Inflammation and Inflammatory Control in Interstitial Cystitis/Bladder Pain Syndrome: Associations with Painful Symptoms

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Introduction

Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is a debilitating chronic condition associated with urinary symptoms including urgency, frequency, nocturia and pelvic pain. Berry and co-authors report the prevalence of IC/BPS at between 3 and 8 million women in the United States [3]. Few patients achieve long-term remission of symptoms despite a range of treatments [1,33]. A substantial body of research has explored bladder pathology and other peripheral tissue abnormalities, however, a large number of IC/BPS patients lack any discernible end organ inflammation, and do not respond to treatment of peripheral tissues (e.g. hydrodistension of bladder) [31]. This suggests that investigation of more systemic factors (e.g., inflammation, inflammatory control) may yield important insights. IC/BPS patients have a high prevalence of comorbid Functional Somatic Syndromes (FSSs), such as irritable bowel syndrome (IBS) and fibromyalgia (FM), which are also characterized by chronic pain in the absence of clearly identifiable peripheral pathology [6,28]. This suggests that altered central pain processing (e.g. hyperalgeisa, allodynia), termed central sensitization [49] could be a contributory factor to the chronic pain seen in IC. Consistent with this notion, a recent study found that IC/BPS patients have reduced pain thresholds and pain tolerance compared to healthy controls [25].

Toll-Like Receptors (TLR), particularly TLR-4, have been identified as critical factors in central pain sensitization. Evidence in animal models of chronic pain suggests that inflammatory signaling secondary to TLR-4 stimulation plays a critical part in the development of hyperalgesia and allodynia [10,14,24]. Preliminary evidence suggests that

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heightened inflammatory responses to TLR stimulation may be a feature of human pain syndromes as well [19], but these have not been evaluated in IC/BPS. Further, no studies have determined if inflammatory responses to TLR stimulation are associated with the magnitude of painful symptoms. Endogenous control of inflammation, one of the central functions of the Hypothalamic-Pituitary-Adrenal (HPA) axis may also be a critical factor in IC/BPS. We previously found that higher levels of morning salivary cortisol, an endogenous HPA axis glucocorticoid with antiinflammatory properties, are associated with less severe symptoms in IC/BPS patients [23]. TLR-mediated inflammation and HPA axis activity may be interactive, as glucocorticoids have been shown to potentiate inflammatory responses in animal models of pain [21]. The goals of the current study were to identify differences in inflammatory processes, including those mediated by TLRs, and HPA axis function as assessed by diurnal cortisol between IC/BPS patients and healthy controls, and to determine whether these factors were associated with IC/BPS symptoms.

Methods

MAPP Study and Recruitment

The Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) study is a multi-site research effort sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases to better characterize syndrome phenotypes, syndrome etiology, and the natural history of chronic urologic pelvic pain patients [50]. The University of Iowa is a participating institution collecting site-specific data on biomarkers of chronic pelvic pain. Inclusion criteria for IC/BPS included being at least 18 years of age and reporting pain, pressure or discomfort associated with the bladder or pelvic region present the majority of the time during the most recent 3 months. Additionally, IC/BPS participants were negative on urine culture for any uropathogens. Exclusion criteria included a history of urethral stricture, neurological disorder affecting the bladder or bowel, cystitis caused by tuberculosis, radiation therapy or Cytoxan/cyclophosphamide therapy, augmentation cystoplasty or cystectomy, active autoimmune or infectious disorder, history of cancer, major psychiatric illness, or cardiac, pulmonary, renal, or hepatic disease, or pregnancy. Of the 98 female Iowa IC/BPS and HC participants enrolled in the broader trans-MAPP study at the University of Iowa, 86 (88%) agreed to an additional blood draw for isolation of PBMCs, and 72 (73%) agreed and were able to collect salivary cortisol. Study participants were 58 (48 for cortisol analyses) IC/BPS patients and 28 (24 for cortisol analyses) HCs who met study criteria. In addition to meeting exclusion criteria, healthy controls had no urinary, pelvic, or bladder symptoms, and met no criteria for common FSSs IBS, FM, or Chronic Fatigue Syndrome [9,12,48]. All participants provided informed consent and all procedures were cleared by the Institutional Review Board of the participating MAPP institutions.

Demographic and Symptom Information—Participants provided demographic information at the time of eligibility screening. Upon study entry, participants had a blood draw, urine collection, physical examination and completed a battery of trans-MAPP questionnaires relating to pelvic and bladder symptoms which have been previously used to assess pain and symptom severity in urologic conditions. These included the 9-item

Genitourinary Pain Index (GUPI)[7], the 19-item Female Sexual Functioning Inventory (FSFI) [34], and the 4-item Interstitial Cystitis Symptom Index (ICSI) [29]. The GUPI includes pain and urinary symptom subscales. The pain subscale contains questions indicating the number of painful areas in the genitourinary region (e.g. the bladder, urethra), the number of activities (i.e. filling, voiding of bladder) that are painful, and two questions relating to the frequency and intensity of pain experience regardless of area or activity. The FSFI contains 3 questions relating specifically to the frequency and intensity of pain during and after intercourse. The ICSI contains questions specific to bladder pain, and to the urgency and frequency of urination and nocturia.

