Research Article

A study of relationship between leucocytospermia and specific seminal function parameters

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Abstract

Introduction: Much controversy surrounds the clinical significance of an increased concentration of white blood cells (WBC) in the male ejaculate. The World Health Organization's classification of leucocytospermia is a concentration >1x10^6 WBC/ml. Considerable evidence indicates that white blood cell concentrations are often elevated in semen of infertility patients and that WBC products can affect sperm function in vitro. Defective sperm function is the most common cause of infertility, and until recently, it was difficult to evaluate and treat. Part of this difficulty was due to our incomplete understanding of the factors contributing to normal and abnormal sperm function leading to male infertility. **Aims and objectives:** To study the relationship between leucocytospermia and specific seminal function parameters. **Material and methods**: The present study was conducted in the Semenology laboratory of department of Physiology, Government Medical College, Nagpur. The study involved 79 subjects with complaint of infertility referred from various Departments of Government Medical College and hospital, Nagpur. These subjects were grouped into three study groups according to leucocytes count in semen. While performing the semen analysis special emphasis was given on Hypo-osmotic swelling test, Acrosome intactness test, Nuclear chromatin decondesation test and sperm mitochondrial activity index.The subject included were in the age group of 21 to 40 yrs, they were all non smokers, non- alcoholics and free of any obvious genital tract abnormalities. The findings of seminal function were compared with the three groups of patients formed according to leucocytes count. **Results:** It was observed that, HOS score (%) was found to be more in case Group I (55.93±3.17) as compared to Group II and III (39.09 ±4.13 and 19.07±4.11 respectively). The Al test score (%) was lowest in group III (17.42±3.92) as compared to group I (54.68±3.79) and II (36.54 ± 3.46). Mean Nuclear Chromatin Decondensation Test score in group I was 55.03±3.70, in group II was 35.36 ± 2.93 and in group III was 18.14±3.35. Mean Sperm mitochondrial activity index was maximum in group I (56.20±3.08) followed by group II and III (35.90 ± 2.94 and 17.03±3.21respectively). When individual groups of leucocytospermia were compared with respect to the seminal function test score, difference in each group was statistically highly significant as compared to the other groups. **Conclusion:** Thus in the end we conclude that various seminal functional tests like Hypo-osmotic swelling test, Acrosome intactness test, Nuclear chromatin decondesation test and sperm mitochondrial activity index were affected by Leucocytospermia.

Key words: Leucocytospermia, infertility, seminal function test.

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INTRODUCTION

Much controversy surrounds the clinical significance of an increased concentration of white blood cells (WBC) in the male ejaculate. The World Health Organization's classification of leucocytospermia is a concentration > 1x10^6 WBC/ml.[1] Considerable evidence indicates that white blood cell concentrations are often elevated in semen of infertility patients and that WBC products can affect sperm function in vitro. Furthermore, recent studies indicate that leucocytospermic semen samples exhibit

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poor motility characteristics and impaired ability to function in sperm penetration assays. The identification and qualification of white blood cells in semen should be an integral component of every semen analysis. If properly diagnosed, a significant number of infertility patients with leucocytospermia may benefit from antibiotic, antiviral, or anti-inflammatory drug treatments.[2] Because of absence of clinical symptoms, the origin of WBC is difficult to determine. Normally, most WBC appear to originate from the epididymis because vasectomized men show very few WBC in semen. On the other hand, leucocytospermic samples show low citric acid levels, pointing to asymptomatic 80% of leucocytospermic samples are microbiologically negative. In some cases Chlamydia trachomatis might have triggered a persistent inflammatory reaction leading to leucocytospermia. Sperm damage by WBC can be mediated by reactive oxygen species, proteases and cytokines. Furthermore, genital tract inflammation facilitates the formation of sperm antibodies. As seminal plasma has strong anti-inflammatory properties and because there is only short contact between sperm and WBC in parostatitis and seminal vasicles, compared to the infections of prostate and seminal vasicles, inflammations of the epididymis and testis are likely to have the largest impact on sperm.[3] Defective sperm function is the most common cause of infertility, and until recently, it was difficult to evaluate and treat. Part of this difficulty was due to our incomplete understanding of the factors contributing to normal and abnormal sperm function leading to male infertility. Mammalian spermatozoa membranes are rich in high unsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid peroxidation. Limited endogenous mechanisms exist to reverse these damages. The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and by contaminating leucocytes (leucocytospermia) has been identified as one of the few defined etiologies for male infertility. In a normal situation, the seminal plasma contains antioxidant mechanisms which are likely to quench these ROS and protect against any likely damage to spermatozoa However, during genitourinary infection/inflammation these antioxidant mechanisms may downplay and create a situation called oxidative stress In addition aging and environmental toxicants are also likely to further induce this oxidative stress. Assessment of such oxidative stress status (OSS) may help in the medical treatment of this male factor infertility by suitable antioxidants.[4]

