Clinical and Biochemical Influence of Prostatic Stones

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\section*{Key Words}
Cytokines · Inflammation · Lower urinary tract symptoms · Prostate · Prostate stones

\section*{Abstract}
\textbf{Introduction:} The study aimed to explore clinical influence of prostatic stones on lower urinary tract symptoms (LUTS), seminal plasma cytokines, and serum biomarkers. \textbf{Materials and Methods:} A total of 70 men aged ≤50 years with LUTS divided into 2 groups: group with stones (GSt) and group without prostatic stones (GNoSt). All subjects completed the International Prostate Symptom Score (IPSS) questionnaire and National Institutes of Health Chronic Prostatitis Symptom Index (NIH-CPSI) scoring questionnaire. Pre- and post-prostate massage test and uroflowmetry were performed. The serum concentration of total prostate specific antigen (PSA), free PSA, and free/total PSA (f/t PSA) ratio, seminal concentration of cytokines interleukin (IL)-1$\beta$, IL-6, IL-8, IL-10, IL-12p70, and tumor necrosis factor-alpha were measured. \textbf{Results:} GSt subjects had significantly more severe symptoms based on IPSS answers ($p = 0.0289$). All domains in NIH-CPSI scores were significantly higher in the GSt group: pain ($p = 0.001$), urinary symptoms ($p = 0.023$), quality of life ($p = 0.008$), and with overall ($p = 0.003$). GSt subjects also had significantly lower maximum urinary flow (Qmax; $p = 0.011$), lower f/t PSA ratio ($p = 0.048$), and higher concentration of IL-1$\beta$ ($p = 0.011$) and IL-8 ($p = 0.001$). \textbf{Conclusions:} Prostatic stones may influence the severity of LUTS and the symptoms of chronic prostatitis. They might reduce Qmax rate and lead to reduction of the f/t PSA ratio and produce more severe inflammation caused increased seminal concentration of IL-1$\beta$ and IL-8.

\section*{Introduction}

The information on etiology and clinical significance of prostatic stones are scarce [1]. In addition, the relationship between prostatic stones and benign or malignant conditions of the prostate gland is unknown. Furthermore, the role of prostatic stones in elicitation of clinical symptoms is not fully explained, particularly in men with high prevalence of prostatic stones, but with no pronounced clinical symptoms, for example, nonspecific lower urinary tract symptoms (LUTS) and chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) related
symptoms [2–5]. It has been assumed that prostatic stones are mostly a consequence of aging in older men, and were associated with inflammation in younger men [1, 6, 7].

In the available literature, we found only one study on correlation of prostate stones and total serum prostatic specific antigen (tPSA) levels, which showed no influence of prostatic stones on tPSA concentration [8]. No study investigated a correlation between prostatic stones and free serum PSA (fPSA) level and/or free/total PSA (f/t PSA) ratio.

Recently published studies showed the importance of immune response in the pathogenesis of chronic prostatitis, while the body of evidence emphasizes the importance of cytokines as inflammatory mediators in CP/CPPS [9, 10]. These studies showed significantly increased levels of cytokines in seminal plasma of men with CP/CPPS [10–16].

The primary goal of this study was to examine the clinical effects of prostatic stones on the severity of LUTS and chronic prostatitis. The second goal was to examine their influence on the maximum urinary flow (Qmax), the serum concentration of tPSA and fPSA, as well as f/t PSA ratio, seminal concentration of interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, and tumor necrosis factor-alpha (TNF-α).

Material and Methods

This prospective observational study was approved by the hospital (Ethical Committee of the General Hospital Zadar, Croatia, No. 01-2095-2/09). From November 2009 to September 2012, 70 men (age 21–49 years) with prostate size up to 40 mL in volume with LUTS were enrolled and consented for the study. The presence of symptoms of LUTS was estimated by the Croatian translation of the internationally recognized questionnaire for the assessment of patients with symptoms of benign prostatic hyperplasia (BPH; International Prostate Symptom Score [IPSS]).

Exclusion criteria were acute infection of the urinary tract, history of lower urinary tract surgery or irradiation, history or evidence of urolological tumor, pyuria, bacteriuria, serum PSA >4 ng/mL, and taking medications for BPH.

