MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Investigating the Connection between Oxidative Stress and Interstitial Cystitis/Bladder Pain Syndrome: A Comprehensive Analysis of Serum Oxidative Markers in Women.

Nadra A. Mohammed[a], Randa I.El-Gammal[b], Ahmed S. El-Hefnawy[c], Om Ali Y. El-Khawaga[a]

[a] *Biochemistry division, Chemistry department, Faculty of Science, Mansoura University.*

[b] *Department of Medical Biochemistry &Molecular Biology , Faculty of Medicine- Mansoura University*

[c] *Professor of Urology, Urology department, Mansoura Urology and Nephrology, Faculty of Medicine, Mansoura University*

*** Correspondence to:** *Omali Y.El-Khawaga. (Elkhawaga70s@mans.edu.eg , 01028464738) Email, Tel)*

Received:11/1/2024
Accepted: 17/1/2024

Abstract: Interstitial cystitis/bladder pain syndrome (IC/BPS), formerly known as interstitial cystitis, is chronic pelvic pain (lasting more than 6 weeks) with symptoms of discomfort, pressure or pain affecting or related to the bladder urinary. The disease is characterized by chronic inflammation and symptoms of the lower urinary tract that are not due to infection or other identifiable causes. Because IC/BPS remains a diagnosis of exclusion, this condition is often underdiagnosed or misdiagnosed. therefore we aim to study The role of oxidative stress in the development of IC/BPS by determination the level of the oxidative markers and it was measured by spectrophotometric assays in serum such as: Malondialdhyde (MDA) (lipid peroxidation marker), Protein carbonyl (PC) (protein oxidation marker) and nitric oxide (antioxidant marker) and then Statistical analysis was performed in order to correlate the level of the measured parameters with the development of IC/BPS in women. Finally, we found an increase in NO, PC serum level of IC patients And there is no significant difference in MDA level in non ulcerative IC compared with the healthy group.

Keywords: interstitial / cystitis / oxidative / stress / biomarker /female.

1.Introduction

Interstitial cystitis/bladder pain syndrome (IC/BPS) is defined as a chronic bladder condition characterized by bladder pain (pelvic pain, bladder pain) and discomfort associated with bladder pressure and/or lower urinary tract filling [1]. Symptoms in patients with IC/BPS often include chronic pelvic pain, including depression, anxiety, insomnia, and sexual dysfunction, which reduces quality of life [2] and interferes with social participation [3]. The patient's subjective perception of pelvic pain is a hallmark of IC/PBS [4]. Depending on the pathological characteristics of the patient, the presence of Hunner ulcers can be classified as IC/PBS Hunner (with ulcers) type IC/BPS (HIC/BPS) or non-Hunner (non-ucerative) IC/BPS (NHIC/BPS) type according to histology, cystoscopy and the characteristics of the bladder biopsy.

However, the pathophysiology of IC/BPD remains unclear and therefore the phenotype is has not been defined completely.

Clinically, IC/BPS can be classified as IC/BPS with Hunner lesions (HIC/BPS) or IC/BPS without Hunner lesions (NHIC/BPS), depending on the histological features on cystoscopy and bladder biopsy. Hunner's ulcer prevalence is approximately 6-8% and is associated with severe symptoms and significant

functional and anesthetic reductions in the capacity of the bladder [5]. The urothelium, peripheral afferent endings, excretory muscles and pelvic vasculature all contribute to the pathophysiological mechanism of IC/PBS. Urothelial cells express many receptors/ion channels, such as receptors for norepinephrine, adenosine, neurotrophins, acetylcholine, endothelin, and various transient receptor potential (TRP) channels [6].

The global concept of "Oxidative Stress" is defined as "an imbalance between oxidants and antioxidants in favor of the oxidants, causing a disruption of redox signaling and control and/or molecular damage" [7].In contrast the redox balance, the mechanism of the defense against damaging oxidants formed of many types of antioxidant enzymes combined with their back-up systems, in addition to antioxidants of low-molecular-mass antioxidant enzymes, forming an antioxidant network [8].

