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Serum Myeloperoxidase as a Biomarker of Asthma Severity Among Adults: A Case Control Study

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Abstract

Background: The contribution of neutrophils is still indistinct in the inflammatory response of bronchial asthma (BAs). Myeloperoxidase (MPO) is an enzyme released from the primary azurophilic granules of the neutrophils. The study aimed to evaluate the levels of serum MPO as a biomarker for the assessment of the level of asthma control.

Methods: The study participants included 94 asthmatic patients and 86 healthy controls. The identification of asthma severity had assessed using the "Global Initiative for Asthma guidelines". Asthmatic adults had divided into three groups: Good (n= 22), partial (n= 30), and poor control (n= 44). Also, patients have been divided again into two groups (treated and untreated) for BAs.

Results: The predicted FEV1% and the peak expiratory flow (PEF/L) of all participants had verified by spirometry. The mean patients' age was 31.9±15.1 year, with a predominance of females. The mean asthma duration was 10.5±8.6 years. Mean spirometric parameters (FEV1 and PEF) were significantly lower among the patients (0.00). Significant higher MPO levels had observed among BAs patients (p-0.00). The MPO levels have not differed significantly with asthma levels and had significant differences with the history of treatment. There was a nonsignificant negative correlation between the mean MPO levels and the spirometry variables among the patients. ROC curves revealed a sensitivity, specificity, accuracy for MPO (80.9%, 72.1%, and 84.3%), respectively to predict asthmatic severity.

Conclusions: There were significantly higher MPO levels compared to healthy controls. Levels of serum MPO had a non-significant positive correlation with levels of asthma control, but a negative non-significant correlation with spirometric results.

Keywords: Asthma, And Neutrophils, FEV1, MPO, PEF.

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Introduction

The universal epidemic of bronchial asthma (BAs) is still ongoing, especially in low/middle-income states, even if some evidence proposes it has decreased in some high-income societies. It had estimated that about 300 million individuals have BAs globally, and by 2025, a further 100 million have expected to be added (1-3). Asthma is an

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illness of the lung characterized by inflammatory and hyperresponsive airways (4-6). The pathophysiology of BAs includes the expression of multipaneled mediators from inflammatory cells. These mediators may act as bronchoconstrictive, proinflammatory, or as chemokinetic (4). Airways inflammation in BAs can evaluate by investigating cells and/or their mediators in the circulation (7). The contribution of neutrophils in inflammation is well-known, but its precise role in BAs is still indistinct. Myeloperoxidase (MPO) is an enzyme released from the primary azurophilic granules of the neutrophils (8). Sputum or blood MPO was elevated in BAs reflecting neutrophil activity in severe asthma in children and adults (9, 10).

For assessment of the clinical value of activated neutrophils in adults with BAs, serum MPO was determined and correlated with pulmonary function tests in this case-control study.

Materials and Methods

Study Participants

This study has encompassed 94 asthmatic adults and 86, sex and aged-matched, healthy control groups selected from patients' accompanists with an age range of 18.9-65.1 years. The asthmatic patients had been diagnosed and followed up over six successive months in 2019 in the Al-Imam Al-Sadiq teaching hospital in Babylon. The asthmatics had carefully preferred from those attending the pulmonology clinic, being non-smokers, and free of pulmonary or systemic infections. The identification of asthma severity had assessed using the "Global Initiative for Asthma guidelines (GINA)", based on the outcomes of spirometry tests (forced expiratory volume/one second FEV1%, and peak expiratory flow rate (PEF/L)) (11). According to the levels of asthma control, adults with BAs had divided into three groups: Good control (n= 22), partial control (n= 30), and poor control (n= 44). Along a similar vein, patients have been divided again into two groups (treated and untreated) depending on whether they were on regular and irregular (untreated) therapy for BAs. Treatment involved systemic or inhaled steroids, oral or inhaled β-agonists, anti-leukotrienes, as well as combined inhalers.

