total
			Present	Absent	
Smoking	Yes	Count	14	33	47
		% within REMARKS	87.5%	54.1%	61.0%
	NO	Count	2	28	30
		% within REMARKS	12.5%	45.9%	39.0%
Total		Count	16	61	77
		% within REMARKS	100.0%	100.0%	100.0%

In the present study, leukocytospermia is also associated with alcohol intake. Of the 39 alcoholics, 10(25.6%) were positive for leukocytospermia and of the 38 non-alcoholics, 6 (15.8%) were leukocytospermic. Ten (62.5%) out of 16 leukocytospermic men are alcoholics and 6 (37.5%) are non-alcoholics. Out of 14 men with risky sexual activities 6 (42.8%) are having leukocytospermia, and of the 63 men with non-risky sexual activities cases only 10 (15.2%) are showing leukocytospermia. In the present study, out of the total of 77 study samples, 57 (74%) were having normal percentage (> 32%) of progressive motility, and 20 (26%) were having lower than normal percentage of progressive motility. (Table 4)

Table 4: distribution of sperm motility in the samples

Total No. of semen samples	77
Semen samples with Normal percentage of Progressive Motility	57 (74%)
Semen samples with Reduced percentage of Progressive Motility	20 (26%)

In the leukocytospermic group, of the 16 samples, 7 (43.8%) had normal percentage of progressive motility and 9 (56.2%) had less than the normal percentage of progressive motility, whereas in the non-leukocytospermic group, out of 61 samples, 50 (82%) had normal percentage of progressive motility and 11 (18%) had less than the normal percentage of progressive motility. From this comparison, it is evident that, samples with leukocytospermia have higher percentage of reduced progressively motile sperm percentage than those found in non-leukocytospermic samples.

When leukocytospermia was brought into the picture, out of 16 leukocytospermic samples, majority i.e. 12 (75%) were diagnosed as Abnormal and remaining 4 (25%) as Normal Study. Whereas in Non-leukocytospermic group, out of 61 samples, 21(34.4%) were diagnosed as Abnormal and 40 (65.6%) as Normal Study. This comparison shows that leukocytospermia is associated with more number and percentage (75%) of Abnormal diagnoses (Final) than that found in non- leukocytospermic group (34.4%)

Table 5: Labdiag * remarks cross tabulation

			Leukocytospermia		Total
			Present	No	
Lab diagnoses	Normal	Count	4	37	41
		% within REMARKS	25%	60.7%	53.2%
	Abnormal	Count	12	24	36
		% within REMARKS	75%	39.3%	46.8%
Total		Count	16	61	77
		% within REMARKS	100.0%	100.0%	100.0%

Table 6: Symmetric measures

		Value	Approx. Sig.
Nominal by Nominal	Phi	0.290	0.011
	Cramer's V	0.290	0.011
N of Valid Cases		77	

Discussion

From the early research on this subject of Leukocytospermia done in the late 1970s, until now, there have been multiple investigators that have tried to better define and interpret the implications of the presence of leukocytes in a semen sample. The oldness of the original data used by the WHO to define leukocytospermia, the lack of a reliable cost-effective method to detect and differentiate white blood cells (WBC) accurately, the significant variation between semen samples of the same individuals and the conflicting results

of research done in this subject are hurdles thatthe scientific community has failed to overcome. Most of the studies published upto this date have been done in small samples and without taking into consideration known and probably unknown confounding variables and the present study is also faced with some of such hurdles and attempts are made to overcome these as for as possible.

There is a wide variation of prevalence of Leukocytospermia depending on different studies by different authors. A review of the available literature supports a prevalence of leukocytospermia from 16% to 60.7% in general population. A largenumber of studies have shown a wide range of leukocytospermic incidence from 2% to 35% in infertile men. Majority of studies with the largest number of cases have estimated the prevalence of leukocytospermia to be 12% to 20% among all infertile men. Potts et al. (1999) found higher sperm DNA damage in smokers than in non- smokers, and Singh et al. (2003) found that there was a significant higher sperm DNA damage in smokers above the age of 36 years. Reina Bouvet et al., in their study in men with idiopathic infertility having habit of tobacco smoking, demonstrated alterations in sperm concentration and morphology with an elevation of immature forms. Lower sperm penetration assay scores and greater numbers of leukocytes in the seminal fluid were also noticed in smokers. [13]

In a prospective study, Saleh et al. compared infertile men who smoked cigarettes with non-smoker infertile men. Smoking was associated with a significantincrease (approximately 48%) in seminal leukocyte concentrations, a 107% ROS level increase and a 10 point decrease in ROS-TAC score. An earlier study also reported anassociation between cigarette smoking in infertile men with increased leukocyte infiltration in their semen. A study by Grygoruk C, Mrugacz G, et al. on 100 semen samples with severe oligospermia and leukocytospermia treated with anti-inflammatory, anti-bacterial and anti-oxidative treatment combined with dietary supplementation for 90 days showed improvement in semen quantity and quality, sperm concentration, motility and morphology with decrease in leukocyte count. [14] Reina Bouvet et al. in their study in men with idiopathic infertility having habit of tobacco smoking demonstrated alterations in sperm concentration and morphology with an elevation of immature forms. Lower sperm penetration assay scores and greater numbers of leukocytes in the seminal fluid were also noticed in smokers. In the present study, out of total of 77 samples under study for infertility investigation, 44 (57.1%) are having a final diagnosis as Normal Study and remaining 33 (42.9%) as Abnormal Study. Thus, majority (57.1%) are showing Normal Study in the routine semen analysis and still they are under infertility investigation. So, there must be probably some other causes for this infertility, perhaps leukocytospermia, sperm DNA defects, immune mechanisms or idiopathic (unexplained male infertility i.e. UMI) which can be disclosed by higher and deeper investigations.

Conclusion

Normal semen analysis does not guarantee the fertilization potential of sperm, and studies have shown significant overlap in semen parameter values between fertile and infertile men. Furthermore, routine semen analysis does not provide information regarding defects in sperm function. Many key aspects of the fertilizationprocess such as transport of the sperm to the oocyte, sperm interaction with the cervical mucus and sperm interaction with the oocyte cannot be assessed by conventional semen analysis. In the present study, most of the leukocytospermia cases are with history of infertility and their routine semen analysis study are showing quite a significant amount of gross abnormalities like reduced semen quantity and quality (Like PH, liquefaction time, etc,.), low sperm density, decreased percentage of motility and increased percentage of abnormal sperm morphologies than those found in non- leukocytospermic cases.

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