This context aims to evaluate The role of oxidative stress in the development of IC/BPS by measuring the level of the oxidative markers by spectrophotometric assays in serum such as: Malondialdhyde (MDA) (lipid peroxidation marker), Protein carbonyl (PC) (protein oxidation marker) and nitric oxide (antioxidant marker).

Subjects and methods

Study population

Study population

The current study was authorized by the Medical Research Ethics Committee, Institutional Review Board, Faculty of Medicine, Mansoura University, code number MS.21.03.1418. All patients signed a written informed agreement before the inclusion in the study.

fourteen females with confirmed diagnosis of non ulcerative IC/BPS and not pregnant. A diagnosis of IC/BPS was established in accordance with the eligibility criteria of Interstitial Cystitis Data Base (ICDB) study. In accordance with the last report ICS/IUGA for standardization of terminology Phenotype selection for the bladder domain was carried out [9]

Only female patients with symptoms for more than 6 months and VAS ≥7 was be enrolled. Exclusion criteria involved patients less than 18 years, pregnancy, previous irradiation, Patients with the evidence of Hunner's ulcer as evidenced by outpatient cystoscopy was not be involved.

An age-matched females; Twenty five females of completely healthy diagnosis and all have negative history of malignant diseases was involved as a control group.

Clinical score:

Patients were evaluated with a complete history and clinical examination, including a brief neurological examination and, for women, a gynecological examination. Laboratory investigation included urinalysis and

urine culture for all patients. In the period after complete drainage, gray scale ultrasound (US) on kidneys and urinary bladder with the full phase after the evacuation to detect post voiding residual urine (PVR) was conducted. A 3-day voiding diary was filled by patients. None invasive uroflowmetry (NIF) was carried out to assess the presence of obstruction.

Sample collection

Serum samples were obtained from patients and healthy females. Five ml of serum samples were collected from all subjects included in the study. It was centrifuged at 2000-3000 RPM for 20 minutes then the supernatant was collected carefully then aliqutes from all samples were made and samples were stored at -20°C.

Serum level of creatinine was used as a measure of kidney function for all participants and it was within normal healthy range (0.6-1.1 mg/dl), excluded any possibility of kidney diseases.

Biochemical analysis:

Evaluation The role of oxidative stress in the development of IC/BPS by measuring the level of the oxidative markers by spectrophotometric assays in serum samples such as: Malondialdhyde (MDA) (lipid peroxidation marker), Protein carbonyl (PC) (protein oxidation marker), Nitric Oxide (NO) (potential antioxidant) and Statistical analysis was

performed in order to correlate the level of the measured parameters with the development of IC/BPS in women.

Serum level of creatinine was used as a measure of kidney function for all participants and it was within normal healthy range (0.6-1.1 mg/dl), excluded any possibility of kidney diseases.

Measurement of serum protein carbonyl:

Protein carbonyl was measured by a modified method of the old one proposed by [10], based on the reaction between 2,4-dinitrophenylhydrazine (DNPH) and carbonyl groups. The hydrazone derivatives of carbonyl groups produced from the reaction of protein carbonyl groups with DNPH in alkaline medium producing hydrazone derivatives of carbonyl groups was measured at 450 nm[11].

2.Measurement of malondialdehyde (MDA):

Serum malondialdehyde (MDA) levels were measured by a method by [12]. Thibarbituric acid (TBA) reacts with MDA at 94 °C in acidic medium for 30 minutes and the product is thiobarbituric acid which is pinkreactive product and measured at 532 nm.

Measurement of Nitric oxide (NO):

Nitric oxide was determined according to the method of using Griess reagent [13].

