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Role of malondialdehyde in the development of interstitial cystitis in Egyptian Patients

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Abstract: Interstitial cystitis is a chronic disease that affects women mostly changing their lifestyle and affecting their ability to live a normal life. It is hard to diagnose as it intersects and comorbid with many diseases so the treatment of that disease is not easy.There are many theories about its pathogenesis ,one of which is oxidative stress. Experts observed anomalies in each subcellular layer of IC/ PBS patients' bladder tissue,some of it could be a result of lipid peroxidation due to high oxidive stress.Lipid peroxidation produces end-products such as malondialdehyde (MDA) which we will estimate to find the possible relationship between lipid peroxidation and IC.

keywords: lipid-peroxidation,interstitial-cystitis,malondialdehyde,bladder

1.Introduction

"Interstitial cystitis" (IC) is a chronic inflammatory pain disease affecting mostly women and causing discomfort in the bladder and pelvic. [1]. It is diagnosed by a sensation of discomfort and pressure in the bladder, as well as symptoms of the lower urinary tract that continue longer than six weeks In absence of illness or other reason [2].

IC was diagnosed according to the presence of Hunner's ulcer or mucosal haemorrhages in the bladder (glomerulations) [3]. Later, in more than 90% of all diagnosed patients of IC/PBS, a non-ulcerative type of IC/PBS was discovered and described. [4] .

The incidence of IC/PBS has been estimated to be in the range of 45 per 100,000 women and 8 per 100,000 males. Because of the female anatomical ureteral morphology, the prevalence of IC/PBS is much greater in women (1 in 4) than in males [5].

The etiology of IC is not fully clear so there are many factors implicated in this disease such as sex hormones, genes, comorbidity with other diseases, nutrition, environmental agents and other factors [6,7].

In most cases, IC/PBS-related dysfunctions begin in and around the bladder, the pelvic organs that surround it, and the neurological tissue that controls the bladder's function [8] .

Many investigators observed anomalies in each subcellular layer of IC/ PBS patients' bladder tissue, including a decreased urothelial layer, impaired storage capacity, atypical smooth muscle cell pattern and a high density of mast cells, high microvascular density, reduced glycosaminoglycans, and nerve fibres [9-11].

There are many pathophysiological theories about the origin of IC, yet no conclusive explanation for the disease. An imbalance between the production of oxygen free radicals and the scavenging of antioxidants is known as oxidative stress. Excessive oxygen free radical generation can induce oxidative damage to biomolecules, resulting in lipid peroxidation. Polyunsaturated fatty acids (PUFA) and certain proteins present in cell membranes are the primary targets of reactive oxygen species (ROS). Malondialdehyde (MDA) and other end-products from the oxidation of macromolecules are produced by defective lipid hydro-peroxides [12].

In this study we aimed to investigate the relationship between MDA as a marker of lipid peroxidation and the development of IC.

2. Subjects, materials and methods.

2.1. S*ubjects:*

The proposal was submitted to the Institutional Research Board (MFM-IRB) in Faculty of Medicine, Mansoura University for the approval (ethical code: MS.22.04.1990.R1). The consents were taken from the patients at Urology & Nephrology Center Mansoura University.

The study included 60 female participants (30 IC patients and 30 normal controls). The medical and surgical history were recorded, the height and the weight were measured and Body mass index (BMI) was calculated and visual analog scale for pain (VAS) was evaluated.

2.2. Sample collection*:*

Three mL of whole blood was collected and allowed to clot at room temperature. The serum was collected after centrifugation at 2000 rpm for 5 minutes, then stored at -80°C till serum MDA was measured.

2.3. Measurement of malondialdehyde concentration in serum:

Draper and Hadley's approach was used to measure malondialdehyde (1990) [13].

a-Principle:

Trichloroacetic acid (TCA) is used to precipitate serum proteins, and malondialdehyde is then condensed with two equivalents of thiobarbituric acid (TBA) to produce a luminous red derivative that can be measured spectrophotometrically at 532 nm .

b-Reagents:

1. TCA (10%):

In 100 mL of distilled water, 10 g of TCA (Sigma-Aldrich, Germany. Product number:T4885) was dissolved.

2. Sodium sulphate solution(2 M):

By boiling 28.4 g anhydrous sodium sulphate(Sigma-Aldrich , Germany.product number: 239313) in 90 m1 distilled water, the volume was increased to 100 mL.

3. TBA (0.67%):

0.67 g of TBA (Sigma-Aldrich, Germany.Product number: T5500) was dissolved in l00 mL of prepared sodium sulphate solution (2 M) by heating.

4. H_2SO_4 (0.05 M):

2.84 mL of conc. H_2SO_4 (Sigma-Aldrich, Germany.Product number: 339741) was completed to 1 L with distilled water.

5.Tetramethoxypropane (TMP) (l nmol/mL):

• Stock standard (10 μmol/mL):

1.65 mL TMP (Sigma-Aldrich, Germany.product number: 108383) was completed to 1 L with 0.05 M H_2SO_4.

• Diluted standard (10 nmo1/mL):

l mL from stock standard solution was completed to 1 L with 0.05 M H_2SO_4.

• Working standard (l nmol/mL):

l mL from diluted standard solution was completed to 10 mL with 0.05 M H_2SO_4.

c. Procedure:

A. Serum proteins precipitation:

- 2.5 mL of TCA was mixed with 0.5 mL of serum of each sample.
- The tubes were placed in a boiling water bath for 5 minutes, and then cooled under running water before being centrifuged at 4000 rpm for 10 minutes.

