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Clinical, laboratory and ultrasonographic correlates of prostate calcifications in patients with chronic prostatitis/chronic pelvic pain syndrome

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Introduction The research aim was to determine the role of clinical, laboratory, immunological and sonographic parameters in the development of an assessment tool for the symptomatic manifestations of prostate calcifications in chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS).

Material and methods All men underwent a transabdominal ultrasonographic examination using a grayscale B-mode and color Doppler mapping, the evaluation of the National Institutes of Health-Chronic Prostatitis Symptom Index and the Patient Health Questionnaire-9, spermogram. Vascular endothelial growth factor (VEGF), serotonin and gamma-aminobutyrate (GABA), interleukins 1β and 10 were determined in blood serum and ejaculate.

Results This study included 102 men aged 18–45 years. Group 1 (n = 34) consisted of patients with CP/CPPS. Group 2 included patients (n = 34) with asymptomatic prostatitis. Group 3 consisted of healthy volunteers (n = 34). More severe symptoms of prostatitis and depression, as well as frequent exacerbations in patients with CP/CPPS, were associated with ultrasound evidence of prostate calcifications, and especially the twinkling artifact (Spearman's r = 0.481; p <0.001; Spearman's r = 0.437; p <0.001, respectively).

The presence of prostate calcifications in both CP/CPPS and asymptomatic prostatitis was accompanied by a significantly higher concentration of pro-inflammatory cytokine IL-1β and a lower concentration of anti-inflammatory cytokine IL-10 in the ejaculate (p < 0.05 in both cases, Kolmogorov-Smirnov test). The clinical manifestations observed in patients with CP/CPPS and asymptomatic prostatitis were not correlated with the leukocyte count in the ejaculate or the levels of VEGF, GABA, and serotonin in both blood and ejaculate.

Conclusions Twinkling artifact potentially could serve as a valuable tool for evaluating the condition of patients with CP/CPPS and prostate calcifications.

Key Words: chronic pelvic pain syndrome ‹› prostate calcification ‹› asymptomatic prostatitis ‹› twinkling artifact ‹› vascular endothelial growth factor ‹› gamma-aminobutyrate ‹› serotonin ‹› interleukins

INTRODUCTION

The pathogenesis of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) combines the mechanisms of chronic pelvic pain and dysuria without an explicit association with bacterial infection. Further elucidation of the mechanisms of this disease is relevant because of its prevalence, morphological heterogeneity, clinical significance, and the need to improve treatment outcomes [1].

Not all cases of CP/CPPS are the same; some patients develop prostate calcifications. Furthermore, large prostate calcifications have been associated with the development of moderate lower urinary tract symptoms (LUTS) [2]; however, they are not considered an independent predictor of severe LUTS [3].

It has been reported that prostate calculi are formed as a result of sedimentation of prostate secretion and desquamated epithelium, and their main constituents are phosphate and calcium carbonate. Oxalate calcifications also occur in clinical practice and have been shown in all age groups; their presence and age of patients are associated with severe LUTS [4].

Many regulatory molecules are involved in the development of symptoms of prostatitis, affecting the course of inflammation, disrupting its protective function and contributing to the chronicity of the disease. In this, the role of changes in the concentrations of pro-inflammatory and anti-inflammatory cytokines (in particular, interleukins 1β and 10) is known [5]. Potentially, these phenomena are associated with vascular endothelial growth factor (VEGF), serotonin and gamma-aminobutyrate (GABA). These molecules are found in the tissue of the prostate gland, and the elucidation of their role in normal and pathological conditions remains relevant. Changes in the metabolism of GABA, serotonin, and VEGF have been associated with in various pathological processes, including such as chronic pain, anxiety-depressive disorders, altered immune responses, and variable treatment results [6, 7, 8, 9]. It can be postulated that the participation of regulatory molecules in chronic inflammation of the prostate gland is associated with the formation of calcifications and should be reflected in their levels in the blood and/or ejaculate.

Today, prostate calcifications are diagnosed by identifying (through ultrasonography and CT tomography) dense inclusions in the prostate parenchyma, combined with an assessment of the symptoms of prostatitis. A non-uniform classification of prostate stones is based on their size and the presence of an echo-shadow [10]. The twinkling artifact is also known to be an ultrasound sign of parenchymal calcification [11], although it has not yet been used to diagnose prostate stones.

