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ORIGINAL ARTICLE

Can Seminal IL-8 Level Be Used as a Marker of Leukocytospermia and Does It Have Any Correlation with Semen Parameters in Infertile Couples?

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Abstract

Objectives Infection of male genital tract leads to leukocytospermia which may have a detrimental effect on semen quality. This study was conducted to evaluate whether seminal IL-8 level can be used as a marker of leukocytospermia and does it have any correlation with semen parameters in infertile couples?

Methods This **c**ross-sectional study was conducted in an infertility clinic of a tertiary care hospital including 150 male partners of infertile couples who underwent semen analysis (WHO laboratory manual for the examination and processing of human semen, 5th edn, World Health Organization, Geneva, p 271, 2010), semen culture sensitivity and seminal IL-8 levels. Independent *t*-test, Mann–Whitney *U* test and Chi-square test were applied for analysis.

Results Mean seminal plasma IL-8 level of patients with leukocytospermia was significantly higher than patients

without leukocytospermia (1143.67 ± 887.03 vs. 267.174 ± 242.29, p value < 0.001). Strong positive correlation was found between seminal plasma IL-8 levels and pus cells in the semen ($r = 0.950$, $p < 0.001$); AUC for seminal plasma IL-8 was 0.985 (CI 0.972–0.988), and a cutoff value of 399 pg/ml was determined to diagnose leukocytospermia. This value had high sensitivity (91.8%), specificity (94.5%), positive predictive value (94.4%) and diagnostic accuracy (93.2%) for detecting leukocytospermia. Seminal IL-8 levels correlated negatively with sperm motility ($r = -0.29$, $p < 0.001$) and morphology ($r = -0.230$, $p < 0.01$).

Conclusion Seminal plasma IL-8 levels were found to be almost five times higher in male partners with leukocytospermia than in non-leukocytospermia group, and it appears to be a promising tool to detect leukocytospermia. Seminal IL-8 level correlated negatively with semen parameters including sperm motility and morphology.

Keywords Seminal plasma IL-8 · Leukocytospermia · Infertility · Semen parameters

Introduction

In men, clinical and subclinical male genital tract infection and male accessory gland infection (MAGI) may be a leading cause of infertility as infection adversely affects sperm parameters. Infection leads to accumulation and activation of leukocytes in genitals as reflected by leukocytospermia which is defined as the presence of at least 1×10^6 leukocytes/ml in a semen sample [1]. Leukocytospermia leads to increased cytokines (IL-8) and reactive oxygen species (ROS) [2].

Leukocytospermia can be used as a marker of MAGI, but during microscopy, even immature germ cells appear as round cells and may be confused as leukocytes. Currently available methods to differentiate pus cells and immature sperm cells in semen are the following:

1. Direct counting of round cells in a semen sample is a highly inaccurate method of diagnosing leukocytospermia because WBC cannot be distinguished from immature germ cells using light microscopy.
2. Immunocytology using cell-type specific monoclonal antibodies is recognized as the gold standard for diagnosis of leukocytospermia. This method is not feasible for the daily clinical practice because of lack of standardization on the exact immunohistological staining method with the specific monoclonal antibodies to be used. Additionally, these techniques are time-consuming, expensive and are not available in standard commercial laboratories.
3. Peroxidase staining is another option to diagnose leukocytospermia. Peroxidases are enzymes that break down H_2O_2, liberating O_2. This molecule oxidizes a benzidine derivate present in the staining solution, which precipitates in the form of a brown color, allowing these cells to be recognized under light microscopy. In contrast to immunohistological staining (which can recognize any leukocyte depending on the monoclonal antibody that is being used), this method only identifies cells rich in peroxidase such as polymorphonuclear granulocytes and macrophages. Although they constitute the predominant white blood cell types seen in semen (80–90%), T-lymphocytes, which represent 2–5% of semen leukocytes, are not recognized by this staining. The main limiting factors of the peroxidase staining are lack of identification of white blood cells and poor sensitivity. Other practical problem is that this test is not available in most commercial laboratories.

