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RESEARCH ARTICLE

Data do not support the use of elevated urinary nerve growth factor and fractalkine levels as diagnostic and prognostic biomarkers for women with overactive bladder

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Abbreviations used: OAB, overactive bladder; NGF, nerve growth factor; OAB-V8, overactive bladder-validated 8; ICIQ-SF, International Consultation on Incontinence Questionnaire Short Form; UTI, urinary tract infection; BMI, body mass index; Cr, creatinine; ROC, receiver operating characteristic; AUC, the area under the ROC curve; MCP-1, monocyte chemotactic protein-1

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ABSTRACT

OBJECTIVE: This study investigated urinary nerve growth factor (NGF) and fractalkine levels in women with overactive bladder (OAB), as well as diagnostic and/or prognostic roles, and correlation of these urinary biomarkers with symptom severity of patients.

MATERIALS AND METHODS: Twenty-seven women with OAB and 26 healthy subjects were enrolled. Patients were diagnosed with OAB based on symptoms, a 3-day voiding diary and a validated Turkish version of the Overactive Bladder-Validated 8 (OAB-V8) questionnaire. The urinary baseline levels of NGF and fractalkine were compared between OAB patients and control group. The Turkish-validated International Consultation on Incontinence Questionnaire Short Form and OAB-V8 used to categorize patients according to disease severity to assess treatment efficacy. Further urinary NGF and fractalkine levels were compared before and after antimuscarinic treatment.

RESULTS: Urinary NGF/creatinine (Cr) and fractalkine/Cr were significantly elevated in OAB patients (0.40 ± 0.40 ng/mg and 4.63 ± 4.36 ng/mg, respectively) compared to healthy subjects (0.14 ± 0.08 ng/mg and 2.00 ± 1.29 ng/mg, P = 0.002 and P = 0.005, respectively). Sensitivity and specificity were 85.2%, 65.4% for NGF, and 74.1%, 65.4% for fractalkine, respectively. No significant differences in NGF/Cr and fractalkine/Cr compared to baseline (P = 0.063 and 0.162, respectively) were observed after trospium chloride treatment in OAB patients. NGF/Cr and fractalkine/Cr exhibited no correlations with symptom severity levels.

CONCLUSIONS: Increased urinary NGF/Cr and fractalkine/Cr levels were found in OAB women. However, the sensitivity and specificity were not sufficient for diagnostic use. NGF and fractalkine levels were decreased after treatment insignificantly, and there was no correlation with symptom severity; therefore their prognostic worth was limited.

Keywords: chemokine, fractalkine, nerve growth factor, overactive bladder, urinary biomarker

INTRODUCTION

The diagnosis of overactive bladder (OAB), symptom severity grade and patient screening during treatment are based on subjective questionnaires or invasive tests, such as urodynamic study [1]. An objective and noninvasive biomarker for the diagnosis of OAB and the evaluation of therapeutic outcomes is needed. Therefore, recent researches have focused on the investigations of possible biomarkers.

Neurotrophins, such as urinary nerve growth factor (NGF), cytokines and chemokines, are associated with morphological changes in sensory and motor neurons that innervate the bladder and contribute to inflammation and afferent sensitization [2, 3]. NGF is a well-documented candidate biomarker for OAB, but no correlation of NGF with symptom severity has been reported, and the results of antimuscarinic treatment on NGF are not clear. Low sensitivity and specificity have also been reported for urinary NGF [2].

Histological evidence of inflammation in bladder biopsy specimens of OAB patients has demonstrated that chronic inflammatory processes in

the bladder might be responsible for the pathophysiology in some OAB patients, especially women with severe symptoms and who are resistant to therapy [4-6]. Fractalkine (CX3CL1) is an exceptional member of the large family of leukocyte-excreted chemokines portraying unique functional and structural characteristics, and it acts a potential mediator of nociceptive facilitation. Fractalkine has been found to play an important role as a sole membrane-bound chemokine with a mucin-like glycosylated stalk where it mediates both chemotaxis and adhesion of inflammatory cells via its highly selective receptor CX3CR1. Many chemokines show considerable overlap in their receptor binding, although several bind with relative specificity. But fractalkine is the only known ligand for CX3CR1, binding with high affinity and specificity. Due to its role in chemotaxis and inflammatory responses, may contribute to inflammatory-induced sensory changes in OAB [7, 8].

This study primarily evaluated urinary NGF and fractalkine levels in OAB patients compared to normal women and hypothesized that urinary NGF and fractalkine contribute to inflammatory-induced changes in OAB. Secondly, the diagnostic and/or prognostic roles of urinary NGF and fractalkine as noninvasive urinary biomarkers were investigated by analyzing the relationship of these factors with symptom severity.

