

Interleukin 1 β and interleukin 18 and their connection with leukocytospermia in human semen

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Abstract

Inflammation of male accessory glands constitutes one of the causes of infertility and in the same time there is no accurate method to diagnose a silent inflammation. The study was aimed in the evaluation of the levels of interleukins (IL-1 β) and (IL-18) in the seminal plasma and their significance in the diagnostics of semen pathology.

107 men diagnosed in the Andrology Outpatient were included in the study. Patients were divided in the 4 subgroups according to the semen parameters (normospermia, asthenoteratozoospermia, oligoasthenoteratozoospermia, and azoospermia). Next they were divided according to the incidence of leukocytospermia.

The levels of proinflammatory cytokines were measured in the seminal plasma with the ELISA method.

The significant correlation between IL-1 β and IL-18 and the rate of abnormal spermatozoa was observed in the oligoasthenoteratozoospermia.

The increased levels of IL-1 β and IL-18 were found in patients with an elevated leukocyte count in the semen. In addition, the levels of IL-1 β and IL-18 were shown to correlate with the number of leukocytes in semen.

Our results suggest the role of IL-1 β and IL-18 in the genesis of semen pathology that might be responsible for defects of spermiogenesis manifested with impaired morphology of spermatozoa. Moreover, it seems that the increased levels of IL-1 β and IL-18 might be indicators of leukocytospermia and can be useful in diagnostics of male accessory gland inflammation.

Key words: IL-1 β , IL-18, male infertility, semen, leukocytospermia.

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Introduction

Infertility is a growing problem in the modern world. This pathology affects approximately 13 to 18% of couples and the number of patients attending clinics dealing with this problem is increasing [1]. In Poland the problem of infertility affects approximately 20% of couples and a male factor is diagnosed in approximately 40-60% of cases [2].

The inflammation of male accessory glands (male accessory gland infections – MAGI) is a causative factor of almost 15% of cases of male infertility [3, 4].

It is suggested that the impairment of fertility is caused by the direct effect of the inflammation of accessory glands on the fertilizing capacity of spermatozoa [5]. Disorders of spermatogenesis and anomalous transport of semen are

additional important mechanisms related to the inflammatory aetiology of infertility [6].

Male accessory glands inflammation seems to be related to the decreased semen density, decreased number and motility of spermatozoa, and changes in levels of inflammatory markers in the seminal plasma [7]. The immune processes include migration of leukocytes to the site of inflammatory reaction induced by chemotactic stimuli and secretion of cytokines, which are indispensable for transmission of signals between immunocompetent cells [8, 9]. At the next stage, adhesion of leukocytes to endothelium occurs and is followed by their migration into extravascular space, which constitutes the beginning of inflammatory reaction cascade. Leukocyte infiltrate is responsible for disturbing the balance between the pro- and antioxidants,

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in consequence leading to the overproduction of free oxygen radicals and to clinical symptoms of impaired fertility [10].

The most important mediators of inflammatory reaction include: IL-1 α i β , IL-18, IL-2, IL-6, IL-8, TNF- α , and interferons (IFN- α , β i γ). Those cytokines play a role of stimulating factors responsible for lymphocyte activation, expression of adhesive particles, and induction of acute-phase proteins, some of them showing pirogenic activity, as well. They are produced mainly by nuclear macrophages.

Interleukin 1 β is secreted by monocytes, macrophages, keratynocytes, chondrocytes, and endothelial cells. Main factors stimulating its production include: lipopolisaccharides of bacterial cellular walls, viruses, yeast and their metabolic products, such as exotoxins and peptidoglycans [11].

Interleukin 1 β is primarily involved in protecting against infection; moreover it plays an important role in differentiation and proliferation of cells. It is also involved in apoptosis.

Interleukin 1 β also takes part in physiologic control of reproductive system. Some papers report expression of IL-1 and its role in male gonad [12]. It has been shown that IL-1 α is produced constitutively in physiologic conditions by Sertoli cells and has a regulatory function within seminiferous epithelium [13]. It is believed that it acts as a paracrine regulator of growth and differentiation of seminiferous cells during spermatogenesis [3]. In seminiferous tubules, it affects spermatogonies causing inhibition of FSH-stimulated aromatase activity in mature Sertoli cells [14].

Interleukin 18 is a recently discovered cytokine, which was formerly known as INF- γ inducing factor. It has functional properties similar to IL-12 and resembles structural proteins from IL-1 family; however it exerts its influence independently of both aforementioned cytokines.

