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Original Article

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Abstract

Overactive bladder (OAB) is an emergency symptom complex, usually accompanied by frequency (voiding 8 or more times in a 24-hour period) and nocturia (awakening at night to void), with (OAB wet) or without (OAB dry) urinary urgency incontinence (UUI). until recent years The classic biomarker used in the diagnosis OAB was detrusor overactivity (DO), which is measured using filling cystometry, In this study we will reassess the evidence for using DO as biomarker for OAB, by used noninvasive tools as measurements of urinary nerve growth factor and Antioxidant stress marker in female patients who were treated with OAB at the Urology & nephrology Center. The result s revealed that The urinary nerve growth factor (NGF) level was increase in OAB patients than control group , and it was the highest in refractory OAB patients compared to ordinary OAB patients , The catalase activity was significantly decrease in OAB patients compared to control and its activity in refractory patients was lower than the activity in ordinary patients, Malondialdehyde (MDA) concentration showed significant increase in OAB patients compared with controls and revealed more increase in the refractory patients than ordinary patients. Urinary nitrite didn't show any significant difference between OAB patients and controls.and these results may indicate of antioxidant and pro-inflammatory markers used as non-invasive and potential marker for OAB.

1. Introduction

The concept of overactive bladder syndrome (OAB) is urinary urgency, with or without urgent urinary incontinence, which in the absence of urinary tract infection or other apparent pathologies is typically followed by urinary frequency and nocturia **(Hanna-Mitchell *et al*.,2014).**OAB can be sub classified as OAB-dry or OAB-wet with an approximate ratio1to2**(Hsiao *et al*.,2019).**it occurs more frequently in women than men and its incidence increases with age **(Irwin *et al*.,2011).** it has a major impact on quality of life and represents a significant burden on the health care system **(Trived *et al*.,2019).** OABs pathophysiological mechanisms are multifactorial. Three main factors have been proposed regarding the cause of OAB, myogenic, neurogenic and urotheliogenic factors. Myogenic originate from upregulated, spontaneous or involuntary contractions of detrusor muscle cells. Then these contractions spread throughout the bladder wall **(Meng *et al*., 2012).** Neurogenic causes may be seen in patients who have multiple sclerosis, cerebrovascular events and Parkinson's disease **(Griffiths and Tadic,2008).** The classic biomarker used in the diagnosis of OAB has been detrusor overactivity, which is assessed using filling cystometry; filling cystometry has considerable limitations as a clinical tool, with poor reliability and an uncertain relationship to OAB severity **(Cartwright *et al*.,2011).** Also, highly invasive second-line treatments for OAB including intravesical botulinum toxin type A and sacral neuromodulation are used. However, there is no agreed definition of refractory OAB and failure of pharmacotherapy is harder to define and encompasses such wide-ranging factors as lack of efficacy, adverse effects, contraindications and patients' perception and expectation of treatment **(Wong and Tincello ,2016).** Noninvasive alternative to urodynamics, much interest has focused on urinary biomarkers with their potential for bedside testing. Evidence suggests that OAB might be associated with subclinical infection, which has led to the investigation of inflammatory

markers in urine. Many other markers of inflammation detectable in urine have now been noted to be raised in OAB, such as urinary and serum NGF (**Dickson *et al*.,2012).**To date several studies tried to elucidate cause and mechanism of OAB, but it is still poorly understood**(Dickson *et al*.,2012 and Knippschild *et al*.,2012).** Up to now a marker for OAB is lacking. In general a biomarker should be able to indicate and define both the presence and severity of a disease as well as progression and response on therapy; In this study we will reassess the evidence for using DO as biomarker for OAB, by used noninvasive tools as measurements of urinary, biomarker and blood oxidative stress markers.

2. Materials and Methods

Patient Specimens

We retrospectively reviewed the data of 37 female patients who were treated with OAB at the Urology & nephrology Center with average age 52 years [range 31-73]. 16'refractory OAB' includes patients who had an inadequate response or were unable to tolerate and 21 ordinary OAB. First-line medical and behavioral treatments OAB were confirmed by urodynamic.and 20 control subjects were recruited from healthy hospital employees and patients who had no urinary or systemic diseases and who were free of lower urinary tract symptoms. Peripheral blood (4-5 mL total) and urine samples were obtained from OAB patients and control completed the validated questionnaire (OAB V8) for screening of OAB, and had hallmarks of OAB including frequency (voiding q2hr or more), nocturia 2 or more times at night and had post void urgency. Furthermore, all patients in this study showed detrusor overactivity during urodynamics and a maximum functional capacity of 250 mL. All patients had severe frequency, nocturia, and urgency with or without incontinence. Patients will be also evaluated for structural integrity (pelvic prolapse, bladder neck dysfunction, bladder capacity stress incontinence, involuntary bladder contraction).Patients and controls did not have any history of recent infection or inflammatory disease and were not using immunosuppressing or immunomodulating therapy. All samples were collected before any invasive procedures (urodynamic, surgery). Patient consent for specimens and internal review board approval was obtained.

