International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Assessment of Serum Osteopontin and Eosinophil Major Basic Protein Levels in Children with Bronchial Asthma

Balkees Abdel Mageed Mohamed[1], Badr Eldin Mohamed Mesbah[1], Abd Elmoneim Kasem Khashana[1], Hanan Hassan Omar[2], Zeinab Abdelaal Mohamed[1]*

[1]*Pediatrics and Neonatology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*
[2]*Clinical pathology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt*

Abstract

Bronchial asthma (BA) is a chronic inflammatory illness that causes airway narrowing, airway hyperresponsiveness, airway mucous plugging. The purpose of this study was to improve the assessment of severity and degree of control of childhood bronchial asthma and to assess serum level of Osteopontin (OPN) and eosinophil major basic protein (EMBP) for pre-school and school-age children with asthma. This case control work was performed on 90 patients categorized into 2 groups; asthmatic group: involved 45 asthmatic children and control group: included 45 age and sex matched healthy children free from any renal injury or chronic diseases. Laboratory investigations (complete blood count (CBC), analysis of OPN and EMBP) were done after ethical considerations. OPN can significantly predict asthma at cut off >10.86 (P value of <0.001, the area under curve (AUC) of 0.914) with 93.33% sensitivity, 86.67% specificity, 87.5% positive predictive value (PPV), 92.9% negative predictive value (NPV). EMBP can significantly predict asthma at cut off >39.86(P value of <0.001, AUC of 0.702) with 86.67% sensitivity, 44.44% specificity, 60.9% PPV, 76.9% NPV. There was no correlation between OPN and EMBP and between the severity of asthma and markers as OPN and EMBP. OPN (OR=1.154, 95% CI= 1.021 -1.303, P value =0.007), EMBP (OR=1.017, 95% CI= 1.001 -1.011, P value =0.013) and eosinophils (OR=7.254, 95% CI= 1.862 -28.261, P value <0.001) were significant predictors of asthma, while WBCs was not a significant predictor. Asthmatic children had higher OPN and EMBP. The severity of asthma was not significantly correlated with OPN and EMBP. OPN and EMBP can significantly predict asthma. Serum OPN and EMBP could be good biomarkers in the diagnosis of BA in different age groups.

Keywords: Bronchial Asthma, Osteopontin, Eosinophil major basic protein

Full length article *Corresponding Author, e-mail: balkees.1478@gmail.com Doi # https://doi.org/10.62877/7-IJCBS-24-25-19-7

1. Introduction

Bronchial asthma is an extensive general health issue. A diverse illness, asthma frequently characterizes by chronic inflammation of the airways. This inflammation is identified by a history of respiratory manifestations, including wheezing, shortness of breathing, tightening of the chest, and cough, which may vary in frequency and severity. Additionally, asthma is associated with varying limitations in the expiratory airflow [1-3]. The airway in individuals with asthma is marked by chronic inflammation of the airway wall caused by a complex infiltration and interaction of immune cells such as eosinophils, lymphocytes, neutrophils, innate lymphoid cells, dendritic cells (CDs), and mast cells. Inflammation causes the airway to become narrower, increases the sensitivity of the airway, leads to the accumulation of mucus in the airway, and causes airway remodeling [4]. Osteopontin (OPN) is an extracellular matrix protein and immune modulator that has a variety of functions. It is considered a crucial cytokine in Th1 immune reactions. OPN is also referred to as early T lymphocyte activation 1 (ETA-1) and is involved in various pathological and physiological processes [5]. OPN is present in several cell types within the immune system. Research has shown that the production of this substance is mostly carried out by T-cells, B-cells, neutrophils, macrophages, natural killer (NK) cells, eosinophils, CD11c-positive CDs, and bronchial epithelium [6]. OPN has many functions in controlling allergic reactions. It contributes to the migration of eosinophils into the airways, which is a characteristic of allergic airway illness [5]. OPN has significant roles in several processes such as inflammation, biomineralization, cardiovascular disorders, cellular viability, diabetes, cancer, and renal disease caused by stones. These activities are carried out by various mechanisms [7]. OPN is present in numerous types of immune cells, like neutrophils,