Interleukin 18 affects primarily various populations of T lymphocytes and NK cells. It has a stronger effect on induction of IFN- γ than IL-12, increases cytotoxicity of T CD8+ lymphocytes, NK cells, and CD4+ lymphocytes [15]. Moreover, it has proinflammatory properties due to the induction of production of IL-16, IL-6, IL-8 i TNF- α .

Up to now, there has been only one, pioneering report stating the presence of IL-18 in the seminal plasma and investigating its correlation with semen parameters [16]. It is believed, that alike in other infectious pathologies, IL-18 could be also involved in inflammatory reaction in the male accessory glands.

Numerous authors agree that leukocyte count in the semen could be a valuable parameter in the diagnostics of infection and inflammation in male reproductive system [17]. However, the increased number of leukocytes in the semen is also observed in other pathologies. It could be induced by harmful environmental factors (alcohol, tobacco), sexual abstinence or atypical sexual practices [18].

The clinical importance of leukocytospermia, even at the absence of positive results of bacteriologic cultures, is confirmed by the improvement of semen density and reduction of leukocyte count in the semen after anti-inflammatory therapy with Cox-2 inhibitors [19].

In spite of the confirmed value of evaluation of leukocytospermia for the diagnostics of inflammation of male accessory glands, it seems that in many cases clinical consequences of inflammation last longer than the presence of elevated leukocyte count.

In addition, the correlation between leukocytospermia and abnormalities of seminogram is not always present.

That is why there is a growing interest in other markers of inflammation of the male accessory glands that could be helpful in a more precise determination of aetiology of infertility.

The aim of the present study was to evaluate the relation between IL-1beta and IL18 and semen parameters, leukocytospermia and their role in infertility diagnostics.

Material and methods

The study involved 107 men who in years 2004-2006 attended the Andrology Outpatient Clinic at the I Chair and Clinic of Obstetrics and Gynaecology of Medical University of Warsaw.

All patients were admitted for the diagnostics of couple infertility – the evaluation of a male factor. All patients underwent andrological examination.

The semen obtained by masturbation after 3 to 10 days of sexual abstinence was collected to the sterile container. It was left for 30 minutes in the room temperature to liquefy, and then a standard semen examination was performed.

One qualified laboratory assistants performed the semen examination manually in accordance with the 1999 WHO standards.

Sperm density, motility [%] (type A motility – progressive, fast, and linear – minimum 25%; type B motility – slow progressive – minimum 25%; twitching and immobile spermatozoa), sperm morphology expressed as the rate of abnormal spermatozoa in semen sample (up to 70% in normospermia), sperm agglutination level (0, 1, 2, 3), and leukocyte count in 1 ml of semen were evaluated.

According to the results of semen examination, the patients were divided into four subgroups: asthenoteratozoospermia (44 patients), oligoasthenoteratozoospermia (20 patients), azoospermia (12 patients), and the patients with normal semen parameters (15 patients).

The remaining portion of the semen samples were centrifuged at 1000 \times g for 10 minutes and thus obtained seminal plasma was frozen in minus 18 centigrade to prepare it for evaluation of the levels of IL-1 β and IL-18 with commercially available sets (R&D System, USA; MBL, Japan).

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, prolactin were eval-

uated in all patients (data not shown). Patients with abnormal hormonal examination were excluded from the study.

Statistical analysis

All statistical analyses were performed by using SPSS soft-ware. As obtained results did not fulfil t-Student test criteria, statistical significance was performed with Mann-Whitney test. Correlation within the parameters were analysed by Spearman test.

Results

In patients with normospermia, the average sperm density was 49.60 ±1.80 mln/ml. The rate of spermatozoa characterized by type A motility was 27.50 ±0.7% and by type B motility 28.83 ±0.53%. The rate of immobile spermatozoa was 35.83 ±0.97%. The rate of abnormal spermatozoa was 68.00 ±0.43%.

In patients with asthenoteratozoospermia, the average sperm density was 56 ±3.36 mln/ml. The rate of spermatozoa characterized by type A motility was 14.37 ±0.66% and by type B motility 21.25 ±0.56%. The rate of immobile spermatozoa was 53.13 ±0.84%. The rate of abnormal spermatozoa was 74.50 ±0.38%.

In patients with oligoasthenoteratozoospermia, the average sperm density was 5.68 ±0.9 mln/ml. The rate of spermatozoa characterized by type A motility was 9.17 ±1.31% and by type B motility 15.00 ±0.71%. The rate of immobile spermatozoa was 63.33 ±2.08%. The rate of abnormal spermatozoa was 79.00 ±0.80%.

