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CHANGES IN URINE MARKERS AND SYMPTOMS AFTER BLADDER DISTENTION FOR INTERSTITIAL CYSTITIS

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Abstract

Purpose—To evaluate changes in urine markers and symptom scores after bladder distention in interstitial cystitis (IC) patients.

Materials and Methods—Subjects were 33 new patients with no prior IC treatments. Urine specimens were taken before and one month after bladder distention. University of Wisconsin (UW) symptom scores were done the same day as the urine specimen collection. Urine marker levels and symptom scores before and after distention were compared. Changes in markers were tested for associations with changes in symptom scores and other markers. Pre-distention markers and specific pre-distention symptoms were tested for their association with post-distention symptom improvement.

Results—After distention, the median total UW score decreased significantly (28.5 before, 10 after, $p < 0.001$). Twelve patients (36%) had at least 30% improvement in UW score, and eight patients (24%) had at least 50% improvement. No pre-distention markers or symptoms predicted which patients would have a good response. Two of the urine markers improved significantly after distention: anti-proliferative factor (APF) activity (median –96% before, –17% after, $p < 0.001$) and heparin binding-epidermal growth factor-like growth factor (HB-EGF) levels (median 0.34 ng/mg creatinine before, 4.1 after, $p < 0.001$). None of the changes in urine markers associated with changes in symptom scores.

Conclusions—The median symptom score for newly diagnosed IC patients decreased after distention, but only a minority of patients had at least 30% symptom improvement. Bladder distention altered urine APF activity and HB-EGF levels towards normal, but the mechanism of symptom relief after distention is still unknown.

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Keywords

interstitial cystitis, urine; interstitial cystitis, surgery; interstitial cystitis, therapy; interstitial cystitis, physiopathology

INTRODUCTION

Bladder distention is a common interstitial cystitis (IC) treatment, but responses vary.^{1–3} Symptoms may resolve, improve partially, remain unchanged, or worsen. The mechanism of benefit for those who do improve is unknown, and one cannot predict ahead of time which patients will improve.

We analyzed IC patients' symptoms and urine markers before distention and one month after distention. Three questions were analyzed: (1) did any of the urine markers or symptom scores change after distention? (2) did changes in symptom scores associate with changes in urine markers? (3) did any pre-distention marker levels or clinical features associate with symptom response after distention?

MATERIALS AND METHODS

Subjects

The procedures were previously described.⁴ Briefly, subjects in this report were new patients with no prior IC treatments, who met the National Institute of Diabetes, Digestive and Kidney Diseases cystoscopic criteria.⁵ Cystoscopy with bladder distention (80 cm water pressure, 4–8 minutes) was performed under general or regional anesthesia. Subjects gave voided urine specimens and answered University of Wisconsin (UW)⁶ symptom scores before and one month after distention. During this month, the investigators did not start other treatments (patients could have made dietary/behavioral changes on their own).

For correlation with urine markers, the UW score was chosen because it asks about symptoms that day (in contrast to the O'Leary-Sant score, which asks about symptoms the past month). The UW score includes seven bladder items, each scored from 0 (none) to 6 (a lot). Total UW score was the sum of these seven scores (0 to 42).

Urine measurements

Urine was analyzed as previously described.⁷ Markers were chosen to reflect different proposed pathophysiologies of IC.⁸ Briefly, anti-proliferative factor (APF) may reflect bladder epithelial deficiency.⁹ Epidermal growth factor (EGF) and heparin-binding EGF-like growth factor (HB-EGF) are influenced by APF. Interleukins 6 and 8 (IL-6, IL-8) may reflect bladder inflammation. Cyclic guanosine monophosphate (cGMP) synthesis is stimulated by nitric oxide (NO). Since voided urine NO assay is unreliable, cGMP was used as a surrogate measure. Low levels of constitutively produced NO may cause IC symptoms; conversely, high levels may reflect induced NO with bladder inflammation.^{8,10}

APF is expressed as percent inhibition of thymidine incorporation, with urine pH and osmolarity corrected before assay. All other markers are normalized to urine creatinine concentration. Creatinine was measured in the Hershey Medical Center clinical laboratory by autoanalyzer (Roche Diagnostics, Indianapolis, Indiana).

Statistical analysis

The exact Wilcoxon signed-rank test was used to compare pre-distention vs. post-distention marker levels, and to compare pre-distention vs. post-distention symptom scores. For each urine marker, the Spearman correlation coefficient was used to test whether the percent change in the marker after distention was associated with the percent change in total UW score after distention. We also considered that APF activity, measured by bioassay, might not be linearly associated with the amount of APF peptide (see discussion). Therefore, we also dichotomized APF activity into normal (≤ 2 standard deviations of the inhibition seen on the corresponding control cells on the same plate) or abnormal. We compared the dichotomized post-distention APF activity to the percent change in symptom scores using the exact Wilcoxon-Mann-Whitney test.