macrophages, CDs, B cells, and T cells, and its expression levels change over time. It has chemotactic features that facilitate the recruitment of cells to areas of inflammation [8]. The eosinophil major basic protein (EMBP), found in the eosinophil secondary granule, is involved in cytotoxicity and the regulation of allergic-related conditions like asthma. The EMBP level is typically elevated in biological fluids of individuals with asthma and other eosinophil-related conditions [8]. The impact of EMBP on contraction of smooth muscle cell is indirect and most likely mediated by disrupting the airway epithelium's barrier or by changing the release of epithelial mediators, according to studies conducted both in-vivo and in-vitro [9] utilizing direct but short administrations of purified EMBP [10]. The purpose of this study was to enhance the assessment of severity and degree of control of childhood bronchial asthma, to assess serum level of OPN and EMBP for pre-school and school age children with BA and to evaluate the relationship between OPN and E MBP with severity of BA for pre-school and school-age children.

2. Materials and Methods

This cross-sectional work was performed on 45 individuals and 45 healthy children as control group aged from 3 to 18 years old, both sexes, diagnosed with various degrees of asthma according to the GINA for cases and who were coming for follow up of weight, height or those coming for check-ups, who didn't have any history of wheezy chest or any allergic disorder for control children. The work was performed approval from the Ethics Committee Suez Canal University Hospital, Ismailia, Egypt. The relatives of the patients provided a well-informed written consent. Criteria of exclusion were children who had chronic chest diseases other than asthma, like upper airway obstruction, thoracic cage malformation, upper or lower respiratory tract infections, cystic fibrosis, chronic or acute systemic disorders (e.g. Cardiac, renal, hepatic disorders, gastrointestinal diseases) and obesity and those with inflammatory diseases.

Patients were categorized into two equal groups:

Cases (asthmatic group): Children were classified as asthmatic based on their experience of recurring episodes of a minimum of one manifestation of asthma, such as wheezes, coughs, dyspnea, and chest tightening. These manifestations fluctuate in frequency and severity and are often caused by variables like physical activity, irritants, and changes in weather [11].

Control (control group): sex and age matched with asthmatic group and coming for regular check-ups. Each participant had been exposed to: taking of history, clinical examinations and laboratory investigations [full blood picture (CBC), analysis of OPN and EMBP].

2.1. Sample collection

A dry, clean centrifuge tube was used, and three ml of blood were permitted to gently flow along its wall. The blood sample was appropriately labeled with the subject's name. The blood had been incubated at 37°C in a water bath for 30 mins to allow clotting. After that, it was centrifuged at 1000 rpm for 15 mins to separate the serum. The serum was then transferred to a dry, clean tube and stored at -20°C to prevent the degradation of the bioactive OPN.

2.2. Analysis of OPN

It was determined by the ELISA technique. The ELISA kit used a quantitative sandwich enzyme immunoassay technique. A microplate was pre-coated with a monoclonal antibody which specifically targets OPN. The standards and samples were transferred into the wells using a pipette, and any OPN that was exist was attached to the immobilized antibodies. after the removal of any unbound compounds, a polyclonal antibody linked to an enzyme was introduced to the wells. The resulting colors were directly proportional to the quantity of OPN that had bonded during the initial stage. The color's intensity was quantified utilizing a microplate reader at 450 nm. The results were measured based on the standards of OPN [12].

2.3. Analysis of EMBP

The measurement was conducted using human eosinophil MBP specific sandwich ELISAs, following the directions provided by the manufacturers [13].

2.4. Sample Size Calculation

The calculated sample size of the work was 40 participants for each group [13] at 5% level of significance and 80 % power, utilizing the following formula:[$N= (Z1-\alpha/2+Z1-\beta)^2 \sigma1^* \sigma2 / \delta^2$,$Z1-\alpha/2 = 1.96$, $Z1-\beta= 0.842$, σ = common SD (5.38), δ = Expected difference to be detected (mean was 5.54 in control group, 7.14 in mild to moderate cases and 10.31 in severe cases regarding EMBP according to a previous study, α = Level of acceptability of a false positive result (level of significance=0.05), β = Level of acceptability of a false negative result (0.20), β= power (0.80)]. In order to account for incomplete data and enhance the statistical power of the study, the sample size for each group was augmented to 45 individuals.

