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ORGINAL ARTICLE

Salivary Surfactant Protein D as a Biomarker of Asthma Severity In Preschool Children.

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ABSTRACT

Background : Surfactant protein D (SP D) is crucial in controlling the immunological response of the peripheral airways, Surfactant changes caused by airway inflammation may have a role in the pathophysiology of asthma.

Aim of this study: was to assess the value of salivary SP D in Preschool asthmatic children .

Methods: in our case control study including 30 non-asthmatic controls and 30 preschool asthmatic children aged 2-5 years old, we assessed salivary SP D and pulmonary functions using impulse oscillometry (IOS) then the correlations between salivary SP D levels and IOS indices were analyzed.

Results: Salivary SP D levels were increased in asthmatic group with mean ± SD of 32.52±12.88 pg/ml compared to 5.98±2.87 pg/ml in healthy controls. Moreover, it was correlated with poor pulmonary functions using IOS indices with cutoff expression value of SP D >8.19 using ROC curve at the optimal sensitivity 83.3% and specificity 81.0% to differentiate asthmatic children from controls.

Conclusion: Salivary SP D can be a useful marker for childhood asthma and can monitor deterioration in pulmonary functions in preschool asthmatic children.

Keywords: Bronchial asthma, Surfactant protein D, Pediatrics, Impulse oscillometry.

INTRODUCTION

Asthma is a complex, multi-factorial illness characterized by chronic airway inflammation resulting in broncho-spasm with expiratory airflow limitation manifested clinically by recurrent attacks of chest tightness, cough, respiratory distress and chest wheeze with high degree of variability over time and reversibility with bronchodilation.[1]

In children, asthma is the commonest chronic pulmonary disease, with more than 50% of all recorded cases presenting under the age of three. There is a challenge for diagnosing asthma in pre-school group, due to the disease heterogeneity, ongoing developing immune system and lack of diagnostic options such as pulmonary function assessment using spirometry. Early diagnosis and treatment of asthmatic symptoms will improve patients' quality of life and help to reduce disease morbidity.[2]

The impulse oscillometry (IOS) was developed as a rapid, simple, non-invasive approach for measuring respiratory functions by superimposing a multi-frequency oscillation during normal tidal breath. The measured variables include total resistance (*R*), which corresponds to the impedance of signals traveling the airways, and reactance (*X*), which is related to the elasticity and inertia in the respiratory tract. IOS measures pulmonary function during normal tidal breathing, and so it is more favorable than spirometry in young children. Moreover, it has higher sensitivity in detecting changes in peripheral small airways.[3]

Surfactant protein D (SP D) belongs to the collectin molecules which plays a vital function in pulmonary host defence as a part of the innate immune system. SP D inhibits eosinophilic response in mice and induces a Th 1 immune response; additionally, SP D is thought to have a protective effect against pulmonary inflammation and atopy [4]. And so, there is a need to study effect of SP D dysregulation and development of asthma.

Decreased concentrations of SP D in bronchoalveolar lavage and increased serum SP D levels have been linked to a defect in the innate immunity and increased bacterial colonization with airway neutrophilia in severe asthma. [5]. Furthermore, serum SP D concentrations in asthmatics rise in proportion to the severity of the disease [6].

SP D is secreted by salivary glands, type II alveolar epithelial cells, and non-ciliated Clara cells [7]. It is worth noting that glandular tissues in asthmatics' small salivary glands demonstrate airway-like inflammation[8]. Accordingly, a simple and non-invasive approach can be used for sampling SP D in young children's saliva and correlate it with IOS indices and clinical manifestations. We aimed to assess the value of salivary SP D in Preschool asthmatic children.

PATIENTS AND METHODS

This study is a case–control study carried out at department of pediatrics, Zagazig university hospitals on preschool children attending either the outpatient clinic, the emergency room, or admitted to the inpatient ward. Over a period two years from march 2019 to May 2021, 60 child aged 2 – 5 years from both sexes were recruited in our study, classified into 2 groups: group A (case group) included 30 asthmatic children and group B (control group) included 30 non-asthmatic, age and sex matched children. We had excluded children receiving systemic steroids within last two weeks of sampling, those with a history of cardiac illness, immune-compromised patients, and those with other chronic lung disease. Patients were diagnosed using one of the following criteria: more than three attacks of wheezing in the preceding year; or need for hospitalizations for respiratory failure and wheezing in the previous year.

Ethical Consideration:

The approval for the study was obtained from the Pediatrics Departments of Zagazig university hospitals after the approval of the Institutional Review Board (ZU-IRB#5337) and also informed written consent was obtained from patients and/or their caregivers. This research was carried out in compliance with the Ethics code of the World Scientific Association (Declaration of Helsinki) for human studies.

