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Serum Osteopontin Level as Biomarker in the Diagnosis of Pediatric Bronchial Asthma in Different Age Groups

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ABSTRACT

Background: Bronchial asthma is chronic inflammatory disease of the respiratory tract, in which many cells of innate and adaptive immune system in combination with epithelial cells are involved causing the main clinical typical criteria for the disease. Osteopontin (OPN) is identified in many cell types in the immune system. OPN is a protein expressed during the inflammatory processes related to Th2 lymphocyte activity.

Objective: The aim of the current study was to evaluate serum osteopontin level as biomarker in the diagnosis of bronchial asthma in different age groups. **Patients and Methods:** This prospective case-control study was carried out at the Pediatric Department, Zagazig University Hospitals from May 2019 till December 2019. All patients were subjected to full history taking and laboratory investigations including measurement of Human Osteopontin (OPN).

Results: There was high significant positive correlation between osteopontin and S. IgE and AEC. Also, there was high significant negative correlation between osteopontin and forced expiratory volume 1 (FEV1), forced vital capacity (FVC) and peak expiratory flow (PEF) in both age groups. There was significant difference between <5 years severe asthma and 5-12 years severe asthma as regard Osteopontin level.

Conclusion: It could be concluded that there is strong association between OPN concentration and disease severity. OPN shows high significant difference between asthmatic and control group. Serum OPN is a good biomarker in the diagnosis of bronchial asthma at different age groups.

Keywords: Osteopontin, Biomarker, Diagnosis, Bronchial asthma, Pediatric.

INTRODUCTION

Bronchial asthma is chronic inflammatory disease of the respiratory tract, in which many cells of innate and adaptive immune system in combination with epithelial cells are involved causing the main clinical typical criteria for the disease **(1).**

Bronchial asthma more closely resembles a complex of clinical diseases than a single condition. Within the past few years, it has emerged as the most common incommunicable respiratory disease affecting children worldwide **(2).**

According to epidemiological studies, 5–10% children suffer from bronchial asthma (BA) .In most children BA formation is associated with Th2-variant of an immune response, atopy, innate tendency for hyper production of total and specific IgE, reduced functional activity of T-regulatory cells (Tr1, CD4+, CD25+) and Th1/Th2 imbalance **(3).**

The interaction of specific IgE with causally significant allergens on the surface of mast cells and basophils induces the release of preformed and synthesized de novo mediators causing acute inflammation of bronchi accompanied by cell migration in respiratory mucosa and formation of cell infiltrate including eosinophils, basophils, Th2-lymphocytes with the involvement of macrophages, monocytes, mast and epithelial cells, platelets, neutrophils, fibroblasts **(4).**

Osteopontin (OPN) is identified in many cell types in the immune system. It has been shown to be produced especially by T-cells, B-cells, macrophages, neutrophils, eosinophil, natural killer cells, CD11c-positive dendritic cells (DCs) and bronchial epithelium. OPN is a protein expressed during the inflammatory processes related to Th2 lymphocyte activity **(5).** It was demonstrated in previous studies that OPN plays role in asthma **(6),** allergic rhinitis, allergic conjunctivitis and response to venom immunotherapy. However, the role of OPN in pediatric asthma has not been studied.

The aim of the current study was to evaluate serum osteopontin level as biomarker in the diagnosis of bronchial asthma in different age groups

PATIENTS AND METHODS

This prospective case-control study included a total of 152 children with bronchial asthma, and 38 healthy, nonasthmatic children as controls, attending at Pediatric Department, Zagazig University Hospitals. This study was conducted between May 2019 till December 2019.

Inclusion criteria:

- **Cases**: all patients presented by wheezy chest and diagnosed as bronchial asthma by history, clinical picture and investigations in preschool and school age children.
- **Healthy control**: age and sex comparable healthy children.

Exclusion criteria:

1. Age less than 1 year.
2. Terminal stages of disease (malignancy, end-stage liver, or renal diseases).
3. Cystic fibrosis, pulmonary tuberculosis.
4. Immune compromised.
5. Patient or relatives don't consent to participate in the study.
6. All other cases of wheezy chest except asthma.