AIMS AND OBJECTIVES

To study the relationship between leucocytospermia and specific seminal function parameters.

MATERIAL AND METHODS

The present study was conducted in the Semenology laboratory of department of Physiology, Government Medical College, Nagpur. The study involved 79 subjects with complaint of infertility referred from various Departments of Government Medical College and hospital, Nagpur. These subject were grouped into three study groups namely, Group 1, Group 2 and Group 3.

Following inclusion and exclusion criteria was used to select the study subjects.

Inclusion Criteria

- Adult healthy male patients in the age group of 21-40 yrs with complaint of no issue in spite of 1 year of unprotected intercourse.
- Non-smokers and non-alcoholic.
- No prior radiation exposure or exposure to aniline dyes.

Exclusion criteria

- Persons with occupation near hot furnace or in chemical industries using the substances like benzene or aniline dyes, which are known to produce alterations in spermatogenesis.
- Persons with history of drug addiction.
- Persons with previous history of hydrocoele, varicocoele, hernia or operations on the genital tract.

Thus by using above mentioned inclusion and exclusion criteria total 79 patients were enrolled in the study. The details of all the enrolled patients with detail history and clinical examination was recorded on a prestructured proforma. All the patients were explained about the study and semen sample collection.

Collection of sample: [5,6]

An appointment for semen analysis was given to the patients who were referred from various departments for the investigation and enrolled in the study. Instructions regarding abstinence from sexual intercourse as well as masturbation and night emission for period of 3 to 5 days; for full maturation of sperms were given. The importance of abstinence was explained to the patients, as it is known that frequency of ejaculation adversely affects the sperm density and volume. Clean, sterile, warm and dry nonspermicidal, prelabelled, 50 ml container was given to the each patient for collection of the sample. Samples were obtained by masturbation in the proximity of the laboratory. The patients were instructed to wash their hands and genitalia and allow it to dry before sample collection. The first part of the ejaculate contains higher sperm concentration; hence, patients were advised to report if sample collection was improper. The samples were allowed to liquefy at least for 30 minutes protecting

it from extremes of the temperature i.e. below 20 °C and above 40°C. The routine semen analysis was carried out after the liquefaction, as per the guidelines laid down by W.H.O. The semen sample after centrifugation was subjected to detail seminal analysis. Slides were prepared from the semen sample after excluding the seminal plasma. The slides were subjected to Papanicolaou staining, Standard peroxidase staining, May grunwaldGiemsa staining and Hematoxylin- eosin staining, in order to estimate the number of leucocyte in the semen sample. While performing the semen analysis special emphasis was given on Hypo-osmotic swelling test, Acrosome intactness test, Nuclear chromatin decondesation test and sperm mitochondrial activity index

All the patients were divided in three groups according to the leucocytes count.

Group 1: Low WBC number (<1 X 10^5 WBC/ml) (n=29)

Group 2: Intermediate WBC number (10^5 to 10^6 WBC/ml) (n=22)

Group 3: High WBC number (leucocytospermic) (>10^6 WBC/ml) (n=28)

Various functional tests like Hypo-osmotic swelling test, Acrosome intactness test, Nuclear chromatin decondesation test and sperm mitochondrial activity index were compared between the three groups. And the data thus obtained was analyzed using mean, standard deviation, standard error, and Student's unpaired 't' test etc wherever necessary.

