SERUM OSTEOPONTIN LEVEL IN CHILDREN WITH BRONCHIAL ASTHMA

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ABSTRACT

Background: *Asthma is defined as an airway inflammation that results from interactions between various cells, cellular elements, and cytokines, which causes symptoms that include wheezing, breathlessness, and cough. Osteopontin (OPN) is a glycoprotein highly expressed in the bone and in a range of immune cells.*

Aim and objectives: *The study aim to evaluate the serum levels of osteopontin in patients with mild, moderate and severe asthma in comparison with apparently healthy group as a control and it is relation to asthma severity.*

Subjects and methods: *This is a case-control study that conducted at pediatric pulmonology outpatient clinics of Sayed-Galal and Al-Hussein University Hospitals, Faculty of Medicine, Al-Azhar University, Egypt. During the period from September 2021 to February 2022. The case group included 40 asthmatic children while Control group included 40 apparently healthy children.*

Results: *As regard Laboratory data of cases and controls, our results showed that the mean serum osteopontin showed significant elevation in cases compared to controls, severe asthmatic patients exhibit higher serum OPN level than mederate and mild patients with statistically significant difference. Hemoglobin was significantly higher in cases compared to controls. Eosinophilic percentage was significantly higher in cases compared to controls.*

Conclusion: *The present study has shown that asthma patients exhibit higher serum OPN levels than controls and severe asthmatic patients exhibit higher serum OPN level than mederate and mild patients.*

Keywords: *airway inflammation; asthma; eosinophil; osteopontin.*

INTRODUCTION

Asthma is a heterogeneous disease usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest tightness, and cough that vary over time in intensity together with expiatory airflow limitation. Incidence rates for asthma vary according to the age of the population under study and the diagnostic criteria used. Based on the application of standardized methods to measure the prevalence of asthma and wheezing illness in children and adults, it appears that the global prevalence of asthma ranges from 1% to 18% of the population in different countries **(Global Initiative for Asthma [GINA], 2021)** While in Egypt is 8.2% **(Zedan et al.,2009)**.

Airway hyper responsiveness is a feature of asthma, in that an exaggerated bronchoconstrictor response to a wide variety of stimuli is major **(Chapman et al., 2015)**.

Many factors contribute to airway hyper responsiveness as inhaled substances (e.g., methacholine), exposure to cold air, exercise, irritants or with hyperventilation **(Borak J et al., 2016)**.

Osteopontin, also known as early T-cell activation protein of gene-1 product, is a secreted 314 amino acid pleiotropic broadly expressed phosphoglycoprotein that exists both as a component of the non-collagenous bone matrix to regulate biomineralization in the bone tissue as a soluble cytokine **(AL- ayadhi, et al., 2013)**.

Osteopontin participates in wide range of biological processes, including bone remodeling, cancer and immunity to infectious disease **(Zhao et al., 2011)**.

Osteopontin plays an important role in chronic airway remodeling and bronchial hyper responsiveness. The association between Osteopontin protein expression and asthma has been investigated extensively, but the results of these studies are inconsistent **(Xu et al., 2019)**.

The study aim to evaluate the serum levels of osteopontin in patients with mild, moderate and severe asthma and then compare it to the serum levels of osteopontin in a group of healthy matched controls and to determind the relation of serum osteopontin level to asthma severity

Ethical considerations:

1. An informed consent was taken from all parents before getting involved in study.
2. Confidentiality of all data was ensured.
3. The study was done after approval of ethical committees of Pediatrics department & faculty of medicine for Al-Azhar University.
4. The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.
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Sample size:

The sample size was calculated by using the following formula:

$N = (Z/\Delta)^2 \times P (100 - P)$

Z: a percentile of slandered normal distribution determined by 95% confidence level = 1.96.

Δ: the width of the confidence interval = 12

P: the prevalence of disease = 8.2%

$N = (1.96/12)^2 \times 8.2 (100 - 8.2) =$ 20 patients in each group.

The least number of study cases by the equation was 20 in each group (case &control) but we increased the number of cases to include 40 in each group. Case group included 40 asthmatic children and Control group included 40 apparently healthy children.

PATIENTS AND METHODS

The present case-control study included 40 asthmatic patients and 40 apparently healthy children that were recruited from pediatrics pulmonolgy outpatient clinics of Sayed-Galal and Al-Hussein University Hospitals by simple random method during the period from September 2021 to February 2022.