Myeloperoxidase biochemical analyses

The MPO enzyme was measured in the blood by MPO (*Cloud-Clone Corp.*®) ELISA kit, which had an inter-Assay precision of CV< 12% and detection range of 78-5000pg/ml.

Pulmonary Function Test (Spirometry)

The predicted FEV1% and the peak expiratory flow rate (PEF/L) of all participants had been verified via hospital spirometry accessible at respiratory outpatient-unite (Micro-Medical® Spiro). Some included patients who had performed a respiratory function in the private clinics too.

Ethical consideration

The entire work had permitted by the Ethical Committees of local authorities. All participants provided an inscribed informed consent, and the research had conducted in line with the ethical morals identified in the 1975 treaty of Helsinki.

Data Analyses Design

Statistical Package for Social Sciences (SPSS-25.0, IBM) had applied to estimate the attained data on the computer. *Chi*-squared-test had applied for univariate investigation, one way-ANOVA to detect changes among the severity' levels, and *t*-test had completed detecting variations between both the treated/untreated and sex groups. Outcomes had assessed at a 95% confidence interval and had determined as significant for all parameters. The sorting accuracy of MPO had analyzed under the receiver operating characteristic (ROC) curves for their analytical fitness to decide the severity of BAs prediction.

Results

The mean ages of patients were 31.9±15.1year, which is comparable to the mean ages of the controls. The frequency of females was higher

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in the BAs group. The mean duration of asthma among asthmatics was 10.5±8.6 years. The mean pulmonary function parameters (FEV1 and PEF) were significantly lower among the patients' group (0.00). Significant higher levels of MPO had observed among BAs patients (p-0.00) (Table 1).

Table 1. The main characteristics and spirometric parameters of adults with bronchial asthma.

	Total (n= 180) Mean±SD	Asthma patients (n= 94) Mean±SD	Healthy controls (n= 86) Mean±SD	P-*value*
Age/years	34.3±13.5	31.9±15.1	36.9±10.9	0.12
Male sex (No/%)	94/52.2	34/36.2	60/63.8	0.00
Duration/years	10.5±8.6	10.5±8.6		
FEV1	86.9±19.5	77.6±21.2	97.1±10.3	0.00
PEF/L	77.2±24	65.6±22.3	89.9±18.8	0.00
MPO (pg/ml)	2481.2±1386.9	3222.5±1280.8	1670.8±991.6	0.00

The gender shows a paradoxical impact on the study parameters, where there were no significant differences in both means of the duration and FEV1, compared to significantly lower PEF means accompanied by significantly higher means of MPO enzyme levels among males (Table 2).

The levels of myeloperoxidase have not differed significantly with the levels of asthma control, while there were significant differences in MPO levels with the history of treatment among asthmatic patients (Table 3).

A nonsignificant negative correlation between the mean levels of serum MPO and the spirometric variables in the studied BAs patients were observed.

Table 2. Main gender variation of study variables.

	Duration		FEV1		PEF		MPO	
Gender	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
Mean±SD	10.5±7.2	10.4±10.9	84.9±21.1	88.7±17.8	71.1±23.5	82.8±23.1	2711.7±1432.4	2270.3±1316.3
P-Value	0.9		0.19		0.001		0.033	

Table 3. Distribution of Myeloperoxidase according to the levels of asthma and treatment history control and treatment history.

	Levels of asthma control			History of treatment	
	Good	**Partial**	**Poor**	**Treated**	**Untreated**
Number	22	28	44	60	34
Mean	3147.9	2829.9	3509.8	3389	2928.8
Std. Deviation	951.4	1240.1	1397.4	1308.9	1191.9
***P*-value**	0.2			0.09	

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Plasma MPO had further analyzed by the "area under the curve" statistics for their predictive ability to distinguish the poorly controlled BAs analyzed by the "receiver operating characteristic curves". It exposed that the accuracy of MPO, specificity, sensitivity, significance, and 95% CI, as follows: 2138 pg/ml, 84.3%, 72.1%, 80.9%, 0.00, and 0.77-0.89, respectively, to predict severe asthma (Fig. 1).