We also tested the association between distention response and pre-distention markers or other clinical features, including age, presence of Hunner's ulcers, bladder capacity under anesthesia, or symptom characteristics (Table 1). For continuous variables, the Spearman correlation coefficient was used to test association between each variable and the percent change in total UW score after distention. For categorical variables, the exact Wilcoxon-Mann-Whitney test was used to compare the percent change in total UW score for patients in each category.

Significance was established at the $\alpha=0.01$ level to account for possible artifacts due to multiple hypothesis tests. The Kruskal-Wallis test was used to determine whether the percent change in total UW score after distention was significantly different for the three centers. A significant difference was not found ($p=0.80$), so correction for centers was not performed. For continuous variables, results are given as median and 25th and 75th percentiles.

RESULTS

Demographics

Patients included one Caucasian man and 32 women: 25 Caucasian, three African-American, three Hispanic and one Asian. Median age was 36.1 years (range 22 to 72). Median age at symptom onset was 26 years; median symptom duration was 6.2 years.

Changes in symptoms after distention

After distention the median total UW score improved significantly (Table 2). However, rates of clinically significant response were low. Twelve patients (36%) had $\geq 30\%$ improvement in total UW score after distention, eight patients (24%) had $\geq 50\%$ improvement, and two patients (6%) had $\geq 80\%$ improvement. Four patients (12%) had a post-distention total UW score less than 10.

Ten patients had worse symptoms after distention. The median change in total UW score for these patients was 4 points (range 1–5 points) and the median percent change was 13.5% (range 6–16%). Two of these patients scored maximum points (42) after distention; they both had scores of 37 before distention.

Changes in markers after distention

After distention, two urine markers had significant changes. These were decreased APF activity and increased HB-EGF levels (Table 3). Considering APF activity as a dichotomous variable (normal or abnormal), all patients had abnormal activity before distention and 18 had normal activity after distention. The lower limit of HB-EGF levels detectable by our assay was 0.3 ng/ml. HB-EGF levels were below this level for 12 patients before distention, but above this level for all patients after distention.

Since distention may decrease APF activity by increasing HB-EGF (see discussion), we tested whether post-distention APF activity was associated with post-distention HB-EGF levels. A significant association was not seen with the Spearman correlation ($r=0.22$, $p=0.21$) nor by comparing the patients with normal vs. abnormal post-distention APF. The median (25th percentile, 75th percentile) post-distention HB-EGF levels were 4.14 (2.93, 15.52) ng/mg creatinine for the normal APF group. For the abnormal APF group the values were median 3.03 (1.73, 5.81) ng/mg creatinine ($p=0.19$).

The Spearman correlation also was used to test associations between EGF and HB-EGF. The pre-distention levels were not associated with each other ($r=0.12$, $p=0.51$). The post-distention levels were positively associated ($r=0.59$, $p=0.0002$). The percent change in EGF after distention also was positively associated with the percent change in HB-EGF ($r=0.51$, $p=0.0043$).

Marker changes vs. symptom changes after distention

No significant associations were found between the percent change in any marker level and the percent change in the total UW score. The closest association was a weak trend between decreased IL-6 and decreased symptom score ($r=0.34$, $p=0.09$). Percent changes in total UW score were similar for the patients with normal vs. abnormal post-distention APF activity.

Symptom changes vs. clinical features or pre-distention markers

There were no associations between percent change in total UW score and pre-distention markers, nor with any of the other clinical features tested. The closest association was a trend for better symptom relief in patients who answered “no” to seeing blood in the urine with IC flares (three yes responses, median change 13.5 % worse, 29 no responses, median change 21.9 % better, $p=0.05$).

DISCUSSION

Symptom improvement after distention is usually transient^{1–3} and response varies. When defined as any degree of improvement, two recent studies had 50–61% response rates.^{1,2} In another study, patients self-rated improvement as excellent, fair or poor. Response rates were 26% excellent and 29% fair (for bladder capacity under anesthesia <600 ml) and 12% excellent and 43% fair (for larger capacities).³ In contrast, we found no association between capacity and symptom response. However, our rates of what we considered “excellent” response were low: two patients had $\geq 80\%$ decrease in total UW score and four had a total UW score <10.