Cortisol—Salivary cortisol was collected in salivettes by participants at 3 time points (upon waking: 4–9am, afternoon: 4–6:30pm, and bedtime: 8pm-12am) for three consecutive days. Samples collected outside this time frame were excluded to maintain homogeneity. Participants were instructed not to eat, exercise or consume caffeine for thirty minutes prior to collecting a sample. Self-report of collection time has been demonstrated to be reliable and salivary cortisol is stable at room temperature [18]. Salivettes were analyzed by chemiluminescence immunoassay (IBL, Hamburg, Germany) at the Technical University of Dresden. The lower detection limit is 0.41 nmol/L and inter-assay and intra-assay coefficients of variance are less than 10%.

Inflammatory Measures—Blood samples were collected between approximately 11:30am and 12:30pm. PBMCs were separated by Ficoll-paque gradient centrifugation within 30 minutes of blood collection and cultured in RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 ug/ml streptomycin for 3 days at 37°C in a humidified incubator with 5% CO_2 and TLR agonists. TLR-2 and 4 agonists were selected on the basis of the role of these receptors in chronic pain in animal models [2,14]. For stimulation of TLR-4, 50 ng/ml of Lipopolysaccharide (LPS) was used; For TLR-2 stimulation, 0.04 ng/ml of Staphylococcus aureus Cowan I (SAC) was used. Conditioned media was then harvested and frozen at −80°C prior to batch ELISA analysis. Each well contained $1×10^6$ cells in 24 well plates, with one well per subject for TLR-4 and TLR-2 stimulation. Cytokines were assayed in duplicate by DuoSet ELISAs (R&D Systems) according to instructions included with the kit. Plasma cytokines were assayed with high sensitivity Quantikine ELISAs. Similar approaches in populations with complex presentations of pain have been reported and are able to distinguish patients from healthy controls [5,16,17,19].

Statistical analyses—Statistical analyses were performed using SPSS v. 21. Inflammatory variables (plasma interleukin-6 (IL-6), pro-inflammatory cytokine response to TLR stimulation in PBMCs) were log-10 transformed to normalize their distribution. Composite inflammation scores for stimulated cytokine responses were calculated by summing the z-scores ([individual score- group mean]/ group standard deviation) for the IL-6 and interleukin-1 beta (IL-1β) response in PBMCs following either LPS or SAC stimulation. Both cytokines have been identified as mediators of pain amplification by spinal glia and are released following TLR-2 and 4 stimulation, in part, by transcription of nuclear factor-kappaB (NFκB) [24]. Cortisol values were normalized using natural log

transformations. Distributions of transformed variables were examined for confirmation of normality. Salivary cortisol values at each of the collection points were regressed on the time of collection over the three-day period to calculate cortisol slope, a measure of the average hourly decrease in cortisol over the course of the day as described previously [18]. To determine if diurnal patterns of cortisol secretion differed between groups, a repeated measures ANOVA was used with time of cortisol sample (morning, afternoon, nocturnal) and group membership as factors. A significant time by group interaction indicates different patterns of diurnal secretion between groups. Post-hoc comparisons with Sidak adjustment were used to determine which, if any, time-points differed in salivary cortisol concentrations between groups.

BMI was used as a covariate in all analyses of biomarkers due to well-established relationships between adiposity and inflammation [11,47]. While all participants were free of major psychiatric diagnoses, levels of negative affect (i.e. anxious and depressive symptoms) have previously been linked to self-reported pain measures in chronic pain populations so the participant score on the Positive and Negative Affect Schedule (PANAS) negative affect scale was included as a covariate in analyses of symptoms [39,42]. Group differences between IC/BPS and HC in demographic and symptom data were tested by one-way analysis of variance (ANOVA) and chi‾squared tests. Within IC/BPS participants, One-way ANOVAS were used to test mean differences in inflammatory variables for the use of tri-cyclic antidepressants, pentosan polysulfate, opioids, SSRI/SNRIs, and NSAIDs and the presence of a comorbid FSS. Differences in inflammatory variables between IC/BPS and HC were tested with General Linear Models controlling for BMI. Relationships between inflammatory variables and symptom scores were assessed using multiple regression controlling for BMI and negative affect.

Because use of tricyclic anti-depressants and the presence of a comorbid FSS were each associated with marginal differences in TLR responses, these variables were also controlled for in additional analyses to determine if the magnitude of the association between inflammatory variables and self-reported painful symptoms remained statistically significant and comparable to reduced models. Similarly, because duration of symptom in years was associated with TLR-4 inflammatory response, models were also tested which controlled for duration of symptoms. To determine which inflammatory measures had the strongest association with painful symptoms, all inflammatory variables (IL-6, cortisol slope, TLR-2 inflammatory score, and TLR-4 inflammatory score) were used simultaneously as predictors of painful symptom scores in models with BMI and negative affect.

To determine if dysregulated HPA activity and heightened TLR-4 inflammatory responses combine to exacerbate symptoms of pain, we divided patients on median splits of cortisol slope (steep, i.e. healthier; flat, i.e. less healthy) and TLR-4 inflammatory response, and compared measures of pain in the resulting 4 groups by One-Way ANOVA. Post-hoc comparisons were conducted with Sidak adjustment.