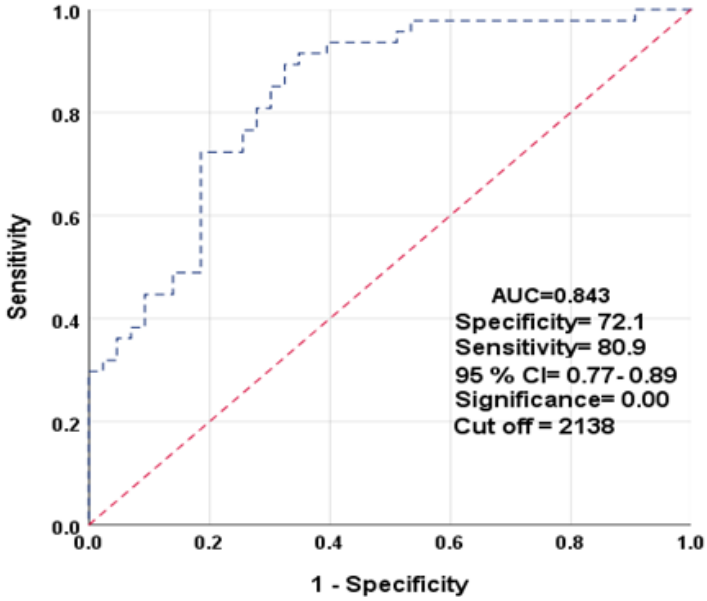

Fig. 1. Curve analyses of MPO in prediction of asthmatic severity (Receiver operating characteristic (ROC) curve).

Discussion

In BAs, and principally in the severe form, many biomarkers had concerned. Several biomarkers had been assumed as inflammatory mediators in BAs and other systemic disorders of inflammatory base, mutually (12-15). These circumstances may increase the circulatory leukocytes as well as systemic levels of pro-inflammatory mediators, like C-reactive protein (16-18), uric acid (19, 20), interleukins (21), tumor necrosis factors (22), and others, which have released in response to cellular damage (4).

Myelo peroxidase (MPO) is one of the neutrophilic-secreted enzymes that had released from the azurophilic granules (5) after oxidative stress and abnormal inflammatory reactions (23). The enzyme MPO had a crucial contribution in microbial killing (24), which approves the proinflammatory activity of MPO. Unfortunately, right now, a supreme model does not present, and there is a real overlap amongst these markers (5). There are increasing shreds of evidence that neutrophils may contribute to severe BAs. A well-known neutrophilic inflammation has been proved in lethal sudden asthma attacks (25). Nevertheless, little is recognized about airway inflammation in severe BAs.

Platelet-derived-growth-factor (PDGF) is a glycoprotein (26, 27) that can affect the bronchial tree during BA causing proliferation of airway smooth muscle cells, their migration into the epithelium, and increased collagen production by pulmonary-fibroblasts (28).

Transforming growth factor-β is a potent profibrogenic factor (29, 30) whose expression is increased in the asthmatic bronchi and is the main candidate for the instigation and persistence of airway-remodeling in BA (31).

In vitro as well as *in vivo* studies have evidenced a dual role for TGF-beta: it can either function as a pro- or anti-inflammatory cytokine on inflammatory cells, participating in the initiation and resolution of inflammatory and immune responses in the airways (32).

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Correlation of serum MPO levels with the levels of asthma control

In this study, the question under discussion is the correlation of serum MPO levels with the levels of BAs control. We speculated that MPO levels will increase with worsening asthma levels. There were higher levels of serum MPO in asthmatics which might suggest a latent role of neutrophils in asthma compared to the control group. However, the higher MPO levels were not correlated with the control levels of asthma significantly in our study. That was consistent with a previous study that assessed serum MPO in children with BAs and found no significant increase of MPO (7). Additionally, it had shown that the higher MPO levels among asthmatic patients had not related significantly to clinical variables in another survey (33), signifying that their neutrophils have a raised tendency to degranulate their proteins (34, 35).