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The included subjects were divided into two age groups ≤ 5 years and > 5 years. Each age group divided into 5 subgroups according to different severities of asthma into intermittent, mild, moderate, and severe persistent asthma with healthy control group.

Sampling technique: Systematic random sampling technique was used.

All patients were subjected to the following:

A. Full history taking.

B. Complete clinical examination:

- General examination.
- Complete chest examination including inspection, palpation, percussion and auscultation.
- Vital data as temperature, heart rate, respiratory rate and blood pressure were recorded.
- Asthma was defined as a clear clinical history with current symptoms plus 15% reversibility in forced expiratory volume in 1 s (FEV1) after two puffs of b2-agonist and/or positive methacholine challenge. All patients had a physician confirmed diagnosis of asthma and were receiving therapy depending on their asthma severity, according to Global Initiative for Asthma guidelines [(7)]**.** Asthma exacerbations were defined as events of severe deterioration of symptoms and rescue medication use that required the use of systemic corticosteroids, or an increase from a stable maintenance dose, foro3 days [(8)]**.**

C. Laboratory investigations: Complete blood count (CBC), erythrocytes sedimentation rate (ESR), c-reactive protein (CRP), Ig-E, liver, and kidney function tests and Human Osteopontin (OPN).

Principle of Human Osteopontin (OPN) test: The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Osteopontin (OPN) in samples**.**

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***Ethical Consideration*:**

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Written informed consent of all the participants' parents was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis:

Analysis of data was done using Statistical Program for Social Science version 20 (SPSS Inc., Chicago, IL, USA). Quantitative variables were described in the form of mean and standard deviation. Qualitative variables were described as number and percent. In order to compare parametric quantitative variables between two groups, Student t test was performed. Qualitative variables were compared using chi-square (X2) test or Fisher's exact test when frequencies were below five. Pearson correlation coefficients were used to assess the association between two normally distributed variables. When a variable was not normally distributed, A P value < 0.05 is considered significant

Operational design:

The researcher introduced himself to parents of all participants included in this study and asked them to participate after illustrating the goal of the study. All selected parents of participants received comprehensive information regarding objective and the expected benefit of the study. All ethical considerations were taken throughout the whole work.

RESULTS

Table 1 shows that there were no statistically significant differences between the five study sub-groups as regard age, BMI, weight, height residence or gender **Table (1)**.

Table (1): Comparison between cases and control as regard Demographic data in (< 5 years) group:

Variable	Control group (n=19)		Intermittent (n=22)		Mild (n=21)		Moderate (n=21)		Severe (n=12)		F	P value
Age: (years):												
Mean±SD Range	3.2± 0.9 (2-5)		3.2± 0.9 (2-5)		3.3±0.7 (2-5)		3.10±0.8 (2-5)		3.01±0.9 (2-5)		0.284	0.887
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	χ^2	**P value**
Gender:												
Female Male	8 11	42.1 57.9	9 13	40.9 59.1	8 13	38.1 61.9	7 14	33.3 66.7	4 8	33.3 66.7	0.520	0.971
Residence:												
Urban Rural	12 7	63.1 36.1	14 8	63.6 36.4	14 7	66.7 33.3	15 6	71.4 28.6	10 2	83.3 16.7	1.83	0.767

F is for one-way ANOVA, X^2 for chi square test. P value is significant if <0.05

Table 2 shows that there were no statistically significant differences between the five study sub-groups as regard age, BMI, weight, height residence or gender.

Table (2): Comparison between cases and control as regard Demographic data in (5-12 years) group:

Variable	Control group (n=19)		Intermittent (n=22)		Mild (n=21)		Moderate (n=21)		Severe (n=12)		F	P value
Age: (years):												
Mean±SD Range	8.0± 2.3 (5-12)		8.1± 2.2 (5-12)		8.2± 2.9 (5-11)		8.1± 1.7 (6-12)		8.0± 2.3 (5-12)		0.609	0.647
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	χ^2	**P value**
Gender:												
Female Male	5 14	26.3 73.7	6 16	27.3 72.7	5 16	23.8 76.2	5 16	23.8 76.2	2 10	16.7 83.3	0.534	0.970
Residence:												
Urban Rural	4 15	21.0 79.0	5 17	22.7 77.3	4 17	19.0 81.0	3 18	14.3 85.7	1 11	8.3 91.7	1.43	0.838

Table 3 shows that there were no statistically significant differences between the five study sub-groups as regard IgE, OPN and AEC.