Results

Participant Characteristics

The mean age of participants was approximately 42 years (range 20–68). IC/BPS patients and healthy controls did not differ on potential confounding variables such as income, education, race, ethnicity, employment status or age (all p>.15). BMI of HCs was significantly elevated compared to that of IC/BPS patients (*p*=.021). As expected, IC/BPS participants reported more genitourinary symptoms including pain and urinary dysfunction, elevated IC symptoms, and more compromised sexual functioning (all *p*<.004) when compared to HCs. In IC/BPS patients the average duration of symptoms was 7.7 (SD=7.3) years. See Table 1. IC/BPS participants on tricyclic antidepressants (n=29, 50%) showed marginally lower IL-6 responses following TLR-4 stimulation (*p*=.056) and lower IL-6 following TLR-2 stimulation (*p*=.044) but no differences in any other inflammatory measure (*p* values>.42). IC/BPS participants on opioids (n=11, 19%), pentosan polysulfate (n=29, 50%), SSRI/SNRIs (n=9, 16%), or NSAIDs (n=7, 12%) did not differ on any inflammatory variable from patients not taking those medications (all *p*>.12). The presence of a comorbid FSS was associated with marginally higher IL-1β following TLR-4 stimulation (*p*=.07) but no differences in any other inflammatory variable (*p* values>.27).

Inflammatory Markers and Cortisol in IC/BPS patients and Healthy Controls

Cortisol slope was significantly flatter (*p*=.010) in IC/BPS participants compared to HCs, indicating a smaller hourly decrease in salivary cortisol for IC/BPS patients. Additionally, the results of the repeated measures ANOVA revealed a significant time by group interaction (*p*=.016). Post-hoc comparisons revealed no significant differences in morning cortisol (*p*=.14) but marginally higher afternoon cortisol (*p*=.07) and significantly higher nocturnal cortisol (*p*=.019) in the IC/BPS group, indicating a blunting of the diurnal cortisol rhythm. See Figure 1. As seen in Table 2, IC/BPS participants had significantly elevated plasma IL-6 compared to HC's (*p*=0.040), suggesting an elevated level of basal inflammation. When stimulated with TLR-2 receptor agonist SAC, PBMCs isolated from IC/BPS participants demonstrated greater IL-1β responses (*p*=.040), but no difference in IL-6 responses (*p*=.10), compared to PBMCs of HC participants. There were no statistically significant differences between IC/BPS and HC participants in the pro-inflammatory cytokine response in LPS stimulated PBMCs (see Table 2.)

Associations between Inflammation and IC/BPS Symptoms

The TLR-4 inflammation score was associated with multiple measures of non-specific pain intensity and frequency. The composite TLR-4 inflammation score (calculated from the response of IL-6 and IL-1β to LPS) was significantly associated with higher total GUPI scores (*p*=.005), GUPI pain subscale scores (*p*=.010), and marginally with GUPI urinary symptom subscale scores (*p*=.062). On the GUPI pain subscale, the relationship with TLR-4 inflammation score was strongest for two items; pain frequency (*p*=.001) and intensity (*p*=.008). The TLR-4 inflammation score was also associated with reduced FSFI sexual functioning (*p*=.001); the strongest relationships were seen with pain variables in this scale: e.g., pain frequency during intercourse (p=.002), pain frequency after intercourse (p=.002), and sexual pain severity (*p*=0.003). The TLR-4 inflammation score was marginally

associated with higher IC symptom index scores (p=.068). However, there were no significant relationships between the TLR-4 inflammation score and the ICSI urinary urgency, frequency, or nocturia items (*p-values* > 0.17) or with the pain/burning specific to the bladder item (*p*=.13). Interestingly, TLR-4 inflammatory response was associated with longer duration of symptoms (years; *p*=.047) and with flatter cortisol slopes (*p*=.043). In contrast to these findings, TLR-2 inflammatory response for SAC-stimulated PBMCs, plasma IL-6, and cortisol slope were not significantly associated with any symptom scale (all *p*>.10). See Table 3 for associations of TLR-4 inflammatory response with scales. See Table 4 for associations of TLR-4 inflammatory response with specific items.

The relationships between TLR-4 inflammation scores and symptom scales were not attenuated or rendered insignificant when analyses adjusted for use of tri-cyclic anti-depressants and presence of comorbid FSSs, nor were either of these associated with differences in painful symptoms (*p* values >.19). Adjusting for the duration of symptoms in years similarly did not affect the relationship between TLR-4 inflammation and painful symptoms and duration of symptoms was not itself associated with pain (*p* values > 0.14). The relationship between TLR-4 inflammation score and painful symptoms was similarly unaffected when all other inflammatory variables were included simultaneously as predictors of painful symptoms, and no other inflammatory variable was associated with pain in these models (*p* values >.16).

Secondary Analyses

Consistent with the hypothesis that HPA dysregulation and TLR-4 inflammation may act synergistically on measures of pain, we found that, amongst the four groups, the most severe pain scores on the GUPI pain subscale, GUPI pain frequency and severity items, and FSFI pain subscale were all reported in the group with flatter cortisol slopes and high TLR-4 inflammatory responses (Table 5). Despite relatively small groups, post-hoc comparisons demonstrated that differences between the flat cortisol slope/high TLR-4 inflammation group and steep cortisol slope/low TLR-4 inflammation group were statistically significant for the GUPI pain frequency and FSFI pain scores (both *p*<.027). See Figure 2.

