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Role of Nitric Oxide in the development of Egyptians urinary bladder patients

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Abstract: Bladder pain syndrome (BPS) is a chronic, debilitating disease characterized by bladder and pelvic pain, irritative voiding symptoms (urgency, frequency, nocturia, dysuria), and sterile urine. Its etiology remains obscure and is thought to be multifactorial and related to a syndrome rather than a specific disease. 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 Reactive oxygen species (ROS). Reactive oxygen species (ROS) are an important byproduct of aerobic respiration and are mostly generated by mitochondria. Nitric oxide radical (NO) has effects on boosting vascular smooth muscle relaxation, which results in vasodilation and increases blood flow to the area of inflammation which we will estimate to find the possible relationship between (ROS) and IC**.**

keywords: Reactive oxygen species, urinary patients, Nitric oxide,bladder

Introduction

Bladder pain syndrome (BPS) is a chronic, debilitating disease characterized by bladder and pelvic pain, irritative voiding symptoms (urgency, frequency, nocturia, dysuria), and sterile urine. Its etiology remains obscure and is thought to be multifactorial and related to a syndrome rather than a specific disease [1, 2]. It occurs primarily in women and remains a diagnosis of exclusion with no distinctive tissue pathology. National Institutes of Health research guidelines for an unequivocal diagnosis of IC require objective findings of glomerulations or a classic Hunner's ulcer during hydrodistension of the bladder, and subjective findings of bladder pain or urinary urgency in the absence of other urogenital pathology. Its prevalence in the United States is estimated to be 2 in 10,000 [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].

Oxidative stress, is defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, it is proved to cause many types of tissue damage [12]. In normal cells, reactive oxidants are counterbalanced by complex antioxidant defense systems regulated by many pathways to ensure its adequacy for the body's needs [13, 14].

In BPS, the disease is more commonly seen in women suffering from fibromyalgia, inflammatory bowel syndrome, and chronic fatigue syndrome triggered by stress with no abnormal laboratory and physical examination findings [15, 16]. Many pathophysiologic mechanisms are still investigated in etiology. However, the evidence that these dysfunctions result from oxidative damage and free radicals originating from decreased blood flow, ischemia, hypoxia, and reperfusion is gradually increasing and gaining attention [17]. Malone et al found that hydrogen peroxide incubation with rabbit bladder cells led to the damage of bladder tissues and the effect was ameliorated by estrogen treatment. Based on these aforementioned data, the oxidative stress in development of BPS worth investigation.

In this study we aimed to investigate the relationship between NO as Reactive oxygen species (ROS) its role in the development of IC.

Supjects Materials and methods

Sample collection

female Each subject

The proposal was submitted to Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB) for the approval (ethical code : MS.22.06.2049) then consents were taken from the patients at Urology & Nephrology Center Mansoura University.

There are 60 female participants in this study, 30 IC patients and 30 normal control. For patients the medical and surgical histories were taken، height and weight were measured and Body mass index (BMI) was calculated and visual analog scale for pain (VAS) was evaluated.

Sample collection:

Subject (patient and controls) had 3 ml of whole blood collected and left at room temperature for clotting, serum separated by centrifugation at 2000 rpm for 5 min, then refrigerated at -40C till serum NO was measured.

Measurement of Nitric oxide (NO)

Nitric oxide was determined according to the method of (Giustarini, Rossi et al. 2008) using Griess reagent.

Principal:

When sulphonamide is added to the nitrites [present in solution] it forms a diazonium salt. When the azo dye agent (N-alpha-naphthyl-ethylenediamine) is added, a pink color develops that can be measured at 540 nm.

Reagents:

1. A typical Griess reagent may contain:

- 0.2% naphthylethylenediamine dihydrochloride (NEDD)
- 2% sulphanilamide in 5% phosphoric acid.

2. Standard sodium nitrite 50 μmol/L

Procedures:

In a well-plate we added:

Reagent blank:100 uL Griess reagent + 100 uL distilled water

Standard:100 uL Griess reagent + 100 uL standard

Samples:100 uL Griess reagent + 100 uL serum\urine of each sample

3. Allowed the mixture to develop color in the dark for 30 min.

4. Measured the absorbance at 548nm using the spectrophotometer.

Calculations

Nitrite in sample (μmol/L) = $\frac{\text{A sample} - \text{A blank}}{\text{A standard} - \text{A blank}} \times 50$

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 $P<0.05$ will be considered to be statistically significant at confidence interval 95%.

Results and Discussion

As demonstrated in Table 1, the mean age for control group was 47.9 years with a standard deviation of 10.54 while for the patient group the mean age was 44.4 years with a standard deviation of 10.16 years.

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

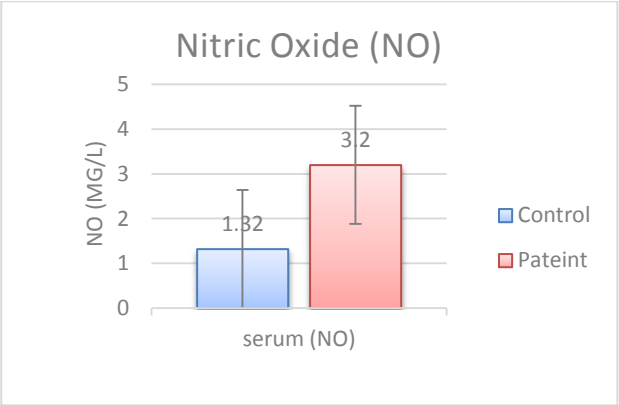

Figure 1: the mean serum NO level in control and patient groups

Table 1: Characterization of patients and control groups at time of testing. Study subjects were characterized according to their age.

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

Table 2: Comparison of serum NO between control and IC groups.

Parameter	Control *n*=30		IC*n*=30		*P*-value
	Medin (Rang)	Mean±D	Median (Range)	Mean ±SD	
Serum NO (mg/L)	1.32	2.523 ±0.460	3.20	2.188±0.399	0.003

According to Table 2 and Figure 1 : a significant increase was found in NO levels in blood samples of IC group as compared to healthy control group (p=0.003).

Table 3: Relation between serum NO the different clinical and prognostic parameters of IC patients.

Parameter	*n*	Serum NO (mg/L)	*P*-value
Age<41 years ≥41 years	11 19	1.72±0.53 2.05±0.64	0.521
Surgery **YesNo**	11 19	1.72±0.27 2.08±0.79	0.322
VAS≤9 >9	20 10	1.87±0.83 1.93 ±0.65	0.840
BMI **Not obese (<30)** Obese (≥30)	9 21	1.81±0.38 1.92±0.63	0.623

As demonstrated in Table 3, the patients were divided in two groups according to each parameter then the mean , standard deviation of serum NO and P-value were calculated.

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

The fact that there was an increase in serum NO levels in IC patients demonstrates that oxidative stress play an important role in the pathogenesis of IC. research.

Conclusion and recommendations

IC is a complex disease which is difficult to diagnose and treat. Many cellular changes occur to the bladder tissue, some of it is due to **Reactive oxygen species (ROS).** which occurs due to high oxidative stress and produces NO. Further research is needed for studying oxidative stress and its possible protective role and treatment trials.

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