The Impact of IL-35, Bacterial Prostatitis in Development Male Infertility in Najaf Province Patients

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ABSTRACT

Background: A case-control study examined the relationship between bacterial prostatitis and IL-35 blood levels in the emergence of male infertility.

Objective: This study aimed to assess the role that bacterial prostatitis and IL-35 play in the progression of male infertility in patients from Najaf province.

Patients and Procedures: 120 patients were seen at AL-Sadder Medical City, Al-Najaf province through the period from January to June, 2021. This number included sixty prostatitis-related infertile patients, 30 prostatitis-related fertile patients, and 30 infertile patients. 30 healthy fertile male subjects served as control group. All subjects provided blood and semen samples and three ml of the blood were placed in a gel tube to separate the serum used to calculate IL-35 level by ELISA. A loop of semen was collected to identify the bacterial culprits as well as semen liquefaction, volume, appearance, and fundamental sperm characteristics in the residual semen (density, motility, viability, and morphology).

Results: Prostatitis patients had considerably lower serum concentrations of IL-35 than infertile patients did, according to the findings (P < 0.05). The concentration of IL-35, which is thought of as a biomarker for a progressive state, decreased with the severity of the disease. The findings showed that *S. aureus* and *E. coli* were the most frequent bacterial causes of prostatitis.

Conclusion: The current study found a correlation between infertility and the infection with prostatitis condition, which is reflected in the body's immune response as a lower level of IL-35.

Keywords: IL-35, Serum level, Infertility, Prostatitis, Men.

INTRODUCTION

Cytokines are multifunctional glycoproteins that are released by several areas of the male genital tract and are active in a variety of processes that may influence steroidogenesis, spermatogenesis, sperm functions, and fertility regulation. Because cytokines' concentrations monitor their release and reflect how they interact with spermatozoa, they can be used to learn more about male infertility ⁽¹⁾. Male semen's pro- and anti-inflammatory ratios may become unbalanced as a result of the inflammation caused by prostatitis, which could have a negative impact on sperm quality and function ⁽²⁾. By controlling inflammatory responses linked to male infertility, cytokines contribute to prostatitis and male infertility ⁽³⁾.

IL-35 is the newest member of the interleukin-12 cytokine family that is produced by a variety of regulatory lymphocytes, and functions as an anti-inflammatory cytokine that is essential for immune suppression ⁽⁴⁾.

CD+8, IL-17, IL-12, and interferon can be inhibited from being secreted, as can the development of CD4+ T cells into Th17 and Th1 cells. IL-35 can also promote the growth of Treg cells and increase the expression of IL-10 ⁽⁵⁾. IL-35 plays an immunomodulatory role by increasing the secretion of IL-10 and TGF- and encouraging the growth of Treg and Breg cells ⁽⁶⁾. Uropathogenic infections in the semen of individuals with acute and chronic bacterial prostatitis impair the vitality and mobility of sperm, resulting in infertility ⁽⁷⁾. Male infertility may develop from chronic prostatitis. The production of its cytokines has been linked to changes in the reproductive system and testicles ⁽⁸⁾. Changes in cytokine levels may affect the physiological mechanisms underlying both male reproductive function and fertility, according to **Mary** *et al.* ^{(9).}

PATIENTS AND METHOD

A hospital-based case-control study included 120 patients who visited AL-Saddar Medical City in the region of Al-Najaf through the period from January to June of 2021. The patients were classified into three groups: 60 prostatitis-related infertile patients, 30 prostatitis fertile patients and 30 infertile subjects. Furthermore, 30 healthy fertile male subjects served as control group. For the ELISA system's evaluation of IL-35 (Elabscience® Company, China), 3 ml of blood from each subject was taken, placed in a gel tube to separate the serum, and maintained at -20 °C. To identify bacterial causes, a loop of semen was collected. The leftover semen was examined to measure its density, motility, viability, and morphology as well as its volume, appearance, and semen liquefaction ⁽¹⁰⁾.

Inclusion Criteria: Infertile men as well as patients with acute and chronic prostatitis were included.

Exclusion criteria: Patients who received ongoing antibiotic treatment and patients who had chronic epididymitis/orchitis, prostate cancer, and benign prostate syndrome.

Ethical considerations:

Kufa University's College of Science and AL-Saddar Hospital Institutional Ethics Committee approved the study. Additionally, before taking part in the study, each individual gave written, informed consent. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The univariate ANOVA in Scheffe's method, the mean and standard deviation were used. The statistical threshold for comparing patients to the healthy was P \leq 0.05.

