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ORIGINAL PAPER

Role of urine glycosaminoglycan levels in the diagnosis and follow-up in men with lower urinary tract symptoms

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Summary ***Objective: The aim of this study was to investigate whether urinary glycosaminoglycans (GAG) levels reflect clinical status in men with lower urinary tract symptoms and if they could be used as a marker in management of overactive bladder (OAB).***
Methods: A total of 34 patients were recruited who were admitted with LUTS and diagnosed as having clinically bladder outlet obstruction (BOO) due to prostate enlargement. These newly diagnosed, never treated patients underwent routine investigation, consisting of history, physical examination, PSA, ultrasound, uroflowmetry, assessment of symptoms scored by both International Prostate Symptom Score (IPSS) and Marmara-Overactive Bladder Questionnaire (M-OBQ). The patients were divided into two groups as those with an initial M-OBQ score < 12 (group 1) and ≥ 13 (group 2). Alfa blocker was initiated in eligible patients. Further evaluations included prostate volume measurement, pre- and post-treatment urinary GAG levels, IPSS and M-QAOB values and maximum urine flow rate (Q_{max}).
Results: Before treatment, urinary GAG level was 21.5 mg/gCr (6.1-45.5) in Group 1, and 23.35 mg/gCr (15.6-32.6) in Group 2 (p =0.845). After the treatment, the GAG level in Group 1 and Group 2 were found to be 19.8 mg/gCr (7.4-70.5) and 18 (7.6-41.7), respectively (p = 0.511). No difference in GAG levels was found in subgroup analysis for patients with or without OAB.
Conclusions: In recent years, there have been many studies investigating the relationship between LUTS and urinary markers. However, in our prospective study, no relationship was found between pre- and post- treatment urinary GAG levels in patients with LUTS with or without OAB.

KEY WORDS: GAG; Bladder outlet obstruction; Overactive bladder; Biomarker.

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INTRODUCTION

Lower urinary tract symptoms (LUTS) are one of the most common conditions in the urology clinic, affecting at least one in four men after 40 years of age (1). Bladder dysfunction (detrusor overactivity or underactivity) and *bladder outlet obstruction* (BOO) are two main pathologies involved in the etiology of LUTS (2). LUTS is often associated with BOO caused by prostate enlargement in men. Moderate and severe LUTS are reported as 26% in men aged 40-49, while this rate doubles in the group aged 70 and older (3). The *International Prostate Symptom Score* (IPSS) from 8 to 19 indicates moderate LUTS (4), while a maximum flow (Q_{max}) of less than 15 ml/sec in uroflowmetry has 82% sensitivity for BOO (5). According to *European Association Urology* (EAU) Guidelines (6), alpha blockers are recommended as the first-line medical treatment in patients clinically diagnosed with BOO (7).
In recent years, many studies have been published on the relationship of urinary biomarkers with LUTS. Nerve growth factor and brain derived neurotrophic factor, which are among most studied markers, have been shown to be closely associated with neurogenic or non-neurogenic detrusor overactivity and significant improvements were observed after treatment (8-11). The relationship between urinary *glycosaminoglycan* (GAG) and *overactive bladder* (OAB) has been demonstrated and it has been reported that the values have decreased after treatment (12). Men with *prostate enlargement* (PE) and LUTS often have symptoms of overactive bladder. However, to our knowledge, there is no previous study in the literature examining the utility of urinary GAG levels in this patient group. The aim of this study was to investigate the relationship between urinary GAG levels and patients with LUTS.

MATERIALS AND METHODS

Patients who were seen in the outpatient clinic due to LUTS and diagnosed as clinically BOO due to PE were included in the study. Ethical approval was obtained from the local ethics committee (2018/53). The study was designed prospectively and submitted to *ClinicalTrials.gov* (Identifier: NCT03955484). Before starting an alpha-blocker medication, patients were enrolled to the study. All patients were evaluated under the guidance of EAU guide-

lines (6). Patients received routine investigation, consisting of medical history taking, physical examination including digital rectal examination, *prostate-specific antigen* (PSA), urinalysis and urine culture, ultrasound imaging, and uroflowmetric study, measurement of *post void residual urine* (PVR) assessment of symptoms scored by both *International Prostate Symptom Score* (IPSS) and *Marmara-Overactive Bladder Questionnaire* (M-OABQ) (13).

Male patients who applied to the urology outpatient clinic with LUTS, and had an IPSS of 8 and above, and a prostate volume greater than 40 ml and a maximum flow of less than 15 ml/sec, were diagnosed with clinically BOO due to prostate enlargement. Of these patients, those who did not receive any medical and/or surgical treatment for LUTS were found to be eligible for the study.