MATERIALS AND METHODS

Study Design

Female OAB patients and healthy women were enrolled to a prospective controlled study after the Institutional Review Board of the hospital and ethics committee approved the study. Informed consent was obtained from each individual. The study was performed in adherence to the Helsinki declaration.

The patients were consecutively selected from outpatients complaining from at least 2 episodes of urgency daily and frequency (≥ 8 voids per day) between April 2012 and October 2012. The diagnosis of OAB was confirmed using a 3-day voiding diary and Turkish validation of the Overactive Bladder-Validated 8 (OAB-V8) questionnaire. Patients were diagnosed with OAB if their OAB-V8 score was ≥ 8. The Turkish-validated International Consultation on Incontinence Questionnaire Short Form (ICIQ-SF) and OAB-V8 discriminated patients according to disease severity at baseline and after treatment to assess treatment efficacy. Positive OAB-V8 scores were categorized in two groups according to symptom severity: 8-13 and ≥ 14 [9]. An ICIQ-SF score of 8 was the cutoff value to define bothersome urinary incontinence, and based on a previous study in Turkish women, patients were classified into two severity levels, < 8 and ≥ 8 [10]. A comparison between categorized groups before and after treatment was performed, and the results were also correlated with urinary NGF and fractalkine levels. Patients were further divided into subgroups according to the presence or absence of urge incontinence based on the patient's voiding diary record as OAB-dry or OAB-wet, age (< 55 years and ≥ 55 years) and body mass index (BMI < 30 and ≥ 30 kg/m²). The control group included age-matched healthy women (within ± 5 years of the OAB patient ages) with negative urine cultures.

All patients underwent urine culture, uroflowmetry and ultrasound for the detection of urinary pathology, including infection, total bladder capacity and post-voiding residual volume. The inclusion criteria were OAB symptoms duration longer than 3 months without antimuscarinic treatment for at least 3 months before; age ≥ 18 years; and the ability to complete a 3-day voiding diary, OAB-V8 questionnaire and ICIQ-SF. Exclusion criteria were a post-voiding residual volume of greater than 50 ml, hematuria, urinary tract infection (UTI), suspected interstitial cystitis, neurogenic bladder, vaginitis, cystocele or pelvic prolapse, urothelial carcinoma, urolithiasis, history of pelvic radiation or urologic surgery, botulinum injection to bladder, the use of intermittent self-catheterization, any disorder (e.g., allergic, neurological, renal, hepatic, psychiatric or metabolic diseases), physiological conditions (e.g., pregnancy, nursing or menstruation), current treatment with potent CYP3A4 inhibitors, macrolide antibiotics, antifungal agents, diuretics or NSAIDS and any condition that is a contraindication for anticholinergic treatment, including uncontrolled narrow-angled glaucoma, urinary retention or gastric retention.

All OAB patients received antimuscarinic therapy using trospium chloride 45 mg/daily, which is routinely used in clinical practice, for 1 month. Urinary levels of NGF and fractalkine were compared between patients and the control group and subgroups of women with OAB at baseline. Urinary NGF and fractalkine levels were compared before and after antimuscarinic treatment in OAB patients.

Biochemical analyses

Midstream urine samples were collected at the first sensation of filling into sterile urine collection tubes in patient and control groups at baseline and 1 month after treatment. Urinalysis was immediately performed on each sample to confirm the absence of complicating factors, such as pyuria, bacteriuria or hematuria. Each urine sample was centrifuged at 3000 g for 10 minutes, and the supernatant was aliquoted in 1.5 ml microcentrifuge tubes and stored at -80°C until analysis. The urinary creatinine (Cr) concentration was determined in each sample using the kinetic Jaffe method in an Olympus 2700 analyzer (Beckman Coulter, Inc., CA, USA). Urinary NGF levels were measured using a β-NGF enzyme-linked immunosorbent assay (ELISA) kit (RayBiotech, Inc., GA, USA), according to the manufacturer's directions. The limit of detection of this test was < 14 pg/ml. The intra- and inter-assay coefficients of variability were < 10% and < 12%, respectively. A commercial ELISA kit (Aviscera Bioscience, Inc., CA, USA) measured urine fractalkine levels. The limit of sensitivity of this assay was 0.15 ng/ml. The intra- and inter-assay coefficients of variability were 4-6% and 8-10%, respectively. The urinary concentrations of β-NGF and fractalkine were normalized to the concentration of urinary Cr, and the results are expressed as ng/mg of Cr.