It was showed that the level of IL-1β was lower in men with azoospermia than in men with normospermia (p < 0.01). No statistically significant differences were found between the level of IL-1β in the seminal plasma of men with normospermia as compared to men with asthenoteratozoospermia and oligoasthenoteratozoospermia (Fig. 1).

It was showed that the level of IL-18 in the seminal plasma of infertile men was similar in all evaluated subgroups (Fig. 2).

The concentrations of IL-1β and IL-18 were shown to significantly correlate with the rate of abnormal spermatozoa in the seminal plasma of men with oligoasthenoteratozoospermia (Tab. 1).

A significantly higher level of IL-1β and IL-18 was found in the seminal plasma of men with an elevated leukocyte count as compared to the control group (Fig. 3, 4).

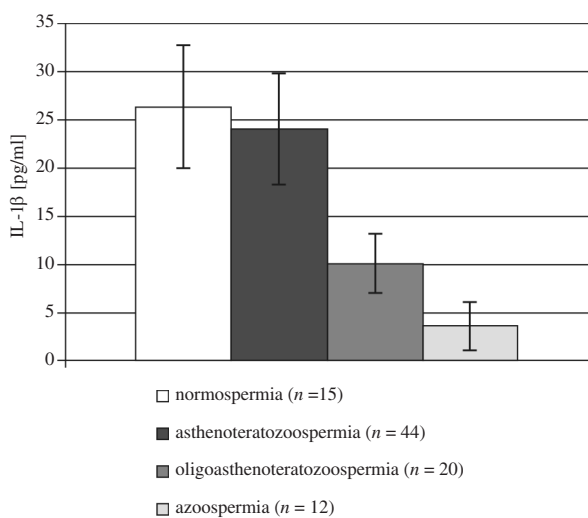


Fig. 1. Interleukin 1β concentration in examined groups

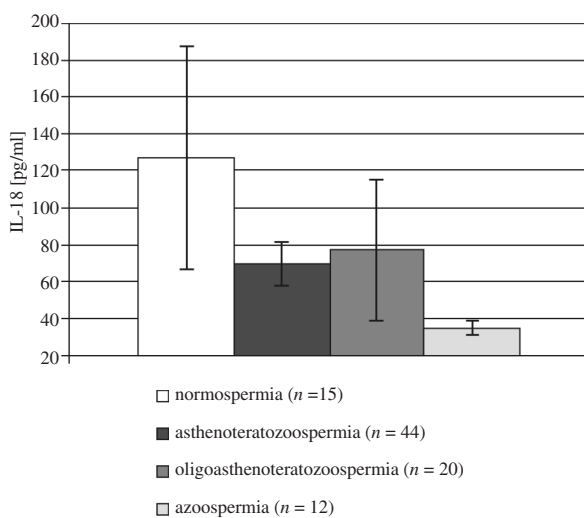


Fig. 2. Interleukin 18 concentration in examined groups

Table 1. Correlations between IL-1β and IL-18 and percentage of abnormal spermatozoa and leukocytes number

Correlation	n	R	p
abnormal spermatozoa vs. IL-1β [pg/ml]	22	0.60	0.01
abnormal spermatozoa vs. IL-18 [pg/ml]	20	0.51	0.04
number of leukocytes/ml vs. IL-1β [pg/ml]	12	0.35	0.05
number of leukocytes/ml vs. IL-18 [pg/ml]	14	0.55	0.04