B. For the blank tube: l mL of 0.67% TBA was added to 2 mL of 0.05 M H_2SO_4.

C. For the standard tube: l mL of 0.67% TBA was added to 2 mL of working standard of TMP.

D. For samples tubes: 1 mL of 0.67% TBA was added to 2 mL of the supernatants.

Then all tubes were transferred to a boiling water bath for 15 minutes, cooled and the absorbance was read at 532 nm using the spectrophotometer.

d. Calculations

Conc. of MDA (nmol/mL) =$(A_{test}/A_{standard})$ × Conc. of standard

Concentration of standard = l nmol/mL.

Conc. of MDA (nmol/m1) = $A_{test}/A_{standard}$ × 1

e. Statistical analysis

Data was analyzed using the SPSS (statistical package for social science) program (SPSS

Inc., Chicago, IL) version 20, and with GraphPad Prism 3.0 program (GraphPad® Software Inc.).

A probable value of P<0.05 was considered statistically significant at confidence interval 95%.

3. Results

As demonstrated in Table 1, The IC patients group mean age was 44.4 years with a standard deviation of 10.16 years, so we choose a control group with mean age of 47.9 years and a standard deviation of 10.54 to be age matched to the patients group.

A statistically significant difference was not established between the two groups in terms of age (P>0.05).

Table 1: Age characterization of IC patients and control groups at time of testing.

Parameters	Control	IC	*P*-value
No. of case	30	30	--
Age (years) Median (range) M ± SD	50.0 (30-64) 47.9±10.54	41.0 (31-69) 44.4±10.16	0.238

According to the data summarized in Table 2 and Figure 1 : a significant increase was found in serum MDA levels of IC group as compared to healthy control group (P<0.001).

Table 2: Comparison of serum MDA levels of control and IC subjects.

Parameter		Control *n*=30	IC *n*=30	*P*-value
Serum MDA (nmol/mL)	Median (Range)	3.58 (1.28-5.54)	7.79 (2.22-10.9)	<0.001
	Mean± SD	1.5±3.75	7.1±2.55	

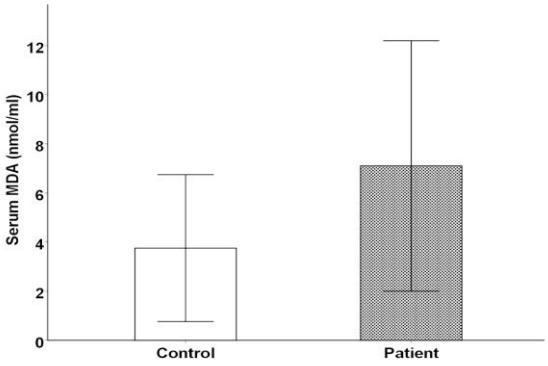

Fig 1: A comparison between the mean serum MDA level in control and patient groups.

A statistically significant increase was found in patients group compared to the control group (P<0.001)

We also studied the relation between serum MDA level and the clinical and prognostic parameters collected about the IC patients group

As demonstrated in Table 3, the patients were classified into two groups according to each parameter then the mean, standard deviation of serum MDA and P-value for each group were calculated.

A statistically significant difference was not established between any two groups in terms of age, surgery, VAS or BMI (P>0.05).

Table 3: Relation between serum MDA levels and different clinical and prognostic parameters of IC patients.

Parameter	*n*	Serum MDA (nmol/ml)	*P*-value
Age <41 years ≥41 years	11 19	6.23±2.77 5.57±2.53	0.487
Surgery Yes No	11 19	7.1±2.38 7.1±2.76	0.996
VAS ≤9 >9	20 10	6.73±2.58 7.34±2.032	0.590
BMI Not obese (<30) Obese (≥30)	9 21	6.32±2.26 5.96±2.97	0.714

So there was no proven relation between patients age, whether they had surgeries in the past or not, their visual analog scale for pain or their body mass index and their serum MDA level

4. Discussion

Approximately 8 million women are thought to be affected by IC/PBS, with many more undiagnosed because of its diagnostic and treatment complications [14, 15]. A better understanding of the disease pathogenesis mechanisms will provide new possible markers to help with its prevention and treatment.

We focused on lipid peroxidation as a possible mechanism for IC pathogenesis as free radicals are thought to play a part in a variety of diseases. Lipid peroxidation begins when free radicals covalently bind to membrane receptors,

changing the unsaturated fatty acid/protein ratio.

Lipid peroxides are unstable and quickly decompose into a variety of chemicals, including aldehydes like MDA. As a result, the amount of MDA measured represents the extent of lipid peroxidation in tissues. [16].

Similar researches were performed on animal models about oxidative stress, antioxidants and their relationship with urinary tract disorders [17].

The fact that there was an increase in serum MDA levels in IC patients demonstrates that oxidative stress and lipid peroxidation play an important role in the pathogenesis of IC.

4. Conclusion and recommendations

IC is a complex multifactorial disease which is difficult to diagnose and treat. Its symptoms are similar to other diseases which makes its diagnosis an exclusion process. Many cellular changes occur to the bladder tissue causing defects in its functions, some of them are due to lipid peroxidation which occurs due to high oxidative stress. Lipid peroxidation produces end-products like MDA. The increased levels of serum MDA in IC patients indicates that oxidative stress and lipid peroxidation play an essential role in the etiology of IC.

Further research is needed for studying free radicals as possible theory in the pathogenesis of IC and antioxidants possible protective role and treatment trials.

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