

Caspase-3 level is associated with bacterial prostatitis in male infertility

Kais Khudhair Al-hadrawi

Radiology Techniques Department, College of Medical Technology, Islamic University, Najaf, Iraq, kaisalhadrawii@gmail.com

Raid Talib ALGarawy

University of Kufa- College of Medicine, Kufa, Iraq

Abstract

Background: Bacterial prostatitis (BP) is an infection of the prostate gland if not treated appropriately it lead to significant morbidity and is associated with some complication like recurrent Urinary Tract Infection (UTI) and infertility.

Objective: The aim of this research was to evaluation of caspase-3 level in serum of infertile males infected with bacterial prostatitis.

Patients and method: The study was done at Infertility Unit, AL-Sadder medical City, Al-Najaf City, Iraq during the period from January to June 2022 included 120 individuals divided as follow: 60 patients were infected with prostatitis and 30 were infertile and 30 healthy as control group. Caspase-3 serum levels were measurement by enzyme-linked immunosorbent assay (ELISA). A loop full of semen has been cultured to bacterial identified.

Results: The results of the current study revealed that Escherichia coli (E.coli) was the first etiological agent caused prostatitis 30(33.2%) and the levels of serum concentration of caspase-3 was significant increase ($P < 0.05$) in patients with infertile (29.3 ± 1.092), prostatitis infertile (41.55 ± 3.452) and prostatitis patients (22.12 ± 1.572) in comparison with a healthy group (10.5 ± 0.4680).

Conclusion: The current study has concluded that prostatitis was associated with multiple etiologies and pathways, most of which resulted from interactions with the immune system and male infertility.

Keywords: *Caspase-3 Serum level, Infertility, Men Prostatitis.*

INTRODUCTION

Over the last several decades, infertility defined as the inability to have a child after trying for a fair amount of time without using birth control has become more common(1). Millions of individuals of reproductive age across the globe struggle with infertility, which has repercussions for their families and communities(2). It is estimated that 48 million couples and 186 million people worldwide

struggle with infertility (3). Many men, especially those between the ages of 30 and 50 years old suffer from prostatitis, an inflammation of the prostate gland that causes a variety of symptoms and indications and may ultimately cause infertility in males(4). Worldwide, prostatitis is a serious health issue for males, with an estimated 8-16% of cases being bacterial in nature (5). Uropathogenic infections in the semen, which define both acute and chronic bacterial prostatitis, have a

deleterious influence on sperm viability and motility, ultimately leading to infertility(6). Progression of motile sperm and changes in reproductive and testicular tissue and genital organ function, as well as an imbalance in the production of cytokines, have been related to chronic prostatitis, which may lead to a number of complications(7). The many components of the male reproductive system release cytokines, which are multifunctional glycoproteins with potential effects on steroidogenesis, spermatogenesis, sperm functions, and fertility control(8). Cytokine concentrations are a proxy for cytokine release and a reflection of cytokine interactions with spermatozoa, and hence may be used to infer the cause of male infertility(9). Therefore, the purpose of this research was to evaluation of caspase-3 level in serum of infertile males infected with bacterial prostatitis.

PATIENTS AND METHOD

Ethical considerations: The study concept for human studies was approved from Kufa University's College of Science and AL-Kufa General Hospital by The Institutional Ethics Committee. 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.

Patients: The study was done at Infertility Unit, AL-Sadder medical City; Al-Najaf City, Iraq during the period from January to June 2022 included 120 individuals divided as follow: 60 patients were infected with prostatitis, 30 infertile and 30 healthy as control group. Caspase-3 serum levels were measurement by enzyme-linked immunosorbent assay (ELISA). A loop full of semen has been cultured to identified of pathogenic bacteria by biochemical testes and VITEK 2 COMPACT(10,11,12).

Statistical analysis:

Data was collected and analysed by using SPSS (Statistical Package for Social Sciences, version 20, IBM, and Armonk, New York). Quantitative data was summarized as mean and standard deviation. Qualitative data were given as number (n) and percentage (%). Correlations were determined by Spearman correlation. P-value <0.05 was considered significant(13,14).