Patients with a history of medical and/or surgical treatment for LUTS, or with a diagnosis of urethral stenosis, prostate cancer, neurologic diseases, spinal cord trauma or an absolute indication for surgical treatment at first admission (macroscopic hematuria, bladder stones, urinary retention, upper urinary tract dilatation) were excluded from the study. Also, patients who required prostate biopsy according to rectal examination and PSA were not included the study. In the initial evaluation, those who were not suitable for alpha-blocker treatment and needed urodynamic examination were excluded from the study. Apart from the study group, 10 healthy adult males were selected as the controls for the assessment of baseline urinary GAG levels.

The study group were divided into two subgroups, according to M-OABQ score before the alpha-blocker medication: a score of 12 and less (group 1) and a score of 13 and higher (group 2). The M-OABQ is a tool to assess OAB symptoms. M-OAB questionnaire consists of eight questions and it has the highest sensitivity (97%) and specificity (94%) for the diagnosis of OAB when a cut off value of 12.5 for the total score is used (13). After one month of alpha blocker treatment, all patients underwent re-evaluated with uroflowmetry, PVR, IPSS, M-OABQ. Pre- and post-treatment urinary GAG values, prostate volumes, IPSS and M-OABQ values and Q_{max} were compared.

Biochemical analysis

Midstream morning urine samples were collected into sterile urine collection tubes and each urine sample was centrifuged at 5000 g for 10 minutes, the supernatant was aliquoted in 1.5-mL microcentrifuge tubes and stored at -800C until further analysis.

Total GAG levels in supernatants were determined using the *Blyscan Sulfated Glycosaminoglycan* assay (*Blyscan Assay, Biocolor Ltd., Northern Ireland, UK*) according to the manufacturer's instructions (14). The *Blyscan Assay* as a direct colorimetric method quantifying urinary glycosaminoglycan excretion exploits the specific binding of 1,9-dimethylmethylene blue that provides a specific label for the sulfated polysaccharide component of proteoglycans or the protein-free sulfated glycosaminoglycan chains (15). The detection limit of assay was 2.5 µg/mL. Urinary *creatinine* (Cr) concentration was determined on each sample using the kinetic Jaffe method in the AU5800 clinical chemistry system (*Beckman Coulter Inc., CA, USA*). The urinary concentrations of GAG were normalized to the concentration of urinary Cr and results were expressed as, milligram per gram of Cr.

Statistical analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences version 22 (*IBM SPSS Statistics for Windows, Chicago, IL, USA*). The normality of the distribution of the variables was evaluated using the Shapiro-Wilk test. As the distribution of continuous variables did not show a normal distribution, continuous data were presented with median, minimum and maximum. Comparison of independent and dependent groups were done with Mann-Whitney U test and Wilcoxon Signed Ranks Test, respectively. The p value < 0.05 was accepted as statistically significant.

Results

Thirty-four patients with a median age of 59.5 (42-74) years were included in the study. There were 22 patients in group 1 and 12 patients in group 2. The median age of the control group was 44.5 (44-52) years. The median age of group 1 was 60 (48-72) years, and of group 2 was 58 (54-74) (p = 0.845). Pre-treatment normalized urinary GAG level was found to be 20.8 (6.1-45.5) mg/gCr in study group and 17.8 (11.5-22.26) mg/gCr in control group (p = 0.183). The normalized urinary GAG level of the study group after the treatment was found to be 19.7 (7.53-70.5) mg/gCr and did not show a statistical difference compared to the pre-treatment level (p = 0.530). Initial normalized urinary GAG value of group 1 and 2 were as 21.5 (6.1-45.5) mg/gCr and 23.35 (15.6-32.6) mg/gCr, respectively (p = 0.845) (Table 1). Both IPSS and M-OBQ score were found to be higher in group 2 compared to group 1 (p = 0.009, p < 0001, respectively) (Table 1). Baseline prostate volume (ml), PSA (mg/dl), PVR (ml), Q_{max} (ml/sec) were found to be similar in both groups (p = 0.136, p = 0.383, p = 0.276, p = 0.790, respectively) (Table 1).

Table 1.
Comparison of age, prostate volume, symptom scores, PVR, Q_{max} and urinary GAG values between the two groups before medical treatment.

Pre-treatment	Group 1 (n: 22) Median (min-max)	Group 2 (n: 12) Median (min-max)	p value
Age (year)	60 (48-72)	58 (54-74)	0.845
Prostate (ml)	47.5 (30-102)	40 (23-93)	0.136
PSA (ng/ml)	1.45 (0.6-40)	1.1 (0.5-13)	0.383
IPSS	11 (5-25)	17.5 (10-30)	0.009
IPSS-QL	3 (0-5)	4 (2-6)	0.037
M-OABQ	6.5 (1-12)	17.5 (14-32)	< 0.001
PVR (ml)	120 (30-350)	76 (14-278)	0.276
Q_{max} (ml/sec)	9 (2-14)	9.5 (4-13)	0.790
GAG (mg/g Cr)	21.55 (6.1-45.5)	23.25 (15.6-32.6)	0.845

PSA: Prostate Specific Antigen; IPSS: International Prostate Symptom Score; QL: Quality of Life; M-OABQ: Marmara-Overactive Bladder Questionnaire; PVR: Post Void Residual Urine Volume; Q_{max}: Maximum Urine Flow Rate; GAG: Glycosaminoglycan.