2.5. Statistical analysis

The statistical analysis had been carried out utilizing SPSS v26 software (IBM Inc., Chicago, IL, USA). The normality of the data distribution was analysed utilizing the Shapiro-Wilks test and histograms. The mean and standard deviation (SD) of the quantitative parametric variables have been displayed and contrasted between the two groups utilizing an unpaired Student's t-test. The quantitative non-parametric data were displayed as the median and interquartile range (IQR) and were assessed utilising the Mann Whitney-test. The qualitative parameters had been displayed as frequencies and percentages (%) and were evaluated utilising the Chi-square test or Fisher's exact test, as appropriate. The Pearson moment correlation equation for linear relations of properly distributed variables was used to conduct correlations between different variables. The area under the curve (AUC) evaluates the overall test performance. A two tailed P value < 0.05 was considered statistically significant.

3. Results and discussion

Sex, age, and residence revealed no substantial variation among the two groups. The mean duration of asthma was 3.82 ± 1.41 years in the asthmatic group. Table 1. Regarding current history of hospitalization, 18 (40%) patients had a current history of hospitalization, The mean number of episodes was 2.47 ± 1.14. 33 (73.33%) patients had a history of passive smoking. Regarding history of medications, 28 (62.22%) patients had a history of steroids intake, 37 (88.10%) participants had a history of receiving bronchodilator, and 22 (52.38%) participants had a history of receiving mast cell stabilizer. Regarding history of allergy, 2 (5.71%) participants had histories of allergic rhinitis, 6 (17.14%) patients had a history of urticarial, 10 (28.57%) participants had histories of drug allergy, and 18 (51.43%) participants had histories of food allergy. 24 (53.33%) patients had no history of hospitalization, 11 (24.44%) patients had a history of hospitalization for asthma and 20 (22.22%) patients had a history of hospitalization for other causes. Regarding family history, 29 (64.44%) patients had a family history of BA, and 29 (64.44%) participants had a family history of allergy. Regarding asthma severity, 37(82.22%) patients had mild asthma and 8 (17.77%) patients' moderate asthma, no one (0%) had severe asthma. Table 2. Patients with eosinophilia, patients with leukocytosis and Anemic patients were substantially greater in asthmatic group contrasted to control group (P value <0.05). OPN and EMBP were substantially greater in asthmatic group contrasted to control group (P value= 0.02). Table 3. There was no correlation between OPN and EMBP and between the severity of asthma and markers as OPN and EMBP. Table 4. In multiple logistic regression, OPN (OR=1.154, 95% CI= 1.021 -1.303, P value =0.007), EMBP (OR=1.017, 95% CI= 1.001 - 1.011, P value =0.013) and eosinophils (OR=7.254, 95% CI= 1.862 -28.261, P value <0.001) were significant predictors of asthma, while WBCs was not a significant predictor. Table 5. OPN can significantly predict asthma at cut off >10.86 (P value of <0.001, AUC of 0.914) with 93.33% sensitivity, 86.67% specificity, 87.5% PPV, 92.9% NPV. EMBP can significantly predict asthma at cut off >39.86(P value of <0.001, AUC of 0.702) with 86.67% sensitivity, 44.44% specificity, 60.9% PPV, 76.9% NPV. Figure 1. Asthma is a diverse condition typically marked by chronic inflammation of the airways [14]. Asthma is characterized by a fluctuating intensity of respiratory manifestations, including wheezes, dyspnea, chest tightness, and coughing, along with a restriction in the expiatory airflow. The global occurrence of asthma varies from 1% to 18% across different countries [15]. Asthma is caused by Th2-mediated inflammation and bronchial hyperreactivity. Unfortunately, there is currently no conclusive biomarker available for precisely recognizing children with high-risk characteristics who will develop persistent asthma [16]. OPN is present in various cell types within the immune system. Research has demonstrated that B-cells, T-cells, macrophages, eosinophils, neutrophils, NK cells, CD11c-positive DCs, and bronchial epithelium mostly carry out the production of this substance. Previous investigations have shown that OPN is involved in BA, allergic conjunctivitis, allergic rhinitis, and the response to venom immunotherapy [6]. EMBP, a component of the eosinophil secondary granule, is involved in cytotoxicity and the regulation of allergic illnesses like BA. The level of EMBP has been found to be elevated in biological fluids of individuals with BA and other conditions linked with eosinophils [17].