Based on the transrectal ultrasonography (TRUS) examination results, the subjects were divided into 2 groups. The first group (GST; n = 35) included subjects with evidence of prostate stones on TRUS and the second group (GNoSt; n = 35) included subjects with no evidence of prostate stones on TRUS. All TRUS were performed by the same urologist with 6.5 MHz E 6509 endorectal transducer (Philips Medical System, Andover, MA, USA). The prostate volume and prostatic stones volume were measured by TRUS using the formula for the ellipsoid volume (π/6 × height × width × length of prostate). The volume of multiple stones was calculated by adding volumes of all stones. Hyperechoic areas without shadowing and tiny stippled calcifications (<3 mm in largest diameter) were not considered as prostatic stones for the purpose of this analysis.

In addition to Croatian versions of the IPSS questionnaire, all patients also completed the National Institutes of Health (NIH) Chronic Prostatitis Symptom Index (NIH-CPSI) score. The NIH-CPSI consists of 9 questions, exploring the 3 major domains of prostatitis, that is, pain (scored 0–21), voiding disturbances (scored 0–10), and impact on quality of life (scored 0–12); the total NIH-CPSI score is 0–43.

Demographic data, history, and physical examination including digital rectal examination (DRE) were recorded for all subjects. All patients had seminal plasma culture and uroradiology. Blood samples were taken before DRE and TRUS. Finally, all the patients’ blood sample tests and values for complete blood counts and concentrations of tPSA (ng/mL), fPSA (ng/mL), and f/t PSA ratio (%) were used for statistical analysis.

Prostatitis syndromes were classified according to the definition and classification system of the National Institute of Diabetes, Digestive, and Kidney Diseases of the US NIH [17]. The pre- and post-massage tests were used for classification of CP/CPPS; patient were given a midstream pre-massage urine specimen (VB2) and a urine specimen (initial 10 mL) after prostatic massage (VB3) [18]. Based on the standard microbiological culture and microscopic analysis of VB2 and VB3, the patients were categorized as having NIH category II chronic bacterial prostatitis (ureapathogenic bacteria were absent in VB2 but cultured in VB3), category IIIA (inflammatory) CP/CPPS (ureapathogenic bacteria were absent in VB2 and VB3), leukocyte count >10 high power field in VB3), and category IIIB (non-inflammatory) CP/CPPS (ureapathogenic bacteria were absent in VB2 and VB3, leukocyte count <10 high power field in VB3).

Fresh semen was obtained by masturbation. All patients were asked to be abstinent for 3 days before providing the sample. Seminal volume was recorded and after the centrifugation (600 g, 10 min) the samples of seminal plasma were stored at −20 °C until the analysis. The concentration of IL-1β, IL-6, IL-8, IL-10, IL-12p70, and TNF-α in seminal plasma were quantified using FlowCytomix Multiplex (Bender MedSystems GmbH, Vienna, Austria) according to manufacturer’s protocol at the flow cytometer FC500 (Beckman Coulter).

The results were expressed as mean ± SE, when normally distributed, and as median (quartiles) for parameters with non-normal distribution. Differences between 2 groups were analyzed by Mann–Whitney U test. Spearman rank correlation analysis was used for assessing correlation between groups. The area under the curve (AUC) of the receiver operating characteristic (ROC) analysis for IL-8 and IL-1β was calculated and used as an index of diagnostic accuracy for CP/CPPS categories. Statistical analysis was performed on SPPS 12.0 for Windows (SPSS Inc., Chicago, IL, USA). p < 0.05 was considered statistically significant.

Results

Out of 70 patients, 64 patients were classified as follows: 3 (4.7%) were considered to be classified as category II chronic bacterial prostatitis, 11 (17.2%) were considered to
have category IIIA (inflammatory) CP/CPPS, and 50 (78.1%) were considered to have category IIIB (non-inflammatory) CP/CPPS. Three patients from the GNoSt group and 3 subjects from the GSt group had a duration of clinical symptoms for less than 3 months and were not classified.

There was no difference in age and prostate volume between groups (Table 1). The concentration of IL-12p70, IL-10, and IL-6 was too low because it was not measurable in most of our examinees. There were significant differences between GNoSt and GSt groups in IL-1β and IL-8 seminal plasma concentrations ($p < 0.05$; Table 1).