RESULTS

According to the type of disease and age, the distribution of patients and healthy is shown below

120 patients were divided into three major groups: 30 (25%) infertile patients, 30 (25%) Prostatitis fertile patients, and 60 (50%) prostatitis infertile patients, which were further subdivided into asthenozoospermia, teratozoospermia, and oligozoospermia, respectively. Asthenozoospermia were 37 (61.7%), oligozoospermia were 15 (25%), and teratozoospermia were 8 (13.3%). 30 healthy individuals served as control group, as seen in Table (1). Patients were categorized into acute prostatitis (35.5%) and chronic prostatitis (58.3%) based on the type of prostatitis they had. (64.5 percent). According to the age distribution, 29 (48.3%) prostatitis infertile, 14 (46.6%) infertile and 13 (43.3%) prostatitis fertile were most common in the age group of 31 to 40 years (Table 1).

| Age | Prostatitis infertile (N=60) | Infertile N= (30) | prostatitis fertile (N=30) | Healthy (N=30) |
|------------------------|------------------------------------|----------------------|----------------------------------|-------------------|
| 20-30 | 11(18.3) | 7(23.4) | 5(16.7) | 4(13.3) |
| 31-40 | 29(48.3) | 14(46.6) | 13(43.3) | 17(56.7) |
| 41-50 | 20(33.4) | 9(30) | 12(40) | 9(30) |
| Oligo- zoospermia | 15(25%) | 17(56.7%) | Nil | Nil |
| Astheno- zoospermia | 37(61.7%) | 9(30%) | Nil | Nil |
| Terato- zoospermia | 8(13.3%) | 4(13.3%) | Nil | Nil |

Table (1): Age and abnormality of sperm of patients

Distribution of patients according to disease grade:

Three grades of infertility among infertile patients were determined including oligospermia. In grades 1, 2, and 3, infertile prostatitis was noted at 23.5%, 29.4%, and 47%.1, respectively. On the other hand, prostatitis patients with teratozoospermia and asthenozoospermia were present in considerable numbers in grade 2 records (53.8% and 53.4%, respectively). While grade 1 appeared to be elevated in all forms of oligospermia, asthenospermia, and teratozoospermia in infertile patients (44.4%, 57.1%, and 53.9% respectively), as shown in figure (1).



Figure (1): Type and infertility grade of patients

Bacterial identification in prostatitis patients:

A total of 90 clinical specimens were found to contain 31 (33.2%) *E coli* isolates, 21 (24.6%) *S aureus* isolates, 15 (15.4%) *K pneumonia* isolates, and 9 (10.2%), 8 (8.8%), and 7 (7.8%) isolates of *Enterobacter spp*, *Pseudomonas aerogenosa*, and *P mirabilis* isolates respectively (Figure 2).



Figure (2): Pathogenic bacteria isolated in the study

Evaluation of IL-35:

Regarding IL-35, patients with prostatitis infertility, prostatitis, and infertility had significantly lower serum concentrations of IL-35 compared to the control group (P < 0.05), as shown in figures (3).



Figure (3): IL-35 level in prostatitis infertile, prostatitis, infertile, and healthy persons

IL-35 level according to the type of prostatitis:

The current results revealed that serum level of IL-35 in acute and chronic prostatitis infertile patients was significantly decreased (P < 0.05) compared to the control group (Figure 4).



Figure (4): IL-35 level (pg/mL) in prostatitis, infertile, and healthy cases

IL-35 level according to the type of sperm anomaly:

Regarding IL-35 levels in infertile patients (Oligozoospermia, Asthenozoospermia, and Teratozoospermia) were 8.3 ± 1.04 , 4.2 ± 0.53 , and 5.3 ± 1.70 pg/mL respectively, which were significantly decreased in all groups compared to healthy control group (P< 0.05). IL-35 levels in prostatitis infertile patients were 2.8 ± 1.48 , 4.1 ± 0.94 , and 3.5 ± 0.88 pg/mL as seen in figure (5).





DISCUSSION

Since the prostate is the main male accessory gland and secretes many substances that are essential for human reproduction. Prostate inflammation is proposed as a potential risk factor for male infertility ⁽¹¹⁾. The most frequent urologic ailment in men under the age of 50 and the third most common in men over the age of 50 is prostatitis, an inflammatory condition of the prostate ⁽¹²⁾. According to the study by **Motrich** *et al.* ⁽¹³⁾, individuals with chronic prostatitis had an average age of 42 and a range of 30-55. Additionally, **Schaeffer** ⁽¹⁴⁾ noted that men's fertility is frequently affected by prostatitis syndrome, with a peak between the ages of 36 and 50. In contrast to acute cases, chronic prostatitis occurred more frequently, accounting for 53% of sperms with increasing

motility and 37% with immotility. Berg et al. (15) reported that both chronic and acute prostatitis were seen to increase in prevalence with age by more than 40 years and to be associated with increased sperm DNA damage, changes in sperm motility, and abnormalities that have an impact on fertility. Asthenozoospermia and oligospermia were important causes of male infertility for 284 contributing patients, according to the findings by Golshani et al. (16), from which oligozoospermia 45 (15.85%), asthenozoospermia 51 (17.96%), and teratozoospermia 15 (5.28%) were significant causes. Low sperm concentration (also known as oligospermia), low sperm motility (also known as asthenospermia), and aberrant sperm morphology are the three most frequent male infertility characteristics, according to WHO (10) (teratozoospermia). Furthermore, Oligozoospermia (56.5%) and asthenospermia were the two most commonly identified reasons for male infertility, according to **Moridi** *et al.* ⁽¹⁷⁾. (24.4%).