Commercially available cultures are mostly aerobic, whereas causative organisms implicated in male genital tract infections are mostly mixed infections and anaerobes. Therefore, many times semen cultures come out to be negative, thus making the decision to treat the patient difficult. Therefore, due to the shortcoming of the above-mentioned tests, there is a need for other reliable, cost-effective methods to detect and differentiate the two accurately so that decision for treatment can be taken.

Interleukin 8 (IL-8) is a pro-inflammatory CXC chemokine and is a potent neutrophil chemotactic and activating factor, which exerts its biological effect by binding to cell surface receptors. Its level in semen increases during infection and may point out toward the diagnosis of leukocytospermia [3]. In this study, levels of IL-8 in semen were measured in male partners of infertile couples with and without leukocytospermia to determine whether seminal IL-8 level be used as a marker of leukocytospermia and does it have any correlation with semen parameters?

Methods

This cross-sectional study was carried out in the departments of obstetrics and gynecology, surgery, and microbiology in a tertiary care hospital. The conduct of the study was in accordance with the principles of ICH-GCP E6 guidelines. A sample size of 146 ± 5 was calculated by assuming the prevalence of leukocytospermia to be 10% [1]. In the infertility clinic, routine husband's semen analysis of all male partners was done. After a written informed consent, consecutively, 150 infertile males were recruited to include 75 males with leukocytospermia and

75 without leukocytospermia. Infertile males with any immunocompromised conditions were excluded.

Detailed history was noted and general physical examination including height, weight, BMI and systemic examination was done for all male partners by a single examiner. Local examination was done in surgery outpatient department. Semen samples were obtained by masturbation after 3–5 days of abstinence in a sterile wide-mouthed calibrated glass container. Semen analysis was performed after 30 min of liquefaction at 37 °C in accordance with the WHO criteria [4]. Smears prepared from semen were stained by Papanicolaou stain and examined under microscope for determining sperm morphology. Head, midpiece and tail defects were specifically examined and recorded. Total and differential leukocyte counts were performed in 20 microscopy fields (40 ×) in a Papinocolaou-stained seminal smear from each sample. The concentration of leukocytes was expressed as millions of cells per milliliter of seminal fluid, calculated from their incidence relative to the average number of observed spermatozoa in the microscopy fields and with reference to the sperm count value. Total sperm count was calculated as the product between sperm concentration and ejaculate volume.

Semen IL-8 levels were measured in all men with and without leukocytospermia. Semen sample of patients was stored at − 25 °C temperature. The Diaclone SAS (BP 1985 25020 Besancon Cedex, France) ELISA kit was used for IL-8 quantification. Semen culture and sensitivity was done for all patients, and all those with positive semen culture were treated and followed as per the reports obtained.

Statistical analysis was performed by the SPSS program for windows, version 19. Continuous variables are presented as mean ± SD, and categorical variables are presented as absolute numbers and percentage. Data were checked for normality before statistical analysis. Normally distributed continuous variables were compared using the independent student *t*-test, whereas Mann–Whitney *U* test was used for those variables that were not normally distributed. Categorical variables were analyzed using either the Chi-square test or fisher's exact test. p value of < 0.05 was taken as statistically significant.

Results

A total of 150 males with mean age of 31.21 ± 4.0 years were enrolled in this study. Mean BMI of the male partners was 25.51 ± 2.92 kg/m^2.

Out of total 150 men recruited, 78 (52%) had derangements in one or the other semen parameters. Mean semen volume was 1.88 ± 0.68 ml. In 33 (22%) participants, ejaculate volume was less than the reference value of 1.5 ml. The mean sperm count of the study population was 53.46 ± 27.21 million/ml. Oligospermia was detected in nine (6%).The mean value of sperm motility in population under study was 49.85 ± 21.54%. In 62 (41.33%) men, sperm motility was less than the reference level of 39%. The mean normal sperm morphology in population under study was 57.3 ± 22.86%. Teratospermia was detected in two (1.33%) patients. Mean sperm vitality of the study population was 66.99 ± 19.45%. In this study, 46 (30.67%) men had sperm vitality below the reference value of 58%. One or more semen parameters were deranged in 52 (69.3%) males of leukocytospermic group as compared to 26 (34.67%) males of non-leukocytospermic group ($p < 0.00$).