Steps of performance:

All children were subjected to complete history taking, full clinical examination and IOS measurement that measure peripheral airway resistance using the Jaeger MasterScreen™ IOS, version 5.2 (VIASYS Healthcare GmbH, Hoechberg, Germany) .It is based on the principle of forced oscillation technique (FOT). IOS was measured during normal, quiet tidal breath while sitting with patient's neck extended with cheeks supported by patient's hands over a period of at least 30 seconds. Children were instructed to breathe exclusively through their mouths surrounding the mouthpieces and not to insert their tongues into the mouthpieces.

Resistance (R) and reactance (X): these variables are measured at different frequencies (ranging from 5 up to 35 Hz) to assess different portions of the respiratory tract. R5 represents the total resistance of the airways, while R20 represents the proximal airway resistance. X5 refers to both the elastic and inertial properties of the lungs, reflecting the elastic recoil of the peripheral airways. Resonance frequency (Fres): it is the point at which the airway inertia properties and the peripheral lung capacitance are equal (X equals zero). Fres is the dividing point between large and small airways based on the mechanical properties of the airways

SP D measurement: Saliva was collected at the time of IOS measurements using ELISA (SP D DuoSet, R&D Systems) to determine the concentrations of SP D in saliva. The saturated sample was placed in the storage tube according to the manufacturer's recommendations with centrifugation for 7 minutes at 4°C at 1200 rpm. After that, samples (1-2 mL) were held at –80°C until salivary SP D was quantified. Collection of saliva was done at least 2 hours apart from the last meal.

We examined SP D levels in both groups then we compared the data to IOS indices during asthma exacerbations.

Statistical analysis:

The collected data during the history, basic clinical examination, laboratory tests, and outcome measures were coded, processed, and analysed using the Microsoft Excel software (SPSS version 20.0). qualitative data represent as number and percentage, whereas quantitative continues data represent by mean ± SD, the following tests were

used to test differences for significance; Chi square test (X2) for difference and association of qualitative data. T test or Mann–Whitney U test for differences between quantitative independent groups. The nonparametric Spearman's rho test was used for correlations between salivary SP D values and IOS parameters. The SP D diagnostic power was assessed using the receiver operating characteristic (ROC) curve to determine the best cut-off values. The level of *P value* <0.05 was considered statistically significant and at ≤0.001 to be highly significant.

RESULTS

We enrolled 30 asthmatic children (male: female 17:13) and 30 healthy controls (male: female 20:10) in the study. Age was distributed as (4.37±0.75years old and 4.06±0.73years old) respectively between cases and control with no significant difference between groups also there was no significant difference regard sex distribution and residence distribution between studied groups. **Table [1]**

Salivary SP D was significantly higher (p-value < 0.001) in asthmatics compared to healthy controls with mean ± SD of 32.52±12.88 pg/ml in asthmatics compared to 5.98±2.87 pg/ml in healthy controls. Eosinophil Count higher in asthmatic with mean ± SD (343.5±95.63) compared to control (198.63±45.63). Asthmatic children demonstrated significantly higher IOS indices (p-value < 0.001) than control (**R5** 174.93±45.99 Vs 100.0±11.1), (**R20** 164.73±44.33 Vs 95.63±10.71), (**X5** 0.11±0.04 Vs 0.03±0.01). **Table [2]**

There was significant positive correlation between SP D with R5 (p-value < 0.001), R20 (p-value < 0.001) and X5 (p-value=0.004) **Table[3]**

ROC analysis revealed that SP D can excellently differentiate asthmatic patients from healthy controls with an area under the curve (AUC) of 0.88 for SP D (95% CI: 0.79 to 0. 96, *P* <0.001). The optimal sensitivity and specificity to differentiate asthmatic children from controls were (83.3% and 81.0% at a cutoff expression value >8.19). **Figure 1**

Table1: Demographic data distribution between studied groups

			Case	Control	t/ X^2	P
Age(years)			4.37±0.75	4.06±0.73	1.609	0.113
Sex	**Female**	**N**	13	10		
		%	43.3%	33.3%		
	Male	**N**	17	20	0.63	0.42
		%	56.7%	66.7%		
Residence	**Rural**	**N**	18	11		
		%	60.0%	36.7%		
	Urban	**N**	12	19	3.27	0.071
		%	40.0%	63.3%		
Total		**N**	30	30		
		%	100.0%	100.0%		

χ^2, Chi-squared test t= Independent-samples-t test

Table2: Surfactant protein D (SP D), Eosinophil Count**,** R5, R20, X5 and Fres distribution between studied groups

