Diagnostic and prognostic values of eosinophil derived neurotoxin in pediatric asthma

By

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Abstract:

Background: Recurrent wheezing is a common condition in pediatric practice, with estimates that one in three children develop at least one episode of wheezing prior to their third birthday.

Objective: to assess the diagnostic and prognostic value of serum eosinophil derived neurotoxin (EDN) levels in children with asthma and preschool wheezes (PSW) in relation to their clinical and laboratory parameters.

Methods: This is a cross-sectional study conducted on a stratified random sample of 60 patients, following up in Pediatric Allergy, Immunology and Rheumatology unit, Children's hospital, Ain Shams university. Patients were compared to age and sex 30 healthy controls. EDN was analyzed using ELISA in the Clinical Pathology Immunology Laboratory, Ain Shams university Hospitals, in the period between June 2022 to November 2022.

Results: There was statistically significant increase in relative eosinophil percentage (P value<0.001), absolute eosinophil count (P value<0.001) and EDN (P value<0.001) among the cases group compared with the controls. Serum EDN was significantly higher in patients with asthma and patients with PSW than in controls. For prediction of allergic respiratory diseases (asthmatic and school wheezers), the best cutoff value is > 7.8 with sensitivity =100% and specificity =96.67%.

Conclusion: EDN is a good biomarker for eosinophilic inflammation in pediatric asthma and PSW with an excellent diagnostic and prognostic value. Therefore, EDN can be used as a screening tool to identify the phenotype of respiratory allergy. Further studies are needed to outline its therapeutic potentials.

Keywords: eosinophil derived neurotoxin; asthma; preschool wheeze

Introduction

Asthma is a polygenic chronic airway inflammation. It is defined according to the recurrence of respiratory symptoms with or without defined triggers, together with variable expiratory airflow limitation **(GINA, 2023)**. Preschool wheezes (PSW) do not necessarily show chronicity or progress to asthma later on, particularly in children with virus-induced wheeze **(Brand et al., 2008).** Wheezing can be episodic, triggered by viral infections with symptom-free intervals, or multi-trigger induced with mild symptoms in between flare ups. The latter phenotype is the suspected to progress into true asthma. Wheezes can also be classified based on time-trend as transient (manifest before the age of 3 years), persistent (manifest early and persist beyond the age of 6 years) and late onset (manifest after the age of 3 years) **(GINA, 2023)**.

Eosinophils are circulating granulocytes manufactured in the bone marrow along with other white blood cells (WBCs) and flood at relatively low levels in the bloodstream, composing 1–3% of WBCs. Eosinophils are fundamentally recruited in response to immunological or inflammatory processes **(Huang et al., 2009, Isobe et al., 2012, Uhm et al., 2012).** The effector function of eosinophils is related to their secretion of toxic granule proteins, reactive oxygen species (ROS), cytokines and lipid mediators **(Liu et al., 2006)**. Levels of eosinophils escalate in the sera and airways of the asthmatic patients, in correlation to the severity of symptoms and pulmonary function tests (PFT) **(Rydell et al., 2019).** Recruited and stimulated eosinophils release eosinophil derived neurotoxin (EDN), eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO) and major basic protein 2 (MBP2) **(Plager et al., 2001).**

EDN is a single-chain polypeptide of approximately 18.6 kDa and it shares approximately 70% sequence homology with ECP. EDN has antimicrobial, antiviral and chemotactic properties **(Hogan et al., 2008).** It is mainly expressed in eosinophils and, to a lower extent in basophils and neutrophils. Changes in serum EDN levels are therefore mostly related to changes in eosinophil activation **(Rydell et al., 2019).** EDN is an alternative biomarker of eosinophilia that can better reflect eosinophil activity than absolute eosinophil count **(An et al., 2020, Rutten et al., 2021).** Yet, its clinical diagnostic value in pediatric asthma and PSW is not well established **(Young et al., 2017).** Therefore, this work aimed to assess the diagnostic and prognostic value of serum EDN levels via ELISA in pediatric patients with asthma and PSW in correlation to the clinical and laboratory parameters.

Subjects and methods:

Ethical considerations: This study has gained approval for the Research Ethics Committee, Faculty of Medicine, Ain shams University (MS 417/2021), and verbal consent was obtained from the guardians of all the participants after explaining all the steps and purpose of the study. Data of the participants was preserved confidentially. Participants had all the right to withdraw themselves from the study at any time without any consequences. Authors declare that they have no conflict of interest. This work did not receive funding from any institute or company.