Table 1. Demographics of the studied groups

		Asthmatic group (n=45)	Control group (n=45)	P
Age (years)		6.74 ± 3.17	7.1 ± 3.1	0.592
Sex	**Male**	24 (53.33%)	27 (60%)	0.671
	Female	21 (46.67%)	18 (40%)	
Residence	**Rural**	17 (37.78%)	20 (44.44%)	0.669
	Urban	28 (62.22%)	25 (55.56%)	
Duration of asthma (years)		3.82 ± 1.41	---	---

Data are presented as mean ± SD or frequency (%), *significant p value <0.05.

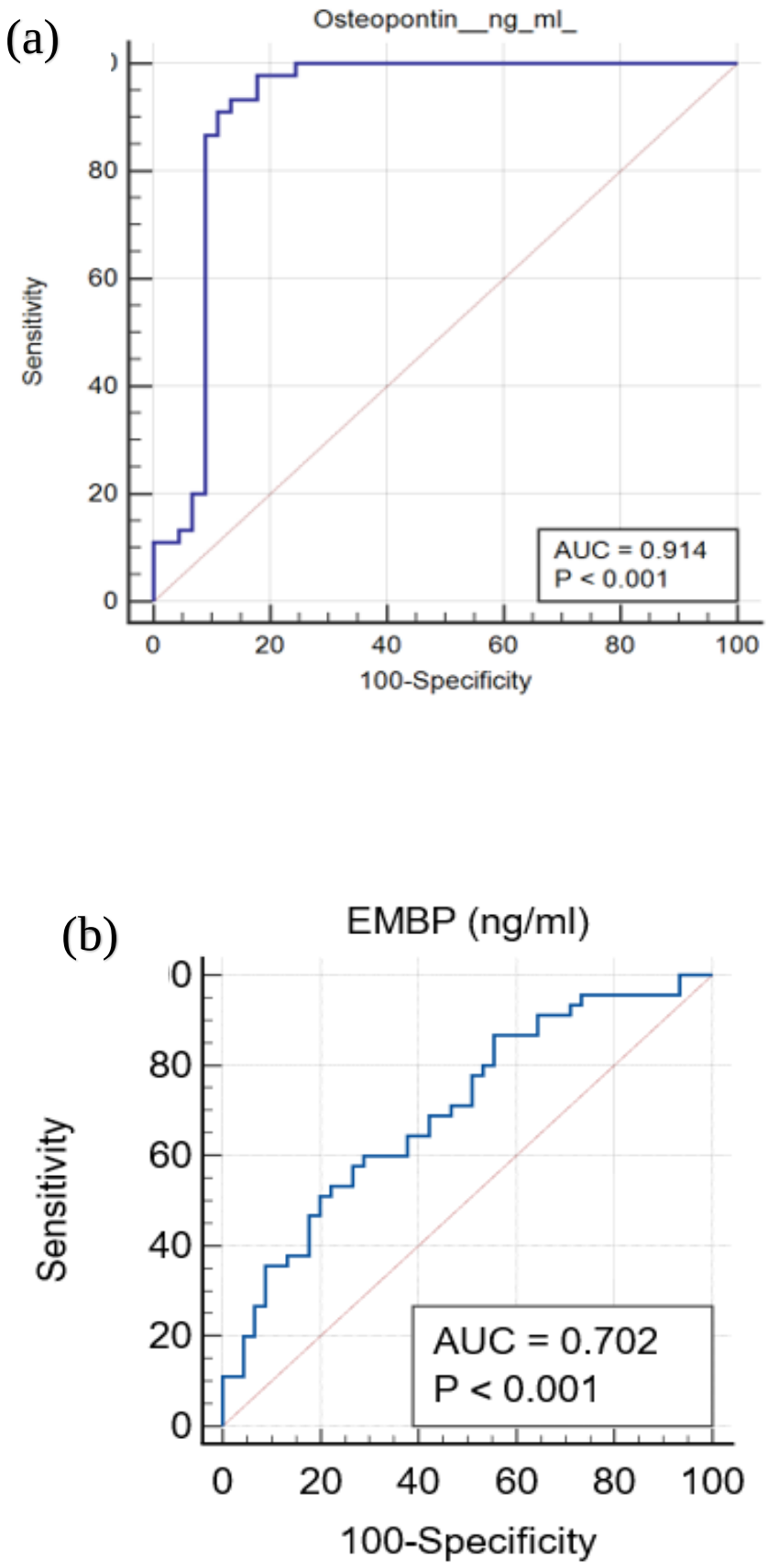

Figure 1. ROC curve of (a) Osteopontin (OPN) and (b) Eosinophil major basic protein (EMBP) for prediction of asthma

Table 2. Medical history of asthmatic group and classification of asthma severity

		Asthmatic group (n=45)
Current hospitalization		18 (40%)
Number of episodes in last year		2.47 ± 1.14
History of passive smoking		33 (73.33%)
History of medications	**No**	0 (0%)
	Steroids	28 (62.22%)
	Bronchodilator	37 (88.10%)
	Mast cell stabilizer	22 (52.38%)
History of allergy	**No**	10 (22.22%)
	Allergic rhinitis	2 (5.71%)
	Urticaria	6 (17.14%)
	Drug allergy	10 (28.57%)
	Food allergy	18 (51.43%)
Past history of hospitalization	**No**	24 (53.33%)
	For asthma	11 (24.44%)
	For other causes	10 (22.22%)
Family history of asthma		29 (64.44%)
Family history of allergy		29 (64.44%)
Classification of asthma severity		