	Case mean ± SD	Control mean ± SD	Test of significant(t test)	p value
SP.D (pg/ml)	32.52±12.88	5.98±2.87	6.254	< 0.001**
Eosinophil Count(cell/µl)	343.5±95.63	198.63±45.63	6.521	< 0.001**
R5 (cm H2O/L/s)	174.93±45.99	100.0±11.1	8.671	< 0.001**
R20 (cm H2O/L/s)	164.73±44.33	95.63±10.71	8.297	< 0.001**
X5 (cm H2O/L/s)	0.11±0.04	0.03±0.01	5.514	< 0.001**
Fres (Hz)	13.07±3.12	12.65±2.54	1.535	0.112

t=student t-test ** High statistical significance

Table3: Correlation between Sp D and pulmonary function

		SP D
R5	**r**	.555**
	P	< 0.001
R20	**r**	.558**
	P	< 0.001
X5	**r**	.363**
	P	.004
Fres	**r**	.235
	P	.070

** High statistical significance

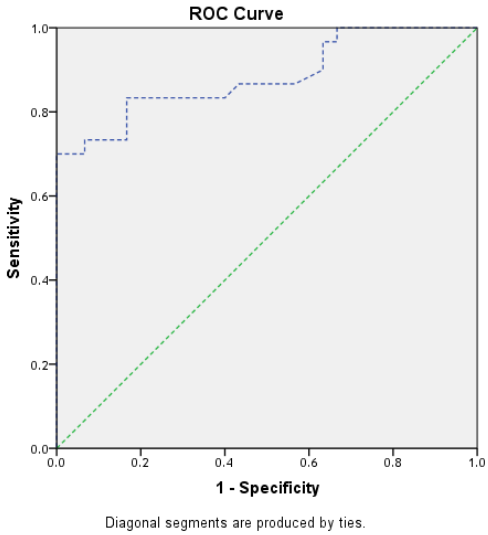

Fig.1 shows area under the receiver operating characteristic (ROC) curve for detecting the cut-off point for SP D >8.19 with sensitivity 83.3% and specificity 81.0%.

DISCUSSION

Asthma is one of the most common chronic childhood diseases, and it is the leading cause of hospitalization in children[9].

IOS has been developed as an alternate approach of pulmonary function evaluation, which is particularly appropriate to younger children with asthma. This is due to the fact that IOS is noninvasive, simple to conduct, and needs little patient cooperation and It is based on the generation of small sound waves that are generated at the mouth and travelled along the airways allowing indirect assessment of the pulmonary function during quiet normal breath[10]. Salivary levels of SP D were measured then correlated with IOS indexes and clinical parameters.

Our study was conducted at the Pediatrics Department of Zagazig university hospitals. The study included 30 patients (17 boys and 13girls) previously diagnosed as being asthmatic and 30 healthy control children (20 boys and 10 girls); their ages ranged from 2 to 5 years old, with mean ages of 4.37±0.75 years among patients and 4.06±0.73 years among controls with no significant difference regarding age, sex and residence.

Regarding eosinophilic count in our study, the mean value was significantly higher in asthmatic patients than the control. This agrees with a one-year prospective interventional trial, enrolling 81 children aged 3 to12 years, who were newly diagnosed cases of asthma visiting asthma clinic in a tertiary care pediatric government hospital found that, the mean eosinophilic count level was higher in cases than control [11].

Regarding IOS results; our study showed that (R5, X5, R20) were significantly higher among cases than Control group. This is consistent with the findings of El-Nemr and Al-Ghndour [12]who found baseline IOS measurements (R5, X5, R20 and Fres.) for asthmatic patients were elevated and compared response before and after bronchodilator administration in asthmatic children using IOS

showed that there was a highly statistically significant difference in response as regards the degree of reversibility. Moreover Shi et al [13]found that increased IOS peripheral airway indices predict asthma exacerbation.

Regarding salivary surfactant protein-D (SP D) our study results revealed that there was highly significant difference between asthmatic and non-asthmatic children as it was distributed as (32.52±12.88) and (5.98±2.87) respectively. Our results are in agreement with Okazaki et al [14]who found that salivary SP D may be a practical marker to identify the peripheral airway inflammation of bronchial asthma. Haczku et al [15]reported that the allergic inflammatory mechanisms whereby alveolar cells release SP D into bronchial alveolar lavage fluid may also enhance SP D secretion from salivary glands, resulting in increased salivary SP D levels in asthmatic patients.

According to our results of both groups; there was significant positive correlation between SP D results and IOS indices (R5,R20,X5). These results were in agree with Okazaki et al [14]who found Salivary SP D may reflect asthmatic inflammation in peripheral small airways and may be a useful prognostic marker for monitoring the degree of exacerbation in childhood asthma.

Our study revealed that SP D can differentiate asthmatic patients from healthy control with an area under the curve (AUC) of 0.88 for SP D (95% CI: 0.79 to 0.96, P<0.001). The optimal sensitivity and specificity were (83.3% and 81.0% at a cutoff expression value >8.19). That mean SP D had a good diagnostic value in asthma.

CONCLUSION

Salivary SP D may indicate asthmatic inflammation in the peripheral small airways and could be used as a lung-specific biomarker for evaluating the severity of exacerbation in children with asthma.

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