Statistical analyses

SPSS 20.0 (SPSS Inc. Chicago, IL, USA) was used for all analyses. The normality of the variables was evaluated using the Kolmogorov Smirnov test. Continuous variables are presented as means and standard deviations. Independent samples t-tests were used to analyze quantitative data. Paired samples t-tests were performed for repeated measurements. Correlation analyses were performed using the Pearson correlation test. The clinical performances of all parameters were measured using receiver operating characteristic (ROC) curves and logistic regression analyses. A P value less than 0.05 was considered statistically significant for all analyses.

RESULTS

Analysis of baseline values

Eleven of the 38 patients were excluded from the study. The reasons for exclusion were treatment discontinuation, drug side effects, and recurrent UTI during treatment. The urine samples of 27 OAB women and 26 control subjects were evaluated for the study. The mean ages were similar between patients (51.67 ± 13.27 years) and healthy individuals (48.42 ± 13.22 years) (P = 0.377). The means of OAB-V8 (28.0 ± 6.86) and ICIQ-SF domain scores (9.93 ± 5.60) at baseline were typically elevated, and these elevations were compatible with the symptom severity in women with OAB.

Respective mean NGF/Cr and fractalkine/Cr levels in urine were 0.40 ± 0.40 ng/mg and 4.63 ± 4.36 ng/mg in OAB patients and 0.14 ± 0.08 ng/mg and 2.00 ± 1.29 ng/mg in healthy subjects. Significantly higher urinary levels of secreted NGF/Cr and fractalkine/Cr were detected in the OAB group compared to controls (P = 0.002 and 0.005, respectively). NGF/Cr and fractalkine/Cr values in urine were significantly elevated 10.36-fold (95% CI =2.86-41.24; P = 0.0001) and 5.40-fold (95% CI = 1.66-17.57; P = 0.005), respectively, in OAB women compared to the control group. No significant differences in questionnaire scores, NGF/Cr and fractalkine/Cr levels according to age, BMI and OAB type (dry or wet) were observed within the OAB group, except significant difference in ICIQ-SF scores between OAB-dry and OAB-wet group (**Table 1**).

The area under the ROC curve (AUC) for NGF/Cr (AUC = 0.753; 95% CI = 0.617-0.889) was higher than the AUC for fractalkine/Cr (AUC = 0.697; 95% CI = 0.553-0.842) (P = 0.002 vs. 0.014) (**Fig. 1**). A cutoff value of ≤0.12 ng/mg for NGF/Cr provided a sensitivity of 85.2% and a specificity of 65.4% (positive predictive value: 71.9%, negative predictive value: 81.0%), and the most effective threshold value was 2.00 ng/mg for fractalkine/Cr, with a sensitivity of 74.1% and a specificity of 65.4% (positive predictive value: 69.0%, negative predictive value: 70.8%). We noted particularly higher sensitivity and positive and negative predictive values for NGF/Cr.

Table 1. NGF/Cr and fractalkine/Cr levels and scores of questionnaires of the OAB group according to age, BMI and OAB type (dry or wet).

		< 55 yr no: 17	> 55 yr no: 10	BMI (< 30) no: 13	BMI (> 30) no: 14	Dry no: 4	Wet no: 23
NGF/Cr (ng/mg)	**Mean ± SD**	0.37 ± 0.4	0.44 ± 0.41	0.27 ± 0.26	0.52 ± 0.48	0.28 ± 0.12	0.42 ± 0.43
	P value	0.940		0.062		0.918	
Fractalkine/Cr (ng/mg)	**Mean ± SD**	4.23 ± 4.04	5.31 ± 5	3.33 ± 2.86	5.84 ± 5.21	3.44 ± 2.42	4.84 ± 4.62
	P value	0.393		0.065		0.785	
ICIQ-SF	**Mean ± SD**	8.35 ± 5.59	12.6 ± 4.74	7.77 ± 5.73	11.93 ± 4.84	0 ± 0	11.65 ± 4.01
	P value	0.101		0.06		0.002	
OAB-V8	**Mean ± SD**	26.94 ± 6.46	29.8 ± 7.48	27.23 ± 7.66	28.71 ± 6.22	28.25 ± 7.27	27.96 ± 6.95
	P value	0.151		0.644		0.973	

Table 2. The comparison of baseline and after treatment levels of NGF/Cr and fractalkine/Cr and scores of questionnaires.