Mild asthma		37(82.22%)
Moderate asthma		8(17.77%)
Severe asthma		0(0%)

Data are presented as mean ± SD or frequency (%).

Table 3. Laboratory investigations and OPN and EMBP levels of the studied groups

		Asthmatic group (n=45)	Control group (n=45)	P
Eosinophils	**Normal**	4 (8.89%)	44 (97.78%)	**<0.001***
	Eosinophilia	41 (91.11%)	1 (2.22%)	
WBCs	**Normal**	29 (64.44%)	32 (71.11%)	**0.036***
	Leukocytosis	15 (33.33%)	7 (15.56%)	
	Leukopenia	1 (2.22%)	6 (13.33%)	
Hb	**Normal**	19 (42.22%)	30 (66.67%)	**0.02***
	Anemia	26 (57.78%)	15 (33.33%)	
OPN (ng/ml)		24.01(18.69– 30.9)	6.49(4.6– 9.28)	**<0.001***
EMBP (ng/ml)		71.75(6.66 – 217.07)	47.56(30.26 – 66.03)	**0.003***

Data are presented as mean ± SD or frequency(%), *: significant as P value ≤0.05, WBCs: white blood cells, Hb: hemoglobin, EMBP: Eosinophil major basic protein, OPN: osteopontin.

Table 4. Correlation between OPN and EMBP and between the severity of asthma and markers as OPN and EMBP in asthmatic patients

	OPN (ng/ml)	
	N=45	
	r	**P**
EMBP (ng/ml)	0.145	0.170
Severity of asthma and markers as OPN and EMBP		
OPN (ng/ml)	-0.086	0.572
EMBP (ng/ml)	0.235	0.120

r: pearson cofficient, *: significant as P value ≤0.05, EMBP: Eosinophil major basic protein, OPN: osteopontin.