Type of the study: a cross-sectional study conducted in the period between June 2022 to November 2022.

Sample size: A total of 90 participants were included, divided into 2 main groups: 60 patients and 30 healthy controls. The sample size was calculated using the PASS 15 program for sample size calculation, adjusting for a 10% dropout rate, a minimum sample size of 50 participants was determined to achieve 90.0% power and according to **Amer et al. (2020)**. This sample size ensures the study's ability to reject the null hypothesis of no effect (effect size = 0) with a significance level (alpha) of 0.050, using a two-sided one sample t-test.

Study population: a sample of 60 wheezy pediatric patients following up in the Pediatric Allergy, Immunology and Rheumatology unit, Ain Shams university Hospitals, Cairo, Egypt. Patients were divided into 2 equal groups by stratified random method based on their ages: 30 patients younger than 6 years with PSW, and 30 school-aged (6-16 years) children with asthma. Diagnosis of asthma and PSW was defined according to the global initiative of asthma (GINA) criteria **(GINA, 2022)**. Another 30 age and sex matched healthy subjects were enrolled as a healthy control group, 15 of them were preschoolers (1- < 6 years) and the 15 children were school-aged (6 – 12 years).

Inclusion criteria:

children and adolescents fulfilling the followings:

- Aged between 1 and 16 years old diagnosed with asthma or PSW,
- Both genders,
- Diagnosis of asthma and PSW was done according to GINA, 2022 diagnostic criteria.
- All patients were in flare up at time of enrollment.

Exclusion criteria for patients:

patients treated with systemic steroids, or with diseases that can be associated with hyper-eosinophilia such as inflammatory bowel disease, hyper IgE. syndrome, hyper-eosinophilic syndrome, malignancy or parasitic infestations.

All the recruited patients were subjected to the following:

1. **Detailed history taking:** age, gender, crowd index (number of persons/ room), duration of allergy, associated atopy, frequency of flare ups/year, controllability of asthma and need for hospital admission due to asthma flare up/year.

Through clinical examination including:

- General examination of the skin, lymph nodes,
- Vital signs including heart rate, respiratory rate, temperature and oxygen saturation were assessed.
- Systemic examination including chest, heart, gastrointestinal tract and neurological examination.

2. **Laboratory studies for all the participants:** complete blood count (CBC) with relative and absolute eosinophil count using colter, serum EDN concentrations were measured using EDN enzyme-linked immunosorbent assay (ELISA) kit, with results expressed in nanograms per milliliter (ng/ mL). ELISA detects human EDN with minimum and maximum detection limits of 6.0 and 400 ng/mL, respectively.

Sample collection: Venous blood samples were withdrawn under aseptic conditions. The collected samples were divided into 2 tubes: 2 ml. of blood were collected on tri-potassium ethylene diamine tetra-acetic acid (K3 EDTA) vacutainer for performing CBC, and 2 ml. of blood were collected into a sterile gel activated vacutainer and was left to clot for 30 minutes in the vacutainer. Serum was then separated by centrifugation at 2000 rpm for 20 minutes for assessment of EDN. Hemolyzed samples were discarded. serum samples were stored at -80°C until assay time. Repeated freezing and thawing were avoided. Samples were analyzed on Sysmex XT 1800i hematology analyzer (Sysmex corporation, Kobe 650-8691, Japan) and quantification of EDN was done using ELISA

Principle of the assay: this ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human EDN. Samples (or standards) were added to the micro ELISA plate wells and combined with the specific antibody, then a biotinylated detection antibody specific for human EDN and Avidin-Horseradish peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. Free components were washed away. The substrate solution was added to each well, only those wells that contain EDN, biotinylated detection antibody and Avidin-HRP conjugate appear blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turns yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

The OD value was proportional to the concentration of EDN and calculation of EDN levels was by comparing the OD of the samples to the standard curve.

Reagents: standard solution (1920 ng/L), pre-coated ELISA plate, biotin conjugate EDN, standard diluent, HRP conjugate diluent, stop solution, substrate solution A, B, wash buffer concentrate (30x) and plate sealer.

Reagent preparation: all reagents were brought to room temperature before use, the standard was centrifuged at 10,000×g for 1 min. One mL of reference standard and sample diluent were added, it was let to stand for 10 min and inverted gently several times. After dissolution, it was mixed thoroughly with a pipette. This reconstitution produces a working solution of 40 ng/mL, then serial dilutions were made as needed. The recommended dilution gradient is as follows: 40, 20, 10, 5, 2.5, 1.25, 0.63, 0 ng/mL and wash buffer: 30 mL of concentrated wash buffer were diluted with 720 mL of deionized or distilled water to prepare 750 mL of wash buffer.