The first goal of this study was to measure changes in urine markers after distention. We found two significant changes: APF activity decreased and HB-EGF increased. This is consistent with previous findings of decreased APF activity and increased HB-EGF at 2–4 hours post-distention and two weeks post-distention.¹¹ Since no direct assay for APF peptide is available, studies to date measured APF activity by bioassay. Therefore, decreased APF activity seen after distention could happen in two ways: (1) decreased APF peptide levels, or (2) a substance that inhibits the antiproliferative effect of APF in the bioassay. For example, HB-EGF inhibits the activity of purified APF on cultured urothelial cells,¹² and detrusor smooth muscle releases HB-EGF when stretched.¹³ If the second mechanism is true, the % change in HB-EGF should associate with the % change in APF activity. We did not find this association, but were limited by the APF bioassay. Inhibition of thymidine incorporation cannot exceed 100%, so a bioassay value approaching 100% could represent a wide range of high APF peptide levels. A lower limit of normal for HB-EGF (ng/mg creatinine) has not been established, so we could not test whether normalized APF activity associated with normalized HB-EGF. It remains possible

that bladder distention decreases APF activity by other mechanisms besides increasing HB-EGF.

Median EGF level for the group was unchanged after distention. Individuals varied; EGF increased in some and decreased in others. Interestingly, post-distention EGF and HB-EGF were significantly associated, as were % increases in EGF and HB-EGF. It remains unknown why distention increased EGF in only a subset of patients, or why this subset had higher post-distention HB-EGF.

Our second goal was to seek associations between biomarker changes and symptom improvement. Such associations might elucidate the mechanisms of symptom relief after distention, allowing development of new, less invasive methods to invoke the relevant mechanism(s). We did not find any such associations, probably because so few patients had significant improvement.

One of our theories was that APF activity in the urine perpetuates epithelial deficiency, and distention decreases APF activity, allowing the epithelium to heal. Our results neither proved nor disproved this theory. Almost all patients had decreased APF activity, but many had persistent symptoms. Their epithelium may have been so severely damaged that it did not heal, even with decreased APF activity, or they may have had neural changes causing persistent pain even if the epithelium healed. It remains unknown what constitutes a significant functional decrease in APF activity, and whether changes in APF activity and/or HB-EGF levels are temporally related to epithelial healing or symptom improvement. Further evaluation would require measurement of APF activity and peptide levels, along with symptom scores and bladder biopsies, both pre-distention and at several time points after distention.

Another theory was that distention has an anti-inflammatory effect. We anticipated this because, in previous studies, good response was associated with evidence of inflammation such as high urine kallikrein activity,¹⁴ severe bladder inflammation,¹⁵ high bladder mast cell counts and high urine prostaglandin E₂.¹⁶ Therefore, we expected the good responders to have high urine levels of inflammatory markers (IL-6, IL-8 and possibly cGMP) before distention, and decreased levels after distention. We did not find these patterns. Pre-distention marker levels had no association with symptom response. Comparing marker changes with symptom changes after distention, we found only a weak trend for association between decreased IL-6 and decreased symptom score. These negative results make it unlikely that distention works by decreasing IL-6, IL-8 or NO, but distention may influence other inflammatory mediators that were not included in this study.

It is also possible that distention improves IC symptoms by other mechanisms. For example, distention may influence neurotransmitter release from bladder sensory nerves or urothelium.^{17,18} Distention may also influence the kallikrein system. Zuraw et al. found that good responders had high urine kallikrein activity before distention, which decreased to normal after distention, while nonresponders had normal kallikrein activity before distention.¹⁴ These intriguing results have not been confirmed in subsequent publications. Rosamilia et al. found a trend towards increased urine kallikrein activity in IC, which did not reach statistical significance.¹⁹ The kallikrein levels in these two studies cannot be compared because the investigators used different assays and normalization procedures.

Our third goal was to find pre-distention markers or other clinical features that were associated with good relief after distention. Such associations would help clinicians improve their outcomes by selecting the patients most likely to respond. In previous studies, good response was associated with low capacity under anesthesia³ and with high mast cell counts¹⁶ and severe inflammation¹⁵ on biopsy. However, these features would not be known before distention. Although high pre-distention urine kallikrein activity¹⁴ and prostaglandin E₂ levels¹⁶ were

predictive in early small studies, none of our pre-distention markers predicted symptom response. We also found no clinical features that predicted good response, consistent with two other recent studies.^{2,3}

A limitation of this study is that only one post-distention time point was used. The one-month point was chosen to avoid the transient symptom flare that often occurs after distention, and to capture good responders before their symptoms recurred. We intended to maximize information while minimizing travel burden on the patients, many of whom live long distances from our centers. However, if we had included more time points, the relationships between markers and symptoms may have been defined more clearly.