RESULTS

Table 1: Comparison of mean Hypo Osmotic swelling score in different groups

Hypo Osmotic swelling score (mean ± SD)	Group I	55.93±3.17
	Group II	39.09 ±4.13
	Group III	19.07±4.11
t value (Student's t test)	Group I vs Group II	15.87 (p< 0.001, significant)
	Group II vs Group III	18.72 (p< 0.001, significant)
	Group I vs Group III	37.76 (p< 0.001, significant)

It was observed that, HOS score (%) was found to be more in case Group I (55.93±3.17) as compared to Group II and III (39.09 ±4.13 and 19.07±4.11 respectively). The HOS score was found to be more in Group II as compared to Group III. When the HOS score was compared among different groups of leucocytospermia. Each score was found to be highly significant among all combinations.

Table 2: Comparison of mean Acrosome intactness test score in different groups

Acrosome intactness test score (mean ± SD)	Group I	54.68±3.79
	Group II	36.54 ± 3.46
	Group III	17.42±3.92
t value (Student's t test)	Group I vs Group II	17.79 (p< 0.001, significant)
	Group II vs Group III	18.26 (p< 0.001, significant)
	Group I vs Group III	36.41 (p< 0.001, significant)

It was observed that the Al test score (%) was lowest in group III (17.42±3.92) as compared to group I (54.68±3.79) and II (36.54 ± 3.46). When individual groups of leucocytospermia were compared with respect to A I test score (%), difference in each group was statistically significant as compared to the other groups.

Table 3: Comparison of mean Nuclear Chromatin Decondensation Test score in different groups

Nuclear Chromatin Decondensation Test score (mean ± SD)	Group I	55.03±3.70
	Group II	35.36 ± 2.93
	Group III	18.14±3.35
t value (Student's t test)	Group I vs Group II	21.13 (p< 0.001, significant)
	Group II vs Group III	19.33 (p< 0.001, significant)
	Group I vs Group III	39.42 (p< 0.001, significant)

It was observed that mean Nuclear Chromatin Decondensation Test score in group I was 55.03±3.70, in group II was 35.36 ± 2.93 and in group III was 18.14±3.35. The difference observed in the three groups also statistically significant.

Table 4: Comparison of mean Sperm mitochondrial activity index score in different groups

Sperm mitochondrial activity index score (mean ± SD)	Group I	56.20±3.08
	Group II	35.90 ± 2.94
	Group III	17.03±3.21
t value (Student's t test)	Group I vs Group II	23.88 (p< 0.001, significant)
	Group II vs Group III	46.91 (p< 0.001, significant)
	Group I vs Group III	21.61 (p< 0.001, significant)

It was seen that Mean Sperm mitochondrial activity index was maximum in group I (56.20±3.08) followed by group II and III (35.90 ± 2.94 and 17.03±3.21 respectively). When individual groups of leucocytospermia were compared with respect to the SMAI score, difference in each group was statistically highly significant as compared to the other groups.

