CORRELATION BETWEEN SERUM PERIOSTIN LEVEL AND BRONCHIAL ASTHMA IN CHILDREN

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ABSTRACT

Background: Asthma is a major public health problem that affects nearly 350 million people worldwide. It is also the most common chronic disease in childhood and adolescence. Periostin is a cell matrix protein secreted by bronchial fibroblasts and epithelial cells, which is involved in fibrogenesis. Significant periostin expression has been noted in the bronchial epithelial cells of children with asthma and could be used as a biomarker of eosinophilic airway inflammation.

Aim of the study: To evaluate the role of measurement of serum periostin level in children with bronchial asthma.

Subject and Methods: This case control prospective study was conducted at Pediatric Department of Zagazig University Hospitals, included 104 cases (eighty-four asthmatic group and twenty healthy children as control group), aged from 5-14 years and excluded cases with other chronic diseases that affect other systems as any Cardiopulmonary diseases, kidney diseases, bone diseases. All patients were subjected to full history taking, meticulous general examination, local chest examination and investigations: included serum periostin level, C-reactive protein, and IGE level.

Results: Serum Periostin concentrations were significantly increased in asthmatic children when compared to controls. Level of Periostin was statistically higher in moderate and severe asthmatics than the other two subgroups. There was a significant positive correlation between asthmatic Periostin level and IgE level, and absolute eosinophilic count, while there were significant negative correlations between level Periostin and pulmonary function tests (FEV1, FVC) as we suggest that the inflammatory response of airways most likely influences the decrease in lung functions.

Conclusions: Periostin level was higher in asthmatic children than controls and correlated with asthma severity. Therefore, serum Periostin can be considered as a possible biomarker for diagnosis, grading of asthma severity and the degree of airway inflammation.

Keywords: bronchial asthma, abnormal perostin level, children, spirometer

I. INTRODUCTION

Asthma is a major public health problem that affects nearly 350 million people worldwide. It is also the most common chronic disease in childhood and adolescence. Asthma is more a syndrome than a disease, exhibiting considerable heterogeneity in its presentation and clinical course (1).

Several asthma phenotypes and endotypes have been identified. While 80% of children with asthma have allergies, the differentiation between TH1 and TH2 immune mechanisms in this group remains unclear. Some studies show a higher percentage of neutrophils in bronchoalveolar lavage (BAL) fluid or induced sputum in children (1).

In recent years, studies on the role of periostin in asthma have been published. Periostin is a cell matrix protein that was first identified in mouse periodontal ligament (hence the name) in 1993. It is secreted by bronchial fibroblasts and epithelial cells, acts as an immunomodulator, repairs connective tissue, and is involved in fibrogenesis. Periostin binds to integrins present on the surface of fibroblasts and epithelial cells. Its main function is to maintain...
tissue structure by binding to fibronectin, tenascin C, and collagen V. Expression of the periostin gene (POST) is regulated by bronchial epithelial cells, IL-13, and IL-4 (2).

Periostin is an extracellular matrix protein expressed in fibroblasts or epithelial cells. Takayama and Izuhara et al. found that T-helper cell 2 (Th2) cytokines induced periostin expression in fibroblasts, which was involved in subepithelial fibrosis in asthma (2).

In fact, significant periostin expression has been noted in the bronchial epithelial cells of children with asthma (3), and a study of severe adult asthma indicated that serum periostin levels could be used as a biomarker of eosinophilic airway inflammation (3).

We aimed to evaluate the role of measurement of serum periostin level in children with bronchial asthma.

II. STUDY DESIGN AND PARTICIPANTS

This is a case control prospective study was conducted at Pediatric Department of Zagazig University Hospitals during the period from August 2018 to August 2019. This study included 104 cases (eighty-four (84) asthmatic groups: intermittent, mild, moderate and severe persistent asthma and twenty (20) healthy children as control group.

This study was ethically approved from Institutional Reviewer Board (IRB) in Faculty of Medicine, Zagazig University Hospital and a written parental consent from every case or their caregivers that participates in this research was taken.

All children aged from 5-14 years with bronchial asthma admitted to Pediatric Department Zagazig University were included into the study. All cases of control group were healthy and free of any disease.

All Any cases with other chronic diseases that affect other system such as cardiopulmonary diseases, kidney diseases, and bone diseases were excluded from the study

All patients were subjected to:

Full history taking, Meticulous general examination, Local chest examination, and lab Investigations

Clinical history and examination:

All the studied children were subjected to a detailed history-taking and thorough clinical examination. History included: demographic data: Age, sex and residency

- Pulmonary function assessment:

All the studied children were evaluated for the pulmonary functions using using the Jaeger Germany Spirometer. Forced vital capacity (FVC), Forced expiratory volume in the first second (FEV1), Percentage of the forced expiratory volume in the first second to the forced vital capacity (FEV1/FVC), Forced expiratory flow 25-75% (FEF 25-75%), and Peak expiratory flow (PFF) were measured and recorded using a spirometer by a trained, experienced chest physician.

