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Unraveling the nature of oxidative stress: a study of diagnostic and pathophysiological biomarkers in women with interstitial cystitis/bladder pain syndrome (IC/BPS)

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Abstract: Bladder pain syndrome (BPS) or interstitial cystitis (IC) is an umbrella term for a group of chronic, incapacitating illnesses that cause lower urinary tract hypersensitivity symptoms and poor quality of life in women with interstitial cystitis. Research indicates that the true prevalence of IC/BPS is between 3% and 7%, notwithstanding the difficulty in determining this due to the heterogeneous classifications and nomenclature. Oxidative stress biomarkers may be utilized as a diagnostic tool for IC/BPS, and urine oxidative stress may also play a role in the pathophysiology of IC/BPS.Therefore, we aim to determine the levels of oxidative stress markers like:malondialdhyde (MDA)(lipid peroxidation marker),protein carbonyl(PC)(protein oxidation marker) and nitric oxide (NO) in urine samples of our study group of women with IC/BPS. The study included 14 women with non ulcerative IClBPS, and the control group included 25 healthy individual females Urine samples were collected in sterile tubes and left to clot at room temperature, then the tubes were centrifuged at 2000-3000 RPM for 10 minutes then the supernatant was collected carefully, and the levels of MDA,PC, and NO were measured by spectrophotometric method. Our results showed that urine Protein carbonyl and urine malondialdehyde were not different between IC/PBS participants and controls, and urine nitric oxide was low in non ulcerative IC/BPS.

keywords: interstitial cystitis, oxidative stress markers, malondialdehyde, Protein carbonyl, nitric oxide.

1.Introduction

Bladder pain syndrome (BPS) or interstitial cystitis is a chronic bladder disorder that lasts for at least six weeks. It is characterized by suprapubic pain, as well as pressure and/or discomfort associated with filling the bladder. Lower urinary tract symptoms, such as urgency and frequency of urination without signs of a Urinary tract infection, are also present [1].

One of the most problematic disorders may be IC/BPS. Its cause is yet unknown, and diagnosis is made by exclusion .The diagnosis is frequently postponed throughout the patient's journey, which has a significant negative influence on the patient's social, psychological, and emotional health [3,4,5].

The quality of life is reduced by IC/BPS symptoms [6]. In IC/BPS, rates of depression, anxiety, and suicidal thoughts are elevated [7, 8]. Based on the existence of Hunner lesions, patients are diagnosed with either non-Hunner-type (nonulcerative) or Hunner-type (ulcerative) interstitial cystitis (HIC) [9].

Living things produce highly reactive molecules called reactive oxygen species (ROS) as a result of regular cellular metabolism and external stimuli. Proteins and nucleic acids can sustain damage from ROS, which alters their functionalities. The body produces antioxidants through several processes to combat oxidative damage. A shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress" [10].

It is commonly acknowledged that oxidative stress has a role in numerous degenerative diseases, either as a cause or a result [11]. Numerous pathophysiologic mechanisms of IC are still investigated in etiology. However, there is mounting evidence that these dysfunctions are caused by free radicals and oxidative damage that arises from reduced blood flow, ischemia, hypoxia, and reperfusion [12].

In this context aimed to determine the levels of oxidative stress markers like malondialdehyde (MDA), protein carbonyl(PC) and nitric oxide (NO) in urine samples of our study group of women with non-ulcerative IC/BPS.

2. Subjects and methods

Study population

The proposal was submitted to the Institutional Research Board (MFM-IRB) in the faculty of medicine, Mansoura University for approval (ethical code: MS.21.03.1419.R1.R2).The consents were taken from the patients at the Urology and Nephrology Center Mansoura University.

This study included 14 women with non ulcerative IC/BPS, in addition to 25 the healthy control subjects of matched age.

Sample collection

Urine samples (3mL) were collected from non-ulcerative IC/BPS patients and control subjects in sterile tubes and left to clot at room temperature, then the tubes were centrifuged at 2000-3000 RPM for 10 minutes then the supernatant was collected carefully, and MDA, PC and NO levels were measured after sample collection.

Biochemical analysis:

Measurement of urine malondialdehyde

Ohkawa et al.'s previously published method was used to measure the levels of malondialdehyde (MDA) in the urine[13]. Thibarbituric acid (TBA) reacts with MDA in the acidic medium at 94 °C for thirty minutes to yield reactive product (thiobarbituric acid) which has a pink color and is measured at wavelength 532 nm.

Measurement of urine protein carbonyl

A modified version of the conventional approach, first proposed by Levine et al. [14], was utilized to detect protein carbonyl. The mechanism employed here was based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH). Measured at 450 nm, the hydrazone derivatives of carbonyl groups were created when protein carbonyl groups reacted with DNPH in an alkaline media.