Thus, the assessment of the clinical and diagnostic significance of these inclusions requires improvement and further study. In this regard, the purpose of this study was to determine the role of clinical, laboratory, immunological and sonographic parameters in the development of an assessment tool for the symptomatic manifestations of prostate parenchyma calcifications in patients with CP/CPPS.

MATERIAL AND METHODS

It was a prospective study, which included 102 men aged 18–45 years. All study participants sign an informed consent and the study protocol was approved by the local Ethics Committee. The study was performed according to the research plan of the State Institution 'Academician O. F. Vozianov Institute of Urology of the National Academy of Medical Sciences of Ukraine'. State registration number 0121U114621. All study participants' sexually transmitted infections were excluded by polymerase chain reaction; pathogenic microorganisms were not found in the ejaculate, and opportunistic microorganisms did not exceed the level of 10^4 CFU/ml. The diagnosis of chronic prostatitis was established according to the classification of Kreiger et al., 1999 [12].

The exclusion criteria were:

1. The history of malignant tumors within the last 5 years.
2. Acute urinary retention within 3 months prior to inclusion in the study.
3. Neurogenic dysfunctions and bladder diverticula.
4. Urolithiasis.
5. Urethral stricture, bladder neck sclerosis.
6. The history of surgical intervention on the pelvic organs.
7. Infections of the genitourinary system in the phase of active inflammation.
8. Systematic intake of medicines.
9. Severe concomitant diseases in the phase of exacerbation or decompensation (including the cardiovascular and nervous systems, renal and hepatic insufficiency).
10. Use of drugs, alcohol more than 2 alcoholic units per day, mental illness of the patient.
11. Legal incapacity or limited legal capacity
12. Participation in other clinical studies within 3 months prior to enrollment in this study.
13. The inability or refusal of the patient to follow the requirements of the study protocol.

To detect prostate calcification, all patients were subjected to a transabdominal ultrasonographic examination with a convex probe using a grayscale B-mode for detecting the presence of echo-positive inclusions (≥3 mm) in the prostate gland, as well as checking

for the presence of a twinkling artifact [13, 14, 15] in the duplex diagnostic mode that is when combining greyscale B-mode and color Doppler mapping. All patients were examined on the Toshiba Xario expert diagnostic ultrasound system. We considered a twinkling artifact as a sign of moderate calcification of the prostate parenchyma, exceeding the intensity of inclusions without an echo shadow, but less dense than inclusions with an echo shadow. It should be noted that the detection of a twinkling artifact was combined with the detection of echo-positive inclusions, with the exception of one case. In addition, patients with echo-shadow inclusions were not included in the study, as they were rare in our practice.

Prostatitis symptoms were assessed using the National Institutes of Health-Chronic Prostatitis Symptom Index (NIH-CPSI) scale [16], depressive manifestations were assessed using the Patient Health Questionnaire-9 (PHQ-9) scale [17]. The frequency of prostatitis relapses in history was defined as the resumption of symptoms of the disease according to the patient. All participants underwent manual ejaculate analysis performed by a laboratory doctor. The abstinence was 2–7 days. GABA, Serotonin, VEGF, interleukin-1β (IL-1β), and interleukin-10 (IL-10) were determined by an enzyme-linked immunosorbent assay in blood serum and ejaculate.

Participants in all groups stated that they received nothing for at least 3 months prior to enrollment in the study. Although it is impossible to completely exclude the episodic use of non-steroidal anti-inflammatory drugs in patients of the Group 1.

The measure of central tendency was specified for each variable (median and interquartile range for quantitative variables; absolute and/or relative frequency for qualitative variables). Data distribution was assessed using the Shapiro-Wilk W test. Statistical analysis was carried out using Kolmogorov-Smirnov, Fisher's exact, Chi-square, ANOVA, Kruskal Wallis tests, Spearman's correlation coefficients. All qualitative variables for statistical calculations were converted to ordinal. Statistical analysis was carried out using SPSS, v. 13.0, the level of significance was taken as $p < 0.05$. Some indicators were evaluated by combining the data of all participants in the study (Group 1+2+3, n = 102).

RESULTS

Group 1 (n = 34) consisted of patients with CP/CPPS (category III according to Kreiger et al., 1999 [12]). Group 2 included patients (n = 34) with asymptomatic prostatitis (category IV [12]). They had no complaints, but there were laboratory signs of prostatitis (pyospermia, leukocytes more than 10 cells per field of view in the ejaculate). Group 3 consisted of healthy volunteers (n = 34). The criterion for inclusion in the group of healthy volunteers was the absence of clinical symptoms and laboratory signs of prostatitis, as well as ultrasonographic signs of prostate calcifications, as well as clinical manifestations of any other diseases.