Table (3): Comparison between cases and control as regard markers of diagnosis in (<5 years) group:

Variable	Control group (n=19)	Intermittent (n=22)	Mild (n=21)	Moderate (n=21)	Severe (n=12)	KW	P value
IgE (IU/ml):							
Mean±SD	53±11.15	99±17.51	191±7.91	271±53.41	403±83.41	64.8	**<0.001**
OPN (ng/mL):							
Mean±SD	12.9±2.81	18.3±4.31	35±7.64	66.8±4.61	201±45.61	66.9	**<0.001**
AEC (cells\mm3):							
Mean±SD	72±16.21	126.5±28.41	299±7.52	330±7.61	391±80.31	69.2	**<0.001**

KW is for Kruskal Wallis test
Similar letters indicate no significant difference between groups
Different letters indicate significant difference between groups

Table 4 shows that there were no statistically significant differences between the five study sub-groups as regard IgE, OPN and AEC.

Table (4): Comparison between cases and control as regard markers of diagnosis in (5-12 years) group:

Variable	Control group (n=19)	Intermittent (n=22)	Mild (n=21)	Moderate (n=21)	Severe (n=10)	KW	P value
IgE (IU/ml):							
Mean±SD	44±8.31	112±23.21	201±46.34	262±58.13	357±8.21	61.0	**<0.001**
OPN (ng/mL):							
Mean±SD	12±2.81	24±5.12	48±9.3	108±25.21	462±95.36	68	**<0.001**
AEC (cells\mm3):							
Mean±SD	80±4.41	150±32.61	302±66.3	331.5±8.31	413±93.69	67.2	**<0.001**

KW is for Kruskal Wallis test.
Similar letters indicate no significant difference between groups.
Different letters indicate significant difference between groups.

Table 5 shows that there is high significant Positive correlation between Osteopontin and S. IgE and AEC, also there is high significant negative correlation between Osteopontin and FEV1, FVC and PEF, there is significant correlation between Osteopontin and age.

Table (5): The correlation between Osteopontin and different parameters in the studded groups:

Variable	Osteopontin	
	r	P
Age (years)	0.412	**0.041 (S)**
Weight(Kg)	0.217	0.312
Height (m^2)	0.131	0.611
BMI (Kg/ m^2)	0.199	0.523
FEV1/L	-0.741	**<0.001 (HS)**
FVC	-0.762	**<0.001 (HS)**
PEF	-0.657	**<0.001 (HS)**
S.IgE (IU/ml)	0.651	**<0.001 (HS)**
ALT(IU/ml)	0.114	0.76
AST(IU/ml)	0.094	0.924
Creatinine	0.037	0.945
(gm/dl)Hb	0.092	0.871
(cells\mm3)AEC	0.662	**<0.001 (HS)**

r is the correlation coefficient of Pearson's correlation

Table 6 shows that at cut off point >14.8, area under the curve was 0.937, sensitivity was 97.8%, specificity 95.7%, PPV 96.4% and NPV 94.3%**.**

Table (6):Statistical performance of ROC curve: ROC curve Analysis for Predictive ability of OPN in detection of brochial asthma (< 5 years)

Area	P	Sensitivity	Specificity	PPV	NPV	Accuracy	Best cut off	95% Confidence Interval	
								Lower Bound	Upper Bound
0.937	**0.00****	**97.8%**	**95.7%**	**96.4%**	**94.3%**	**95.2%**	**>14.8**	**0.919**	**0.957**

Table 7 shows that at cut off point >15.15, area under the curve was 0.961, sensitivity was 98.3%, specificity 92.7%, PPV 95.4% and NPV 94.7%**.**

Table (7):Statistical performance of ROC curve: **ROC curve Analysis for Predictive ability of OPN in detection of bronchial asthma (5-12 years)**

Area	P	Sensitivity	Specificity	PPV	NPV	Accuracy	Best cut off	95% Confidence Interval	
								Lower Bound	Upper Bound
0.961	**0.00****	**98.3%**	**92.7%**	**95.4%**	**94.7%**	**96.8%**	**> 15.15**	**0.919**	**0.957**

DISCUSSION

In this study we found that there was high significant positive correlation between Osteopontin and IgE and AEC.