Measurement of urine nitric oxide

Nitric oxide was measured using the Griess reagent method described by Giustarini et al. [16]. A diazonium salt is created when sulphonamide is mixed with nitrites that are already in solution. When N-alpha-naphthyl-ethylenediamine, an azo dye agent, is added, a pink color develops that can be measured at 540 nm.

Statistical analysis

The Power Analysis and Sample Size (PASS) software (version 15, 2017) was used to calculate the sample size. NCSS Enterprises LLC. USA: Kaysville, Utah.

The sample size for oxidative stress (urine nitric oxide):

Using a one-sided two-sample equal-variance t-test, group sample sizes of 20 and 10 yield 91.58% power to reject the null hypothesis of zero effect size when the population effect size is 1.20 and the significance level (α) is 0.050. Assuming a 20% dropout rate results in a dropout-inflated sample size of 25 in the healthy control group vs. 13 in IC group.

Power analysis for oxidative stress (urine nitric oxide):

Using a two-sided two-sample equal-variance t-test with a significance level (α) of 0.050 and a population effect size of 1.24 (as determined from our data), group sample sizes of 25 and 14 achieve 95.1% power to reject the null hypothesis of zero effect size.

3. Results

Table 1: Comparison of demographic data among patients and control group.

Variable	Group		Test statistic	p-value
	Healthy control (n=25)*	Non-ulcerative IC (n=14)*		
Age (years)	37.89 ± 10.65	36.42 ± 3.72	T= -0.875	0.385#
Body mass index (kg/m2)	29.29 ± 2.39 A	27.24 ± 4.07 B	T= 2.630	**0.011#**
Marital status: Single: Married: Divorced: *Missed data:	0 (0%) 24 (96%) 0 (0%) 1 (4%)	2 (4.3%) 42 (91.3%) 1 (2.2%) 1 (2.2%)	χ^2-= 1.861	0.602**
creatinine (mg/dl)	0.87 ± 0.03	0.85 ± 0.02	T= 0.614	0.541#

Data are shown as mean ± standard deviation or count (percent). #p value by independent sample-t test, ** p value by Chi-Square Test. Bold values indicate significant p values (≤0.05).

The Current study was conducted on 14 patients, In addition to 25 healthy control subjects of matched age. There were no significant statistical differences between non-ulcerative IC/BPS

patients and control subjects in terms of age , marital status and creatinine. (P>0.05). there was statistically significant difference in body mass index between the two groups, it was lower in non-ulcerative IC.

Table 2: Comparison of Urinemalondialdehyde (MDA) marker in the study groups.

Variable	Group		Test statistic	P-value
	Healthy control (n=25)*	Non-Ulcerative IC (n=14)*		
Urine malondialdehyde (MDA) (nmol/ml)	10.77 ± 2.35	13.98 ± 11.10	-1.069	0.304

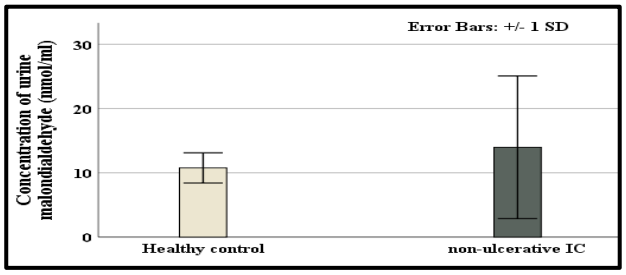

Figure (1): Concentration of urine malondialdehyde in the healthy control and non-ulcerative interstitial cystitis group.

As shown in (table 2) and (Figure 1), there was no statistically significant difference in urine malondialdehyde (MDA) between the control group and the Non-ulcerative IC group.

Table 3: Comparison of Urine nitric oxidemarker in the study groups**.**

Variable	Group		Test Statistic	p-value
	Healthy control (n=25)*	Non-ulcerative IC (n=14)*		
Urine Nitric oxide (nmol/ml)	0.0203± 0.0207	0.0090± 0.0047	2.623	**0.014**

Data are shown as mean ± standard deviation. Significant p values (≤0.05) are indicated by bold values in the independent sample t-test P value.

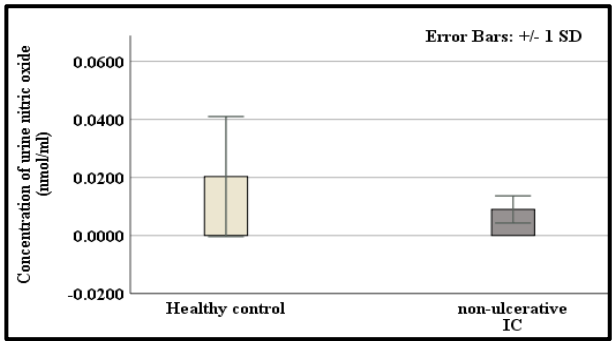

Figure (2):*concentration of urine nitric oxide in the healthy control and non-ulcerative interstitial cystitis group.*

As shown in (table 3) and (Figure2),there was a statistically significant difference in Urine nitric oxide between the healthy control group and Non-ulcerative IC group , it was lower in non ulcerative IC.