		Baseline	After Treatment	*P* value
NGF/Cr (ng/mg)	Mean ± SD	0.40 ± 0.40	0.23 ± 0.18	0.063
Fractalkine/Cr (ng/mg)	Mean ± SD	4.63 ± 4.36	3.23 ± 2.29	0.162
OAB-V8	Mean ± SD	28.0 ± 6.86	16.93 ± 9.27	<0.0001
ICIQ-SF	Mean ± SD	9.93 ± 5.60	4.74 ± 4.25	<0.0001

Analysis of therapeutic outcome

No significant differences in NGF/Cr and fractalkine/Cr levels were observed after treatment compared to baseline levels (P = 0.063 and 0.162, respectively). In contrast, a statistically significant difference in both OAB-V8 and ICIQ-SF scores was found between the baseline and after treatment groups (**Table 2**). Urinary values of NGF/Cr and fractalkine/Cr decreased slightly with treatment, but this reduction was not correlated with significant improvements in questionnaire scores (**Fig. 2A-D**). Treatment-related changes in OAB-V8 scores correlated significantly with changes in ICIQ-SF scores (**Fig. 2E**). Significant correlations were observed in urinary NGF/Cr and fractalkine/Cr levels at baseline and after 4 weeks of treatment (**Fig. 2F**). NGF/Cr and fractalkine/Cr exhibited no correlations with symptom severity levels in subcategorized groups of OAB-V8 and ICIQ-SF questionnaires in terms of differences and changes between the baseline and after

treatment (**Table 3**).

DISCUSSION

The search for biomarkers for the diagnosis and assessment of therapeutic outcomes in OAB has been emphasized. However, several factors need to be investigated prior to the application of any reliable urinary biomarker: the role of neuronal and nonneuronal pathways; the underestimated role of inflammation; the use of present urinary biomarkers for diagnosis and follow-up; patient classification according to questionnaires, symptom severity, urinary biomarkers and risk factors; the lack of response to medical treatment in some women; the role of inflammation in the resistance to antimuscarinic therapy. No ultimate solutions to these issues presently exist.

Table 3. The correlation of NGF/Cr and fractalkine/Cr with symptom severity levels in subcategorized groups of OAB-V8 and ICIQ-SF questionnaires in terms of differences and changes between the baseline and after treatment.

		OAB-V8 (8-13)	OAB-V8 (≥ 14)	*P* value	ICIQ-SF (< 8)	ICIQ-SF (≥ 8)	*P* value
NGF/Cr (ng/mg)	Baseline	0.32 ± 0.28	0.45 ± 0.47	0.435	0.37 ± 0.32	0.47 ± 0.60	0.607
	After treatment	0.22 ± 0.18	0.24 ± 0.18	0.796	0.25 ± 0.19	0.15 ± 0.11	0.203
	Difference	-0.11 ± 0.34	-0.21 ± 0.52	0.559	-0.12 ± 0.40	-0.31 ± 0.59	0.340
	Difference *P*	0.302	0.124		0.436	0.211	
Fractalkine/Cr (ng/mg)	Baseline	4.00 ± 2.83	5.07 ± 5.20	0.553	4.77 ± 4.00	4.25 ± 5.60	0.794
	After treatment	2.87 ± 2.14	3.48 ± 2.42	0.502	3.48 ± 2.44	2.53 ± 1.76	0.357
	Difference	-1.14 ± 3.68	-1.58 ± 5.93	0.862	-1.29 ± 5.05	-1.72 ± 5.45	0.850
	Difference p	0.330	0.330		0.268	0.200	

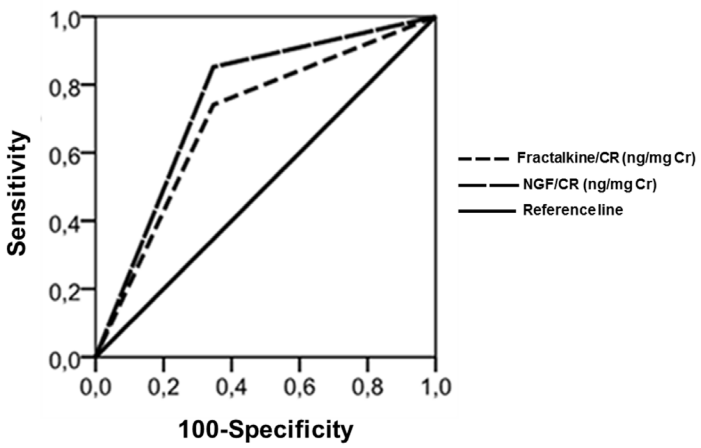

Figure 1. The area under AUC for NGF/Cr and fractalkine/Cr.

Not all women with OAB demonstrate detrusor overactivity in the urodynamic test, and the invasiveness of this test restricts its routine use. In one study, 44% of women with urgency had detrusor overactivity, but 58% of women with urgency and urge incontinence had detrusor overactivity [1]. Measurement of bladder wall thickness was suggested as a noninvasive alternative test. However, studies on the potential value of bladder wall thickness measurements are contradictory [11, 12].