RESULTS

1. Study subjects are distributed based on the type of disease and age: The study showed in (Table 1) that 120 male patients were divided into three main groups, 60 (50%) prostatitis infertile patients that further divided into Asthenozoospermia with 37(61.7%), Teratozoospermia 8(13.3%) and Oligozoospermia 15 (25%) and 30 (25%) prostatitis fertile patients, 30(25%) infertile patients that divided as 9(30%) Asthenozoospermia, 4(13.3%) Teratozoospermia and 17(66.7%) Oligozoospermia.

According to the definition of prostatitis, the 90 patients were split into two categories: acute prostatitis, which affected 32 (35.5% of the patients), and chronic prostatitis, which affected 58 (64.5% of the patients).

The age distribution indicated that the age range 31-40 years records a high frequency in prostatitis infertile patients, prostatitis fertile patients, and infertile patients correspondingly, as shown in Table. This age group records a high frequency of 29 (48.3%), 13 (43.3%), and 14 (46.6%). (1).

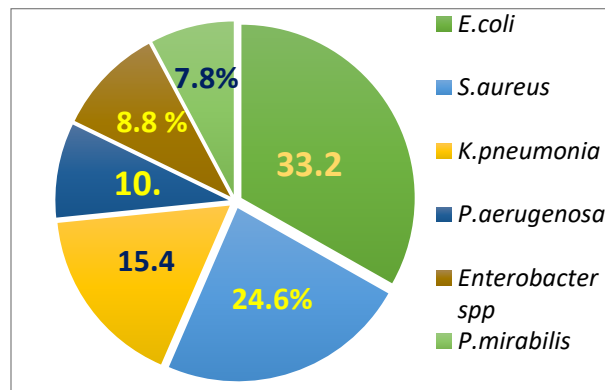
Table (1): Patients were categorized according to the kind of sickness they were suffering from, as well as their age and the abnormalities in their sperm.

Age abnormalities sperm	Prostatitis infertile (N=60)	Infertile N=(30)	Prostatitis fertile (N=30)
20-30	11(18.3)	7(23.4)	5(16.7)
31-40	29(48.3)	14(46.6)	13(43.3)
41-50	20(33.4)	9(30)	12(40)
Oligozoospermia	15(25%)	17(56.7%)	Nil
Asthenozoospermia	37(61.7%)	9(30%)	Nil
Teratozoospermia	8(13.3%)	4(13.3%)	Nil

2- Bacterial identification in prostatitis patients:

According to the results of this study, out of a total of 90 clinical specimens, approximately 30 (33.2%) isolates belonged to Escherichia coli, 21(24.6%) isolates were Staphylococcus.aureus, 15 (15.4%) isolates belonged to Klebsiella.pneumonia, and 9 (10.2%), 8(8.8%), 7(7.8%) isolates were Enterobacter spp., Pseudomonas.aerogenosa and Proteus.mirabilis, respectively as shown in Figure (1).

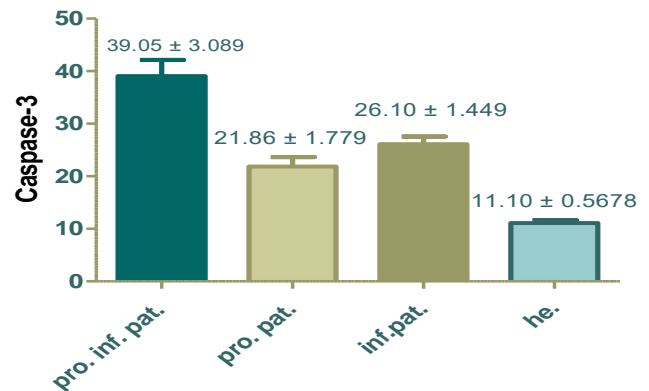
Figure (1): The identified bacterial species from the research



3- Evaluation serum level of Caspase-3 in patients and healthy controls.

From the results of the present study as shown in figure (2), the results showed a significant increase ($p < 0.05$) of Caspase-3 level in both prostatitis infertile patients (39.05 ± 3.089) and infertile men (26.10 ± 1.449) compared with the control group (11.10 ± 0.5678), also significant increase ($p < 0.05$) of Caspase-3 level prostatitis infertile compared to both prostatitis patients (21.86 ± 1.779) and infertile men, there was non-significant difference ($p > 0.05$) between infertile patient and prostatitis patient and between prostatitis patient and control group.