Table 4: Comparison of Urine protein carbonyl marker in the study groups.

Variable	Group		Test statistic	p-value
	Healthy control (n=25)*	Non-ulcerative IC (n=14)*		
Urine Protein carbonyl (nmol/ml)	6.62 ± 1.86	5.93 ± 2.31	1.019	0.315

Data is shown as mean ± standard deviation. Significant p values (≤0.05) are indicated by bold values in the independent sample t-test P value.

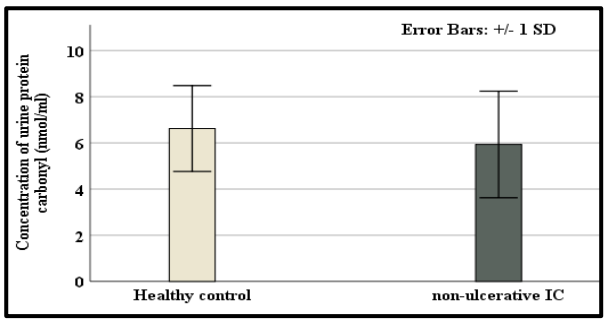

Figure (3): concentration of urine protein carbonyl in the healthy control and non-ulcerative interstitial cystitis group.

As shown in (table 4) and (Figure 3), there was no a statistically significant difference in Urine protein carbonyl between the healthy control group and Non-ulcerative IC group.

4. Discussion

In the present study, we have determined some oxidative stress biomarkers such as (MDA, PC and NO) in urine of women with interstitial cystitis and healthy volunteers.

Based on the results obtained in this study, it showed that the level of nitric oxide in urine was significantly lower in IC group compared to control group ,this can be explained by its decrease production due to decreasing in NOS activity in urine of patients with IC than normal controls, this matches with the results found in research by Bouchard et al [17] which showed that L-arginine is a semi-essential amino acid that leads to increased nitric oxide (NO) and its precursor nitric oxide synthase (NOS).

Non-ulcerative interstitial cystitis may share a neurophysiological process with commonly known Functional Somatic Syndromes (FSSs) that cause central sensitization such as irritable bowel syndrome, and fibromyalgia [18].

In this study the levels of (MDA) show non-significant difference between IC and healthy group.

This agrees with the study of Eisinger et al [19] which showed that MDA values are unchanged in faibromylagia when compared to controls.

This disagree with the study of Tanik et al [20] which showed that the highest MDA level was determined in the cystitis group and the lowest in the control group and Some earlier papers [21] showed an increased urinary MDA level in human interstitial cystitis.

PC assessment is a helpful technique to look into the oxidative stress-induced damage to proteins [22].In this study the levels of (pc) show non-significant difference between IC and healthy group.

This disagree with the study of Hatala et al [23] which showed that concentration of PC was elevated in the case of the highest concentration (1000 μM) of NE treatment

Numerous researchers believe the etiology of IC varies among distinct subsets of its patients, making IC a heterogeneous condition. This is one reason why there is a noticeable overlap in most

IC urine indicators between IC patients and controls [24].

Conclusion and recommendations

IC/BPS is a chronic illness that affects women more than men and affects the quality of life, rates of suicide thoughts are elevated in IC/BPS. Etiology of IC is still unclear. Urine Protein carbonyl and urine malondialdehyde were not different between IC/PBS participants and controls. Urine nitric oxide was low in non ulcerative IC/BPS. Further research is needed for studying more free radical as possible theory in the pathogenesis of interstitial cystitis.

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4.References

1. Doggweiler,R.;Whitmore, K.E.; Meijlink, J.M.; Drake, M.J.; Frawley, H.; Nordling, J.; Hanno, P.; Fraser, M.O.; Homma, Y.; Garrido, G.; et al (2017) A standard for terminology in chronic pelvic pain syndromes: A report from the chronic pelvic pain working group of the international continence society. *Neurourol. Urodyn.***36**: 984–1008.
2. Homma, Y., Akiyama, Y., Tomoe, H., Furuta, A., Ueda, T., Maeda, D., & Lee, K. S. (2020). Clinical guidelines for interstitial cystitis/bladder pain syndrome. *International Journal of Urology* **27**(7): 578-589.
3. Peng YC, Yueh-Hsia Chiu S, Feng M, Liang CC (2020) The effect of intravesical hyaluronic acid therapy on urodynamic