Measurement of urinary NGF levels

Measurement of urinary NGF levels was performed by the ELISA method using nondiluted urine samples. Voided urine was put on ice immediately and centrifuged at 3000g for 10 minutes at 4°C. The supernatant was separated into aliquots in 1.5 mL tubes and preserved in a −80°C freezer. The urinary NGF concentration was determined using the EmaxImmuno Assay System (Promega, Madison, WI, USA) with a specific and highly sensitive ELISA kit, which has a minimum sensitivity of 7.8 pg/ mL. The amount of NGF in urine samples which had levels below the detection limits of the NGF assay was extracted from an NGF standard curve. We ran samples in triplicate. If the urinary NGF levels were not consistent after three measurements, the assay was repeated and the values averaged. When the urinary NGF concentration was higher than the upper detection limit (250 pg/mL), the urine samples were diluted to fit the detection limit. For urine samples with NGF concentrations lower than the detectable limit but above zero, a concentration method was performed using a column-protein concentration kit (Amicon Ultra- 15; Millipore, Billerica, MA USA).

Antioxidant assessment

Malondialdehyde (MDA) level, catalase (CAT) activity and nitric oxide (No) concentrations were measured in serum, plasma and urine samples respectively using kits purchased from (Bio diagnostic Co., Giza, Egypt). Determination of the biomarkers was done according to manual instructions of those kits.

Statistical analysis

Data are expressed as means±standard deviation of the mean. The Student t-test and Mann-Whitney U-test were used for between- group comparisons for parametric and nonparametric data, respectively. The chi-squared test was used for categorical data, and a ROC curve was used to analyze the diagnostic performance of biomarkers for the diagnosis of OAB. Statistical significance was set at $P<0.05$ and all statistical tests were 2-sided. IBM SPSS Statistics ver. 20.0 (IBM Co., Armonk, NY, USA) was used to perform all statistical analyses

3. Results

The urinary NGF level was significant high in OAB patients than control group ($p<0.0001$; table 1), and it was the highest in refractory OAB patients compared to ordinary OAB patients ($p<0.05$; fig.1).

Table (1): Level of urinary NGF, in Control, OAB, ordinary and refractory samples

marker	control (n=20)	OAB (n=37)	P value*	ordinary (n=21)	P value*	refractory (n=16)	P value*
NGF (pg/mL)	1.5 (1.5-5.21)	33.2(8.9-78)	0.000	25 (8.9-35)	0.000	61.5(40.8-78)	0.000

The results expressed in Median and range
*Mann-Whitney test compared to control with significance consider at $p<0.05$.

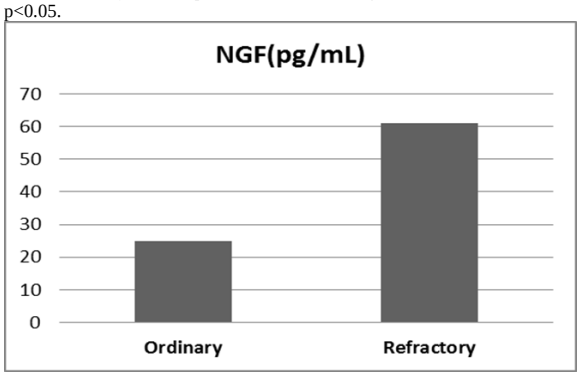

Fig. (1): Level of urinary NGF, in ordinary and refractory OAB patients.

The catalase activity was significantly reduced in OAB patients compared to controls ($p<0.0001$; table 2). Moreover, its activity in refractory patients was lower than the activity in ordinary patients ($p<0.05$; fig.2).

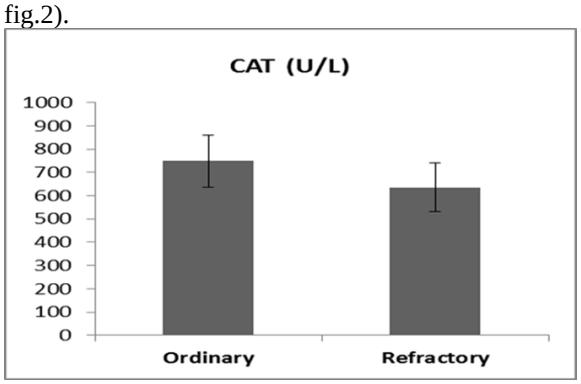

Fig (2): the level of plasma CAT in ordinary and refractory OAB patients.

Table (2): Level of serum MDA, plasma CAT and urinary Nitrite in Control, OAB, ordinary and refractory samples.

Marker	control	OAB	P	ordinary	P	refractory	P
	n=20	n=37	value*	n=21	value*	n=16	value*
MDA	0.3765± 0.352	5.2 ±2.21	0.000	3.62±1.23	0.000	7.30±1.26	0.000
nmol/ml)							
CAT(U/L)	856.9±100.58	689 ±135.58	0.000	749.15±111.25	0.002	635 ±104.54	0.000
NO(µmol/l)	4.48 ±0.96	4.37±1.6	0.765	4.11±0.99	0.232	4.70±2.15	0.693

The results expressed in Mean ± SD.