The difference in age distribution among groups (Table 1), including healthy volunteers, was not statistically significant (Chi-square, p = 0.269). The age-related features of prostate calcification were observed. The presence of a twinkling artifact, in contrast to echo-dense inclusions and excessive level of white blood cells in the ejaculate, was observed significantly more often in patients with CP/CPPS (Group 1) over the age of 30 years (Fisher's Exact Test, p = 0.024, p = 0.692, p = 0.714, respectively) (Table 2). A similar association between age and the presence of twinkling artifact and echo-dense inclusions was not observed in patients with asymptomatic prostatitis (Group 2) (Fisher's Exact Test, p = 0.280, p = 0.465, respectively).

Table 1. *Age structure of study participants*

Age	Group 1	Group 2	Group 3
Under 30 years old	10 (29.4 %)	11 (32.4 %)	16 (47.1%)
Over 30 years old	24 (70.6%)	23 (67.6%)	18 (52.9%)
Chi-square, p	0.269		

Table 2. *Age distribution of ultrasonographic symptoms and ejaculate leukocytosis*

Age	Group 1 n = 34 Echo-dense inclusions '+'	Group 1 n = 34 Twinkling artefact '+'	Group 1 n = 34 Sperm leukocytosis '+'	Group 2 n = 34 Echo-dense inclusions '+'	Group 2 n = 34 Twinkling artefact '+'	Group 2 n = 34 Sperm leukocytosis '+'
Under 30 years old	6 (17.7 %)	1 (2.9 %)	4 (11.8 %)	2 (5.9%)	0	11 (32.4 %)
Over 30 years old	17 (50 %)	13 (38.2 %)	8 (23.5 %)	9 (26.5 %)	4 (11.8 %)	23 (67.7 %)
Fisher's Exact Test, p	0.692	0.024	0.714	0.465	0.280	not applicable

The prevalence of calcifications (≥3 mm) of the prostate detected by transobdominal ultrasonography as echo-dense inclusions was 67.7% in patients with CP/CPPS and 35.3% in patients with asymptomatic prostatitis (Chi-square test, p = 0.008; Table 3). Twinkling artifact was significantly less common than echo-dense inclusions and was detected in 41.2% of patients with CP/CPPS (Chi-square test, p = 0.029) and in 11.8% of patients with asymptomatic prostatitis (Chi-square test, p = 0.023). An elevated level of leukocytes in the ejaculate was more often observed in Group 2, which is largely due to the inability to routinely detect a non-inflammatory variant of asymptomatic prostatitis.

The concentrations of GABA, serotonin and VEGF differed significantly in serum and ejaculate (Table 4). In all groups, the concentration of serotonin was significantly ($p < 0.05$) higher in the blood serum, and the concentration of GABA and VEGF was significantly ($p < 0.05$) higher in the ejaculate. No differences were found between the groups.

Table 3. *Structure of ultrasonographic signs of prostate calcification and ejaculate leukocytosis in patients with CP/CPPS and asymptomatic prostatitis*

Indicators	Group 1	Group 2	p
Twinkling '+'	14 (41.2%)	4 (11.8%)	<0.05†
Twinkling '-'	20 (58.8%)	30 (88.2%)	
Echo-dense inclusions '+'	23 (67.7%)	12 (35.3%)	0.008♦
Echo-dense inclusions '-'	11 (32.3%)	22 (64.7%)	
Leukocytes in ejaculate ≥10 per field of view	14 (41.2%)	34 (100%)	<0.05†
Leukocytes in ejaculate <10 per field of view	20 (58.9%)	0	

The significance of the difference between groups was determined using the Chi-square test – ♦; Fisher's exact test – †.