IL-1β significantly positively correlated with the concentration of IL-8, coefficient of correlation was 0.479 ($p < 0.05$), as well as with coefficient of TNF-α with coefficient of Spearman rank correlation ($r$) 0.266 ($p < 0.05$). Coefficient of correlation between concentrations of IL-8 and other examined variables indicated that IL-8 concentration significantly positively correlated with age, coefficient of correlation ($r$) was 0.317 ($p < 0.05$), as well as with the concentration of IL-1β, the coefficient of correlation ($r$) was 0.479 ($p < 0.05$) and with concentration of TNF-α with coefficient of correlation ($r$) 0.278 ($p < 0.05$) and negatively correlated with f/t PSA ratio, coefficient of correlation ($r$) was 0.358 ($p < 0.05$; Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>No stones (GnoSt)</th>
<th>Stones (GST)</th>
<th>$p$ value (Mann–Whitney test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>35</td>
<td>35</td>
<td>0.178</td>
</tr>
<tr>
<td>Age, years</td>
<td>41 (34–46)</td>
<td>44 (36–48)</td>
<td>0.074</td>
</tr>
<tr>
<td>Prostate volume, mL</td>
<td>19 (16–27)</td>
<td>24 (18–30)</td>
<td>0.029</td>
</tr>
<tr>
<td>IPSS</td>
<td>8 (4–13)</td>
<td>13 (5–22)</td>
<td>0.003</td>
</tr>
<tr>
<td>NIH-CPSI total score</td>
<td>16 (11–21)</td>
<td>21 (16–28)</td>
<td>0.001</td>
</tr>
<tr>
<td>NIH-CPSI pain domain</td>
<td>7 (2–9)</td>
<td>9 (7–12)</td>
<td>0.023</td>
</tr>
<tr>
<td>NIH-CPSI void domain</td>
<td>3 (1–5)</td>
<td>4 (2–8)</td>
<td>0.008</td>
</tr>
<tr>
<td>NIH-CPSI quality of life</td>
<td>6 (3–8)</td>
<td>8 (5–10)</td>
<td>0.011</td>
</tr>
<tr>
<td>Qmax, mL/s</td>
<td>20 (14–24)</td>
<td>16 (12–20)</td>
<td>0.796</td>
</tr>
<tr>
<td>PSA, ng/mL</td>
<td>0.87 (0.53–1.07)</td>
<td>0.88 (0.5–1.16)</td>
<td>0.489</td>
</tr>
<tr>
<td>Free PSA, ng/mL</td>
<td>0.3 (0.20–0.50)</td>
<td>0.3 (0.20–0.50)</td>
<td>0.065</td>
</tr>
<tr>
<td>Free/total PSA ratio, %</td>
<td>39 (33–53)</td>
<td>33 (25–45)</td>
<td>0.011</td>
</tr>
<tr>
<td>IL-8, pg/mL</td>
<td>735 (506–1,236)</td>
<td>1,088 (842–1,869)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-1β, pg/mL</td>
<td>0 (0–13)</td>
<td>17 (0–44)</td>
<td>0.011</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>10 (0–12)</td>
<td>10 (0–15)</td>
<td>0.359</td>
</tr>
</tbody>
</table>

Table 2. Correlation between cytokines and variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation coefficient of IL-1β</th>
<th>Correlation coefficient of IL-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.022</td>
<td>0.317*</td>
</tr>
<tr>
<td>Prostate volume</td>
<td>0.103</td>
<td>0.058</td>
</tr>
<tr>
<td>Total PSA</td>
<td>0.220</td>
<td>0.132</td>
</tr>
<tr>
<td>Free PSA</td>
<td>−0.132</td>
<td>−0.127</td>
</tr>
<tr>
<td>Free/total PSA ratio</td>
<td>−0.475*</td>
<td>−0.358*</td>
</tr>
<tr>
<td>IPSS</td>
<td>0.101</td>
<td>0.095</td>
</tr>
<tr>
<td>NIH-CPSI total score</td>
<td>0.004</td>
<td>0.063</td>
</tr>
<tr>
<td>NIH-CPSI pain domain</td>
<td>0.012</td>
<td>−0.012</td>
</tr>
<tr>
<td>NIH-CPSI void domain</td>
<td>0.162</td>
<td>0.151</td>
</tr>
<tr>
<td>NIH-CPSI quality of life</td>
<td>−0.007</td>
<td>0.063</td>
</tr>
<tr>
<td>Qmax</td>
<td>−0.173</td>
<td>−0.106</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.479*</td>
<td>0.479*</td>
</tr>
<tr>
<td>IL-8</td>
<td>−</td>
<td>0.479*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.363*</td>
<td>0.278*</td>
</tr>
</tbody>
</table>

* $p < 0.05$. Spearman rank correlation.