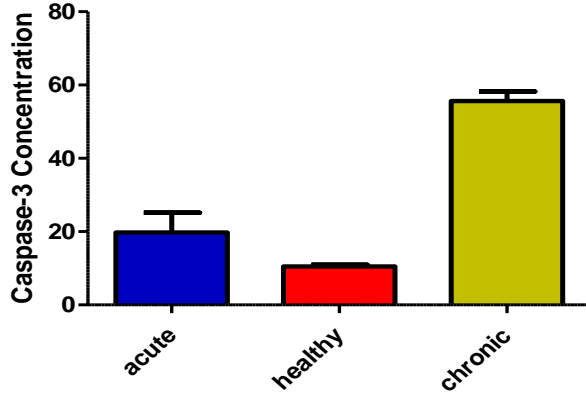
Figure: (2) Serum level Caspase-3 in prostatitis infertile, prostatitis, infertile patients, and healthy.



4 – The level of caspase-3 according to type of prostatitis:

The results of the present study revealed that the levels of serum concentration of caspase-3 were significant increase ($P < 0.05$) in acute and chronic prostatitis (19.80 ± 5.392 ; 55.65 ± 2.576) in comparison with healthy group (10.50 ± 0.4680) as shown in figure(3).

Figure: (3) Serum caspase-3 levels were compared between men with chronic prostatitis, those with acute prostatitis, and healthy men.

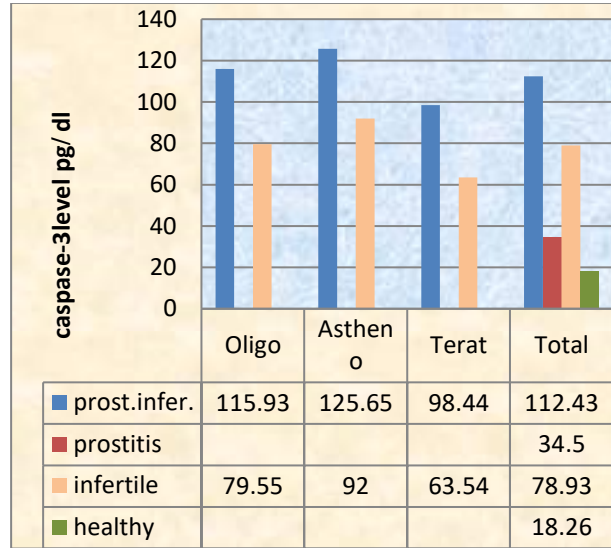


5 - Incidence of illness and severity of sperm abnormality are correlated with caspase-3 activity:

The present study revealed that serum level of caspase-3 significantly highly increased in prostatitis infertile patients with (112.43 ± 35.87) pg/ml, in prostatitis patients (35.60 ± 11.28) pg/ml, in infertile patient (78.93 ± 26.00) in compare to healthy fertile group (11.09 ± 2.48) as show in figure (4).

It seems from the sperm analysis data that individuals with prostatitis who are sterile have a higher caspase-3 level: Oligozoospermia, Asthenozoospermia, and Teratozoospermia were 115.93 ± 38.69 , 125.65 ± 33.58 and 98.42 ± 26.39 pg/ml respectively, while in the infertile patients were (79.55 ± 31.98) , 92 ± 26.88 and 63.54 ± 19.43 pg/ml.

Figure: (4) Caspase-3 level according to type of infertility and sperm anomaly.



DISCUSSION:

The current study showed that mean prostate infections affect above 20 years with a peak incidence between 30-40 age group, this age who seek for fathering this agree with Xiong(13) . they observed that chronic and acute bacterial prostatitis (ABP) generally affects men in age 30 - 40 years but also has a second peak of incidence in men over the age of 60 year. While other studies found a high incidence of prostatitis in the older age group more than 50, it may be due to a decrease in sexual potentials in such age group Montes (14) .

According to studies Nesheim (15) , prostate inflammation lowers the quality of sperm and raises the likelihood that sperm will die, making it a substantial risk factor for male infertility.

La Vignera (16) they noted that the pathogenic bacteria, leukocytes disorder, cytokines, and reactive oxygen species (ROS) might be the primary mechanisms of infertility resulting from male accessory gland infection.