Inclusion criteria: Children aged 6-16 y and patients with diagnosis of bronchial asthma by pulmonogist ([mild], [moderate] and [severe]) according to **(GINA, 2021)** guidelines.

Exclusion criteria: Children aged >6 years or <16 years, any pulmonary disease other than bronchial asthma for example (bronchiectasis, cystic fibrosis), and refusal to participate in the study.

Tools of assessment: For all patients who fulfilled the inclusion criteria in the absence of the exclusion criteria, the following

baseline data will be collected as follows:

History taking: Detailed history of the child with emphasis on age, gender, residence, history of wheezes, cough and shortness of breath, seasonal variation of symptoms, nocturnal symptoms, previous history of similar attacks, history of other atopic manifestations and family history of atopy.

Physical examination: General examination: with stress on, heart rate, respiratory rate and temperature. Local chest examination: Inspection: Shape of chest, respiratory movements, trachea, and palpation: Tenderness, trachea and chest movement, percussion: For dullness or hyper resonance and auscultation: Air entry, type of breathing and rhonchi.

Investigations:

1. **Complete Blood Count:** with stress on complete absolute eosinophilic count, total leucocytic count and eosinophilic percentage using sysmex Kx 21N.
2. **Osteopontin level:** Assay of serum levels of osteopontin was performed as follows: Three milliliters of venous blood will be withdrawn from each candidate under complete aseptic condition. The blood sample will be centrifuged at 1000 x g and serum will be stored in plastic tubes at -20° C till assay will performed after all samples collected. Osteopontin will be analyzed using commercial enzyme-linked immunosorbent assay (ELISA)
3. **Pulmonary function tests** were done for all included children by spirometry with stress on FEV1 and FEV1: FVC ratio.

Statistical Methods: Statistical analysis will be performed using IBM Statistical Package for Social Sciences (SPSS) version 25 (IBM Inc. - USA).

The following tests were used: Frequency distributions, percentage distributions, Means ± standard deviation, t-tests, chi-square test, Fisher's exact test, tests of correlation and receiver operating characteristic (ROC) analysis. P-values less than 0.05 were considered significant. Confidence intervals (95% CI) were calculated when appropriate.

RESULTS

Our results will be demonstrated in the following tables and figure:

Table (1): Demographic and clinical data of cases and controls:

	Cases (40)	Controls(40)	95% CI	P-value
Age (years)	6.17 ± 2.34	5.21± 3.52	-2.37, 0.29	0.124
Residence (urban/rural)	24/16	21/19	-	0.652
Gender Female/male	23/17	22/18		0.673
Heart rate	135.4 ± 20.8	99.2 ± 10.8	28.72, 43.58	**<0.001**
Respiratory rate	44.48 ± 9.98	27.10 ± 4.33	13.92, 20.83	**<0.001**

This table shows significant increase in heart rate and respiratory rate in cases than controls

Table (2): Laboratory data of cases and control

Variable	Cases (40)	Controls(40)	95% Cl	P-value
Hemoglobin	11.82±1.25	10.62±2.47	0.32,2.07	0.008
Total leukocytic count	9.51±3.84	11.27±4.29	-3.57,0.05	0.057
Esinophils % Count	3.12±2.62 313±451	1.72±2.01 181±232	0.09,2.70 -29.3,292.00	0.037 0.107
Osteopontin (ng/ml)	5.88±0.86	4.66±0.66	0.87,1.57	<0.001

