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Original Research Article

The Study of Prevalence of Leukocyto Spermia in PMCH Patna

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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., 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., Study duration of 18 Months. 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.,

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 by many 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; White blood corpuscle

Introduction

Infertility and childlessness is a major health and family problem in the world especially 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 after 12 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 general population is estimated to be 15% to 20%. Of this, male factor alone is responsible for 20 to 40%. [3] In Indian couples seeking treatment for infertility, male factor is the cause in 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 in study 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 semen analysis. It has an incidence of 10 to 20% in the general population and is especially common in infertile men. Hence, the present "Study of Prevalence of Leukocytospermia" is taken up.

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.

Material and Method

The Cross sectional study. The present study was carried out in the department of Pathology, Patna Medical College and Hospital Patna Bihar. Study duration of 18 Months. Semen samples of subjects were referred from department of OBG, PMCH and department of Surgery.
Observation method of primary source of information was used on these semen samples.
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

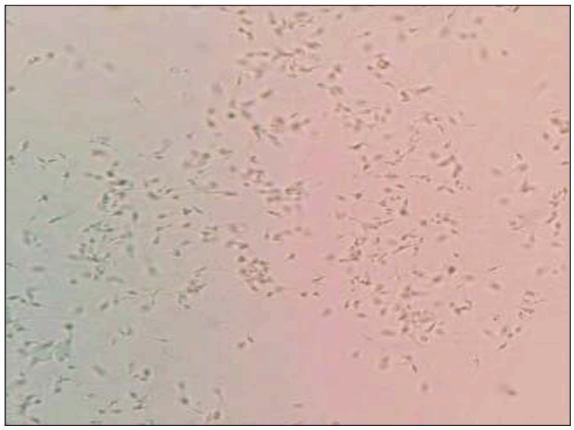
All Male patients sent for semen analysis for infertility investigation of the couples, for their follow up and also for assessing the quality and quantity of semen in various pathological conditions of a patient.

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.

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 various parameters of each semen sample. Semen samples were collected in fresh after 3-5 days of ejaculatory abstinence in a wide mouthed plastic bottle with graduations and labelling for patients' details over it, emptying the bladder and washing the urethral part of glans before masturbating.

Before proceeding to microscope, 2 smears were made from the semen sample and kept for air-drying for staining and morphological examination (described in detail later).

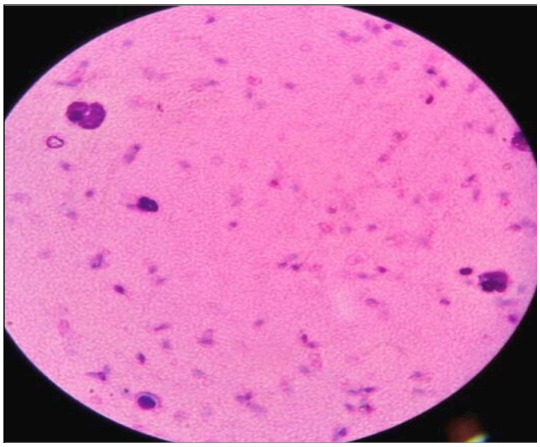


A Wet-Mount View 40x

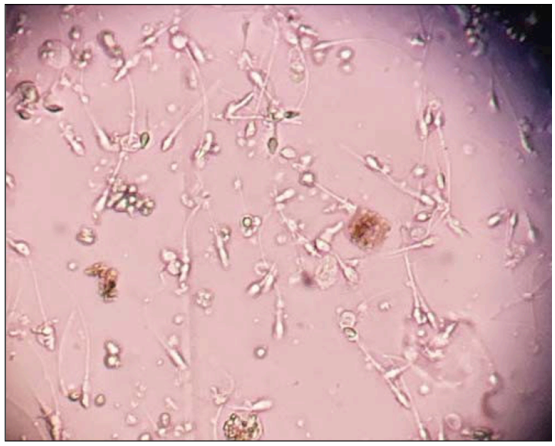
The semen sample was mixed well.
An aliquot of semen was removed immediately after mixing, allowing no time for the spermatozoa to settle out of the suspension. The volume of semen and the dimensions of the coverslip were standardized, so that the analyses are carried out on a preparation of fixed depth of about 20 µm, which allows the spermatozoa to swim freely. 10 µl of the semen was placed onto a clean glass slide.

Sperm Morphological Study 100x

Leukocytes are seen as round cells as mentioned earlier. In order to count these cells, first they have to be distinguished from other cells such as germ cells. This is achieved by the commonly used Peroxidase staining technique. This technique is based on identifying intracellular peroxidase enzyme, a characteristic of Polymorphonuclear granulocytes and also macrophages.



Leukocytes, Immature Spermatocytes (Round Cells) and Epithelial Cells in the Smear



Brown Coloured Peroxidase Positive PMNLeukocytes

These cells are counted as per the routine WBC counting by Haemocytometer method, i.e. stained cells are counted in outer 4 large squares and the total leukocyte count is calculated using the formula, C = N/4 X 10^4 leukocytes per mL,

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 88 these 88 samples studied, 24 (27.3%) cases were found to be positive for leukocytospermia. The 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.

Age group of the men presented (in years): (total 77 cases)

Lowest age	25
Highest age	43
Mean (Average) age	32.4

Majority of the men with infertility investigation were in the age group of 30- 34 years.

Age-range of presentation

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

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

It is evident from the table 6 and graph 2 that, smoking is the most prevalent habit in the study population and all the 3 habits are more common in men under infertility investigation, and still more common in men with leukocytospermia.

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. In the present study, out of all the 77 samples, 49 (63.6%) had normal total sperm count (above 39 millions per ejaculate) and 28 (36.4%) had reduced total sperm count than normal.

Distribution of sperm count in the samples

Total No. of samples	**77**
Samples with Normal Total Sperm Count	49 (63.6%)
Samples with Reduced Total Sperm Count	28 (36.4%)

Final diagnoses of semen analyses of individual sample

Total No. of samples	**77**
Normal Study	44
Abnormal Study	33

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.

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 that the 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. [12] A large number 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. In the present study, the prevalence of leukocytospermia in the general (men's) population is 22.8% and 20.8% in the men under infertility investigation, which is almost in concordance with the previous studies. A study by Grygoruk C, Mrugacz G, et al. on 100 semen samples with severe oligospermia and leukocytospermia after treatment 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. [13] A and leukocytospermia treated with anti-inflammatory, anti-bacterial and anti-oxidative study by Grygoruk C, Mrugacz G, et al. on 100 semen samples with severe oligospermia 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.[81] 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. [15] A number of studies have shown a relationship between human sperm DNA damage and various semen parameters including sperm concentration, motility, morphology, leukocyte concentration. [14] 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. 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. This observation is in

concordance with previous studies on UMI, [6] which have mentioned that UMI cases show Normal semen analysis study.

Conclusion

The prevalence might have been more if the men with symptoms of uro-genital tract infections had frank leukocytospermia Also, if all the semen samples were subjected to some better method for leukocytospermia detection like flow-cytometry, immunoflourescence methods, the prevalence number would have been still more. So, the value of prevalence of leukocytospermia is not to be neglected and studied in more detail with more sophisticated methods to detect leukocytospermia, the cause for leukocytospermia and the effective treatment for it. If the cause for infertility is leukocytospermia in these men, if they are diagnosed early before the leukocytes can cause significant damage, and effectively treated as seen in the meta-analysis of 12 studies, a significant percentage of infertility cases will be very thankful to this leukocytospermia detecting personnel.

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