Table 2.
Comparison of symptom scores, PVR, Q_{max} and urinary GAG values between the two groups after medical treatment.

Post-treatment	Group 1 (n: 22) Median (min-max)	Group 2 (n: 12) Median (min-max)	p value
IPSS	8 (1-16)	10 (3-30)	0.204
IPSS-QL	2 (0-5)	3 (0-5)	0.631
M-OABQ	5 (1-13)	12 (5-27)	< 0.0001
PVR (ml)	43 (6-300)	77 (0-177)	0.309
Q_{max} (ml/sec)	10.5 (4-17)	9.5 (4-13)	0.245
GAG (mg/g Cr)	19.8 (7.4-70.5)	18 (7.6-41.7)	0.511

IPSS: International Prostate Symptom Score; QL: Quality of Life; M-OABQ: Marmara-Overactive Bladder Questionnaire; PVR: Post Void Residual Urine Volume; Q_{max}: Maximum Urine Flow Rate; GAG: Glycosaminoglycan.

After the alpha-blocker treatment, the normalized urinary GAG levels in group 1 and 2 was found to be statistically similar as 19.8 (7.4-70.5) mg/gCr and 18 (7.6-41.7) mg/gCr, respectively (p = 0.511) (Table 2). Interestingly, only M-OBQ score was still found to be higher in group 2 compared to group 1 (p < 0.0001), but IPSS, PVR and Q_{max} were found to be statistically similar (p=0.204, p = 0.309, p = 0.245, respectively) (Table 2).

Discussion

Recently, several urinary biomarkers have been frequently studied in the assessment of LUTS. In general, urinary biomarker levels are frequently studied in various disease as they can be measured both noninvasively and easily. GAGs, a polysaccharide molecule produced in every cell in the human body, exist in two main structures as sulfated and non-sulfated (16, 17). Non-sulfated GAG contains hyaluronic acid, while sulfated GAGs include chondroitin sulfate, dermatan sulfate, keratan sulfate, heparan sulfate, and heparin. GAGs are in the structure of the basal lamina of the urethra, and damage to the GAG layer can affect the basal functions of the urothelium (17, 18). This process may result in the bladder surface being exposed to microcrystals, proteins, calcium, toxic metabolic products, and carcinogens of the urine (19). Thus, GAGs have been studied in *overactive bladder* (OAB) and found to have an association with OAB (12).

Intriguingly, urinary GAG levels are affected differently in patients with LUTS symptoms. In a study conducted with 45 patients with OAB, urinary GAG levels were found to be higher in the patients with OAB compared to the healthy subjects. One month of solifenacin treatment did help to decrease GAG levels but no significant difference was found between responders and non-responders (12). *Yet*, in another study involving 25 patients with LUTS (mean age 65.75 years) and urodynamically proven detrusor overactivity, urinary GAG values were shown to be low compared to the healthy control group (20). In interpreting this finding, the authors speculated that high-amplitude and prolonged overactive detrusor contractions may be associated with the ischemic process in the bladder epithelium, which in turn could reduce urinary GAG excretion.

The relationship between *interstitial cystitis* (IC), another pathology, and urinary GAG level has also been studied. Like in OAB, levels of urinary GAG were differently reported. *Lokeshwar et al.* reported urinary GAG levels to be higher in patients with IC compared to healthy controls and even higher in patients with severe IC symptoms compared to those with mild symptoms (21). Controversially, *Lucon et al.* reported urinary GAG levels to be low in patients with IC compared to patients with stress urinary incontinence. However, these authors also evaluated GAG in tissue samples and could not show a decrease in the GAG content of the urothelium in any groups (22). Nocturnal enuresis appears to be another pathology associated with changes of urinary GAG values. Researchers have shown that urinary GAG excretion is increased in children with nocturnal enuresis compared to healthy children (23, 24). Urinary GAG has also been reported to be associated with other urological pathologies such as renal cell carcinoma, acute renal failure, and bladder cancer, apart from lower urinary tract pathologies.

In the current study, no significant difference was found between urinary GAG values in BOO patients with and without OAB symptoms. Most significant limitation of our prospective study is the small sample size; it could be responsible for the failure to show statistical difference in various situations. Another limitation is the lack of urodynamics to diagnose detrusor over activity and BOO. Additionally, the control group was younger and was not formally assessed as the study group with medical history taking, physical examination including digital rectal examination, PSA, ultrasound imaging, and uroflowmetric study, measurement of PVR and assessment of symptoms by IPSS and M-OABQ.

Conclusions

No potential role of measurement of urinary GAG was found in the evaluation of patients with LUTS in this study. We consider that this result may have been affected by the small number of the sample of patients and should be confirmed by studies with a higher number of subjects.

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