and clinical outcomes among women with interstitial cystitis/bladder pain syndrome. *Taiwan J Obstet Gynecol*.**59**:922–926.
4. Tsai CP, Yang JM, Liang SJ, Lin YH, Huang WC, Lin TY, et al (2021) Factors associated with treatment outcomes after intravesical hyaluronic acid therapy in women with refractory interstitial cystitis: a prospective, multicenter study. *J Chin Med Assoc*. **84**:418–422.
5. Lee WL, Lee FK, Wang PH (2021) Application of hyaluronic acid in patients with interstitial cystitis. *J Chin Med Assoc*. **84**:341-343.
6. Windgassen S, McKernan L (2020) Cognition, emotion, and the bladder: psychosocial factors in bladder pain syndrome and interstitial cystitis (BPS/IC). Curr Bladder Dysfunct Rep. **15**:9-14.
7. McKernan LC, Nash M, Carr ER(2016) An integrative trauma-based approach toward chronic pain: specific applications to interstitial cystitis/bladder pain syndrome. *J Psychother Integr*. **26**(4):378-393.
8. Tripp DA, Nickel JC, Krsmanovic A, et al (2016) Depression and catastrophizing predict suicidal ideation in tertiary care patients with interstitial cystitis/bladder pain syndrome. *Can Urol Assoc J*. **10**(11-12):383-388.
9. Fall, M.; Logadottir, Y.; Peeker, R (2014) Interstitial cystitis is bladder pain syndrome with Hunner's lesion. *Int. J. Urol*. **21** (Suppl. 1): 79–82.
10. Gupta RK, Patel AK, Shah N, Choudhary AK, Jha UK, Yadav UC, et al (2014) Oxidative Stress and Antioxidants in Disease and Cancer **15**: 4405–4409.
11. Andrew R Collins (2005) Assays for oxidative stress and antioxidant status: applications to research into the biological effectiveness of polyphenols, *The American Journal of Clinical Nutrition* **81**: 261S–267S.
12. Malone L, Schuler C, Leggett RE, Levin RM (2014) Effect of estrogen and ovariectomy on response of the female rabbit urinary bladder to two forms of in vitro oxidative stress. *Int Urogynecol J* **25**(6):791–798.
13. Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry **95**(2):351-358.
14. Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A. G., ... & Stadtman, E. R. (1990). [49] Determination of carbonyl content in oxidatively modified proteins. In Methods in enzymology **186**: 464-478.
15. Mesquita, C. S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., & Marcos, J. C. (2014). Simplified 2, 4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Analytical biochemistry **458**: 69-71.
16. Giustarini, D., Rossi, R., Milzani, A., & Dalle-Donne, I. (2008). Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization. Methods in enzymology **440**:361-380.
17. Bouchard, B., & Campeau, L. (2023). Quality, Value, and Efficacy of Complementary and Alternative Medicine in the Treatment of Interstitial Cystitis/Bladder Pain Syndrome. Current Bladder Dysfunction Reports, **18(1)**, 51-58.
18. Akiyama, Y. (2021). Biomarkers in interstitial cystitis/bladder pain syndrome with and without Hunner lesion: A review and future perspectives. Diagnostics, 11(12), 2238.
19. Eisinger, J., Ayavou, T., Plantamura, A., Lawson, K., & Danneskiold-Samsoe, B. (2002). Lipid and protein peroxidations in fibromyalgia. Myalgies. Intl, ***2***, 37-42.
20. Tanik, S., Zengin, K., Albayrak, S., Gurel, A., Atar, M., Sahin, S., ... & Gurdal, M. (2017). Assessment of therapeutic effect of human choriogonadotropin in a chemical cystitis model. *The Kaohsiung Journal of Medical Sciences*, ***33*****(5)**, 229-235.
21. Dokumacioglu, E., İskender, H., Yenice, G., Kapakin, K. A. T., Sevim, C., Hayirli,

A., ... & Comakli, S. (2018). Effects of astaxanthin on biochemical and histopathological parameters related to oxidative stress on testes of rats on high fructose regime. Andrologia, ***50*(7)**, e13042.

22. Dalle-Donne, I.; Rossi, R.; Giustarini, D.; Milzani, A.; Colombo, R. (2003)Protein carbonyl groups as biomarkers of oxidative stress. Clin. Chim. Acta , **329**, 23–38.

23. Hatala, P., Lajos, A., Mackei, M., Sebők, C., Tráj, P., Vörösházi, J., ... & Mátis, G. (2023). Feline Uroepithelial Cell Culture as a Novel Model of Idiopathic Cystitis: Investigations on the Effects of Norepinephrine on Inflammatory Response, Oxidative Stress, and Barrier Function. Veterinary Sciences, **10(2)**, 132.

24. Erickson, D. R. (2001). Urine markers of interstitial cystitis. Urology, **57(6),** 15-21.