Discussion

One of the major findings of this study is that TLR-4 stimulated inflammatory cytokine responses are robustly associated with measures of painful symptoms in women with IC/BPS. These findings were not altered when controlling for multiple other measures of inflammation and potential confounding variables. The symptoms most strongly associated with TLR-4 stimulated inflammatory responses were non-specific pain severity and pain frequency. Further, IC/BPS is marked by inflammatory dysregulation, indicated by flattened diurnal cortisol rhythmicity, elevated plasma IL-6 and an exaggerated IL-1β response to TLR-2 stimulation in PBMCs. TLR-2 stimulated IL1-β responses in PBMCs were recently shown to be capable of differentiating a population of chronic pain patients from HCs [19]. This study extends that finding to a chronic pelvic pain population. More generally, these findings are in agreement with the results of other studies demonstrating that inflammation from TLR stimulated PBMCs are able to differentiate patients with Inflammatory Bowel

Disease, recent onset rheumatoid arthritis, and persistent fatigue from healthy controls [5,16,17]. Importantly, none of the aforementioned studies determined if the magnitude of the response to TLR stimulation was associated with the magnitude of reported painful symptoms, or examined HPA activity in conjunction with TLR responses. Ours is the first study we know of to report these associations between pain and TLR inflammatory response in a clinical population; importantly, this sample was screened extensively for medical comorbidities and conditions which could simultaneously affect both inflammation and pain (e.g. recurrent bacterial infection) and many potential confounding variables were controlled. For instance, while the use of tri-cyclic anti-depressants was associated with lower TLR inflammatory responses, its inclusion as a covariate had no effect on the relationship between TLR-4 mediated inflammation and pain. In models controlling for all other measures of inflammation, the relationships between TLR-4 inflammatory responses and pain scales were not attenuated; this provides strong evidence that TLR-4 mediated inflammation specifically plays a critical role in IC/BPS pain symptoms. These findings may be related to recent work demonstrating that experimental induced endotoxemia via LPS administration reduces pain thresholds in healthy subjects [8].

The present findings indicate that HPA dysregulation is a feature of IC/BPS. Compromised GC diurnal variation has been associated with a reduced ability to respond to acute stressors with rapid GC release [37]. This may be of particular importance in IC/BPS, where symptom flares are often preceded by acute psychosocial stress, and these findings suggest one potential process by which this may occur [35]. Thus, HPA dysregulation may limit the ability of IC/BPS sufferers to control pain-promoting inflammation; the association of flattened cortisol slope with greater TLR-4 inflammatory cytokine responses suggests that this is true. Early life traumatic events, such as sexual trauma, are associated with HPA axis dysregulation, and there is evidence that chronic pain patients, including pelvic pain patients, report more early life trauma [30].

It is now well established that pro-inflammatory TLR-4 activation of spinal cord glial cells is a key factor in the development and maintenance of chronic pain [24,10,14]. It has been hypothesized that PBMC cytokine responsiveness to TLRs may mirror the cytokine responsiveness to TLRs in the glial cells of the spinal cord, and a recent study in a sciatic constriction animal model of pain demonstrated concordance between TLR-2 and TLR-4 stimulated PBMCs and inflammation assayed in the supernatant of lumbar spinal cultures [19,20]. Therefore, it is possible that the difference in TLR-2 inflammation between IC/BPS and HCs reflects underlying processes in the spinal cord. That TLR mediated inflammation in PBMCs would distinguish pain populations as different in presentation as IC/BPS patients and the pain group used in Kwok, et al. (primarily chronic back, shoulder and leg pain, and osteoarthritis) is noteworthy [19]. Inflammation in PBMCs may therefore serve as a useful biomarker of persistent pain in clinical settings and may help phenotype pain patients. In animal models, TLR-4 stimulation specifically in spinal glial cells has been shown to lead to amplified ascending pain signaling via, in part, the release of pro-inflammatory cytokines This TLR-4-stimulated glial activation is thought to underlie the initiation of chronic pain and its extension beyond the original site [24]. However, recent experiments in preclinical models of pain call into question the role of TLR-4 in female pain. These found that TLR-4 stimulation in the spinal cord only produced a heightened pain response in male mice, an

effect possibly mediated by testosterone [40], and that TLR-4 knockout did not reliably attenuate the pain response in female mice [41]. As administration of TLR-4 agonists to the brain and hindpaw still produced heightened pain in both female and male mice, this sex difference may be limited to direct stimulation of the spinal cord [40]. Clearly, further mechanistic investigation with an animal model of IC/BPS would be required to determine relevant sex differences in TLR activity for IC/BPS.

That TLR-4 mediated inflammation was a robust predictor of painful symptoms in female IC/BPS patients is interesting, as interactions between TLR-4 mediated inflammation and sex hormones have been posited as a one potential mechanism in the greater prevalence of pain conditions in women [27]. Glial cells are known to express estrogen receptors [27], and chronic estrogen stimulation *in vivo* enhances pro-inflammatory gene expression in microglia following LPS stimulation [4]. Further, IC/BPS symptoms are known to differ according to phases of the menstrual cycle [32]. Therefore, exploring the interaction between TLR mediated inflammation and sex hormones/phases of the menstrual cycles may reveal insight into the painful symptoms experienced by female IC/BPS patients.