Table 4. *Laboratory and clinical parameters of study participants*

Indicators	Group 1	Group 2	Group 3	Intergroup difference, p
	Me (Q25–Q75)	Me (Q25–Q75)	Me (Q25–Q75)	
GABA, serum, ng/ml	284.3* (222.8–415.3)	292.0* (184.5–402.9)	267.3* (161.6–375.6)	0.846 (Kruskal Wallis test)
GABA ejaculate, ng/ml	2361.7 (1619.0–3922.6)	2415.7 (1811.9–3062.0)	2995.6 (1907.6–4160.1)	0.430 (Kruskal Wallis test)
VEGF serum, pg/ml	178.7* (108.1–238.9)	173.9* (110.6–196.5)	151.7* (87.4–207.0)	0.307 (Kruskal Wallis test)
VEGF ejaculate, pg/ml	1745.8 (1669.0–1783.7)	1757.5 (1736.3–1792.9)	1749.2 (1715.4–1784.8)	0.320 (Kruskal Wallis test)
Serotonin serum, ng/ml	170.8* (98.3–222.1)	154.6* (123.9–251.2)	170.5* (137.6–225.7)	0.846 (Kruskal Wallis test)
Serotonin ejaculate, ng/ml	24.6 (18.8–33.0)	23.8 (17.0–29.8)	19.43 (15.8–28.4)	0.146 (Kruskal Wallis test)
IL-1β serum, pg/ml	4.9* (3.6–6.4)	5.4* (3.7–6.9)	4.0* (3.1–5.7)	0.125 (ANOVA)
IL-1β ejaculate, pg/ml	184† (162.9–204.7)	102.8 (83.6–122.0)	55.8 (32.2–77.5)	<0.001 (ANOVA)
IL-10 serum, pg/ml	4.5* (3.2–6.7)	4.8* (3.5–6.0)	4.1* (2.8–5.6)	0.244 (ANOVA)
IL-10 ejaculate, pg/ml	72.8 (26.1–87.9)	60.3 (44.7–81.8)	183.4 (124.7–215.0)	<0.001 (Kruskal Wallis test)
NIH-CPSI, points	14.5 (9.0–17.0)	1.0 (1.0–2.0)	1.0 (1.0–2.0)	<0.001 (Kruskal Wallis test)
PHQ-9, points	9.0 (6.75–12.0)	2.0 (1.0–2.0)	1.0 (1.0–2.0)	<0.001 (Kruskal Wallis test)

† – the indicator is significantly different from those in other groups (Dunkan test); * – the indicator is significantly different from that in the ejaculate ($p < 0.05$)
GABA – gamma-aminobutyrate; VEGF – vascular endothelial growth factor; IL – interleukin. NIH-CPSI – National Institutes of Health-Chronic Prostatitis Symptom Index; PHQ-9 – Patient Health Questionnaire-9; Me – median

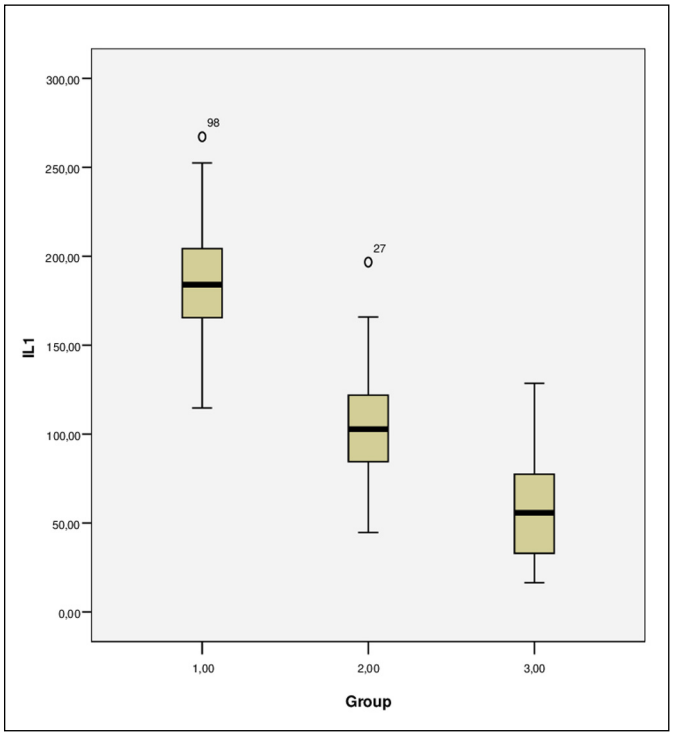

Figure 1. *Concentration of interleukin 1ß in the ejaculate of study participants (pg/ml).*