Table 5. Logestic regression of OPN and EMBP in the prediction of asthma

	OR	P value	95% CI
OPN (ng/ml)	1.154	**0.007***	1.021 - 1.303
EMBP (ng/ml)	1.017	**0.013***	1.001 - 1.011
Eosinophils (%)	7.254	**<0.001***	1.862 – 28.261
WBCs (*103 / µl)	0.774	0.298	0.478 - 1.254

CI: confidence interval, *: significant as P value ≤0.05, EMBP: Eosinophil major basic protein, WBCs: white blood cells, OPN: osteopontin.

The present study demonstrated that asthmatic group were substantially greater in patients with eosinophilia (P <0.001), leukocytosis (P value=0.036), anemia (P =0.02) contrasted to control group. Similar to our results, El-Toraky et al. [15] showed substantial rise in eosinophilic percentage among cases contrasted to controls, while they differed from our results in the increased hemoglobin level and decreased leukocytic count in asthmatic children than controls. Also, Kumar et al. [18] stated that the asthmatic group had a statistically higher eosinophilia contrasted to the control group in their prospective, cross-sectional work on 93 children, 51 of individuals with BA and 42 of healthy controls. In the current study, OPN was considerably greater in asthmatic group contrasted to control group (P values <0.001). in line with our findings, El-Toraky et al. [15] demonstrated that OPN was considerably greater in asthmatic group (P= 0.0054) contrasted to control group (p=0.04), respectively. Also, Akelma et al. and Toema et al. [18, 19] stated that OPN was considerably greater in asthmatic group compared to control group. In the current study, EMBP was considerably greater in asthmatic group contrasted to control group (P value= 0.003). Rasheed et al. [20] found that the EMBP levels among individuals with various atopic illnesses (n=395) had been considerably greater in contrast to non-atopic healthy pediatric controls (n=410) (p<0.001). In addition, Lee provided evidence indicating the concentration of EMBP is increased in biological fluids of individuals suffering from asthma and other disorders related with eosinophils [21]. Our study revealed no correlation between OPN and EMBP (P value 0.170). Also, we found that the severity of asthma was not significantly correlated with OPN (P value 0.170) nor EMBP (P value 0.120). in line with our findings, Toema et al. [19] stated that no association was existed among concentration of OPN and the severity of the disease. Also, Nacaroglu et al. [22] observed no substantial difference among OPN levels and severity of asthma. In multiple logistic regression, our study revealed that OPN (OR=1.154, 95% CI= 1.021 -1.303, P value =0.007), EMBP (OR=1.017, 95% CI= 1.001 - 1.011, P value =0.013) and eosinophils (OR=7.254, 95% CI= 1.862 - 28.261, P value <0.001) were substantial predictors of asthma (P value =0.022 and 0.004 correspondingly) while WBCs was not a significant predictor. This study demonstrated that OPN can significantly predict asthma at cut off >10.86 (P value of <0.001, AUC of 0.914) with 93.33% sensitivity, 86.67% specificity, 87.5% PPV, 92.9% NPV. Also, we found that EMBP can significantly predict asthma at cut off >39.86(P value of <0.001, AUC of 0.702) with 86.67% sensitivity, 44.44% specificity, 60.9% PPV, 76.9% NPV. El-Toraky et al. [23] revealed that at a cutoff point of 4.7 ng/ml OPN had 84% sensitivity and 60% specificity in prediction of pediatric BA. Nagiub et al. [24] reported that at cut off point >15.15 ng/ml with AUC 0.961, OPN can significantly predict asthma with sensitivity 98.3%, specificity 92.7%, PPV 95.4% and NPV 94.7%. Rasheed et al. [13] reported that EMBP is a valuable tool for assessing the activity of AD, AR, or BA diseases. It can also be used to predict the progression of these atopic disorders in children. Additionally, EMBP levels in the blood were substantially higher in individuals with severe BA contrasted to those with mild to moderate BA ($p < 0.05$). Our findings found in multiple logistic regression (OR=7.254, 95% CI= 1.862 -28.261, P value <0.001) that eosinophilia could significantly predict asthma. Similar to our results, Medrek et al. [25] demonstrated that blood eosinophilia, measured either by the percentage of eosinophils in the blood or the absolute count of eosinophils, is a widely utilized biomarker for predicting the T2 high asthma phenotype.