Importantly, TLR-4 responsiveness was associated most strongly with pain frequency and intensity, rather than specific urologic symptoms (i.e. pain on filling of the bladder) or anatomical regions (i.e. the urethra). This suggests that peripheral tissue inflammation or damage is not solely responsible for the initiation of painful signaling, and is consistent with the hypothesis that some pain in IC/BPS may be mediated by central pain amplification. The marginal difference in IL-1β response to TLR-4 stimulation between IC patients with pelvic pain only and comorbid FSSs is intriguing and requires further investigation. That TLR-4 stimulated inflammatory cytokine responses are associated with longer symptom duration and flattened cortisol slopes raises the possibility that that TLR-4-mediated pain may be progressive. Other studies have demonstrated that longer symptom duration is associated with a greater number of problematic symptom domains in IC/BPS [26]. These findings suggest possible parallel physiologic processes. Glucocorticoid signaling is known to mediate sensitization of microglial proinflammatory responses, a pathway that may additionally provoke stress-induced symptom flares in IC/BPS [21]. Analyses demonstrating that the most severe pain was experienced by those with flat cortisol slopes and high TLR-4 inflammatory responses provides some evidence that inflammatory responses and poor endogenous inflammatory control may contribute to pain exacerbation synergistically, though longitudinal analyses are required to clarify this relationship. Compromised inflammatory control may permit sensitization of TLRs in immune cells and the spinal cord, and repeated inflammatory insults from reactive immune cells may disrupt HPA activity; more likely, the relationship is bi-directional.

These novel findings offer compelling evidence that the inflammatory response to TLR-4 stimulation is associated with pain in IC/BPS patients, and suggest that PBMC responsiveness to TLR-2 stimulation holds promise as a biomarker for IC/BPS pain. Importantly, these findings also suggest that TLR-4 may be a therapeutic target in IC/BPS. Animal research has demonstrated that TLR4 antagonism can reverse neuropathic pain in a sciatic nerve constriction model [14]. Further, recent work has demonstrated that TLR-4

antagonism may increase the effectiveness of opioids while reducing tolerance and dependence [13].

Limitations

These analyses were cross-sectional and thus do not provide information about causality or about the longitudinal course of IC/BPS symptoms, nor can temporal precedence for any measure of inflammatory dysregulation be established. We used self-report measures of pain; future work should incorporate quantitative sensory pain tests. We used a single concentration of LPS; thus it is possible that examination of dose-response kinetics with higher concentrations may have revealed that TLR4 cytokine response can differentiate between IC/BPS and HCs as reported by Kwok et al. in other chronic pain populations [19].

Future Directions

Understanding the longitudinal course of TLR-4 mediated pain is a critical endeavor. In particular, it would be important to determine if TLR-4 mediated pain is an early or late feature of IC/BPS pain, and if it is only an important mechanism in a subset of patients. Animal models need to be developed to allow for greater mechanistic investigation of TLR-mediated pain in IC/BPS, and to confirm if PBMC cytokine responsiveness is reflective of glial cytokine responsiveness in the spinal cord. Additionally, the role of TLR-2 mediated inflammation, which differentiates IC/BPS patients from HCs, but does not predict painful symptoms, needs to be elucidated. Characterizing TLR-2 and TLR-4 receptor expression in IC/BPS patients may provide important insights, as might investigation of cytokine and TLR polymorphisms. Finally TLR-4 antagonists may provide an important avenue of future treatment for those suffering from IC/BPS.

Acknowledgments

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Inflammatory dysregulation marks Interstitial Cystitis/Bladder Pain Syndrome. Inflammatory responses in Peripheral Blood Mononuclear Cells following Toll-Like Receptor 4 stimulation predict the magnitude of painful symptoms.