In line with the findings of Khan (17) , which found that acute bacterial prostatitis is caused

by uropathogenic bacteria, the most frequent causes are *E. coli* (responsible for more than 50 percent of cases), followed by *Klebsiella* spp, *Enterobacter* spp, *Serratia* spp., *P.aeruginosa*, and *Proteus* spp. The results showed that *E. coli* was the most common

On the other hand, Dome (18) found that bacterial infections of the male reproductive tract lead to an impairment of the secretory capacity of the prostate. This may have a negative effect on all semen parameters, such as morphology and motility, and they discovered that *E. coli* was responsible for 58% of bacterial causes.

According to Heras (19), they verified that *E. coli* was thought to be the primary cause of chronic bacterial prostatitis (CBP), followed by gram-positive bacteria, mainly *E. faecalis*, as the causative agents. *E. coli* was regarded to be the primary cause of CBP.

It has been demonstrated by Shang (20) that bacterial infections of the male reproductive tract have the side effect of inhibiting the prostate's secretory capacity. This side effect might result in an undesirable impact on all semen parameters, which could ultimately result in infertility.

Ma (21) found a positive correlation between apoptosis and serum level of testosterone and spermatozoa responsible for male infertility. Harchegania (22) observed that several factors like hyperviscosity, oxidative stress impairment of seminal plasma can be associated with sperm DNA damage, sperm chromatin instability that leads to apoptosis, and male infertility.

According to Liu(23) , the seminal plasma of infertile males contains high levels of inflammatory cytokines, which have a toxic effect on the quality of sperm. They also explain that caspase-1, caspase-3, and apoptosis-associated speck-like protein form inflammasome, which is a multiprotein complex known as the master switch of the

innate immune response. Inflammasome significantly higher expresses 9 folders. Also, Camargo(24) Showed that seminal plasma inflammasome activity included IL-1 β , IL-18, caspase-3, caspase-1, and (Speck-like protein involved in apoptosis) were highly increased in varicocele patients. when compared to control men.

Said (25) revealed that increased caspases in peripheral blood impaired spermatogenesis decreased sperm motility and increased levels of sperm DNA fragmentation, testicular torsion, varicocele, and immunological infertility.

Given that male fertility depends on the prostate gland for spermatogenesis and the other accessory glands of the male genital canal for coordinated functioning, these findings may help explain why spermatogenesis is so important to the process. There is mounting evidence that prostate infection and inflammation cause prostate dysfunction, which in turn reduces sperm quality and male fertility Motrich (26).

The present study also showed that patients with acute and chronic prostatitis showed significantly elevated levels of caspase-3 in serum and decreased semen quality in patients with chronic prostatitis, which may be associated with infertility Hu (27) .

The current study agreed with the study of Eggert (28) they found that cytokines that can be elevated showed to negatively affect sperm motility and this may be lead to infertility. Also agreed with the study of La Vignera(16) they found prostatitis may cause ejaculatory dysfunction and this leads to male infertility, sexual dysfunction, which includes ejaculatory and erectile dysfunction.

As a consequence of this, the generation of sperm may be reduced, and conditions such as asthenozoospermia and teratozoospermia may be connected to caspase-3 as the major effector. The results showed an increase in

caspase-3, in addition to a degradation in normal morphology and rapid progressive motility. This is similar with the research, which found an increase in caspase-1 Asadi (9).

Oehninger (29) conducted research on the existence of and relevance of programmed cell death (apoptosis) in spermatozoa and found that it causes poor rates of fertilization and implantation, which in turn reduces fertility. Incomplete maturation of human ejaculated spermatozoa is associated with an increase in initiator and effector caspase activity, which activates apoptosis signaling and results in a decreased fertilizing capacity of the sperm. This is due to the high impact that caspases have on the fertilizing capacity of sperm Grunewald(30).

The authors of the study, Aziz (31) looked at the connection between sperm morphology and apoptosis.

The activity of caspase-9 and caspase-3 was found to have a significant negative correlation with sperm count, sperm motility, sperm velocity, and sperm normal morphology, according to the findings of Zalata(32). These researchers made their observations after comparing the sperm of oligoasthenoteratozoospermia males to the sperm of healthy men. Infertile men, in general, have lower sperm variables that are produced by greater ROS levels in semen, which has a positive link with increased sperm damage and higher levels of caspases 9 and 3, suggesting positive apoptosis. This was observed by Wang (33).

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