Calculation of results: A standard curve was constructed by plotting the average optical density for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and a best fit curve was drawn through the points on the graph.

Statistical analysis: the collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 25). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. Descriptive statistics included mean, standard deviation (± SD) and range for parametric numerical data, while median and interquartile range (IQR) for non-parametric numerical data and frequency and percentage of non-numerical data. Analytical statistics included student T test and Mann Whitney Test (U test) for comparing 2 groups. Chi-square test was used to examine the relationship between two qualitative variables, fisher's exact test was used to examine the relationship between two qualitative variables and the ROC Curve (Receiver Operating Characteristic) was used to evaluate the sensitivity and specificity for quantitative diagnostic measures. P>0.05: non-significant and P< 0.05: significant.

RESULTS

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

Table (1): Demographic, clinical and laboratory data of all the studied participants

		Groups		Test of significance	
		Controls (n=30)	Cases (n=60)		
		Mean ± SD N (%) Median (IQR)	Mean ± SD N (%) Median (IQR)	Value	p-Value
Age		6.7 ± 3.09	6.4 ± 3.18	t= 0.426	0.671
Sex	Male	13 (43.33%)	32 (53.33%)	X^2= 0.8	0.371
	Female	17 (56.67%)	28 (46.67%)		
Crowd Index	2	30 (100%)	47 (78.33%)	X^2= 7.597	0.022
	3	0 (0%)	11 (18.33%)		
	4	0 (0%)	2 (3.33%)		
Eosinophil percentage		2 (1 - 3)	6 (4 - 8)	z= -6.365	<0.001
Absolute eosinophil count (cells/uL)		0.1 (0.1 - 0.2)	0.7 (0.3 - 1)	z= -5.776	<0.001
EDN (ng/ml)		5.52 ± 1.4	17.96 ± 4.95	t= -18.07	<0.001

EDN: eosinophil derived neurotoxin, *Student t-test (t). *Chi-Square test (X2). *Mann-Whitney test (z)

This table shows that relative and absolute eosinophil counts, in addition to serum EDN levels were significantly higher in allergic patients than in controls.

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Table (2): Demographic and laboratory data of the PSW patients in comparison to their matched controls

		Subgroups		Test of significance	
		Matched controls for PSW (n=15)	PSW (n=30)		
		Mean ± SD N (%) Median (IQR)	Mean ± SD N (%) Median (IQR)	Value	p-Value
Age		4 ± 0.85	3.63 ± 0.85	t= 1.366	0.179
Sex	Male	6 (40%)	19 (63.33%)	X^2= 2.205	0.138
	Female	9 (60%)	11 (36.67%)		
Crowd Index	2	15 (100%)	23 (76.67%)	Fisher's Exact test	0.105
	3	0 (0%)	6 (20%)		
	4	0 (0%)	1 (3.33%)		
% Eosinophil		2 (2 - 3)	6 (3 - 8)	z= -4.325	<0.001
Absolute Eosinophil (cells/uL)		0.1 (0.1 - 0.1)	0.65 (0.2 - 0.9)	z= -3.925	<0.001
EDN (ng/ml)		5.14 ± 1.39	16.97 ± 4.24	t= -13.867	<0.001

*Student t-test (t). *Chi-Square test (X2). *Mann-Whitney test (z).

Eosinophil percentage, absolute eosinophil count and EDN were significantly higher in patients with PSW than in their matched peers.

Table (3): Demographic and laboratory data of asthmatic patients in comparison to their matched controls

		Subgroups		Test of significance	
		Matched controls (n=15)	Asthmatics (n=30)		
		Mean ± SD N (%) Median (IQR)	Mean ± SD N (%) Median (IQR)	Value	p-Value
Age		9.4 ± 1.84	9.17 ± 2.02	t= -0.376	0.709
Sex	Male	7 (46.67%)	13 (43.33%)	X^2= 0.045	0.832
	Female	8 (53.33%)	17 (56.67%)		
Crowd Index	2	15 (100%)	24 (80%)	Fisher's Exact test	0.201
	3	0 (0%)	5 (16.67%)		
	4	0 (0%)	1 (3.33%)		
% Eosinophil		2 (1 - 3)	6 (4 - 8)	z= -4.662	<0.001
Absolute Eosinophil (cells/uL)		0.2 (0.1 - 0.2)	0.75 (0.5 - 1)	z= -4.347	<0.001
EDN (ng/ml)		5.89 ± 1.35	18.95 ± 5.47	t= 12.341	<0.001

*Student t-test (t). *Chi-Square test (X2). *Mann-Whitney test (z).