Another limitation is the finite number of markers in the panel. Since we could not include every urine component that might be an IC marker, we included only markers that had been previously published as being abnormal in IC. Also, we excluded markers with very expensive or labor-intensive assays, or that are unstable in frozen urine. Therefore, certain possible mechanisms of distention (e.g. related to neuropeptides) were not explored. Also, we could not determine why our inflammatory markers (IL-6 and IL-8) did not show the same relationship with symptom response that was previously described for kallikrein.¹⁵ These markers may reflect different aspects of the inflammatory response, which are not relevant to distention, or our patient population may have been different from the population previously studied.¹⁵ Originally we included methylhistamine to reflect bladder mast cell activation, but the antibody became unavailable and our interim results did not justify developing a new assay.²⁰

Another limitation is that symptoms may be influenced by factors unrelated to biomarker levels, including bias of the physician selecting the patients, the patients' perceptions of the clinicians' expectations, the stress of undergoing the procedure, and/or baseline symptom variability unrelated to treatments. These factors may have influenced symptom scores to such extent that any associations between symptom scores and objective biomarkers were masked. We attempted to minimize symptom variability, and the confounding effects of treatments on symptom scores and marker levels, by including only new patients with no prior IC treatments. However, this choice decreased our sample size, and also may have biased our outcomes. For example, patients with severe inflammation and small bladder capacities, who may be the best responders, are unlikely to present as new untreated patients.

Only 36% of our patients had at least 30% symptom improvement. These results, plus the transient nature of symptom relief,¹⁻³ cast doubt on the role of distention as an initial treatment for new patients.

CONCLUSIONS

After bladder distention for IC, urine APF activity decreased and HB-EGF levels increased. Median symptom scores decreased significantly, but only 36% of patients had $\geq 30\%$ improvement. The mechanism by which distention improves symptoms remains unclear.

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Glossary

KEY OF DEFINITIONS FOR ABBREVIATIONS

IC

Interstitial cystitis

UW

University of Wisconsin

APF

Anti-proliferative factor

HB-EGF

Heparin binding-epidermal growth factor-like growth factor

EGF

Epidermal growth factor

IL-6

Interleukin-6

IL-8

Interleukin-8

cGMP

Cyclic guanosine monophosphate

NO

Nitric oxide

TABLE 1**Questions to Characterize IC Symptoms**

Did IC symptoms start suddenly versus gradually?
Do any foods or drinks make IC symptoms worse? (If yes, subjects are then asked to respond yes or no to six individual foods/drinks.)
Have you been diagnosed by a doctor with irritable bowel syndrome? (yes versus no)
Allergies to medications (yes versus no; if yes, list them)
Allergies to air-borne particles (e.g., dust, pollen, animal hair) (yes versus no; if yes, list them)
Skin contact allergies (yes versus no; if yes, list them)
Does bladder pain improve even temporarily after urinating? (yes versus no)
Do your IC symptoms include burning? (yes versus no)
Do your IC symptoms include bloating? (yes versus no)
Does the IC pain feel sharp or stabbing? (yes versus no)
Does the IC pain feel like a dull ache? (yes versus no)
Does the IC pain improve with standing up? (yes versus no)
Is the main area of IC pain the right lower quadrant? (yes versus no)
Does visible hematuria occur with IC flares? (yes versus no)
Do you feel a constant urge to urinate that never goes away? (yes versus no)

TABLE 2
UNIVERSITY OF WISCONSIN SYMPTOM SCORES BEFORE AND ONE MONTH AFTER BLADDER DISTENTION

Symptom	N	Before		After		P-value
		Median	p25, p75*	Median	p25, p75*	
Bladder discomfort	32	4.0	3.0, 5.0	3.0	2.0, 5.0	0.21
Bladder pain	32	3.5	2.0, 4.5	2.0	0.0, 4.0	0.04
Nocturia	33	5.0	3.0, 6.0	4.0	2.0, 6.0	0.003
Daytime frequency	33	6.0	4.0, 6.0	4.0	3.0, 5.0	< 0.001
Difficulty sleeping	33	5.0	3.0, 6.0	3.0	1.0, 6.0	0.004
Urgency	33	5.0	3.0, 6.0	4.0	1.0, 6.0	0.003
Burning	33	2.0	0.0, 4.0	1.0	0.0, 3.0	0.13
Total UW score	32	28.5	23.5, 33.5	20.0	14.0, 30.0	< 0.001

* 25th percentile, 75th percentile

TABLE 3

URINE MARKERS BEFORE AND AFTER BLADDER DISTENTION

Marker	N	Before		After		P-value
		Median	p25, p75*	Median	p25, p75*	
APF**	33	-96.0	-97.0, -91.0	-17.0	-37.0, -20.0	< 0.001
HB-EGF (ng)	33	0.3	0.2, 0.7	4.1	2.2, 10.4	< 0.001
EGF (ng)	33	25.0	17.6, 48.9	22.3	5.5, 27.0	0.04
IL-6 (pg)	31	3.1	1.8, 7.0	4.5	1.9, 11.8	0.10
IL-8 (pg)	20	5.5	0.0, 26.3	5.4	0.0, 21.4	0.28
cGMP (nmoles)	21	235	154, 401	245	126, 389	0.92

* 25th percentile, 75th percentile

** APF is expressed as % inhibition of thymidine uptake. The other markers are expressed as the unit in parentheses per mg creatinine.