Table 1 Seminal IL-8 levels in patients with and without leukocytospermia

Serial number	Category	Seminal plasma IL-8 levels		p value
		Mean	SD	
1	Leukocytospermia	1143.67	887.03	0.000
2	Non-leukocytospermia	267.174	242.29	

Seminal IL-8 levels were estimated for all except for four patients. Semen samples of these four patients had excessive amount of mucous. Therefore, seminal IL-8 levels could not be assessed.

Mean seminal plasma IL-8 level was measured for participants with and without leukocytospermia, and the results are shown in Table 1. Comparison of semen parameters in males with and without leukocytospermia is depicted in Table 2.

In men with leukocytospermia, 23 (30.67%) had positive semen culture whereas in men without leukocytospermia, three (4%) patients had positive semen culture report ($p < 0.000$). In males with leukocytospermia, most common organisms grown in semen culture were MRSA (8, 10.67%) followed by *E.coli* (7, 9.33%), Klebsiella spp. (6, 8%) and *Enterococcus* spp. (2, 2.67%).

Urine routine microscopy was done for all men recruited in the study. Out of 150 men, 14 had history of urinary tract infection. However, none of the male partner was having active urinary tract infection at the time of recruitment in the study.

In leukocytospermia group, USG scrotum picked up abnormalities in 20 (16%) participants.

Discussion

Infertility has become a major public health problem globally, and its magnitude has increased tremendously over past few decades. Silent and symptomatic male

Table 2 Semen parameters in male partners with and without leukocytospermia

Serial no.	Semen parameters	Without leukocytospermia		With leukocytospermia		*p* value
		Mean (median)	SD	Mean (median)	SD	
1	Semen volume	1.88 (2)	0.65	1.90 (2)	0.70	0.873
2	Sperm counts	54.99 (56)	25.13	52.52 (46)	28.98	0.583
3	Sperm motility	57.11 (65)	21.92	43.13 (38)	18.79	< 0.001
4	Sperm morphology (normal)	61.88 (66)	23.84	53.11 (56)	21.07	0.02
5	Vitality	70.29 (75)	21.06	63.77 (66)	17.23	0.042

genital tract infections may be a leading cause of male infertility. Leukocytospermia leads to increase in pro-inflammatory cytokines and ROS. Cytokines, especially IL-8 and IL-6, have been found to be higher in semen of men with genital tract inflammation in various studies [1–3]. Interleukin 8 (IL-8) is a pro-inflammatory CXC chemokine which causes chemotaxis of leukocytes to the inflammatory sites. It recruits and activates neutrophils for phagocytosis of bacteria to clear up infection.

Subnormal semen parameters were twice as common in male partners with leukocytospermia as compared to males without leukocytospermia (69.3% vs. 34.6%; $p < 0.001$). On comparing semen parameters in males with and without leukocytospermia, no significant difference was found in the mean semen volume ($p = 0.873$) and sperm count ($p = 0.583$). Mean sperm motility was considerably lower in patients with leukocytospermia ($p < 0.001$). Mean normal sperm morphology ($p = 0.02$) and mean sperm vitality ($p = 0.04$) were also significantly lower in population with leukocytospermia (Table 2). Fraczek et al. [5] evaluated standard semen analysis and nonconventional sperm parameters including subcellular changes in sperm membranes (phospholipid scrambling, peroxidative damage and phosphatidylserine (PS) externalization), mitochondria (mitochondrial transmembrane potential, ΔYm and oxidoreductive capability) and DNA fragmentation in healthy young normozoospermic males with asymptomatic bacteriospermia and leukocytospermia. As seen in our study, bacteriospermia and leukocytospermia had a harmful effect on sperm concentration, motility and morphology. They observed that bacteria mainly participated in intrinsic mitochondria-dependent apoptotic cell death mechanisms. Leukocytospermia increases oxidative stress which has adverse effect on routine sperm parameters. They commented that these observations may support the development of new diagnostic biomarkers for infertile males with infections in the reproductive tract.