The presence of elevated levels of proteins and inflammatory biomarkers in urine suggests a role for inflammation in OAB. Therefore, studies have focused on C-reactive protein (CRP), prostaglandins, neurotrophins, cytokines and chemokines. Urinary prostaglandins, which are triggered by detrusor muscle stretch, mucosal damage or inflammation, modulate the activity of bladder nerves. But, the role of urinary prostaglandins is controversial. CRP is also hardly detectable in urine. Prostaglandins and CRP correlate with OAB symptom severity, but these molecules do not show any independent prognostic benefit [2, 13-15].

NGF is the most investigated urinary biomarker. Higher levels of NGF were measured in animal models of detrusor overactivity, and acute and chronic intravesical NGF administration provoked OAB, and NGF inhibition decreases the frequency of bladder contractions in experimental bladder inflammation models [16]. Urinary NGF levels are higher (12-fold) in women with OAB compared to normal controls, and an 8-fold increase was calculated in women complaining of urgency urinary incontinence compared to women without urinary incontinence [2, 17]. However, another study reported an opposite conclusion; urinary NGF/Cr was higher in OAB patients compared to healthy volunteers, but the difference was not statistically significant, and the sensitivity and specificity were lower [18, 19]. Birder and colleagues did not observe elevated NGF levels in bladder biopsies, and they failed to show an association between elevated NGF concentrations in urothelium and detrusor overactivity [20]. Although there is a verified interaction between urinary NGF and sensory fibers, the levels of NGF in urine might not correlate well. Up to one-third of OAB patients exhibit normal urinary NGF values at baseline in some cohorts. Therefore, significant proportions of OAB patients without raised urinary NGF levels reduce the sensitivity of NGF as a potential biomarker [21]. Another possible reason for low sensitivity and specificity of urinary NGF levels might be as a result of varying whether the urine was collected from an empty or a full distended bladder [2]. Additionally, NGF is released from various cells; including urothelial cells, smooth muscle cells, and mast cells caused by inflammation, obstruction, or denervation. Moreover, the precise mechanisms by which urinary NGF promotes OAB are not yet defined, and its levels might be related to activities of P2X3 and TRPV1 receptors that modulate bladder function. The sensitivity

and specificity of NGF and fractalkine were not high enough for ideal diagnostic urinary biomarker in our study, but ROC analysis indicated that NGF was more valuable than fractalkine clinically.

A suitable biomarker should be correlated with OAB severity. Liu and coworkers classified patients' severity of urgency as 0-4, and reported significantly higher levels of NGF/Cr in women with scores of 4 compared to scores of 3 or lower [17]. Antimuscarinic treatment and botulinum injection decrease urinary levels of NGF significantly, and this reduction is correlated with reduced symptom severity. Withdrawal of the therapy also reversed treatment-induced changes [18, 22, 23]. Significant reductions in OAB-V8 and ICIQ-SF domain scores were observed after treatment in our study, but these differences were not correlated with NGF/Cr and fractalkine/Cr levels.