Toll-Like Receptors (TLR) are known to play a role in chronic pain from animal models and limited research in humans, but their role in Interstitial Cystitis/Bladder Pain Syndrome (IC/BPS) is unknown. Similarly, alterations of the Hypothalamic Pituitary Adrenal axis have been reported in some pain conditions. Our objectives were to identify inflammatory processes which might distinguish IC/BPS sufferers from healthy controls (HC) and to examine their associations with IC/BPS symptoms. Female participants (58 IC/BPS and 28 HC) completed pain and urinary symptom questionnaires and collected saliva for cortisol as part of the Multidisciplinary Approach to Pelvic Pain study. Inflammatory cytokines were assayed in plasma, and in TLR2 and TLR4- stimulated peripheral blood mononuclear cells. Controlling for BMI and negative affect, between group differences were analyzed by General Linear Models and relationships between symptoms and inflammatory variables were analyzed by regression. Compared to HCs, IC/BPS patients had higher levels of plasma interleukin-6 (p=.040), greater interleukin-1β responsive to TLR2 stimulation (p=.040) and flatter diurnal cortisol slopes (p=.010), indicating inflammatory dysregulation. In IC/BPS, inflammation following TLR4 stimulation was associated with multiple symptoms, including genitourinary pain (p=.010), sexual pain (p=.002), and marginally with urinary symptoms (p=.068). Genitourinary pain severity (p=.008), frequency (p=.001) and pain with intercourse (p=. 002) were strongly associated with TLR4 inflammatory response. TLR4 appears to play a central role in painful symptoms of IC/BPS sufferers, which may be linked to poor endogenous inflammatory control. These findings may help identify new mechanisms in IC/BPS and lead to new therapeutic approaches.
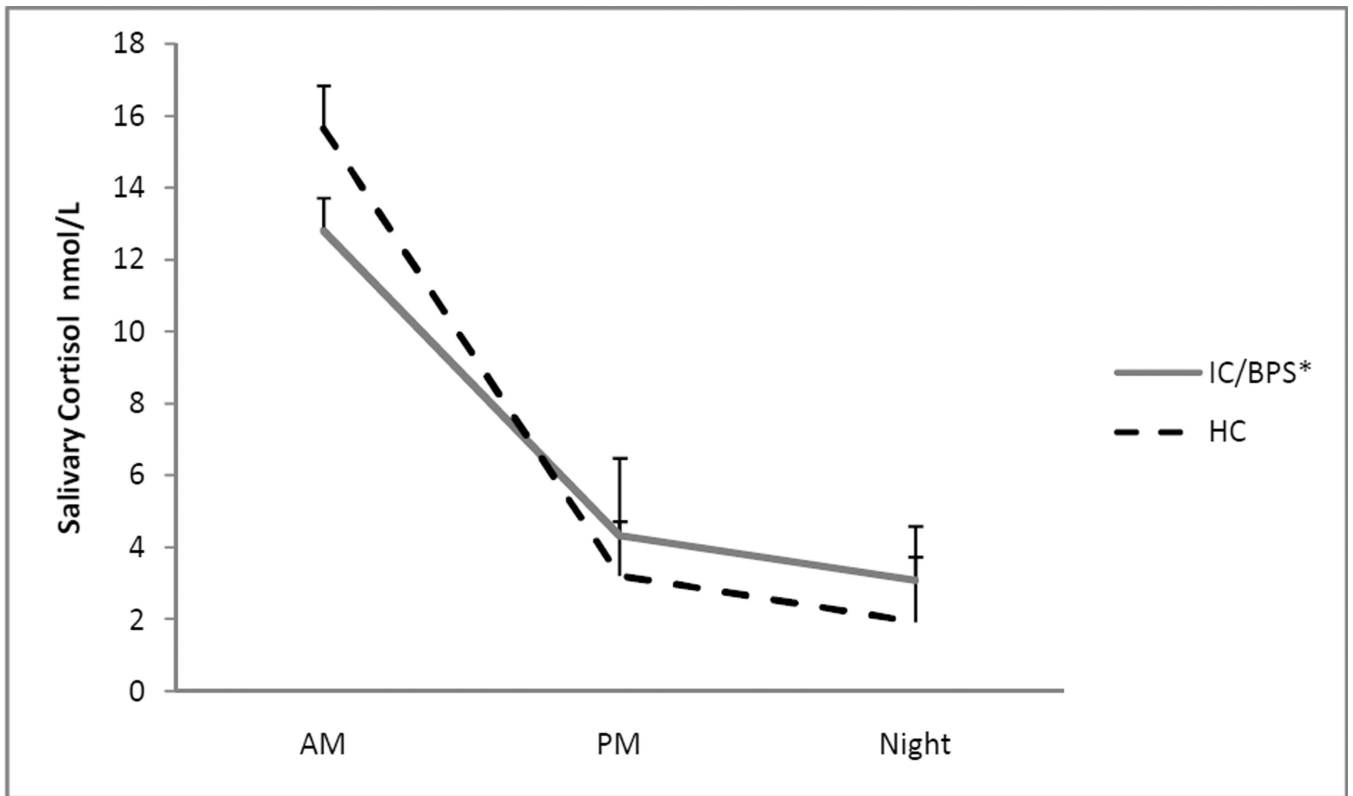
Figure 1.
Means and standard deviations of diurnal salivary cortisol concentrations in IC/BPS participants and HCs. Natural log-transformed values are back transformed.
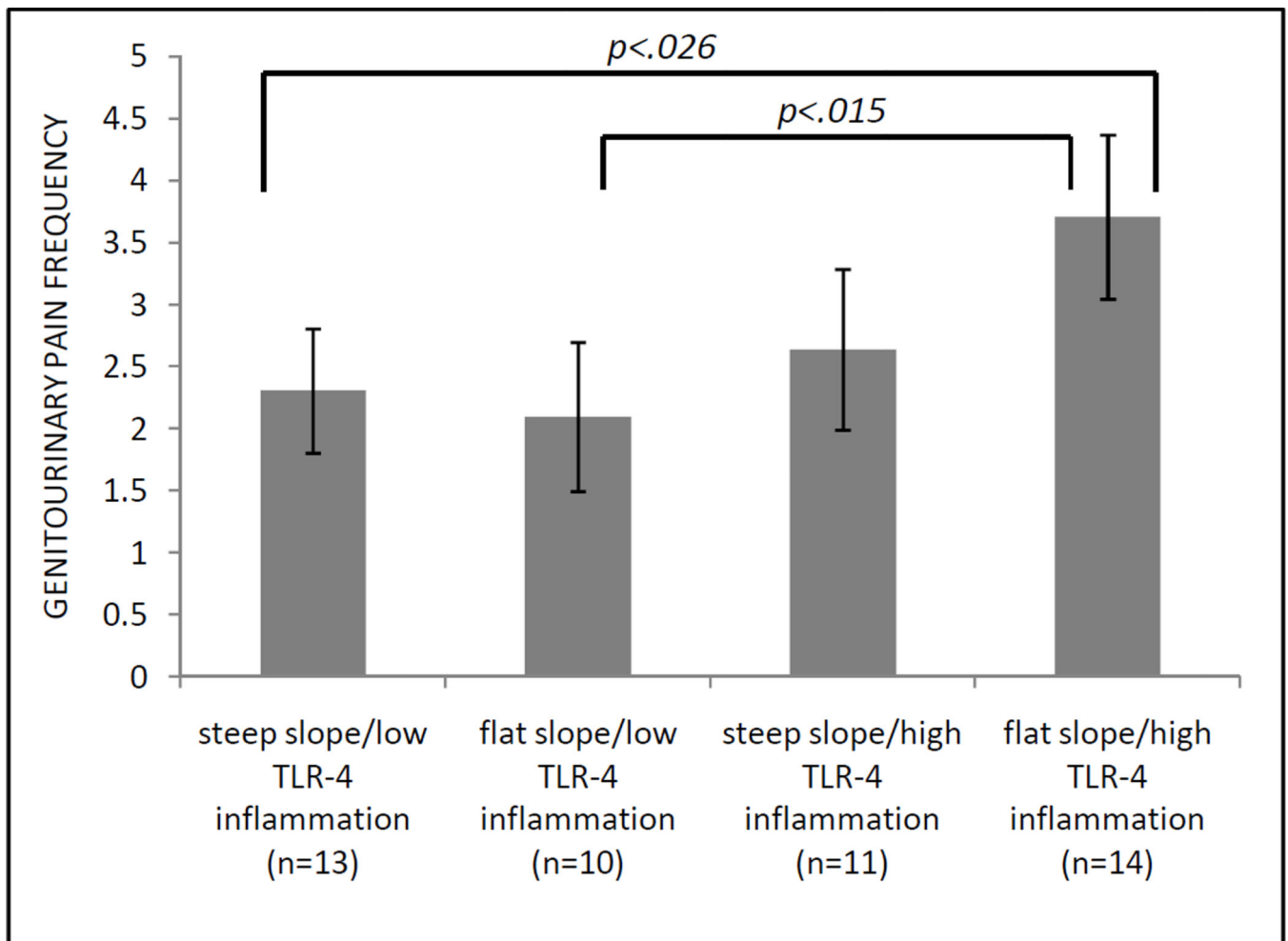*Significant time x group interaction in repeated measures ANOVA ($F_{2,68}$ =4.414, p=.016)