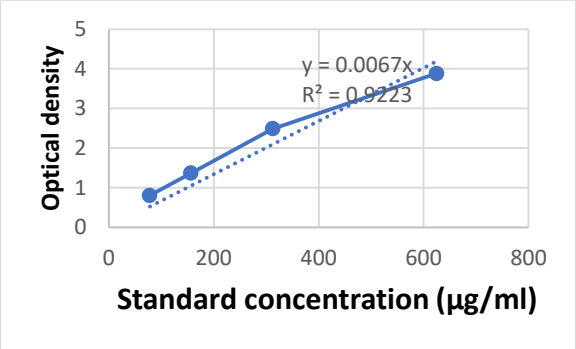

Figure 1: Standard curve of nitric oxide

Statistical analysis

IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used for Data were entered and analyzed. Shapiro-Wilk's test was used for Quantitative data and they were initially tested for normality with data being normally distributed if p>0.050 and vice versa.

Examining boxplots was used for testing Presence of significant outliers was tested for by. Quantitative data were expressed as mean ± standard deviation (SD) when normally-distributed, and median (interquartile range or range) when not normally-distributed. Qualitative data were expressed as count (percentage).

Independent sample t-test test was used when data are normally-distributed for comparing quantitative data between two groups. For any of the used tests, results were considered as statically significant if p value ≤0.050

Bar charts, box plots and scatter plots were used To to graphically present the results whenever needed.

3.Results and Discussion

Oxidative stress markers in serum protein carbonyl from the study groups:

As shown in **(Table 1)** and **(Figure 2)**, there was a highly significant difference in serum protein carbonyl between the two groups; Healthy control and non-ulcerative IC.

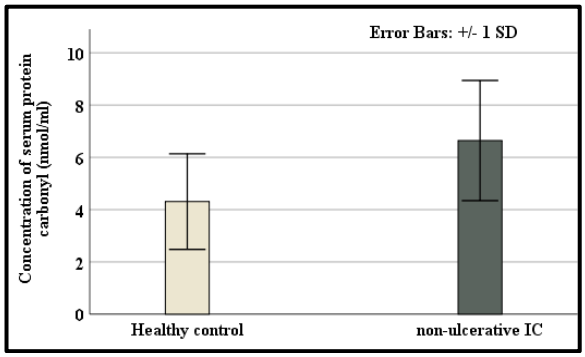

Figure (2): Concentration of serum protein carbonyl in the healthy control and non-ulcerative interstitial cystitis group

Table 1: Oxidative stress markers in serum protein carbonyl from the study groups

Variable	Healthy control (n=25)*	Non-ulcerative IC (n=14)	P	T
Serum protein carbonyl (nmol/ml)	4.31 ± 1.83	6.64 ± 2.30	0.001	-3.485

data are presented as mean ± standard deviation. P value by independent sample t-test, bold values indicate significant p values (≤0.05)

Oxidative stress markers in serum Malondialdehyde from the study groups:

As shown in **(Table 2)** and **(Figure 3)**, there was non significant difference in serum malondialdehyde between the two groups; Healthy control and non-ulcerative IC.

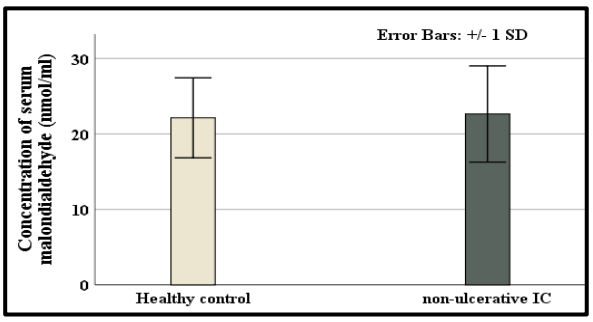

Figure (3): Concentration of serum malondialdehyde in the healthy control and non-ulcerative interstitial cystitis group.