NIH-CPSI, National Institutes of Health Chronic Prostatitis Symptom Score; IPSS, International Prostate Symptom Score; Qmax, maximum flow rate; PSA, prostate-specific antigen; IL-8, interleukin-8; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor alpha.
ROC analysis showed the best diagnostic efficacy of IL-8 to distinguish GSt and GNoSt groups, AUC = 0.740, 95% CI was from 0.622 to 0.838 and \( p = 0.001 \); at the limit concentration of 798 pg/mL, the diagnostic sensitivity was 83% and specificity 63% (Fig. 1).

ROC analysis showed a lower diagnostic accuracy of IL-1β to distinguish between 2 groups, GSt and GNoSt, AUC = 0.662; 95% CI from 0.539 to 0.771 with \( p = 0.007 \); at concentration limit of 13 pg/mL, the diagnostic sensitivity was 54% and specificity 77% (Fig. 2).

ROC analysis also showed good diagnostic efficacy of IL-8 to distinguish category IIIA CP/CPPS and category IIIB CP/CPPS, AUC = 0.805; 95% CI from 0.684 to 0.896; \( p = 0.001 \); at the limit concentration of 1,379 pg/mL, the diagnostic sensitivity was 78% and specificity 82% (Fig. 3).

ROC analysis showed the best diagnostic accuracy of IL-1β to distinguish category IIIA CP/CPPS and category IIIB CP/CPPS, AUC = 0.889; 95% CI from 0.782 to 0.955; \( p = 0.001 \); at concentration limit of 20 pg/mL, the diagnostic sensitivity was 84%, and specificity 91% (Fig. 4).

**Discussion**

Prostatic stones are frequently encountered in urologic practice; however, their clinical significance remains obscure. Kim et al. [4] demonstrated that presence of large prostatic stones (large masses of multiple echoes, much coarser) was significantly associated with moderate LUTS and the likelihood of an IPSS \( \geq 8 \) was related to the large stones group of patients with a 1.784-fold lower risk for IPSS \( \geq 8 \) in patients with no or small prostatic stones. According to the study by Geramoutsos et al. [6], patients with symptoms (LUTS and CP/CPPS-related symptoms) have fold higher probability of having large stones compared with asymptomatic patients. In contrast, Park et al. [3] did not show any significant correlation between IPSS score and the presence of prostatic stones in patients who have LUTS. In this study, the mean age of the patients with prostatic stones was over 70 years, and overlapping symptoms by concurrent BPH was an important confounding factor. Our study demonstrated that subjects with prostatic stones had significantly more symptoms based on the IPSS test, and significantly worse scores in...
all category of the NIH-CPSI scores: pain, urinary symp-
toms, and quality of life.

We also found that Qmax was significantly lower in
patients with prostatic stones when compared with pa-
tients without prostatic stones. The effect of prostatic
stones on LUTS and Qmax are unclear, but several expla-
nations were proposed. Park et al. [3] proposed that pro-
static calcification probably affects the relaxation of the
prostatic urethrae, and interferes with urination. Some
studies suggested that prostatic stones induce prostatitis,
and they were frequently found in patients with CPPS [1,
2, 5]. Possible explanation could be that prostatitis prob-
ably causes LUTS and lower Qmax by contraction of the
smooth muscle of the prostate and bladder neck. Pro-
static stones might cause changes in prostatic blood flow.
Alteration of PBF or vascular resistance is associated with
LUTS [19].

Our study confirmed the results of the only pub-
lished study where correlation between PSA serum con-
centration and presence of prostatic stones were exam-
ined [8].

The presence or volume of prostatic calculi had no sig-
nificant effect on serum concentration of PSA. That result
suggested that the influence of prostatic calculi was not
relevant in men with elevated PSA [8].

In our study, we also reported that the f/t PSA ratio was
significantly lower in subjects with prostatic stones com-
pared to patients without prostatic stones. A possible ex-
planation could be that prostatic stones can lead to
flammation in which cytokines initiate proliferation and
destruction of glandular epithelium [9]. Our results sup-
port this theory because our subjects with prostatic stones
had significantly higher concentration of cytokines (IL-1β
and IL-8) in seminal plasma, compared to those without
prostate stones. In addition, we have shown that the con-
centration of TNF-α in seminal plasma significantly cor-
related with higher levels of serum tPSA and lower f/t PSA
ratio. Furthermore, IL-1β and IL-8 concentration signifi-
cantly correlated with lower f/t PSA ratio, found in this
study. Average tPSA level was 0.91 ng/mL in patients with
prostatic stones, and 0.90 ng/mL in patients without pro-
state stones (mean total age in all patients was 40 years); so
it is questionable whether significantly reduced f/t PSA ratio in our patients with prostatic stones might have clinical relevance.