Figure 2.
Means and standard deviations of GUPI pain frequency item for IC/BPS participants grouped by median split of cortisol slope and TLR-4 inflammation response. GUPI=Genitourinary Pain Index. TLR=Toll-Like Receptor.

Table 1

Participant Characteristics. IC/BPS=Interstitial Cystitis/Bladder Pain Syndrome. BMI=Body Mass Index.

	IC/BPS *n*=58	Healthy Controls *n*=28	*p*
Age Mean(SD)	41.1 (14.8)	42.9 (13.1)	.59
BMI Mean(SD)	27.2 (5.8)	30.5 (6.9)	.021
Race % (*n*)			
White	97 (56)	100 (28)	.61
Asian	2 (1)	0	
Multi Race	2 (1)	0	
Ethnicity % (n)			
Non-Hispanic	100 (58)	100 (28)	n/a
Education % (*n*)			
High School or GED	14 (8)	11 (3)	.93
Some College	24 (14)	25 (7)	
Graduated College	33 (19)	39 (11)	
Graduate Degree	29 (17)	25 (7)	
Employment % (*n*)			
Employed	60 (35)	90 (25)	.15
Unemployed	12 (7)	4 (1)	
Disabled	9 (5)	0 (0)	
Retired	7 (4)	4 (1)	
Full Time Homemaker	10 (6)	4 (1)	
Annual Income/$ % (*n*)			
<10,000	14 (8)	7 (2)	.92
<25,000	7 (4)	7 (2)	
<50,000	21 (12)	18 (5)	
< 100,000	36 (21)	46 (13)	
> 100,000	17 (10)	18 (5)	
Prefer not to answer	5 (3)	4 (1)	
Comorbid Conditions % (*n*)			
None	33 (47)	0 (100)	N/A
Irritable Bowel Syndrome	23 (40)		
Fibromyalgia	2 (3)		
Chronic Fatigue Syndrome	8 (14)		

Table 2

Symptoms and Biomarkers in IC/BPS patients and Healthy Controls. Inflammatory variables are adjusted for BMI. GUPI=Genitourinary Pain Index, IC= Interstitial Cystitis, BPS=Bladder Pain Syndrome, FSFI=Female Sexual Functioning Inventory. IL-6=Interleukin-6. IL-1β=Interleukin-1 beta. LPS= Lipopolysaccharide. SAC= Staphylococcus aureus Cowan.