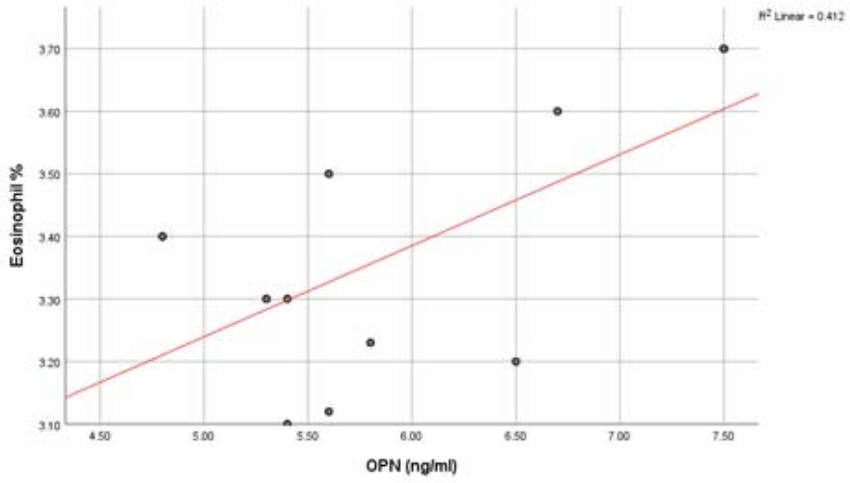
This table shows significant increase in hemoglobin, Eosinophilic percentage and osteopontin level in cases than controls.

Table (3): Relation between serum osteopontin and severity of bronchial asthma:

	Mild asthma	Moderate asthma	Severe asthma	95% CI	P- value
Serum osteopontin	4.7± 0.26	5.37 ± 0.32	7.05± 0.48	1.94, 1.42	<0.001

This table shows positive correlation between osteopontin level and asthma severity.

Correlation between OPN and eosinophils in cases:



This figure shows Positive correlation between OPN and eosinophils in cases.

Table (4): Pulmonary function tests of cases and controls

PFT	Cases	Control	P Value
FEV1	63.5 ±13.6	95.8± 2.0	<0.0001
FEV1: FVC	76 ± 4.6	89 ± 3.7	<0.0001

FEV1 and FEV1: FVC were significantly lower in cases compared to controls

Table (5): Diagnostic accuracy of serum osteopontin in pediatric bronchial asthma

Area under the ROC curve (AUC)	0.886
95% Confidence interval	0.796 to 0.946
P-value	<0.001
Cutoff point	>4.7
Sensitivity	**84%**
Specificity	60%
Positive predictive value	71.4%
Negative predictive value	**93%**

Osteopontin had good sensitivity and fair specificity in prediction of pediatric bronchial asthma.

Table (6): Diagnostic accuracy of serum osteopontin in prediction of severity of bronchial asthma in pediatric patients

Area under the ROC curve (AUC)	1.00
95% Confidence interval	0.912 to 1.000
P-value	<0.001
Cutoff point	>5.8
Sensitivity	**94%**
Specificity	**92%**
Positive predictive value	**87%**
Negative predictive value	**89%**

Osteopontin had excellent sensitivity and specificity in prediction of severity of bronchial asthma in pediatric patients.

DISCUSSION

Osteopontin (OPN) is a phosphorylated acidic glycoprotein that was originally regarded as a structural component of the extracellular matrix; it can bind proteins and most types of collagens **(Icer and Gezmen-Karadag, 2018)**.

OPN regulates the immune system at many different levels. It serves as a pro-inflammatory cytokine and can modulate the immune response by enhancing expression of Th1 cytokines and matrix degrading enzymes. OPN plays a pivotal role in T cell and macrophage responses during cell mediated immune responses against bacterial and viral pathogens. OPN has also been shown to modulate dendritic cell responses and neutrophil chemotaxis **(Challis et al., 2009)**.

The main aim of this study was to determine the serum levels of osteopontin in patients with mild, moderate and severe asthma and then compare it to the serum levels of osteopontin in a group of healthy matched controls, Also, to find the relation between the serum level of osteopontin and the severity of asthma.

The present case-control study was conducted at pediatrics pulmonology outpatient clinics of Sayed-Galal and Al-Hussein University Hospitals, Faculty of Medicine, Al-Azhar University, Egypt. It was conducted from September 2021 to February 2022. The case group included 40 asthmatic children. Control group included 40 apparently healthy children who were recruited from the outpatient clinics after taking consent from their caregivers.

Regarding the sociodemographic data of the studied group, the current study showed that the mean age was 6.17±2.34 years with 57% females; the majority (60%) of the studied cases was Urban.

The current study was in agreement with the study by **Toema et al.,** aimed to measure the serum OPN level in asthmatic children to clarify its relation to bronchial asthma.

As well, the prospective, cross-sectional study by **Akelma et al.,** aimed to investigate whether OPN levels change due to allergic inflammation in pre-school and school-age children.