Similarly, this tables shows that relative and absolute eosinophil counts, in addition to EDN were significantly elevated in asthmatic school-aged children compared to their healthy age-matched peers.

Table (4): Demographic and laboratory data of the PSW patients in comparison to asthmatics

		Subgroup		Test of significance	
		PSW (n=30)	Asthmatics (n=30)		
		Mean ± SD N (%) Median (IQR)	Mean ± SD N (%) Median (IQR)	Value	p-Value
Age		3.63 ± 0.85	9.17 ± 2.02	t= -13.837	<0.001
Sex	Male	19 (63.33%)	13 (43.33%)	X^2= 2.411	0.121
	Female	11 (36.67%)	17 (56.67%)		
Hospitalization	No	14 (46.67%)	16 (53.33%)	X^2= 0.74	0.691
	Yes	10 (33.33%)	7 (23.33%)		
Crowd Index	2	23 (76.67%)	24 (80%)	Fisher's Exact test	1.00
	3	6 (20%)	5 (16.67%)		
	4	1 (3.33%)	1 (3.33%)		
% Eosinophil		6 (3 - 8)	6 (4 - 8)	z= -0.805	0.421
Absolute Eosinophil (cells/uL)		0.65 (0.2 - 0.9)	0.75 (0.5 - 1)	z= -1.241	0.215
EDN (ng/ml)		16.97 ± 4.24	18.95 ± 5.47	t= -1.562	0.124

*Student t-test (t). *Chi-Square test (X^2). *Mann-Whitney test (z).

This table shows that absolute eosinophil count, eosinophil percentage and EDN levels were comparable between all patients, regardless their age with insignificant difference.

Table (5): Relation between EDN levels and demographic and laboratory data of patients with PSW

Preschool wheezers		EDN (ng/ml)	Test of significance	
		Mean ± SD	Value	p-Value
Sex	Male	17.21 ± 4.48	t= 0.393	0.698
	Female	16.57 ± 3.96		
Current Hospitalization	No	18.35 ± 3.4	f= 12.191	<0.001
	Yes	13.05 ± 2.9		
ICU admission	No	16.14 ± 4.12	t= -2.303	0.029
	Yes	20.3 ± 3.07		
Crowd Index	2	17.16 ± 4.23	f= 0.159	0.854
	3	16.11 ± 4.92		
	4	17.82		
Absolute eosinophil count (cells/uL)		0.65 (0.2 - 0.9)	z= -0.158	0.406

*Student t-test (t). *One Way ANOVA test (f)

This table shows that levels of EDN were significantly higher among patients with PSW who needed hospitalization than their peers who received home treatment.

Table (6): Relation between EDN levels and demographic and laboratory data of patients with asthma

Asthma		EDN (ng/ml)	Test of significance	
		Mean ± SD	Value	p-Value
Sex	Male	19.85 ± 4.18	t= 0.785	0.785

	Female	18.26 ± 6.32		
Current Hospitalization	No	17.27 ± 4.87	f= 11.487	<0.001
	Yes	16.21 ± 1.82		
ICU admission	No	16.95 ± 4.16	t= -4.82	<0.001
	Yes	25.51 ± 3.94		
Crowd Index	2	19.07 ± 5.9	f= 0.33	0.722
	3	19.25 ± 3.34		
	4	14.49		
Absolute eosinophil count (cells/uL)		0.75 (0.5 - 1)	P= 0.113	0.554

*Student t-test (t). *One Way ANOVA test (f)

According to this table, asthmatic patients who needed hospitalization and PICU admission had significantly higher levels of EDN compared to the patients who received home treatment.