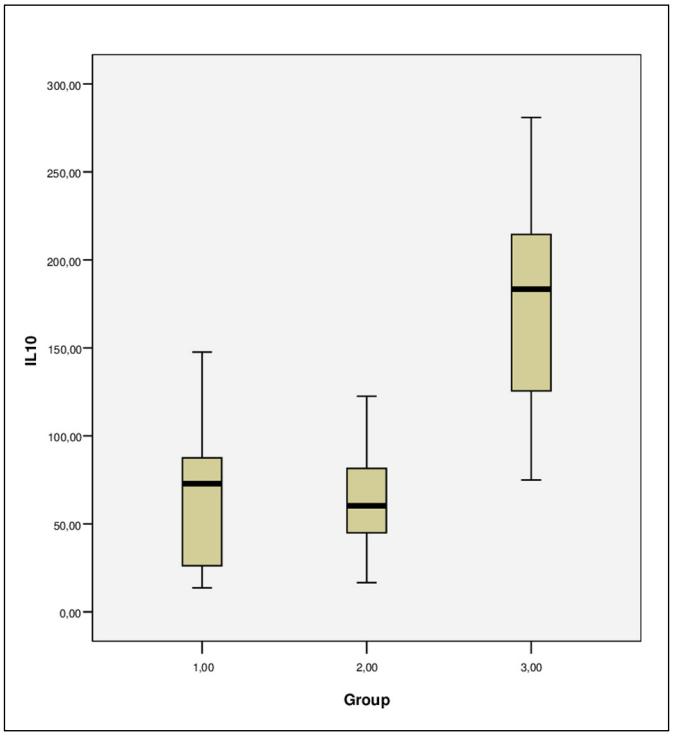

Figure 2. *Concentrations of interleukin 10 in the ejaculate of study participants (pg/ml).*

The concentrations of pro-inflammatory IL-1β (ANOVA, p <0.001) and anti-inflammatory IL-10 (Kruskal Wallis test, p <0.001) in the ejaculate of study participants differed significantly among the groups (Figures. 1, 2). The content of IL-1β in the ejaculate of patients in Group 1 was significantly higher than in Groups 2 and 3 (Duncan test). A significant difference in their concentrations was found in study participants (Group 1+2+3) with and without signs of prostate calcification (Table 5) (Kolmogorov-Smirnov, p <0.05). The difference in interleukin levels in the ejaculate of study participants with different ultrasound signs of prostate calcification was not significant. No significant difference in the concentrations of IL-1β and IL-10 in blood serum between groups 1, 2 and 3 (ANOVA, p = 0.125, p = 0.244, respectively) was also found.

The results of the survey of patients according to the NIH-CPSI and PHQ-9 scales differed significantly depending on the presence of ultrasonographic signs of prostate calcifications. However, there was no significant difference in the activity of symptoms of prostatitis and depression depending on the degree of calcification.

Correlations have broadly covered the obtained data. The frequency of exacerbation of symptoms of prostatitis (Table 6) in Group 1 significantly correlated with the presence of echo-dense inclusions and twinkling artifact on ultrasonography of the prostate (Spearman's r = 0.580, p <0.001; r = 0.745, p <0.001, respectively). At the same time, there was no relationship between the frequency of relapses and age, pyospermia, prostate volume.

Clinical symptoms of prostatitis directly correlated with the presence of dense inclusions without echo-shadow in the prostate tissue (Spearman's r = 0.531; p <0.001), with twinkling artifact (Spearman's r = 0.481; p <0.001), as well as with the symptoms of depression (Spearman's r = 0.703; p <0.001). The depression also directly correlated with the presence of inclusions in the prostate tissue (Spearman's r = 0.452; p <0.001) and the presence of a twinkling artifact (Spearman's r = 0.437; p <0.001). In turn, a direct correlation was also found between the presence of dense inclusions and the presence of a twinkling artifact in the prostate tissue (Spearman's r = 0.573; p <0.001). The detection of twinkling artifact, as opposed to echo-dense inclusions, was associated with the age of study participants (Spearman's r = 0.296; p = 0.003).