Wong *et al.* ⁽¹⁸⁾ study showed that oligospermia, which is caused by hormonal imbalances, occurs at a high rate in patients with a high number, movement, shape, and degree of activity of semen leading to a defect in the pituitary gland or the hypothalamus, and weakness in the process of sex. **Fainberg** and **Kashanian** ⁽¹⁹⁾ demonstrated significant differences between various types of infertility. The patients with asthenozoospermia had the highest rate of infertility, according to the findings of **Ugwuja** *et al.* ⁽²⁰⁾. Additionally, **Correa** ⁽²¹⁾ reaffirmed that asthenozoospermia is one of the causes of infertility that affects men in various age groups, particularly those between 30 and 50. According to **Curi** *et al.* ⁽²²⁾, more than 20% of infertile males have asthenozoospermia.

Infertile patients with male genital tract infections have significantly lower ejaculate volume, concentration, sperm motility, morphology, and viability (23) Similarly, Ikechebelu ⁽²⁴⁾ discovered that oligozoospermia (35.9%) and asthenozoospermia (32.3%) were the most prevalent aetiological factors causing male infertility. They also noted that genital tract infections brought on by inadequately treated sexually transmitted diseases were the root cause of these abnormalities. According to Alaimary ⁽²⁵⁾, there were statistically significant differences between the different types of infertility, with oligozoospermia being found in the greatest number of patients, 117 men (36%), followed by asthenozoospermia, 97 men (30%), teratozoospermia, 73 men (22%), and azoospermia, 41 men (12%).

According to our findings, E. coli was the most frequent cause of disease. According to Khan et al. (12), acute bacterial prostatitis is brought on by uropathogenic bacteria, with E. coli accounting for more than half of all cases. Other common causes included Kleibsiella spp., Enterobacter spp., Serratia spp., P. aeruginosa, and Proteus spp. However, Domes (26) reported that E. coli accounted for 58% of bacterial causes and confirmed that bacterial infections of the male reproductive tract result in the reduction of the prostate's secretory ability, which may harm all semen parameters, including morphology and motility. Acute and chronic prostatitis are among the main causes of male infertility according to **Dutta** ⁽²⁷⁾. who also noted that infection raised the levels of proinflammatory mediators in the male reproductive tract to a higher-than-normal value. This opens the door to affecting male reproductive processes through the common inflammatory mechanisms. According to Khanmohammad et al. (28), E. coli lipopolysaccharide (LPS) stimulates the immune system to release proinflammatory cytokines, which cause an immunological response in the testis and ultimately results in deficits in sperm parameters, primarily reduced sperm motility. According to **Huina** *et al.* ⁽²⁹⁾, bacterial infection in the vaginal canal caused aberrant expression of IL-17, which had a negative impact on the quality of male sperm and may contribute to infertility in men. Gram-positive bacteria, particularly E. faecalis, were found to be the second most common cause of chronic bacterial prostatitis (CBP), according to **Heras** *et al.* ⁽³⁰⁾. According to **Xiong** *et al.* ⁽³¹⁾, bacteria that build biofilms may improve the potential of strains that cause acute prostatitis to remain in the prostatic secretory system and induce recurrent urinary tract infections (UTIs), which are indicative of chronic bacterial prostatitis.

The results of the current study demonstrated that IL-35 is crucial for regulating immune-related conditions such as infectious illnesses. This supports the theory put by Ye et al. (32), who explain that immunoregulatory cell populations and immunosuppressive cytokines are responsible for maintaining the equilibrium between inflammatory and anti-inflammatory immune responses. Additionally, according to Tsiporenko⁽⁴⁾, IL-35 inhibits the differentiation of Th17 cells, and in individuals with chronic prostatitis, lower levels of IL-35 are associated with the onset of infertility. Bello (33) discovered that variations in IL-35 percentage are critical in suppressing immunological defenses in the lower genital tract, suppressing cell-mediated immune responses. Infertile men and patients with both infertility and a symptom of inflammatory prostatitis (CP type IV) had considerably lower levels of anti-inflammatory cytokines including IL-10 and IL-35, as well as TGF-, in the healthy group, according to Li et al. (34). Similar to this, He and Li (35) noted that all forms and stages of chronic prostatitis (CP) are caused by inflammatory cells and cytokines and that CP develops and progresses as a result.

CONCLUSION

IL-35 levels decreased with prostatitis infection, resulting in infertility and decreased immune response.

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