Table (7): ROC curve to assess diagnostic and prognostic value of EDN for allergic patients, PSW children and asthmatics

Group	AUC	95% CI	Sig.	Cut-off value	Sensitivity	Specificity	+PV	-PV
All patients	0.999	0.958 to 1.00	<0.001	>7.8	100	96.67	98.4	100
PSW	1.00	0.921 to 1.00	<0.001	>7.4	100	100	100	100
Asthmatics	0.998	0.917 to 1.00	<0.001	>8.7	96.67	100	100	93.8

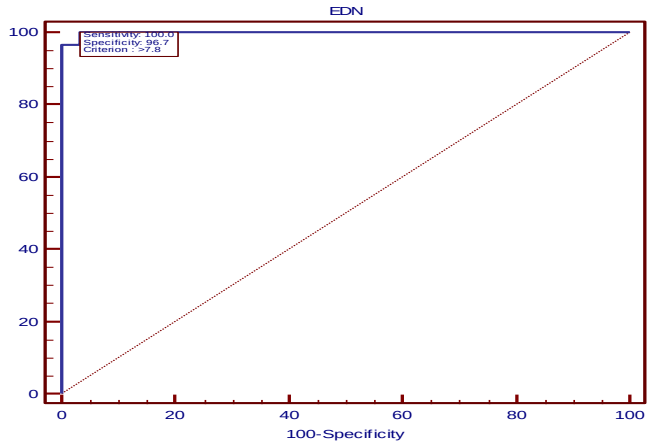

Figure (1): ROC curve showing the predictive value of EDN for pediatric respiratory allergy

EDN worked as an excellent predictor of pediatric respiratory allergy, asthma and PSW as proved with ROC curve and the area under the curve (AUC). A cutoff value for EDN level > 7.4 ng/L had sensitivity of 100%, specificity 100%, positive predictive value 100 % and negative predictive value 100% for PSW (P value < 0.001). The best cut-off value for EDN that discriminate between asthma patients and healthy controls was > 8.7 ng/L, with sensitivity of 96.67%, specificity 100%, positive predictive value 100 % and negative predictive value 93.8% (P value < 0.001).

DISCUSSION

In the current work, eosinophil derived neurotoxin (EDN) levels as well as relative and absolute eosinophils were significantly elevated in patients with respiratory allergy, regardless the age of the patients, either being preschoolers with recurrent wheezes or school-aged with asthma. The potent correlation with the hospital and intensive care admission reflects the significance of EDN as a marker of

wheeze and asthma severity. Moreover, the excellent predictive value of EDN to discriminate asthma and preschool wheeze (PSW) from healthy control refer to the valued importance of EDN as a diagnostic and prognostic biomarker for both pediatric asthma and PSW. It can work also as a potential therapeutic target in difficult-to-treat and uncontrolled cases. The lack of correlation between EDN and eosinophil count suggests the sensitivity and importance of EDN in childhood wheeze more than eosinophils, therefore, it is thought that EDN as an asthma predictor would be more informative and accurate than absolute eosinophil count. Value of EDN can be attributed to its link to eosinophil activation as a part of type 2 inflammation and T helper (Th)-mediated inflammation, which has become the most widely accepted explanation to asthma pathogenesis. Another explanation could be attributed to the increased eosinophil extracellular traps that promote the degranulation of eosinophils **(Choi et al., 2018)**.

Such findings are in agreement with **Amer et al.'s (2020)** study, conducted on 85 asthmatic pediatric cases. EDN concentrations were significantly increased in asthmatic children when compared to controls (30.74 ± 30.05 vs. 1.465 ± 0.56 ng/ml, respectively), with significant correlation with asthma severity. The aforementioned data approximates the results of **Rydell et al., (2019)**. Another study conducted on 171 wheezy children revealed higher levels of serum EDN in wheezers than non-wheezers (48.8± 71.4 ng/ml and 13.3±5.1 ng/ml, respectively) (**Kim et al., 2020).** Interestingly, EDN levels were more elevated in patients with uncontrolled asthma than well-controlled peers **(Mansour et al., 2022)**, which approximates our findings that EDN was correlated to the wheeze-related hospital and intensive care admission. EDN was reported in elevated levels in school-aged children with different forms of allergy such as food allergy, atopic dermatitis and allergic rhinitis, which adds to its biomedical value in allergy. EDN levels higher than 0.790 ng/ml showed 81.2% sensitivity and 69.8% specificity for childhood allergy **(Kim et al., 2022).** Interestingly, a study conducted on infantile virus-triggered wheeze revealed higher serum levels of EDN than healthy controls, in addition to its elevated levels among asthmatics compared to controls with 66% sensitivity and 86% specificity **(Kim et al., 2010)**.

Conclusion:

In conclusion, EDN was significantly more elevated in asthmatic children regardless their age. There was significant elevation of EDN levels among hospitalized children due to asthma flare up.

Recommendations:

We recommend conduction of more studies on larger numbers of wheezy children, with consideration of asthma phenotypes and modality of treatment including immunotherapy and biological agents to outline the precise role of EDN in childhood respiratory allergies.