In the present study, mean seminal plasma IL-8 level of patients with leukocytospermia was almost five times higher than patients without leukocytospermia (1143.67 ± 887.03 versus 267.174 ± 242.29, p value < 0.001). A positive correlation was found between seminal plasma IL-8 levels and pus cells in the semen ($r = 0.950$, $p < 0.001$) (Fig. 1). A seminal plasma IL-8 cutoff value of 399 pg/ml was determined to diagnose leukocytospermia which in turn indicated a state of male genital tract infection. In the leukocytospermic group, 67 (92%) men had seminal plasma IL-8 value ≥ 399 pg/ml. In non-leukocytospermic group, 73 (97.3%) patients had seminal plasma IL-8 levels below this cutoff. Sensitivity and specificity of seminal plasma IL-8 as marker of leukocytospermia are shown in Table 3. Area under the receiver operating characteristic curve for seminal plasma IL-8 level measurement for the diagnosis of leukocytospermia is depicted in Fig. 2. The area under the curve was 0.985 (CI 0.972–0.988).

Aghazarian et al. [2] evaluated leukocyte threshold values in semen to detect inflammation involving seminal interleukin IL-6 and IL-8. The 75th and 90th percentiles of seminal IL-6 and IL-8 were considered as "high" and "very high" concentrations, respectively. They observed that leukocytospermia demonstrated sensitivity of 94% and specificity of 92% to predict very high levels of IL-8. On the basis of very high levels of IL-6 or IL-8, leukocytospermia is a sensitive and specific marker to predict acute seminal inflammation [2].

Correlation of seminal IL-8 levels was done with different semen parameters. Statistically, no significant correlation of seminal plasma IL-8 levels was found with semen volume ($r = -0.024$, $p = 0.778$), sperm counts ($r = -0.126$, $p = 0.13$) or with sperm vitality ($r = -0.132$, $p = 0.112$). A statistically significant negative correlation was found between seminal plasma IL-8 levels with sperm motility ($r = -0.290$, $p < 0.001$) and sperm morphology ($r = -0.230$, $p < 0.01$). Similar findings were observed in another study by Seshadri et al. [6]. A strong positive correlation was found between seminal plasma IL-8 levels and pus cells in the semen ($r = 0.950$, $p < 0.001$).

Twenty-three (30.67%) male partners had positive semen culture in leukocytospermia group as compared to only three (4%) men in non-leukocytospermia group ($p < 0.000$).

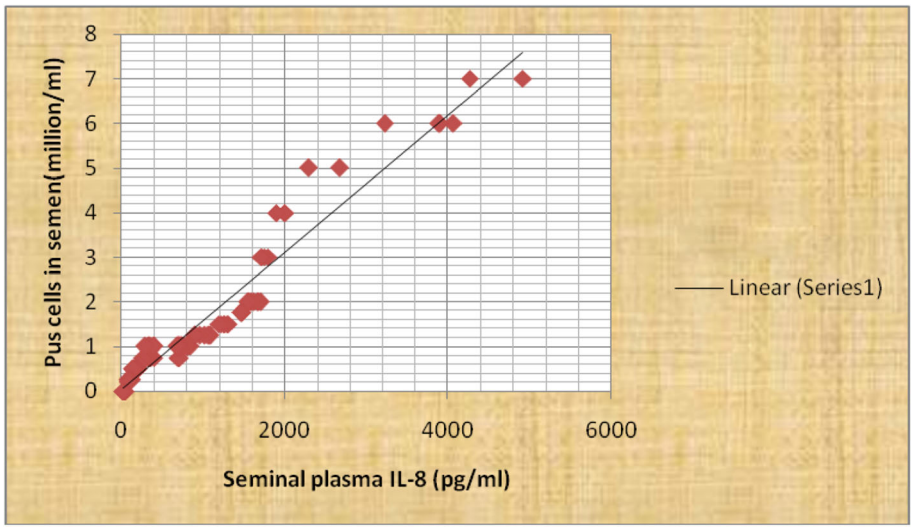

Fig. 1 Correlation of pus cells with seminal IL-8 levels

Table 3 Sensitivity and specificity of seminal plasma IL-8 as marker of leukocytospermia