As a rebuttal to this point, it might be (convincingly) argued that MPO values were similarly higher in patients with moderate BAs than in healthy subjects. Amongst asthmatics, the MPO values were also greater in patients with severe than in mild BAs (25) as well as in other research conducted on BAs patients in Basra (36). Wang et al. also stated meaningfully varied MPO levels in asthmatics throughout attacks, but not in asthmatic patients in remission (34).

For the sake of discussion, I would like to argue that plasma MPO levels (a biomarker of activated neutrophils) do not influence the inflammatory process in BAs. Few revisions have recommended that neutrophils and/or MPO are not the main factors in asthma inflammation (37, 38). It seems that higher serum MPO values in asthmatic adults might be related to an infection. Neutrophils-activation causes their granules to defend the body from bacterial invasion. It is well-known that respiratory infections exacerbate BAs (25, 39). Hence, the higher MPO values stated by other studies in BAs could be due to the coincident infection in their patients. Moreover, serum MPO levels did not correlate with spirometric functions (34, 38), signifying that plasma MPO does not contribute to the extent of asthmatic inflammation.

Sex variations of the study variables

The gender impact on the study parameters is to some extent similar to what had been exposed by several other studies in animal models that had proposed the contribution of sex hormones in the pathogenesis of pulmonary inflammation and BAs (40). Investigations in mouse models of BAs have also revealed that sex hormones can disturb the inflammatory process of related macrophages' polarization (41). Other scholars had reported the effects of these hormones on asthmatic symptoms in post-pubertal women, even though their influence on men and children has not yet been recognized (41).

Variation of the study variables with history of treatment

The serum MPO values had not inclined by different treatment modalities as reported by Tauber E. et al. (7) contradict our results of significant differences of MPO values with history of treatment among BAs patients. Neutrophilic inflammation in severe BAs patients may probably be due to high corticosteroid doses. The corticosteroids can increase neutrophilic survival by inhibiting apoptosis (25). However, in another survey, the researchers found that an increase in neutrophils of respiratory passages exists only after oral but not inhaled steroid therapy in patients with mild asthma (42). In this study, the authors could not establish a significant correlation between clinical markers of asthma severity such as FEV1 and PEF, with higher levels of serum MPO might be due to the potential confounding effect of corticosteroid treatment (25). Another study proposes that immune-therapy significantly modifies the MPO release of neutrophils from allergic asthma (43). Nonetheless, our patients had no history of immunotherapy- consumption. The anti-inflammatory effect of β2-agonists against interleukin-8 and neutrophil activation in asthma is another probable explanation (44).

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Of note, the prevalence of BAs has increased substantially in current decades all over the world, particularly in developed states. There is an unmet requirement for developing a novel diagnostic marker for the clinical valuation of asthma severity. It seems that MPO is not the ideal biomarker for this purpose. Unfortunately, at present, an ideal marker doesn't present, and the overlay among the biomarkers is a truth. Using multi-panel biomarkers can improve the detection of asthma endotypes in the era of state-of-art medicine. Many future works in this field are required to elucidate the exact inflammatory pathogenesis and postulate the diagnostic biomarkers for BAs.

In asthmatic patients, there were significantly higher serum levels of MPO compared to healthy controls. Levels of serum MPO had a nonsignificant correlation with FEV1 and PEF, but a significant correlation with levels of asthma control.

There are some limitations to this study. First, the presence of bacterial infection, which cannot be excluded completely. Second, the study should include measurement of neutrophils count and eosinophils count to differentiate the atopic from non-atopic asthma. Third, the study should also include the measurement of C-reactive protein.

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