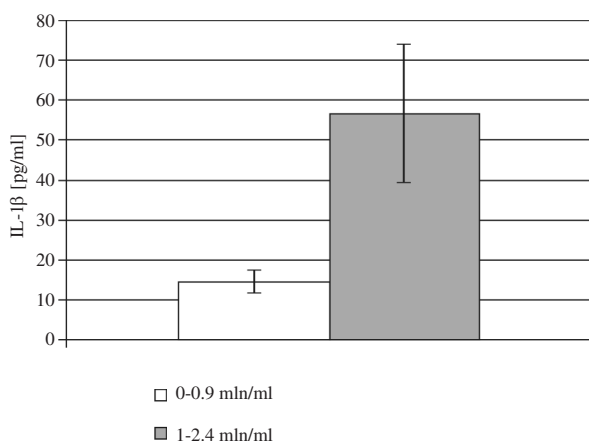


Fig. 3. Interleukin 1β concentration depending on leucocytes count

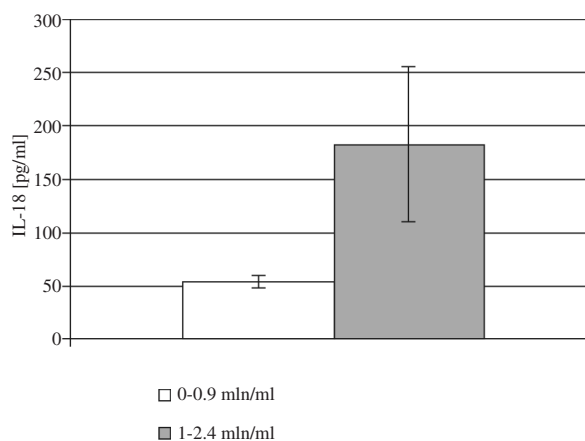


Fig. 4. Interleukin 18 concentration depending on leucocytes count

There was also a significant correlation between leucocyte count and concentrations of IL-1β and IL-18 in the seminal plasma of men with leukocytospermia (IL-1β on the border of statistical significance) (Tab. 1).

Discussion

Male infertility remains an important clinical and diagnostic problem. It seems that the evaluation of concentration of proinflammatory cytokines in the seminal plasma of infertile men could constitute a valuable complement of diagnostics of male accessory glands inflammation resulting in an impaired semen quality.

Interleukin 1 takes part in physiologic processes of spermatogenesis functioning as an autocrine regulator [20]. There is a great deal of data pointing to the role of this cytokine in the pathology of male reproductive system.

Our study was aimed into evaluation of significance of IL-1β for semen pathology leading to impaired fertility.

The concentration of IL-1β in the seminal plasma of men with abnormal results of seminogram was not found to be changed as compared with control group with the exception for azoospermic men.

Our observations agree with the results of Papadimas, who examined relation between the level of IL-1β and parameters of seminogram in subgroups of patients with various clinical diagnoses of pathology in the male reproductive system and also did not found any differences [21].

We observed that increased level of IL-1β correlates with the rate of spermatozoa with abnormal morphology. Those relations have not been described previously.

At present, it is believed that IL-1β could damage spermatozoa via stimulation of lipid peroxidation in their cellular membranes [22]. According to our results, namely the

observed correlation between the concentration of IL-1β and the rate of pathologic spermatozoa, and data from other reports it seems that those relations could be due to the induction of oxidative stress and excess of free oxygen radicals related to the formation of leukocyte infiltrate.

Lack of the differences between IL-1β in control group and groups with semen pathology may be explained by the fact that IL-1β is produced mainly at the very beginning of the inflammation cascade. Thus it is possible that in case of pathology lasting longer concentrations of IL-1β was already lower than in the very beginning of infection/inflammation.

However we found that the concentration of IL-1β is significantly higher in men with leukocytospermia and correlates positively with the number of leukocytes in the semen. Our observations seem to be confirmed by clinical reports of elevated IL-1β levels in men diagnosed with chronic prostatitis and pelvic pain syndrome [23].

As leukocytospermia is widely accepted not highly specific but good indication of inflammation our results together with data from other publications suggest that the evaluation of IL-1β concentration could have a practical significance in diagnostics of male accessory glands inflammation with the leukocyte count in semen sample.

Our study did not show any differences in IL-18 concentrations in the seminal plasma of men with abnormal results of semen examination. The only relation was found between the concentration of IL-18 and the rate of pathologic spermatozoa in patients with oligoastheno-teratozoospermia.

However, we found a highly significant increase in the concentration of IL-18 in leukocytospermia and also a correlation between IL-18 concentration and the number of leukocytes in the semen.

As yet, only one paper evaluating the significance of IL-18 for the pathology of male reproductive system has been published [16]. That study showed higher concentrations of IL-18 in the seminal plasma of infertile men with male accessory glands inflammation as compared to control subjects (fertile men), men with varicocele, and patients with Klinefelter syndrome.

Since that paper analyzed IL-18 concentrations with reference to the etiologic factor of infertility and our study was focused on abnormalities in semen examination, direct comparison of both studies could not be made. However, it seems that the group with infection could to a large extent correspond to the group of patients with leukocytospermia in our material. Thus, both reports seem to confirm the role of IL-18 in the pathogenesis of male accessory glands inflammation and its possible significance in the diagnostics of this pathology [24].

In conclusion the correlation between the concentrations of IL-1 β and IL-18 and the rate of pathologic spermatozoa in the group of men with oligoasthenoteratozoospermia suggests the role of these cytokines in the aetiology of teratozoospermia. The increased concentrations of IL-1 β and IL-18 in the seminal plasma of men with leukocytospermia and correlation between concentrations of IL-1 β and IL-18 and the number of leukocytes in the semen in this group of patients points to the relation of these cytokines to the mobilization of inflammatory infiltrate in male reproductive system.