The following activities have been noted prior to spirometric study: chest medications were not allowed for 12 hours before the test. The child was relaxed, wearing loose – fitting clothes with no belts or girdles that may make it harder for him to breath. It was clearly illustrated to the patients. A nose clip was applied and the patient closed his lips around the mouth piece

The system receives the child's data including name, age, sex, height and weight in Kg, and automatically calculates the predicted normal values for the ventilatory function parameters from regression equation.

The child was asked to perform three repetitive attempts. Data from best attempt was recruited in the study. The flow- volume loop was plotted by having the subject inspire maximally, and then expire as forcefully and rapidly as possible, then reinspire as forcefully and rapidly as possible.
**Interpretation of Pulmonary Function Tests:**

FEF 25-75% values range from 50-60% and up to 130% is considered normal. It is advocated that lung function tests are considered abnormal only when the value deviates by 20% or more from the mean normal value (100%) (3).

- **Laboratory investigations**

  **Sample preparation:** Serum coagulation at room temperature for 10-20min, centrifuge at speed of 2000-3000 rpm for 20 min. Remove supernatant, if precipitation appeared, centrifuge again.

  **Sample Collection:** All blood samples were collected and processed within 2 hours and stored at -20 °C until assayed.

- **Measurement of blood cell count:**

  Two ml of peripheral blood were collected in EDTA tubes and assayed by coulter on the same day. Peripheral blood eosinophil count was calculated by multiplying the total white blood cells count by the percentage of eosinophils obtained from the differential white cells count. Count over 400 cells/cmm was considered eosinophilia.

- **Measurement of serum IgE level:**

  Venous blood samples were obtained from each subject under study, from the antecubital vein under complete aseptic conditions. For the preparation of serum, 2ml of blood were collected into sterile serum separating plastic tubes, allowed to clot at room temperature for sixty minutes, centrifuged; serum was separated and stored at -20 degree Celsius until assayed.

  Using the DiaMedEurogen IgE Quantitative which is a monoclonal antibody-based enzyme immunoassay for the quantitative determination of human total IgE in human serum.

- **Human Periostin(POSTN):**

  Serum periostin was Quantitatively measured using an enzyme-linked immunosorbent assay ELISA

**Principle**

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Periostin(POSTN) in samples. Adding Periostin to monoclonal antibody Enzyme well which is pre-coated with Human Periostin monoclonal antibody, incubation; then, add Periostin(POSTN) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The Chroma of color and concentration of the Human Substance Periostin(POSTN) of Sample where positively correlated.

**Assay procedures**

First, 50μl of standard, and Streptavidin-HRP were added, then 40μl of sample, and both POSTN –antibody 10 μl and Streptavidin-HRP 50μl were added. The sealing membrane was sealed, gently shook, and incubated 60 minutes at 37 c.

For 30×washing concentrate, the wells were dilute 30 times with distilled water and the membrane was carefully removed and drained with the liquid to shake away the remaining water.

The chromogen solution A 50μl, then chromogen solution B 50μl were added to each well with Gentle mixing and was incubated for 10 min at 37°C away from light. 50 ml of stop solution were added to each well. Finally, the optical density of each well was determined within 15 minutes, using a microplate reader set to a dual wavelength at 450 nm.

- **Statistical analysis:**

  The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 24. Armonk, NY: IBM Corp.). Data were
presented and suitable analysis was done according to the type of data obtained for each parameter. Differences between groups were analyzed with one-way analysis of variance (ANOVA) test Mean, Standard deviation (± SD) for parametric numerical data, while Median and range for non-parametric numerical data. 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 when the expected count is less than 5 in more than 20% of cells. Receiver operating characteristic curves (ROC) were used to identify sensitivity, specificity and determine optimal cut-off points of periostien serum level. A statistically significant difference was defined as a P-value of less than 0.05.

III. RESULTS

Regarding socio-demographic data: Age was distributed as 8.1±2.6 in intermittent asthma, 7.1±2.3 in mild asthma, 8.4±2.4 in moderate asthma, 8.9±1.9 in severe asthma, while the mean age of control group is 7.5±2.6. There was no statistically significant difference between the studied groups in age, sex and residence (Table 1).

Regarding controller drugs, there was statistically significant difference in controller drugs mainly between control and asthmatic children. (Table 2).

In Comparison of Periostin level among the studied groups: there was a higher level of periostin among severely asthmatic children than other asthmatic levels and the control group. There was statistically significant difference in periostin mainly between control and asthmatic children (Figure 1) (Table 3).