Comparison between cases and control groups showed that there was no statistically significant difference between studied groups as regard age, sex and residence, but heart rate and respiratory rate were significantly higher in cases compared to controls.

In agreement with our results, **Toema et al., & Akelma et al.,** reported that there was no statistically significant difference between patient and control groups as regard age and sex.

Heart rate and respiratory rate were significantly higher in cases as compare with controls, this can be explained as asthma is a chronic respiratory disease characterized by episodic and reversible attacks of wheezing, chest tightness, shortness of breath, and coughing **(Scarlata and Incalzi, 2022)**, so it was predictable by definition that patients with asthma had higher heart rate and respiratory rate, and also as any inflammation asthma can cause elevation in temperature.

As regard Laboratory data of cases and controls, our results showed that Hemoglobin was significantly higher in cases compared to controls. Eosinophilic percentage was significantly higher in cases compared to controls. The mean serum osteopontin showed significant elevation in cases compared to controls.

In agreement with our results, **Toema et al., (2018)** reported that the serum OPN level was significantly higher in asthmatic patients during stable asthma conditions and during exacerbations than the control group (P= 0.0054). Also reported that there was significant positive correlation between serum OPN level and Hb, Esinophilic count.

As well, the current study was supported by **Yang et al.,** who reported that the serum level of OPN in the asthma group was

significantly higher than in the control group (P<0.05). In addition, they reported that both the atopic and non-atopic subgroups showed increased serum levels of OPN compared with the control group (P<0.05). The serum levels of OPN showed no significant differences in asthmatic children with different genotypes.

Furthermore, in agreement with our results **Samitas et al.,** showed that Serum and BALF (bronchoalveolar lavage fluid) OPN and eosinophils levels were significantly increased in all asthmatics in the steady state, whereas serum levels decreased during exacerbations.

Also, in harmony with the current study **Akelma et al.,** reported that eosinophilia and osteopontin were significantly higher in asthmatic patients as compared with controls.

The Serum osteopontin was significantly higher in cases with bronchial asthma than healthy as OPN is expressed in a variety of cells, including bronchial epithelial cells and inflammatory cells around airways, such as T cells, CD11c-positive DCs, and eosinophils in asthmatic airways. Although the factors responsible for the higher expression of OPN in asthma have not been elucidated, it is probable that several pro-inflammatory cytokines, such as TNF-α and IL-1β, are involved in the induction of OPN in the airway **(Miethe et al., 2018)**.

The current study showed that Serum osteopontin was significantly higher in cases with severe asthma compared to cases with mild and moderate asthma.

Our results can be supported by **Samitas et al.,** who concluded that OPN expression is up regulated in human asthma and associated with remodeling changes, and its sub epithelial expression correlates with disease severity.

However, the meta-analysis by **Xu et al.,** reported that 3 out of 9 studies reported an association between OPN protein expression and the severity of asthma. Analysis of the pooled data showed correlation but not reach significance between OPN protein expression and severity of asthma (SMD = 0.28, 95% CI: -0.23–0.79), but significant heterogeneity was detected among these three studies (I2 = 65.8%, P = 0.054).

Also, **Toema et al.,** reported that there was no association was observed between OPN concentration and disease severity.

As well, in adults **Delimpoura et al.,** and **Zhao et al.,** reported that the increase in OPN does not seem to correlate with the severity or control of asthma.

The current study showed that at a cutoff point of 4.7 osteopontin had excellent sensitivity and fair specificity in prediction of pediatric bronchial asthma and at a cutoff of 5.8 Osteopontin had excellent sensitivity and specificity in prediction of severity of asthma in pediatric patients.

The Serum osteopontin was significantly higher in cases with severe asthma compared to cases with mild and moderate asthma as OPN is associated with mediators involved in both inflammatory and remodeling processes, such as TGF-β1, IL-13 and cysteinylleukotrienes, only in severe asthmatic patients **(Brusselle and Koppelman, 2022)**.

CONCLUSION

The present study has shown that:

1. Asthmatic children exhibit higher serum OPN levels than healthy.
2. Severe asthmatic children exhibit higher serum OPN level than moderate and mild patients suggesting a role for this protein in the pathogenesis of asthma.
3. Serum OPN is a promising biomarker in the diagnosis of bronchial asthma. The data suggest a role for OPN in the pathogenesis of asthma.

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