Prostate volume and maximum flow rate of urine of study participants corresponded to normal values or were clinically insignificantly different from the norm. At the same time, there was no significant intergroup difference in prostate size indicators (Table 7, p = 0.054, Kruskal Wallis Test), and all

groups differed significantly in maximum urine flow rate (Group 1 <Group 2 <Group 3; p <0.01 ANOVA, Duncan test). In Groups 1 there were no significant correlations of prostate volume and maximum urination rate with the presence of echo-dense inclusions (Spearman's r = - 0.160; p = 0.365; r = 0.115, p = 0. 516, respectively) and a twinkling artifact (Spearman's r = - 0.098; p = 0. 583; r = 0. 186, p = 0. 293, respectively). In Group 2, there were also no significant correlations of prostate volume and maximum urination rate with the presence of echo-dense inclusions (Spearman's r = 0.003; p = 0.986; r = 0.012, p = 0.945, respectively) and twinkling artifact (Spearman's r = - 0.107; p = 0.547; r = 0.084, p = 0.638, respectively). The presence of echo-dense inclusions, in contrast to the twinkling artifact, was associated with an increased level of leukocytes in the ejaculate (Spearman's r = 0.196; p = 0.048).

The content of IL-1β in the ejaculate of the study participants was significantly inversely correlated with the content of IL-10 (Spearman's r = -0.475; p <0.001) and was associated with prostate volume (Spearman's r = 0.349; p <0.001). Also, interleukins 1β and 10 were associated with the presence of echo-dense inclusions (Spearman's r = 0.469; p <0.001; r = -0.433; p <0.001, respectively) and twinkling artifact (Spearman's r = 0.382; p <0.001; r = -0.372; p <0.001, respectively), the presence of an increased level of ejaculate leukocytes (Spearman's r = 0.232; p <0.001; r = -0.445; p <0.001, respectively). There was also a correlation between the content of interleukins 1β and 10 in the ejaculate with the intensity of symptoms of prostatitis (Spearman's r = 0.681; r = -0.344, p <0.001, respectively) and symptoms of depression (Spearman's r = 0.688; r = -0.324, p <0.01, respectively).

No relationship was found between the concentrations of regulatory molecules GABA, serotonin, VEGF in blood serum and ejaculate and scale assessments of the clinical symptoms of the disease. At the same time, the level of VEGF in the blood serum was associated with the level of serotonin in the ejaculate (Spearman's r = 0.227; p = 0.022), and the level of GABA in the ejaculate was associated with the level of leukocytes in it (Spearman's r = -0.243; p = 0.014).

Table 5. *Distribution of laboratory and clinical parameters depending on the ultrasound structure of the prostate*

Indicators	Twinkling artefact '+'	Twinkling artefact '-'	Echo-dense inclusions '+'	Echo-dense inclusions '-'
	Me (Q25–Q75)	Me (Q25–Q75)	Me (Q25–Q75)	Me (Q25–Q75)
GABA serum, ng/ml	375.0 (252.0–461.3)	265.3 (169.6–367.5)	262.3 (163.9–385.0)	292.0 (183.0–449.0)
GABA ejaculate, ng/ml	3009.5 (1814.0–4457.1)	2457.0 (1723.8–3742.8)	2288.7 (1661.3–4017.6)	2487.5 (1901.7–3826.6)
VEGF serum, pg/ml	175.5 (104.7–255.1)	170.2 (102.8–209.0)	177.0 (120.5–232.5)	169.5 (89.2–210.4)
VEGF ejaculate, pg/ml	1740.6 (1652.0–1780.3)	1752.0 (1721.0–1793.0)	1745.8 (1667.8–1780.7)	1753.0 (1718.4–1795.2)
Serotonin serum, ng/ml	141.0 (105.0–209.0)	171.7 (125.0–245.9)	141.8 (108.5–219.7)	173.5 (127.3–235.2)
Serotonin ejaculate, ng/ml	20.1 (17.0–42.5)	23.6 (17.1–29.2)	24.0 (14.5–30.2)	22.4 (17.0–30.0)
IL-1β serum, pg/ml	4.5 (3.2–6.0)	4.9 (3.4–6.6)	5.3 (3.8–6.7)	4.7 (3.3–6.4)
IL-1β ejaculate, pg/ml	177.7 (119.2–197.9)	90.3* (61.7–134.8)	165.7 (110.9–194.2)	81.0* (53.4–122.1)
IL-10 serum, pg/ml	4.2 (2.3–6.6)	4.5 (3.3–5.9)	4.8 (3.0–6.9)	4.3 (3.3–5.8)
IL-10 ejaculate, pg/ml	27.8 (25.4–78.7)	88.6* (63.3–149.4)	56.6 (25.8–86.8)	94.2* (67.9–190.2)
NIH-CPSI, points	16.0 (7.3–18.3)	1.5* (1.0–2.0)	13.5 (2.0–17.0)	1* (1.0–2.0)
PHQ-9, points	4.5 (1.0–10.0)	1.0* (1.0–2.0)	7 (2.0–11.8)	2.0* (1.0–3.0)