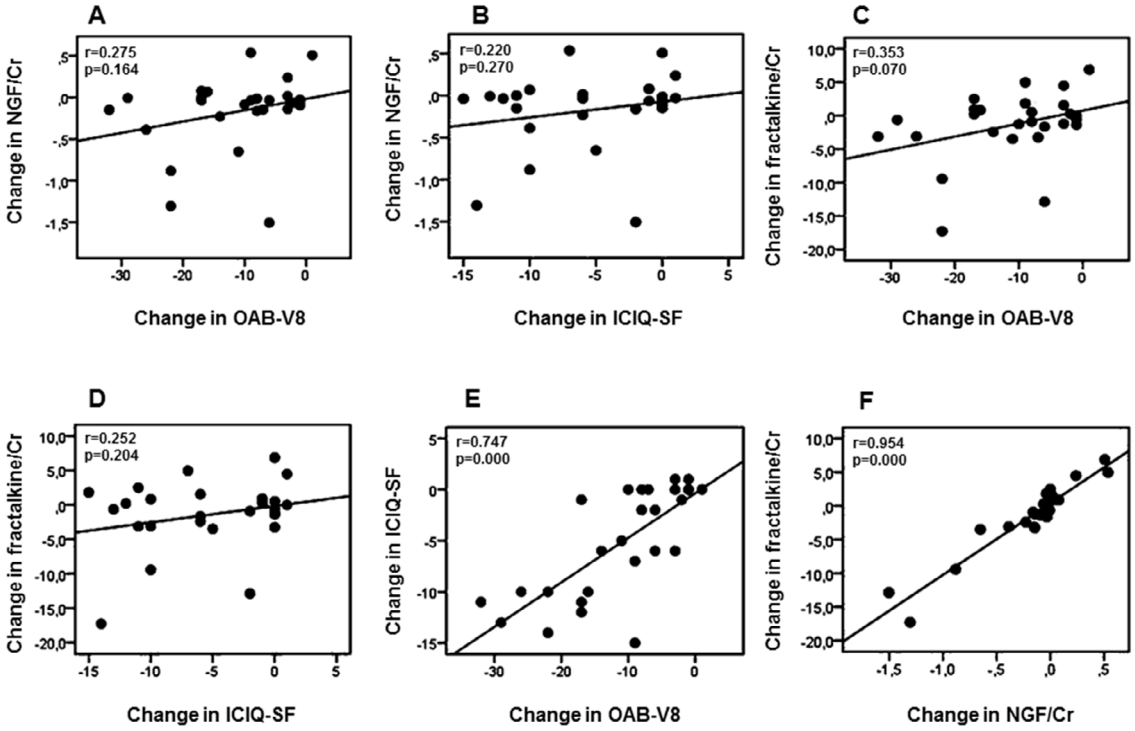

Figure 2. Urinary values of NGF/Cr and fractalkine/Cr decreased slightly with treatment, but these values did not correlate with significant improvements in questionnaire scores (A-D). Treatment-related changes in OAB-V8 scores correlated significantly with changes in ICIQ-SF scores (E). Significant correlations were observed in changes in urinary NGF/Cr and fractalkine/Cr levels between the baseline and after 4 weeks of treatment (F).

Urinary NGF levels may be a potential biomarker for the diagnosis of OAB. However, a recent review noted that NGF alone could be insufficient as a urinary marker for OAB due to the numerous sensory neurotransmitters and inflammatory substances that also likely play important roles [21]. Another systemic review revealed a tendency to produce overly optimistic estimates of NGF test accuracy [24].

NGF modulates urothelial responses to inflammation by altering pain signaling. Therefore, increased urinary NGF levels in women refractory to antimuscarinic treatment may be related to chronic inflammation [25]. Bladder biopsies have demonstrated histological evidence of inflammatory changes in OAB in the absence of urinary tract infection [5]. Basic research has implied that bladder inflammation might be augmented and could cause sensitization via the release of chemokines in the suburothelium [3, 4]. Gap junction interactions between suburothelial myofibroblasts, urothelium and detrusor smooth muscle cells may be altered by cytokine expression, and OAB can be an inflammatory process in the bladder [3, 4]. The presence of elevated urine cytokine and chemokine levels may support the relationship between inflammation and afferent nerve sensitization [26, 27].

Approximately 50 different types of human chemokines that are responsible for the autocrine, paracrine and endocrine signaling in the bladder have been investigated at basal levels, but some of these factors are expressed at higher levels in urinary tract inflammation [2, 11, 26]. Tyagi et al. suggested that different inflammatory pathways were activated following UTI vs. OAB according to their findings of elevated levels of monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein, CXCL1 and CXCL8 in the urine of OAB patients without UTIs [3]. Ghoniem et al. measured urinary cytokines in OAB patients compared to normal subjects and UTI patients [27]. These authors hypothesized that the secretion of these substances from smooth muscle cells in detrusor overactivity and the production of proinflammatory cytokines in response to high level of MCP-1 may irritate the detrusor interstitial cell of Cajal-like cells located in the urothelium. The relative fold-change of fractalkine in OAB patients compared to control group was as low as 0.99 in their study ($P = 0.64$) [27]. Baseline urinary NGF (10-fold) and fractalkine (5-fold) levels were highly expressed in our OAB patients compared to controls. No statistically significant decrease was noted after treatment, but an insignificant difference in NGF levels ($P = 0.06$) compared to fractalkine was observed. This result may due to an insufficient number of OAB patients in our study.

The increased expression of fractalkine in urinary bladder after cyclophosphamide-induced cystitis has been observed previously [8]. These authors hypothesize that chronic bladder inflammation may alter micturition reflexes and sensory processing via the release of fractalkine, in addition to its roles in chemotaxis and inflammatory responses [8]. The therapeutic potential of targeting fractalkine or its single and highly selective chemokine-receptor (CX3CR1) has been reported, but no therapeutics are in use clinically [7].

Some investigations have focused on urinary NGF levels in patients refractory to antimuscarinic treatment as a potential biomarker to assess therapeutic outcomes objectively. This type of tool may also be a prognostic indicator and could identify patients who may respond to certain treatments [21, 23]. Responders to antimuscarinic therapy exhibit significantly improved urinary symptom scores and a corresponding decrease in urinary NGF levels, but the reduced NGF levels remained significantly higher than controls [21]. These authors stated that the complex pathophysiology of OAB may be multimodal and could involve several neurotransmitter and inflammatory pathways [21, 25]. Our results were not compatible with the literature and changes before and after therapy in OAB-V8 and ICIQ-SF questionnaires were not correlated with NGF/Cr and fractalkine/Cr levels.