Correlation between periostin level and all laboratory data in the asthmatic group: there was statistically significant positive correlation between periostin with IgE, CRP and AEC (increased periostin level is associated with increased IgE, CRP and AEC) (Figure 2) and there was statistically significant negative correlation between periostin with FVC1, FEV and PEF (increased periostin level is associated with decreased FVC1, FEV and PEF). However, regarding other variables, there was no statistically significant correlation in the asthmatic group (Table 4).

Regarding the accuracy of periostin in detection of asthma severity: the accuracy of periostin level in detection of asthma was 87.0% with 89.0% ability to detect positive cases (sensitivity) and 86.0% ability to exclude negative cases (specificity) (Table 5,6) (Figure 3).

Table 1. Comparison between the studied groups regarding socio-demographic data:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control NO (20)</th>
<th>Intermittent asthma NO (21)</th>
<th>Mild asthma NO (21)</th>
<th>Moderate asthma NO (21)</th>
<th>Severe asthma NO (21)</th>
<th>test</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years) mean ± SD</td>
<td>7.5±2.6</td>
<td>8.1±2.6</td>
<td>7.1±2.3</td>
<td>8.4±2.4</td>
<td>8.9±1.9</td>
<td>F=1.8</td>
<td>0.1</td>
</tr>
<tr>
<td>range</td>
<td>(5-14)</td>
<td>(5-13)</td>
<td>(5-12.5)</td>
<td>(5-12)</td>
<td>(6-13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex Male</td>
<td>15(75.0%)</td>
<td>12(57.1%)</td>
<td>16(76.2%)</td>
<td>19(90.5%)</td>
<td>18(85.7%)</td>
<td>χ²=7.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Female</td>
<td>5 (25.0%)</td>
<td>9 (42.9%)</td>
<td>5(23.8%)</td>
<td>2 (9.5%)</td>
<td>3(14.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence Rural</td>
<td>14(70.0%)</td>
<td>16(76.2%)</td>
<td>15(71.4%)</td>
<td>15(71.4%)</td>
<td>16(76.2%)</td>
<td>χ²=0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Urban</td>
<td>6(30.0%)</td>
<td>5(23.8%)</td>
<td>6(28.6%)</td>
<td>6(28.6%)</td>
<td>5(23.8%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Relation between controller drugs and disease severity among studied groups:

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Control NO(20)</th>
<th>Intermittent NO(21)</th>
<th>Mild NO(21)</th>
<th>Moderate NO(21)</th>
<th>Severe NO(21)</th>
<th>Test (x²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>no</td>
<td>20</td>
<td>14</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>percentage</td>
<td>100%</td>
<td>66.7%</td>
<td>9.5%</td>
<td>0.0%</td>
<td>4.8%</td>
</tr>
<tr>
<td>I.C.S (dose)</td>
<td>no</td>
<td>0.0</td>
<td>7</td>
<td>19</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>percentage</td>
<td>0.0%</td>
<td>33.3%</td>
<td>90.5%</td>
<td>100.0%</td>
<td>95.0%</td>
</tr>
<tr>
<td></td>
<td>dose</td>
<td>0</td>
<td>0</td>
<td>100-200</td>
<td>200-400</td>
<td>400</td>
</tr>
<tr>
<td>LABA</td>
<td>no</td>
<td>0.0</td>
<td>0.0</td>
<td>2</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>percentage</td>
<td>0.0%</td>
<td>0.0%</td>
<td>9.5%</td>
<td>9.5%</td>
<td>76.2%</td>
</tr>
<tr>
<td>LTRA</td>
<td>no</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>percentage</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>9.5%</td>
<td>38.1%</td>
</tr>
</tbody>
</table>

Table 3. Comparison between the studied groups in Periostin.

<table>
<thead>
<tr>
<th>Periostin (ng/ml)</th>
<th>Control NO(20)</th>
<th>Intermittent Asthma NO(21)</th>
<th>Mild Asthma NO(21)</th>
<th>Moderate Asthma NO(21)</th>
<th>Severe Asthma NO(21)</th>
<th>K.W Test</th>
<th>P. value</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean ± SD</td>
<td>(a) 7.1±5.4</td>
<td>(b) 14.8±23.3</td>
<td>(C) 16.7±29.5</td>
<td>(d) 24.2 ±35.9</td>
<td>(e) 29.6±36.9</td>
<td>4.8</td>
<td>0.003**</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>3.9-29</td>
<td>3.6-105.9</td>
<td>4.2-35.9</td>
<td>5.3-133.7</td>
<td>4.6-153.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Correlation between periostin level and all data in the asthmatic group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Periostin r</th>
<th>p</th>
<th>SIG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.04</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>0.2</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>FVC1</td>
<td>-0.6</td>
<td>0.001**</td>
<td>S</td>
</tr>
<tr>
<td>FEV</td>
<td>-0.53</td>
<td>0.01*</td>
<td>S</td>
</tr>
<tr>
<td>PEF</td>
<td>-0.48</td>
<td>0.02*</td>
<td>S</td>
</tr>
<tr>
<td>IgE</td>
<td>0.4</td>
<td>0.02*</td>
<td>S</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>0.4</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>AEC</td>
<td>-0.2</td>
<td>0.001**</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0.03*</td>
<td>S</td>
</tr>
</tbody>
</table>
Table 5. Accuracy of periostin in detection of asthma severity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cut off</th>
<th>AUC</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periostin (ng/ml)</td>
<td>6.1</td>
<td>0.85</td>
<td>&lt;0.001**</td>
<td>(0.6-0.9)</td>
</tr>
</tbody>
</table>