* – the difference in indicators in the presence and absence of an ultrasound sign of prostate calcification is significant (Kolmogorov-Smirnov, p <0.05)
GABA – gamma-aminobutyrate.; VEGF – vascular endothelial growth factor; IL – interleukin; NIH-CPSI – National Institutes of Health-Chronic Prostatitis Symptom Index; PHQ-9 – Patient Health Questionnaire-9; Me – median

Table 6. *Relapse rate in patients with CP/CPPS (Group 1)*

Frequency	Group 1
Once a year or less	5 (14.7%)
2 times a year	13 (38.2%)
3 times a year	7 (20.6%)
Persistent symptoms	9 (26.5%)

Table 7. *Prostate volume and maximum flow rate of urine of study participants*

Indicators	Group 1 M ±SD	Group 2 M ±SD	Group 3 M ±SD	Intergroup difference, p
Prostate volume, ml	20.7 ±3.7	19.9 ±3.6	18.5 ±3.1	0.054 (Kruskal Wallis Test)
Maximum flow rate of urine, ml/sec	23.6 ±7.0	27.4 ±7.0	32.2 ±6.5	<0.01 (ANOVA) Group1 <Group2 <Group3 (Duncan test)

DISCUSSION

The prevalence of prostate calcifications demonstrated in this study during transabdominal ultrasonography in the form of echo-dense inclusions corresponded to the data reported by other authors. Specifically, in CP/CPPS, echo-dense inclusions with a diameter of 3 mm or more were revealed by ultrasonography in 47–48.9% of cases (transabdominally and transrectally, respectively) [18, 19].

Studies by other authors have shown a relationship between the severity of prostate tissue calcification and the age of patients with CP/CPPS [20]. According to our data, the presence of ultrasound signs in the prostate tissue and pyospermia in patients with CP/CPPS and asymptomatic prostatitis was also associated with age. The detection of a twinkling artifact, in contrast to echo-dense inclusions, significantly correlated with the age of the study participants.

Notably, the relationship between the signs of calcification of the prostate tissue and the severity of the symptoms of the disease was both revealed by some researchers [21, 22, 23] and denied by others, indicating the leading role of other factors [24]. Small stones were considered as a non-pathological ultrasonographic finding. At the same time, coarser prostate stones may be associated with inflammation and require treatment [25].

In our study, the association between symptoms of prostatitis and comorbid depression with prostate calcifications, both in the form of echo-dense inclusions and as a twinkling artifact, appeared obvious. Echo-dense inclusions and twinkling artifact were significantly more common in CP/CPPS than in asymptomatic prostatitis (Table 3). At the same time, a significant relationship between prostate calcification and the frequency of relapses of prostatitis and the absence of a relationship between the degree of calcification and the activity of symptoms of prostatitis and depression in CP/CPPS are indicative. In other words, it might be plausible that deep calcification of the prostate contributes more to the development of an exacerbation of the disease than corresponds to the severity of its manifestations.

We did not find a relationship between calcifications and the volume of the prostate gland and the maximum flow rate of urine. A possible explanation for this discrepancy is the limitation in enrolling patients under 45 years of age, which excludes older men with benign prostatic hyperplasia. In addition, there were no cases with severe bladder emptying disorders in this study.

The previously shown predominance of the inflammatory form of CP/CPPS in prostate calcification [23] was not confirmed in our study. A non-inflammatory form of CP/CPPS was more likely observed in our study. Additionally, no significant association between elevated white blood cells with the severity of disease symptoms was also found here. At the same time, an increased level of leukocytes in the ejaculate was associated with the detection of echo-dense inclusions in the prostate, in contrast to the twinkling artifact.

One of the dramatic features of chronic prostatitis, depleting the nervous system, is the frequent relapses of the disease. So, according to a survey by Nickel at al. about two-thirds of men with symptoms of prostatitis reported persistence or recurrence of symptoms within 1 year [26]. In our study, in Group 1 patients, relapses were noted 2 times a year and more often in 29 out of 34. The association of the presence of echo-dense inclusions and twinkling artifact with an increase in the frequency of relapses of CP/CPPS revealed in this study indicates a more persistent course of the disease in the presence of prostate stones.