This study has some limitations. The enrollment numbers were small due to high cost of ELISA kits which is a common problem of similar studies, and follow-up after treatment was short. Number and the distribution of wet and dry patients were not sufficient to make any conclusion. We did not investigate asymptomatic urinary tract infection during anticholinergic treatment, and any asymptomatic urinary tract infection may alter anticholinergic responses and urinary NGF and fractalkine levels.

CONCLUSIONS

In summary, we found higher NGF and fractalkine levels in OAB women, which demonstrates the role of alterations in the sensitivity of afferent nerves and bladder inflammation. However, the sensitivity and specificity were not sufficient for routine use in OAB diagnosis. An ideal biomarker must have a high level of specificity and sensitivity and a clear association with OAB severity and outcome. Patients' symptoms improved following antimuscarinic therapy, but NGF and fractalkine levels were not correlated with symptom severity, and their prognostic and follow-up usages were restricted. No ideal biomarker for use in clinical practice can be recommended. Therefore, a multimarker approach that combines several biomarkers, such as neurotrophins, cytokines and chemokines, might be an option for the detection and monitoring of OAB due to the alterations in urinary levels of these factors under different conditions and across patients.

References

1. Hashim H, Abrams P (2006) Is the bladder a reliable witness for predicting detrusor overactivity?. J Urol 175: 191-194. doi: 10.1016/S0022-5347(05)00067-4. PMID: 16406907
2. Antunes-Lopes T, Carvalho-Barros S, Cruz C, Cruz F, Martins-Silva C (2011) Biomarkers in overactive bladder: a new objective and noninvasive tool?. Adv Urol 2011: 382431. doi: 10.1155/2011/382431. PMID: 21687625
3. Tyagi P, Barclay D, Zamora R, Yoshimura N, Peters K, et al. (2009) Urine cytokines suggest an inflammatory response in the overactive bladder: a pilot study. Int Urol Nephrol 42: 629-635. doi: 10.1007/s11255-009-9647-5. PMID: 19784793
4. Bouchelouche K, Alvarez S, Horn T, Nordling J, Bouchelouche P (2006) Human detrusor smooth muscle cells release interleukin-6, interleukin-8, and RANTES in response to proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha. Urology 67: 214-219. doi: 10.1016/j.urology.2005.07.049. PMID: 16413378
5. Compérat E, Reitz A, Delcourt A, Capron F, Denys P, et al. (2006) Histologic features in the urinary bladder wall affected from neurogenic overactivity--a comparison of inflammation, oedema and fibrosis with and without injection of botulinum toxin type A. Eur Urol 50: 1058-1064. doi: 10.1016/j.eururo.2006.01.025. PMID: 16517054
6. Apostolidis A, Jacques TS, Freeman A, Kalsi V, Popat R, et al. (2008) Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. Eur Urol 53: 1245-1253. doi: 10.1016/j.eururo.2008.02.037. PMID: 18343564
7. D'Haese JG, Demir IE, Friess H, Ceyhan GO (2010) Fractalkine/CX3CR1: why a single chemokine-receptor duo bears a major and unique therapeutic potential. Expert Opin Ther Targets 14: 207-219. doi: 10.1517/14728220903540265. PMID: 20055718
8. Yuridullah R, Corrow KA, Malley SE, Vizzard MA (2006) Expression of fractalkine and fractalkine receptor in urinary bladder after cyclophosphamide (CYP)-induced cystitis. Auton Neurosci 126-127: 380-389. doi: 10.1016/j.autneu.2006.02.030. PMID: 16651033
9. Cheung WW, Borawski D, Abulafia O, Vincent MT, Harel M, et al. (2011) Characterization of overactive bladder in women in a primary care setting. Open Access J Urol 3: 29-34. doi: 10.2147/OAJU.S15712. PMID: 24198633
10. Cetinel B, Demirkesen O, Tarcan T, Yalcin O, Kocak T, et al. (2006) Hidden female urinary incontinence in urology and obstetrics and gynecology outpatient clinics in Turkey: what are the determinants of bothersome urinary incontinence and help-seeking behavior?. Int Urogynecol J Pelvic Floor Dysfunct 18: 659-664. doi: 10.1007/s00192-006-0223-6. PMID: 17164988
11. Cartwright R, Afshan I, Derpapas A, Vijaya G, Khullar V (2011) Novel biomarkers for overactive bladder. Nat Rev Urol 8: 139-145. doi: 10.1038/nrurol.2011.7. PMID: 21321572
12. Chung S, Chiu B, Kuo H, Chuang Y, Wang C, et al. (2009) Transabdominal ultrasonography of detrusor wall thickness in women with overactive bladder. BJU Int 105: 668-672. doi: 10.1111/j.1464-410X.2009.08927.x. PMID: 19793377
13. Chung S, Liu H, Lin H, Kuo H (2011) Elevation of serum c-reactive protein in patients with OAB and IC/BPS implies chronic inflammation in the urinary bladder. Neurourol Urodyn 30: 417-420. doi: 10.1002/nau.20938. PMID: 21284020
14. Kupelian V, McVary KT, Barry MJ, Link CL, Rosen RC, et al. (2009) Association of C-reactive protein and lower urinary tract symptoms in men and women: results from Boston Area Community Health survey. Urology 73: 950-957. doi: 10.1016/j.urology.2008.12.012. PMID: 19394490
15. Cho KJ, Kim HS, Koh JS, Kim JC (2012) Changes in urinary nerve growth factor and prostaglandin E2 in women with overactive bladder after anticholinergics. Int Urogynecol J 24: 325-330. doi: 10.1007/s00192-012-1854-4. PMID: 22717785
16. Frias B, Allen S, Dawbarn D, Charrua A, Cruz F, et al. (2013) Brain-derived neurotrophic factor, acting at the spinal cord level, participates in bladder hyperactivity and referred pain during chronic bladder inflammation. Neuroscience 234: 88-102. doi: 10.1016/j.neuroscience.2012.12.044. PMID: 23313710
17. Liu HT, Chen CY, Kuo HC (2011) Urinary nerve growth factor in women with overactive bladder syndrome. BJU Int 107: 799-803. doi: 10.1111/j.1464-410X.2010.09585.x. PMID: 20804479
18. Liu H, Chen C, Kuo H (2010) Urinary nerve growth factor levels in overactive bladder syndrome and lower urinary tract disorders. J Formos Med Assoc 109: 862-878. doi: 10.1016/S0929-6646(10)60133-7. PMID: 21195884
19. Antunes-Lopes T, Pinto R, Barros SC, Botelho F, Silva CM, et al. (2012) Urinary neurotrophic factors in healthy individuals and patients with overactive bladder. J Urol 189: 359-365. doi: 10.1016/j.juro.2012.08.187. PMID: 23174241
20. Birder LA, Wolf-Johnston A, Griffiths D, Resnick NM (2007) Role of urothelial nerve growth factor in human bladder function. Neurourol Urodyn 26: 405-409. doi: 10.1002/nau.20372. PMID: 17266135
21. Seth JH, Sahai A, Khan MS, van der Aa, (Frank) , de Ridder D, et al. (2013) Nerve growth factor (NGF): a potential urinary biomarker for overactive bladder syndrome (OAB)?. BJU Int 111: 372-380. doi: 10.1111/j.1464-410X.2012.11672.x. PMID: 23444927