3.1. Limitations of our study

The sample size was relatively small. The work was in a single center. Therefore, we suggest conducting additional investigations with a larger sample size and follow-up are required to clarify the correlation between asthma severity and OPN and EMBP. Serum OPN and EMBP could be promising biomarkers in the diagnosis of bronchial asthma at various age groups.

4. Conclusions

The severity of asthma was not significantly correlated with OPN and EMBP. OPN can significantly predict asthma at cut off >10.86 (P value of <0.001, AUC of

0.914) with 93.33% sensitivity, 86.67% specificity. EMBP can significantly predict asthma at cut off >39.86(P value of <0.001, AUC of 0.702) with 86.67% sensitivity, 44.44% specificity. Serum OPN and EMBP could be good biomarkers in the diagnosis of BA in various age groups.

Financial support and sponsorship

Nil.

Conflict of Interest

Nil.

References

[1] J.H. Lee, L.C. Wang, Y.T. Lin, Y.H. Yang, H.H. Yu, Y.C. Hu, B.L. Chiang. (2023). Differentially expressed microRNAs in peripheral blood cell are associated with downregulated expression of IgE in nonallergic childhood asthma. Scientific Reports. 13 (1) 6381.

[2] L.A. Conrad, M.D. Cabana, D. Rastogi. (2021). Defining pediatric asthma: phenotypes to endotypes and beyond. Pediatric research. 90 (1) 45-51.

[3] R. Tesse, G. Borrelli, G. Mongelli, V. Mastrorilli, F. Cardinale. (2018). Treating pediatric asthma according guidelines. Frontiers in pediatrics. 6 234.

[4] I. Howell, A. Howell, I.D. Pavord. (2023). Type 2 inflammation and biological therapies in asthma: Targeted medicine taking flight. Journal of Experimental Medicine. 220 (7) e20221212.

[5] S. Konno, M. Kurokawa, T. Uede, M. Nishimura, S.K. Huang. (2011). Role of osteopontin, a multifunctional protein, in allergy and asthma. Clinical & Experimental Allergy. 41 (10) 1360-1366.

[6] Y. Zhou, Y. Yao, L. Shen, J. Zhang, J.H. Zhang, A. Shao. (2020). Osteopontin as a candidate of therapeutic application for the acute brain injury. Journal of Cellular and Molecular Medicine. 24 (16) 8918-8929.

[7] M. Mrag, Y. Khalji, A.A. Alhodhodi, S. Elsayed, Y. Ayed, A. Kassab. (2023). Adjunctive systemic antibiotic effect on periodontal state, salivary enzyme activity, and glycemia imbalance in type-2 diabetics after non-surgical periodontal management. Libyan Journal of Medicine. 18 (1) 2222449.

[8] A. Del Prete, S. Scutera, S. Sozzani, T. Musso. (2019). Role of osteopontin in dendritic cell shaping of immune responses. Cytokine & Growth Factor Reviews. 50 19-28.

[9] T. Omari, M.P. Sparrow, M.K. Church, S.T. Holgate, C. Robinson. (1993). A comparison of the effects of polyarginine and stimulated eosinophils on the responsiveness of the bovine isovolumic bronchial segment preparation. British journal of pharmacology. 109 (2) 553.

[10] T. Homma, J.H. Bates, C.G. Irvin. (2005). Airway hyperresponsiveness induced by cationic proteins in vivo: site of action. American Journal of Physiology-Lung Cellular and Molecular Physiology. 289 (3) L413-L418.

[11] P. Venkatesan. (2023). 2023 GINA report for asthma. The Lancet Respiratory Medicine. 11 (7) 589.