Sensitivity	91.8%
Specificity	94.5%
PPV	94.4%
NPV	92.0%
Accuracy	93.2%

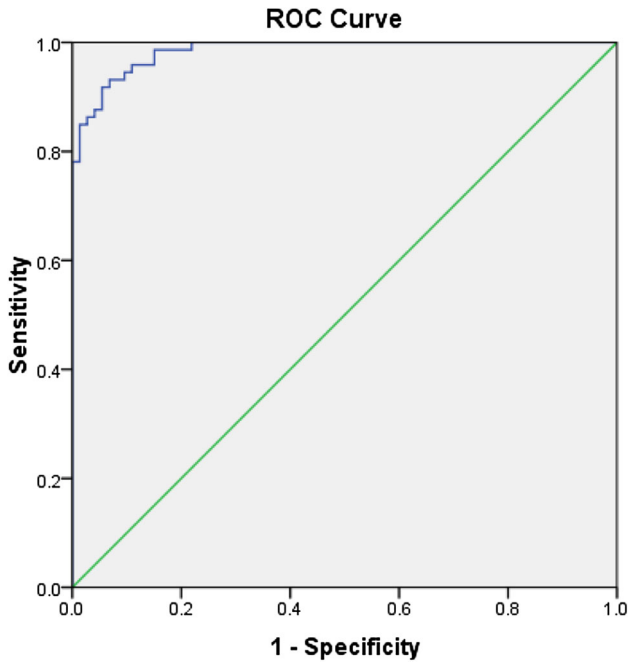

Fig. 2 Area under ROC curve for seminal IL-8 level for diagnosis of leukocytospermia

In leukocytospermia group, USG scrotum picked up abnormalities in 20 (16%) participants including hydrocele in 11 (14.66%) patients, varicocele in four (5.33%), subependymal cyst in three (4%), chronic testicular inflammation in one (1.30%) and chronic testicular infarct in one (1.30%) patients.

In our study, 40 (53.33%) patients with leukocytospermia were diagnosed with male accessory gland infection (MAGI) [7] as per WHO criteria. Out of 75 males with leukocytospermia, 40 (53.3%) had MAGI, 23 (57.5%) men had positive semen culture with potentially pathogenic organism, 14 (35%) had history of recurrent urinary tract infection, two had chronic pelvic pain while one had high semen viscosity with altered semen appearance. They were treated with appropriate antibiotics and anti-inflammatory drugs according to the culture sensitivity reports.

Few other studies have suggested that IL-8 may be used as a marker of MAGI [2, 3]. Lotti et al. [3] have suggested that seminal plasma IL-8 (sIL-8) appears a reliable and predictive surrogate marker of prostatitis. They observed that sIL-8 is involved in inflammation not only of the prostate but also of other organs of the male genital tract (MGT) like seminal vesicles, epididymis and indicate male accessory gland infection (MAGI). An association between sIL-8 levels and color Doppler ultrasound characteristics of the MGT suggestive of inflammation has been recently reported [8].

The limitation of this study was that transrectal color Doppler could not be performed to detect inflammation-induced long-term changes in male genital tract due to financial and technical constraints. In its absence, USG scrotum was done. A drawback of USG scrotum was inability to comment on prostate to detect prostatitis or benign hyperplasia of prostate as well as on seminal vesicle lesions. However, this is the first Indian study to evaluate correlation between leukocytospermia and seminal plasma IL-8 which may serve as a surrogate marker of MAGI in the absence of sophisticated investigations like color

Doppler. To conclude, mean seminal IL-8 levels were five times higher in patients with leukocytospermia as compared to patients without leukocytospermia. A cutoff value $\geq$ 399 pg/ml of seminal IL-8 depicted high diagnostic accuracy to detect leukocytospermia. Instead of using cumbersome and expensive techniques to distinguish between pus cells and round cells in semen, a simple, cost-effective (costs approximately Rs 120–150 per test) determination of IL-8 levels in the semen can be used to guide further treatment. In this study, higher seminal IL-8 had a negative impact on semen parameters including sperm motility and morphology.

Compliance with Ethical Standards

Conflict of interest Authors have no financial conflict of interest to declare in the findings of this study.

Ethical statements This study was initiated after approval from the Ethics Committee of Human Research of the Institute. Informed consent was obtained from all individual participants included in the study. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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