22. Liu HT, Chancellor MB, Kuo HC (2009) Urinary nerve growth factor levels are elevated in patients with detrusor overactivity and decreased in responders to detrusor botulinum toxin-A injection. Eur Urol 56: 700-6. doi: 10.1016/j.eururo.2008.04.037. PMID: 18472208

23. Kuo HC, Liu HT, Chancellor MB (2010) Can urinary nerve growth factor be a biomarker for overactive bladder. Rev Urol 12: 69-77. PMID: 20811555

24. Rachaneni S, Arya P, Latthe P (2013) Urinary nerve growth factor: a biomarker of detrusor overactivity? A systematic review. Int Urogynecol J 24: 1603-1609. doi: 10.1007/s00192-013-2104-0. PMID: 23649686

25. Liu HT, Lin H, Kuo HC (2011) Increased serum nerve growth factor levels in patients with overactive bladder syndrome refractory to antimuscarinic therapy. Neurourol Urodyn 30: 1525-9. doi: 10.1002/nau.21118. PMID: 21826717

26. Tyagi P, Killinger K, Tyagi V, Nirmal J, Chancellor M, et al. (2012) Urinary chemokines as noninvasive predictors of ulcerative interstitial cystitis. J Urol 187: 2243-2248. doi: 10.1016/j.juro.2012.01.034. PMID: 22503040

27. Ghoniem G, Faruqui N, Elmissiry M, Mahdy A, Abdelwahab H, et al. (2011) Differential profile analysis of urinary cytokines in patients with overactive bladder. Int Urogynecol J 22: 953-961. doi: 10.1007/s00192-011-1401-8. PMID: 21487829