[12] B. Koletzko, K.M. Godfrey, L. Poston, H. Szajewska, J.B. Van Goudoever, M. De Waard, EarlyNutrition Project Systematic Review Group. (2019). Nutrition during pregnancy, lactation and early childhood and its implications for maternal and long-term child health: the early nutrition project recommendations. Annals of Nutrition and Metabolism. 74 (2) 93-106.

[13] Z. Rasheed, K. Zedan, G.B. Saif, R.H. Salama, T. Salem, A.A. Ahmed, A.A. Alzolibani. (2018). Markers of atopic dermatitis, allergic rhinitis and bronchial asthma in pediatric patients: correlation with filaggrin, eosinophil major basic protein and immunoglobulin E. Clinical and Molecular Allergy. 16 1-9.

[14] A. Corlateanu, I. Stratan, S. Covantev, V. Botnaru, O. Corlateanu, N. Siafakas. (2021). Asthma and stroke: a narrative review. Asthma Research and Practice. 7 1-17.

[15] A.R.I. El-Toraky, H.A. Aly, H.I. Mohamed, M.A. Salah. SERUM OSTEOPONTIN LEVEL IN CHILDREN WITH BRONCHIAL ASTHMA.

[16] K. Smejda, A. Brzozowska, D. Podlecka, K. Polanska, J. Jerzynska. (2019). Maternal stress and allergic diseases in children: a systematic review. Journal of Dermatology and Skin Science. 1 (1).

[17] A. Vatrella, A. Maglio, C. Pelaia, L. Ciampo, G. Pelaia, C. Vitale. (2022). Eosinophilic inflammation: An appealing target for pharmacologic treatments in severe asthma. Biomedicines. 10 (9) 2181.

[18] A.Z. Akelma, M.N. Cizmeci, M.K. Kanburoglu, D. Bozkaya, F. Catal, E. Mete, M. Namuslu. (2014). Elevated level of serum osteopontin in school-age children with asthma. Allergologia et immunopathologia. 42 (4) 275-281.

[19] O.H. Toema, N.M. El-Esawy, M.A. Saad. (2018). Study of serum osteopontin levels in children with bronchial asthma in Egypt. Tanta Medical Journal. 46 (3) 210-214.

[20] Z. Rasheed, K. Zedan, G.B. Saif, R.H. Salama, T. Salem, A.A. Ahmed, A.A. Alzolibani. (2018). Markers of atopic dermatitis, allergic rhinitis and bronchial asthma in pediatric patients: correlation with filaggrin, eosinophil major basic protein and immunoglobulin E. Clinical and Molecular Allergy. 16 1-9.

[21] Y. Lee, J.H. Lee, E.M. Yang, E. Kwon, C.G. Jung, S.C. Kim, H.S. Park. (2019). Serum levels of eosinophil-derived neurotoxin: a biomarker for asthma severity in adult asthmatics. Allergy, asthma & immunology research. 11 (3) 394.

[22] H.T. Nacaroglu, O.B. Gayret, M. Erol, O. Buke, O. Zengi, M. Tasdemir, O. Yigit. (2017). Biomarkers of airway and systemic inflammation in obese

asthmatic paediatric patients. Allergologia et immunopathologia. 45 (6) 534-540.

[23] R.S. Tepper, R.S. Wise, R. Covar, C.G. Irvin, C.M. Kercsmar, M. Kraft, A. Togias. (2012). Asthma outcomes: pulmonary physiology. Journal of Allergy and Clinical Immunology. 129 (3) S65-S87.

[24] M.S. Nagiub, E.M. Rasheed, A.G. Hussein, A.I.E.H. Atya. (2022). Serum Osteopontin Level as Biomarker in the Diagnosis of Pediatric Bronchial Asthma in Different Age Groups. The Egyptian Journal of Hospital Medicine. 88 (1) 2704-2709.

[25] S.K. Medrek, A.D. Parulekar, N.A. Hanania. (2017). Predictive biomarkers for asthma therapy. Current allergy and asthma reports. 17 1-13.