White blood cells (WBCs) count in expressed prostate secretion has been considered for a long time as a marker of prostatitis. However, it does not appear to be optimal marker of inflammation in IIIB category of CP/CPPS and in asymptomatic inflammation. WBC count appears to offer little clinically useful information [20, 21]. Recently, cytokines as mediators in the pathogenesis of CP/CPPS have attracted more attention [9, 21, 22]. Previous studies reported that patients with CP/CPPS show higher levels of seminal plasma pro-inflammatory cytokines, such as IL-1β, IL-6, and TNFα [13–15] and chemokines such as IL-8 compared to controls [11, 12, 16].

To our knowledge, this study is the first one where cytokines in men with prostatic stones were measured. We showed that patients with prostatic stones had significantly higher concentration of IL-1β and IL-8 than those without prostatic stones, while there was no significant difference in the concentration of TNF-α between the groups. In this study, IL-1β and IL-8 concentrations can clearly discriminate between inflammatory (IIIA) and non-inflammatory subtypes (IIIB) of CP/CPPS, what is consistent with previously published data findings. We did not analyze concentrations of IL-12p70, IL-10, and IL-6 since they were not detectable in our samples. Among all cytokines, IL-8 appears to be the most reliable and predictive biomarker in chronic prostatic inflammatory conditions, potentially applicable in diagnosis, prognosis, and treatment due to its elevated concentration in seminal plasma in CP/CPPS patients, as well as its capacity to discriminate CP/CPPS category IIIA from category IIIB [12].

Based on our observational study, we could conclude that patients <50 years who have prostatic stones and LUTS also have greater inflammation compared to patients without prostatic stones. Shoskes et al. [1] suggested that prostatic calcification could contribute to obstruction of intraprostatic ducts and bacterial biofilm production with predominated chronic inflammation. In addition, some prostatic stones can cause atrophy of the glands in certain areas of the prostate and lead to chronic inflammation. On the other hand, some older studies showed no association between infection and/or inflammation with presence of the prostatic stones [23, 24]. This discrepancy may be due in part to new and more appropriate methods available nowadays that allow the detection of chronic inflammation and infection with greater precision. Recently published studies showed that acute phase proteins predominated in the structure of prostatic stones [25, 26]. In addition, bacterial biofilm was found in the prostatic stones and high frequency of bacterial imprints was found in these stones by electron microscopy scanning [27–29]. Results of the study by Kim et al. [4] support these findings, since they showed that prostatic stones could be influenced by antimicrobial therapy in patients with chronic bacterial prostatitis. Namely, after 4 weeks of antimicrobial therapy and follow-up, a greater percentage of organisms were continuously eradicated in patients without prostatic calculi compared to patients with prostatic calculi. Similarly, patients without prostatic calculi resulted in significantly higher symptoms improvement compared to those with prostatic calculi [30]. This opens a new field of research of chronic prostatitis and defines the essence of calcification as a bacteria and bacterial resistance, with consequent chronic inflammation in these patients. Limitation of this study is a lack of histological examination of the prostate in our patients, in order to demonstrate histological changes in men with prostatic stones. However, we considered that it could be unnecessary and not ethical. DREs were performed and PSA was below 4 ng/mL, so prostate biopsy was not justified for patients in our study. We did not determine metabolic syndrome in our patients, but Fu et al. [31] showed in their study that metabolic syndrome in patients with LUTS symptoms is associated with higher IPSS and lower Qmax.

Although the effects of prostatic stones on LUTS are probably different regarding types and locations of prostatic stones, we did not classify prostatic stones in that manner, which is also a limitation of this study.

Conclusions

Our study revealed a correlation between prostatic stones, severity of LUTS, and symptoms of chronic prostatitis. Furthermore, the stones reduced the urinary flow rate and lead to the reduction the f/t PSA ratio. Prostatic stones could also influence inflammation causing increased secretion of IL-1β and IL-8.

Disclosure Statement

There are no financial or commercial interests for any of the authors.
References