Variable Mean (95% CI)	IC/BPS *n*=58	Healthy Controls *n*=28	*p*
GUPI score total	24.7 (22.7,26.8)	1.4(.5,2.3)	<.001
GUPI score pain	12.0(10.8,13.1)	.3(−.1,.6)	<.001
GUPI score urinary subscale	5.6(4.8,6.4)	.7(.3,1.1)	<.001
IC Symptom Index	10.5(9.3,11.6)	2.5(1.6,3.5)	<.001
FSFI total	16.5(13.78,19.1)	25.3(21.2,29.5)	<.001
FSFI Pain	6.00(4.5,7.4)	11.9(9.7,14.1)	<.001
Morning Cortisol[a] nmol/L	2.55 (2.40, 2.71)	2.75 (2.54, 2.96)	.14[c]
Afternoon Cortisol[a] nmol/L	1.48 (1.28, 1.67)	1.17 (.90, 1.44)	.07[c]
Nocturnal Cortisol[a] nmol/L	1.13 (.91, 1.36)	.66 (.35, .97)	.019[c]
Cortisol Slope[a] nmol/L, hourly decrease	−.10(−.12, −.08)	−.14(−.17, −.12)	.010
IL-6 plasma[b] pg/mL	.45(.39,.52)	.34(.25,.43)	.040
IL-β + LPS[b] pg/mL	3.34(3.16,3.50)	3.53(3.27,3.77)	.24
IL-β + SAC[b] pg/mL	1.22(.93,1.52)	.67(.24,1.10)	.039
IL-6 + LPS[b] pg/mL	4.20(4.09,4.31)	4.13(3.96,4.29)	.48
IL-6 + SAC[b] pg/mL	1.71(1.35,2.07)	1.15(.63,1.67)	.09

[a] natural log transformed

[b] log 10 transformed

[c] post-hoc comparison by Sidak adjustment

Table 3

Relationship of TLR-4 inflammatory response with symptom scores (controlling for BMI and negative affect), and inflammatory variables (controlling for BMI) in IC/BPS participants (n=58). GUPI=Genitourinary Pain Index, IC= Interstitial Cystitis, BPS=Bladder Pain Syndrome, FSFI=Female Sexual Functioning Inventory. IL-6=Interleukin-6. LPS= Lipopolysaccharide. SAC= Staphylococcus aureus Cowan. TLR=Toll-Like Receptor.

TLR-4 (LPS) inflammation score		
	β	*p*
GUPI score total	.333	.005
GUPI score pain	.310	.010
GUPI score urine	.252	.062
FSFI total	−.407	.001
FSFI pain	−.393	.002
IC symptom Index	.246	.068
Cortisol slope	.299	.043
Duration of symptoms (years)[a]	.256	.047
TLR-2 (SAC) inflammation score	.236	.080
Plasma IL-6 pg/mL	.037	.78

[a] adjusted for age

Table 4

Relationships between TLR-4 inflammatory response and scale items controlling for MI and negative affect. Lower FSFI scores reflect worse symptom severity. GUPI=Genitourinary Pain Index, ICSI= Interstitial Cystitis Symptom Index, BPS=Bladder Pain Syndrome, FSFI=Female Sexual Functioning Inventory.

Item	β	*p*
GUPI pain frequency	.401	.001
GUPI pain intensity	.339	.008
FSFI pain frequency during intercourse	−.405	.002
FSFI pain frequency after intercourse	−.389	.002
FSFI pain severity	−.368	.003
Urinary Urgency (ICSI Q1)	.174	.18
Urinary Frequency (ICSI Q2)	.117	.40
Nocturia (ICSI Q3)	.182	.18
Burning/Pain in Bladder (ICSI Q4)	.201	.13

Table 5

Means and standard deviations of selected scales and items by patient groups divided on median splits of cortisol slope and TLR-4 inflammatory response. Steeper cortisol slopes indicate more normal diurnal cortisol profiles. GUPI=Genitourinary Pain Index. FSFI=Female Sexual Functioning Inventory. TLR=Toll-Like Receptor.

Group	GUPI pain scale	GUPI pain frequency [**]	GUPI pain severity	FSFI pain scale [**][+]
steep slope/low TLR-4 inflammation (n=13)	11.055 (5.29)	2.31 (1.03)[*]	4.38 (2.63)	10.54 (4.86)[*]
flat slope/low TLR-4 inflammation (n=10)	10.50 (4.35)	2.1 (1.2)[*]	4.00 (1.89)	4.4 (5.03)
steep slope/high TLR-4 inflammation (n=11)	11.42 (4.45)	2.64 (1.29)	4.82 (1.89)	4.36 (4.18)
flat slope/high TLR-4 inflammation (n=14)	13.81 (3.32)	3.71 (1.33)	5.86 (1.46)	3.00 (4.22)

[**] omnimbus test, $p<.05$

[*] post-hoc comparison vs. flat slope/high TLR-4 inflammation group, $p<.05$

[+] lower scores indicate greater pain