The levels of interleukins 1β and 10 in the ejaculate, as well as their direct relationship with the volume of the prostate, reflect the state of inflammation and its own immunological mechanisms [27]. It seems logical the increase in the concentration of pro-inflammatory IL-1β, and the relative decrease in the concentration of anti-inflammatory IL-10 in patients with inflammation of the prostate, both in the presence of symptoms and in the asymptomatic form. Similar results have also been observed in the past [28]. Other authors also showed increased levels of IL-1β in the urine in patients with CP/CPPS; however, this was accompanied by an increase in the

concentration of IL-10 rather than a decrease [29]. A significant increase in the ejaculate concentration of IL-1β was shown in the presence of prostate stones in LUTS patients [30]. Along with this, the revealed absence of a significant intergroup difference in the concentration of IL-1β and IL-10 in the blood serum, in our opinion, indicates a predominantly local inflammatory process in the prostate gland without systemic involvement.

It is known that prostate neuroendocrine cells secrete serotonin and vascular endothelial growth factor, and also express serotonin receptors [31, 32, 33, 34, 35]. A regulatory effect of VEGF, GABA, and serotonin on sperm function is plausible; in particular, a dose-dependent effect on fertility parameters has been reported [36, 37, 38].

Here, we showed the presence of VEGF, GABA and serotonin in the blood serum and ejaculate, but did not find a significant difference in their concentrations in patients with CP/CPPS, asymptomatic prostatitis and in healthy men. Also, there was no significant relationship between their concentrations and the presence of signs of prostate calcification. However, it cannot be excluded that serum and ejaculate concentrations of these mediators may not be reflective of the structural and/or functional difference in the state of the receptor apparatus to VEGF, GABA and serotonin within the prostate gland tissue per se.

To summarize, the results of our study and those reported by other authors may reflect the balance and the relationship between the pathogenic mechanisms of chronic pain and the actual changes in the prostate gland. In other words, central and peripheral sensitization of the nervous system developed to varying degrees, reactive muscular-tonic disorders in combination with inflammatory and degenerative processes in the prostate gland create a unique clinical picture of a particular patient. In the early stages of the development of the disease, the inflammatory process in the prostate gland prevails, while in the final stages, the processes characteristic of chronic pain predominates. Therefore, if we assume that in the early stage there are echo-dense inclusions, and at later stage- a twinkling artifact, then at the early stage we observe a connection between prostate calcification and an inflammatory reaction (pyospermia), and as the pathology progresses, this connection disappears.

In addition, a greater strength of the correlation between interleukins 1β and 10, as well as the intensity of symptoms of prostatitis and depression with the presence of echo-dense inclusions, compared with cases with a twinkling artifact, should be noted. This does not allow to highlight the leading role of individual pathogenic factors, including regulatory molecules, but underscores the need for a comprehensive/multimodal approach to the patient.

The limitations of the study are:

1) a small number of participants, which reduces the confidence in the extrapolation of the results;
2) age restrictions of participants (18–45 years); this approach leaves older patients out of the focus of the study , but it provides data on patients with CP/CPPS and asymptomatic prostatitis;
3) the selection of patients with CP/CPPS and asymptomatic prostatitis without the presence of sexually transmitted infections; this takes cases of chronic prostatitis of infectious origin out of the scope of our study;
4) due to the lack of documentary evidence of the first episode of chronic prostatitis, we were unable to establish the number of years when patients were diagnosed with chronic prostatitis.
5) the prospective study was conducted without preliminary sample calculation. Determination using Post-hoc Statistical Power Calculator for a Student t-Test showed that with a moderate effect (0.5); p-value: 0.05; groups size (34) observed power (one-tailed hypothesis): 0.65.

CONCLUSIONS

Ultrasound evidence of prostate calcifications, and especially the twinkling artifact, is associated with more severe symptoms of prostatitis and depression, as well as with frequent exacerbations in patients with CP/CPPS. A significantly higher concentration of pro-inflammatory IL-1β and a lower concentration of anti-inflammatory IL-10 cytokines in the ejaculate accompanies prostate calcification in both CP/CPPS and asymptomatic prostatitis. The level of leukocytes in the ejaculate and the concentration of VEGF, GABA and serotonin in the blood and ejaculate in patients with CP/CPPS and asymptomatic prostatitis are not associated with clinical manifestations. Twinkling artifact potentially could serve as a valuable tool for evaluating the condition of patients with CP/CPPS and prostate calcifications.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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