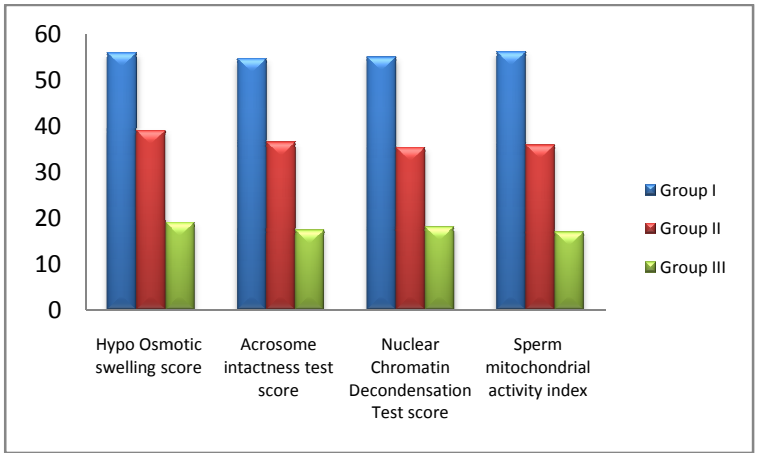

Figure 1: Comparison of seminal function test

DISCUSSION

The mean Hypo-Osmotic Swelling Test scores in three study groups i.e. group I, group II and group III were 55.93 ±3.17, 39.09 ±4.13, and 19.07 ± 4.11 respectively. The HOS scores were significantly lower ($p<0.001$) in group III as compared to group II and group I. The HOS scores in group II were significantly lower ($p<0.001$) as compared to group I. Omu AE *et al.*[7] found that sperm membrane integrity was worse in leucocytospermia. Arata de Bellabarba *et al.*[8] found positive relationship between leucocytospermia and decreased HOS score. However, Kaleli *et al.*[9] found negative relationship between leucocytospermia and decreased HOS score. He also found that increased sperm concentration and enhanced acrosome reaction were closely related to leucocytospermia. The mean values of Acrosome lntactness Test Score in the group I, group II and group III were 54.68±3 79, 36.54±3.46 and 17.42±3.92 respectively. The leucocytospermia and Al score showed a consistent trend of decreasing ($p<0.001$) Al score with increasing leucocytospermia with statistically significant difference. However in a study conducted by Aitken RJ *et al.*[10], the author observed that leucocytospermia did not significantly influence any component of the semen profile. Also the findings of Kaleli S *et al.*[9] revealed exactly opposite trend e.g. enhanced acrosome reaction associated with leucocytospermia. They also found that increased hypo-osmotic swelling test score and higher sperm concentration were closely related to leucocytospermia.

The NCDT score is an indicator of nuclear function and intact chromatin network which is pivotal for good nuclear function was adversely affected by leucocytospermia. The values of NCDT scores in three study groups i.e. group I, group II and group III were 55.03 ± 3.70, 35.36 ± 2.93 and 18 14 ± 3 35 respectively. And the difference observed in the three groups was also statistically significant. Alvarez JG *et al.*[11] and Saleh R A *et al.*[12] also found that leucocytospermia was associated with a significant increase in DNA damage. The mean Sperm Mitochondrial Activity Index in group I was 6.20 ± 3.08 whereas in group II and III was 35.90±2.94 and 17.03±3.21 respectively. The SMAI scores were significantly lower ($p<0.001$) in group III as compared to group II and group I. The SMAI scores in group II was also significantly lower ($p<0.001$) as compared to group I. Thus the present study proves that leucocytospermia due to reactive oxygen species damages the mitochondrial enzyme machinery of the sperms which is revealed by poor SMAI scores. Although no parallel study revealing effects of leucocytospermia on SMAI score could be traced, the relationship is evidence towards demonstrating the effects of leucocytospermia on sperm motility. This is especially important considering indispensable nature of mitochondrial enzyme machinery in determining the progressive sperm motility. There is ample evidence to prove the relationship between leucocytospermia and poor sperm motility. Therefore, the present finding is a strong argument in favour of decreasing sperm motility with increased leucocytospermia. Sikka SC[4] has attributed the damaging effect of leucocytospermia in male infertility to their capacity to generate the reactive oxygen species (ROS). He postulates that mammalian spermatozoal membranes are rich in high unsaturated fatty acids and are sensitive to oxygen induced damage mediated by lipid peroxidation. Limited endogenous

mechanisms exist to reverse these damages. The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and by contaminating leucocytes (leucocytospermia) has been identified as one of the few defined etiologies for male infertility. In a normal situation, the seminal plasma contains antioxidant mechanisms, which are likely to quench these ROS and protect against any likely damage to spermatozoa. However, during genitourinary infection/inflammation these antioxidant mechanisms may downplay and create a situation called oxidative stress. In addition, aging and environmental toxicants are also likely to further induce this oxidative stress. Assessment of such oxidative stress status **(OSS)** may help in the medical treatment of this male factor infertility by suitable antioxidants. Under normal conditions a balance is maintained between the pro- oxidant and the anti-oxidant mechanisms present in the semen sample. These substances include ROS(reactive oxygen species) viz O2.H2O2.OH and ONOO act as the pro-oxidants. These are derived either directly from the leucocytes or as a result of lipid membrane peroxidation secondary to the activation of chemokines like IL8 and GRO (pro-inflammatory chemokine). The various anti-oxidants present in the seminal plasma like SOD, Vit C, Vit E (3-carotene and GSH (Glutathione peroxidase and reductase system) oppose this membrane destabilising and DNA damaging actions of leucocyte and their activation products. Testicular inflammation or epididymal inflammation, however, because of the lack of protective effect of seminal plasma lead to extensive sperm damage. Thus the results of the present study highlight the role of leucocytospermia in male infertility.

CONCLUSION

Thus in the end we conclude that various seminal functional tests like Hypo-osmotic swelling test, Acrosome intactness test, Nuclear chromatin decondesation test and sperm mitochondrial activity index were affected by Leucocytospermia.

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