Table 2: Oxidative stress markers in serum malondialdehyde (MDA) from the study groups:

Variable	Healthy control (n=25)*	Non-ulcerative IC (n=14)	P	T
Serum malondialdehyde (MDA) (nmol/ml)	22.13 ± 5.30	22.64 ± 6.37	0.793	-0.265

Oxidative stress markers in serum Nitric oxide (NO) from the three study groups:

As shown in **(Table 3)** and **(Figure 4)**, there was a highly significant difference in serum Nitric oxide (NO) between the two groups; Healthy control and non-ulcerative IC

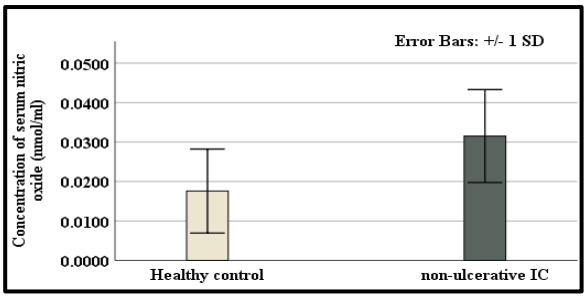

Figure (4): Concentration of serum nitric oxide in the healthy control and non-ulcerative interstitial cystitis group.

Table 3: Oxidative stress markers in serum Nitric oxide (NO) from the study groups:

Variable	Healthy control (n=25)*	Non-ulcerative IC (n=14)	P	T
Serum nitric oxide (nmol/ml)	0.017 6 ± 0.0106	0.031 5 ± 0.0118	0.001	-3.779

Sample size and power analysis

Software:

Power Analysis and Sample Size (PASS) Software (version 15, 2017). NCSS, LLC. Kaysville, Utah, USA was used for Sample size calculation.

Sample size for oxidative stress:

Hypothesis: We expect a significant increase in serum protein carbonyl in IC compared with healthy control with a large effect size, Cohen's d = 1.2).

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

Discussion

Interstitial cystitis/bladder pain syndrome (IC/BPS) remains unobvious. affect more than 8 million women globally [14].

The current study is an observational case-control study that included 39 female participants (14 IC patients and 25 healthy controls). A diagnosis of IC/BPS was established in accordance with the eligibility criteria of Interstitial Cystitis Data Base (ICDB) study.

IC patients group were outpatient clinic with complaint suggestive of IC in the Mansoura University Urology and nephrology center.

Serum samples of control were collected from individuals admitted to Mansoura Urology and Nephrology as a donor for kidney transplantation operation.

The imbalance between produced oxygen free radicals and the scavenging ability of antioxidants is known as the oxidative stress. We focused on oxidative stress as a possible mechanism for IC pathogenesis as free radicals are thought to play a part in a variety of diseases. Increased oxygen free radical generation can cause oxidative damage to

biomolecules, resulting in lipid peroxidation, protein peroxidation.

Nitric oxide (NO) is a neurotransmitter that can affect bladder activity which is higher in IC group compared to control group, it is also a product of the activation of inflammation response, it also has a role in increasing oxidative stress.

Our results show that the levels of nitric oxide in serum were significantly higher in IC group compared to control group, this could be explained by its increased production due to inflammation in the bladder, this matches the results found in research by Hosseini et al in which they used it as a marker for treatment response [15].

In this study the level of protein carbonyl (PC) were significantly increased in IC group compared to control group, which could be explained by its nature as protein oxidation marker.

Malondialdehyde (MDA) is one of the final products of the peroxidation of polyunsaturated fatty acids in the cell. MDA level is often known as a marker of oxidative stress and the antioxidant status in patients [16]. In our study the levels of (MDA) show non-significant difference between IC and healthy group.

1.Conclusion and recommendations

The presented results indicate an increase in NO, PC serum level of IC patients. And there is no significant difference in MDA level in non ulcerative IC compared with the healthy group. Further researches more recommended for studying the correlation between another free radicals serum levels and IC patients