On the other hand, MDA concentration showed significant increase in OAB patients compared with controls ($p<0.0001$; table 2) and revealed more increase in the refractory patients than ordinary patients ($p<0.05$; fig.3).

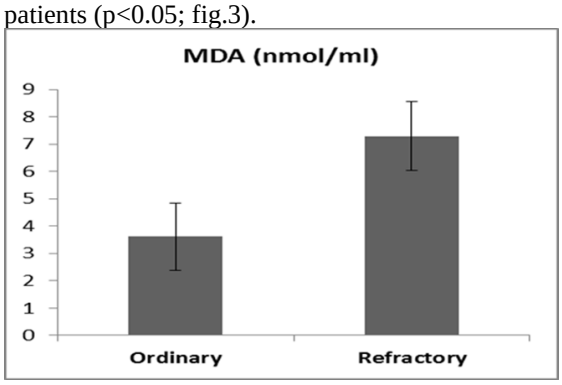

Fig (3): level of serum MDA in ordinary and refractory OAB patients.

Whereas, there was no statistically significant relationship in NO concentration between the OAB patients and control ($p>0.05$; table 2) and neither between ordinary and refractory patients ($p=0.227$; fig. 4).

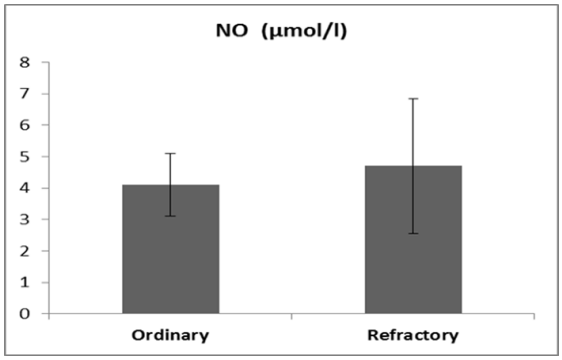

Fig (4): the level of urine NO in ordinary and refractory OAB patients.

4. Discussion

Diagnosis and management of overactive bladder remains a challenge to date there are no definitive blood or serum-based test available for disease confirmation **(Chen *et al* .2003and 2006).**Urodynamics remains the sole objective test for diagnosis of OAB. This approach is not available in all clinics **(Cheung *et al*.,2011).**The term "biomarker" typically refers to anything that can be used as a predictor of a specific disease state, including its existence (diagnostic biomarker), severity and/or reaction to a specific treatment (predictive biomarker); Biomarkers may be specific cells, enzymes, hormones, genes or products of genes that can be detected and measured in parts of the body, such as blood, urine or **(Bhide *et al*.,2013)** .NGF is considered a key regulator of neurogenic inflammation in many different tissues, it produced by urothelium and bladder muscles. It is widely documented, however, that the amount of pro-inflammatory mediator NGF inside OAB patients' bladder and urine is increasing **(Furuta *et al*.,2018).**Obtained results of the present study show highly significantly increase of urinary NGF in OAB than control .this result corresponds with similar observation made by Qu eta al., (2014), he reveled that the level of urinary NGF higer in patients with OAB than healthy people .This result suggested that urinary NGF levels could be a potential biomarker for the diagnosis of OAB **(Qu *et al*.,2014).**Nevertheless These result are consistent with previous study which showed, the urinary and serum NGF levels were significantly higher in OAB group compared with control group **(Trivedi *et al*.,2019).**Increased levels of NGF could lead to reduced thresholds or increased excitability of bladder afferent fibres, and an enhanced spinal reflex**(Vijaya *et al*.,2016).** Also In our study the level of Urinary NGF is significantly increase in refractory OAB than in control .this .In the present study, we measured three of the most commonly used and accepted markers of the presence of oxidative stress namely; MDA, CAT and No. MDA, which is one of the most important products of lipid peroxidation, showed significant increase in OAB patients than controls and its concentration became

higher in refractory OAB than ordinary. That was in agreement with Alexandre et al. 2016 findings (**Alexandre *et al*.,2016).** Moreover, CAT antioxidant activity showed significant decrease in OAB patients compared to controls and more reduction was found in refractory OAB than ordinary which was in accordance with Topol et al. 2011 study **(Topol *et al*.,2011)** . However, urinary nitrite didn't show any significant difference between OAB patients and controls. Excessive free radicals induce destructive changes in the cellular and subcellular components, including lipids, protein, and DNA, ultimately leading to cellular degeneration. Therefore, ROS may be a pathogenic factor in inflammation of the dysfunctional bladder. Inflammation is also characterized by the formation of ROS **(Nomiya *et al*.,2012).**

Conclusion

These promising first data are indicating the helpfulness of antioxidant and pro-inflammatory markers in OAB. Those non-invasive markers seem to have the potential for OAB defining the presence, severity and response to therapy and relapse. Future investigations have to evaluate the relevance of those biomarkers to diagnose and monitor patients with OAB.

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