References

- Iammarrone E, Balet R, Lower AM, et al. (2003): Male infertility. *Best Practical Res in Clin Obstetr Gynaecol* 17: 211-229.
- Sanocka D, Kurpisz M (2003): Infertility in Poland – present status, reasons and prognosis as a reflection of Central and Eastern Europe problems with reproduction. *Med Sci Monitor* 9: 16-20.
- Diemer T, Hales DB, Weidner W (2003): Immune – endocrine interactions and Leydig cell function: the role of cytokines. *Andrologia* 35: 55-63.
- Keck C, Gerber-Schafer C, Clad A, et al. (1998): Seminal tract infections: impact on male fertility and treatment options. *Hum Reprod Update* 4: 891-903.
- Comhaire FH, Mahmoud AM, Depuydt CE, et al. (1999): Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist's viewpoint. *Hum Reprod Update* 5: 393-398.
- Purvis K, Christiansen E (1993): Infection in the male reproductive tract. Impact, diagnosis and treatment in relation to male infertility. *Int J Androl* 16: 1-13.
- Weidner W, Jantos C, Schiefer HG, et al. (1991): Semen parameters in men with and without proven chronic prostatitis. *Arch Androl* 26: 173-183.
- el Demiry MI, Hargreave TB, Busuttill A, et al. (1985): Lymphocyte sub-populations in the male genital tract. *Brit J Urol* 57: 769-774.
- el Demiry MI, Young H, Elton RA, et al. (1986): Leucocytes in the ejaculate from fertile and infertile men. *Brit J Urol* 58: 715-720.
- Ochsendorf FR (1999): Infections in the male genital tract and reactive oxygen species. *HumReprod Update* 5: 399-420.
- Dinarelo CA (2005): Interleukin 1beta. *Critical Care Medicine* 33 S: 460-462.
- Fiszer D, Rozwadowska N, Lukaszzyk A, et al. (2003): Quantitative mRNA analysis of IL-1 gene system in human testis. *Am J Reprod Immunol* 50: 389-398.
- Soder O, Sultana T, Jonsson C, et al. (2000): The interleukin-1 system in the testis. *Andrologia* 32: 52-55.
- Khan SA, Nieschlag E (1991): Interleukin-1 inhibits follitropin-induced aromatase activity in immature rat Sertoli cells *in vitro*. *Mol Cell Endocrinol* 75: 1-7.
- Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H (2001): Interleukin-18 regulates both Th1 and Th2 responses. *Ann Reproduct Immunol* 19: 423-474.
- Matalliotakis IM, Cakmak H, Fragouli Y (2006): Increased IL-18 levels in seminal plasma of infertile men with genital tract infections. *Am J Reproduct Immunol* 55: 428-433.
- Rodin DM, Larone D, Goldstein M (2003): Relationship between semen cultures, leukospermia, and semen analysis in men undergoing fertility evaluation. *Fertility and Sterility* 79: 1555-1558.
- Anderson DJ, Hill JA (1998): Cell-mediated immunity in infertility. *Am J Reproduct Immunol* (17): 22-30.
- Lackner JE, Herwig R, Schmidbauer J, et al. (2006): Correlation of leukocytospermia with clinical infection and the positive effect of anti-inflammatory treatment on semen quality. *Fertility and Sterility* 86: 601-605.
- Huleihel M, Lunenfeld E, Horowitz S, et al. (2000): Production of interleukin-1-like molecules by human sperm cells. *Fertil Steril* 73: 1132-1137.
- Papadimas J, Goulis DG, Sotiriades A, et al. (2002): Interleukin-1 beta and tumor necrosis factor-alpha in normal/infertile men. *Arch Androl* 48: 107-113.
- Buch JP, Kolon TF, Maulik N, et al. (1994): Cytokines stimulate lipid membrane peroxidation of human sperm. *Fertility and Sterility* 62: 186-188.
- Alexander RB, Ponniah S, Hasday J, Hebel R (1998): Elevated levels of proinflammatory cytokines in the semen of patients with chronic prostatitis/chronic pelvic pain syndrome. *Urology* 52: 744-749.
- Kohno K, Kurimoto M (1998): Molecule of the month. Interleukin-18, a cytokine which resembles IL-1 structurally and IL-12 functionally but exerts its effect independently of both. *Clin Immunol Immunopathol* 86: 11-15.