4. References

1. Homma Y., Akiyama Y., Tomoe H., Furuta A., Ueda T., Maeda D., Lin A.T., Kuo H.C., Lee M.H., Oh S.J., et al. (2020);Clinical guidelines for interstitial cystitis/bladder pain syndrome. *Int. J. Urol. Off. J. Jpn. Urol. Assoc.* **27**:578–589. doi: 10.1111/iju.14234.
2. Nickel J.C., Tripp D.A., Pontari M., Moldwin R., Mayer R., Carr L.K., Doggweiler R., Yang C.C., Mishra N., Nordling J. (2010) ;Psychosocial phenotyping in women with interstitial cystitis/painful bladder syndrome: *A case control study. J. Urol*. **183**:167–172. doi: 10.1016/j.juro.2009.08.133.
3. Homma Y., Ueda T., Tomoe H., Lin A.T., Kuo H.C., Lee M.H., Lee J.G., Kim D.Y., Lee K.S., (2009) ;Interstitial Cystitis Guideline Committee Clinical guidelines for interstitial cystitis and hypersensitive bladder syndrome. *Int. J. Urol.* **16**:597–615. doi: 10.1111/j.1442-2042.2009.02326.x.
4. Abrams P., Cardozo L., Fall M., Griffiths D., Rosier P., Ulmsten U., van Kerrebroeck P., Victor A., Wein A., (2002);Standardisation Sub-committee of the International Continence Society The standardisation of terminology of lower urinary tract function: Report from the Standardisation Sub-committee of the International Continence Society. Neurourol. Urodyn. 21:167–178. doi: 10.1002/nau.10052.
5. Malde S., Palmisani S., Al-Kaisy A., Sahai A. (2018);Guideline of guidelines: Bladder pain syndrome. BJU Int. 122:729–743. doi: 10.1111/bju.14399.
6. Messing E., Pauk D., Schaeffer A., Nieweglowski M., Nyberg L.M., Jr., Landis J.R., Cook Y.L., Simon L. J(1997);. Associations among cystoscopic findings and symptoms and physical examination findings in women enrolled in the Interstitial Cystitis Data Base (ICDB) Study. Urology. 49:81–85. doi: 10.1016/S0090-4295(99)80336-7.
7. Birder L., Andersson K.E. (2013);Urothelial signaling. Physiol. Rev. 93:653–680. doi: 10.1152/physrev.00030.2012.
8. Homma Y., Ueda T., Tomoe H., Lin A.T., Kuo H.C., Lee M.H., Oh S.J., Kim J.C., Lee K.S. (2016);Clinical guidelines for interstitial cystitis and hypersensitive bladder updated in 2015. *Int. J. Urol*. **23**:542–549. doi: 10.1111/iju.13118
9. Sies H., Berndt C., Jones D.P. (2017);Oxidative stress. Annu. Rev. Biochem. **86**:715–748. doi: 10.1146/annurev-biochem-061516-045037.
10. Sies H. (1993) Strategies of antioxidant defense. *Eur. J. Biochem*.; **215**:213–219. doi: 10.1111/j.1432-1033.1993.tb18025.x.
11. O'Leary MP, et al. (1997); The interstitial

cystitis symptom index and problem index. Urology. **49**(5A Suppl):58–63.
12. Daniela Giustarini , Ranieri Rossi, Aldo Milzani, Isabella Dalle-Donne (2008) Affiliations PMID: 18423230 ;440:361-80. doi: 10.1016/S0076-6879(07)00823-3.
13. R L Levine, D Garland, C N Oliver, A Amici, I Climent, A G Lenz, B W Ahn, S Shaltiel, E R Stadtman 1990;186:464-78 PMID: **1978225** DOI: 10.1016/0076-6879(90)86141-h
14. 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. Anal. Biochem. **458**:69–71.
15. Daniela Giustarini ., Ranieri Rossi., Aldo Milzani., Isabella Dalle-Donne. Nitrite and nitrate measurement by Griess reagent in human plasma: evaluation of interferences and standardization PMID: 18423230 DOI: 10.1016/S0076-6879(07)00823-3
16. Hosseini A., Ehren I., Wiklund N.P. (2004) Nitric oxide as an objective marker for evaluation of treatment response in patients with classic *interstitial cystitis. J Urol* **172**: 2261–2265