Limitations:

This study was limited with the small sample size, the non-phenotypic classification of the patients, and the underestimation of the possible role of presence of other form allergy.

REFERENCES

1. **Amer OT, Naguib MS, Allam AA, Fouad EMI. (2020):** Evaluation of Serum Eosinophil-Derived Neurotoxin Level in Children with Bronchial Asthma and its Relation to Disease Severity. Egypt J Hosp Med. 80(3):951-7.

2. **An J, Lee JH, Sim JH, Song WJ, Kwon HS, Cho YS, et al. (2020):** Serum eosinophil-derived neurotoxin better reflect asthma control status than blood eosinophil counts. J Allergy Clin Immunol: In Practice. 8(8):2681-8.

3. **Brand PLP, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, et al. (2008):** Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 3:1096–110.

4. **Choi Y, Le Pham D, Lee DH, Lee SH, Kim SH, Park HS. (2018):** Biological function of eosinophil extracellular traps in patients with severe eosinophilic asthma. Exper Mol Med. 50(8):1-8.

5. **Global Initiative for Asthma. (2023):** Global Strategy for Asthma Management and Prevention. Updated May 2023. Available from: www.ginasthma.org

6. **Global Initiative for Asthma. (2022):** Global Strategy for Asthma Management and Prevention. Available from: www.ginasthma.org

7. **Hogan SP, Rosenberg HF, Moqbel R, Phipps S, Foster PS, Lacy P, et al. (2008):** Eosinophils: biological properties and role in health and disease. Clin Exper Allergy. 38(5):709-50.

8. **Huang FY, Wang CC, Zhou SL, Huang YH, Wang H, Chen F, et al. (2009):** Antisense interleukin-5 reduces eosinophil infiltration and hyperresponsiveness in an allergic asthma model. Asian Pac J Allergy Immunol. 27:35–41.

9. **Isobe Y, Kato T, Arita M. (2012):** Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. Front Immunol. 3:270.

10. **Kim CK, Callaway Z, Fletcher R, Koh YY. (2010):** Eosinophil-derived neurotoxin in childhood asthma: correlation with disease severity. J Asthma. 47(5):568-73.

11. **Kim H, Kwon GE, Kim YH, Callaway Z, Han YS, Seo JJK, et al. (2020):** Comparison of serum eosinophil-derived neurotoxin levels between wheezing and non-wheezing groups in children with respiratory tract infection. J Asthma. 57(11):1211-5.

12. **Kim HS, Yang HJ, Song DJ, Lee YJ, Suh DI, Shim JY, et al. (2022):** March. Eosinophil-derived neurotoxin: An asthma exacerbation biomarker in children. Allergy Asthma Proc. 43(2):133-9.

13. **Liu YH, Chou HH, Jan RL, Lin HJ, Liang CC, Wang JY, et al. (2006):** Comparison of two specific allergen screening tests in different patient groups. Acta Pediatr Taiwan. 47:116–22.

14. **Mansour SAEAA, El-Sayed AH, Mohamed A, Mohmoud A, Abd El-Ghaffar MAS. (2022):** Evaluation of serum eosinophil-derived neurotoxin in children as a biomarker for asthma diagnosis and control. Al-Azhar J Ped. 25(3):2879- 94.

15. **Plager DA, Adolphson CR, Gleich GJ. (2001):** A novel human homolog of eosinophil major basic protein. Immunol Rev. 179(1):192-202.

16. **Rutten B, Young S, Rhedin M, Olsson M, Kurian N, Syed F, et al. (2021):** Eosinophil-derived neurotoxin: A biologically and analytically attractive asthma biomarker. PlosOne.

17. **Rydell N, Nagao M, Ekoff H, Sjölander A, Borres M, Fujisawa, T. (2019):** Development of an automated Immuno CAP research assay for eosinophil derived neurotoxin and its use in asthma diagnosis in children. Prac Lab Med. 17:e00138.
18. **Uhm TG, Kim BS, Chung IY. (2012):** Eosinophil development, regulation of eosinophil-specific genes, and role of eosinophils in the pathogenesis of asthma. Allergy Asthma Immunol Res. 4(2):68-79.
19. **Young S, Tigerstrom A, Olsson M, Rhedin M, Hidi R, Platt A, et al. (2017):** Point of care measurement of eosinophil derived neurotoxin (EDN) as a biomarker of eosinophilic asthma. Euro Respir J. 50:PA1131.