This was explained by **Del Prete *et al.* [9] and Puxeddu *et al.* [10]** as they noticed that OPN plays multiple roles in the regulation of allergic responses. It plays a role in the migration of eosinophils into the airways which is a feature of allergic airway disease.

In this study we found that there was high significant negative correlation between Osteopontin and FEV1, FVC and PEF.

This was in agreement with **Delimpoura *et al.* [11] and Akelma *et al.* [12]** as they noticed high significant negative correlation between **patients** with asthma of different underlying severity and healthy controls as regard Osteopontin and FEV1, FVC and PEF.

Also, **Kanemitsu *et al.* [13]** also conducted a study to investigate how osteopontin and periostin were related to the decline of pulmonary function, on twenty patients with asthma, with bronchial biopsy suggest that osteopontin and periostin in vivo are linked to the decline of pulmonary function in asthma.

on the other hand, **Toema *et al.* [14]** demonstrated that there was no statistically significant correlation observed between pulmonary functions and serum OPN level either in group I (during acute exacerbation) or group II (stable condition) and this may be due to small number of patients in this study.

This study showed that OPN show high significant difference between asthmatic and control group.

This was similarity with **Samitas *et al.* [15]** who found OPN in asthma patients were higher than the control group.

We also reported that there was high significant difference between the control and asthmatic as Osteopontin in <5 age group and in 5-12 age group.

Also, in our study we verified that there was significant difference between <5 years and 5-12 years of different severities of asthma as regard Osteopontin level. Patients with severe persistent asthma show higher level of OPN than moderate and mild cases.

Delimpoura *et al.* [11] who conducted a study to measure OPN levels in sputum of patients with asthma of different underlying severity and healthy controls verified that OPN levels in patients with a co morbidity of asthma and allergic rhinitis were significantly higher than in patients with allergic rhinitis without asthma in the same study.

In several other studies conducted in adult patients with asthma, OPN levels were found to be increased in serum, saliva and BAL, also they found that OPN levels (pg/ml) were significantly higher in patients with SRA than in patients with moderate asthma, those with steroid-naive asthma or healthy control subjects ($p<0.001$). Patients with moderate asthma had significantly higher OPN levels than steroid-naïve patients and healthy subjects ($p=0.01$).

Also, **Liu *et al.* [16]** verified that The OPN protein levels may serve as predictors of disease severity in childhood asthma and appear to be promising targets for modulating bronchial asthma allergic rhinitis.

This can be explained by incomplete activation of OPN producing cells according to different severities of asthma.

This was in contradict with **Xu *et al.* [17]** who carried out a meta-analysis was to determine the relationship between OPN protein expression and asthma, concluded that OPN protein expression is significantly up regulated in patients with asthma, but that it does not correlate with the type or severity of asthma.

Also, **Zhao *et al.* [18]** found no association was present between OPN concentration and disease severity.

At the present study at cut off point >14.8, area under the curve was 0.937, sensitivity was 97.8%, specificity 95.7%, PPV 96.4% and NPV 94.3%.

The high validity for Osteopontin can be justified by the high difference in its level between cases and control.

The limitation of the current study was the lack for follow up to demonstrate role of treatment on alteration of OPN level and To clarify this, the relationship between OPN and the development of persistent wheezing should be investigated in long-term follow-up studies, another limitation was that also in our study we did not examine bronchial biopsies, which would have allowed the more precise analysis of the presence and location of OPN protein and to detect its cellular source via immune histochemistry.

CONCLUSION

It could be concluded that there is strong association between OPN concentration and disease severity. OPN show high significant difference between asthmatic and control group. Serum OPN is a good biomarker in the diagnosis of bronchial asthma at different age groups.

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Author contribution: Authors contributed equally in the study.

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