Table 6. Predictive value of periostin in detection of asthma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PVP</th>
<th>PVN</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periostin</td>
<td>89%</td>
<td>86%</td>
<td>85.5%</td>
<td>86.5%</td>
<td>87.0%</td>
</tr>
</tbody>
</table>

Figure 1. Box plot chart for periostin level among the studied groups
Asthma is a major public health problem that affects nearly 350 million people worldwide. It is also the most common chronic disease in childhood and adolescence. Asthma is more a syndrome than a disease, exhibiting considerable heterogeneity in its presentation and clinical course (1).

Periostin is a cell matrix protein that was first identified in mouse periodontal ligament (hence the name) in 1993. It is secreted by bronchial fibroblasts and epithelial cells, acts as an immunomodulator, repairs connective tissue, and is involved in fibrogenesis. Periostin binds to integrins present on the surface of fibroblasts and epithelial cells. Its main function is to maintain tissue structure by binding to fibronectin, tenascin C, and collagen V. Expression of the periostin gene (POST) is regulated by bronchial epithelial cells, IL-13, and IL-4 (2).

This study aimed to assess periostin serum level in children with bronchial asthma and to determine the relation of these levels to the severity of bronchial asthma in children.

Figure 2. Scatter plot with line chart for correlation between periostin level and IgE among the studied groups

Figure 3. ROC curve for the role of periostin level in detection of asthma severity

IV. DISCUSSION

Asthma is a major public health problem that affects nearly 350 million people worldwide. It is also the most common chronic disease in childhood and adolescence. Asthma is more a syndrome than a disease, exhibiting considerable heterogeneity in its presentation and clinical course (1).

Periostin is a cell matrix protein that was first identified in mouse periodontal ligament (hence the name) in 1993. It is secreted by bronchial fibroblasts and epithelial cells, acts as an immunomodulator, repairs connective tissue, and is involved in fibrogenesis. Periostin binds to integrins present on the surface of fibroblasts and epithelial cells. Its main function is to maintain tissue structure by binding to fibronectin, tenascin C, and collagen V. Expression of the periostin gene (POST) is regulated by bronchial epithelial cells, IL-13, and IL-4 (2).

This study aimed to assess periostin serum level in children with bronchial asthma and to determine the relation of these levels to the severity of bronchial asthma in children.
This study showed that, there was no statistically significant difference between cases and controls regarding age and gender.

This agreed with (4) who aimed to assess the serum periostin levels in the diagnosis of pediatric bronchial asthma. They found that no significant differences in age and gender were observed between the two groups.

In the present study, 23% of our cases were females and 77% were males.

The exact reason for male predominance is not known, but several explanations have been offered. Airway in boys are smaller in comparison to their lung sizes compared to girls which may contribute to increased risk of wheezing after a cold or other viral infections (5).

In our study, there was no statistically significant difference between asthmatics and controls as regards residence. In this study most of asthmatic children were from urban residence but also most of controls were so.

This study agreed with Guner SN et al, (6) - Shimwela M., et al. (7), they found that more asthma sufferers lives in urban than rural areas.

This could be explained as the traditional rural lifestyle and early childhood exposure to infectious agents in rural areas are believed to be protective against asthma and allergic diseases (8).

**Periostin** is an extracellular matrix (ECM) protein belonging to the fasciclin family (9). Periostin also acts as a matricellular protein by binding to cell-surface receptors belonging to the integrin family, followed by transducing the signals in cells. The roles of periostin as both an ECM and a matricellular protein contribute together to developing or maintaining various tissues or organs. For example, periostin is involved in the process of wound healing in skin as both an ECM and a matricellular protein; a genetic deficiency of periostin in mice delays the closure of wounds (10, 11)

Our study shows that there was statistically significant difference (P ≤ 0.05) in periostin between control and asthmatic children with higher level among severely asthmatic children.

This agreed with Inoue et al., (4) who found that Serum periostin levels were significantly higher in children with asthma and this agreed with J.H. Cho et al., (12) found that serum periostin level in asthmatic children group is greater than that in control group.

The dual functions of periostin as an ECM and a matricellular protein are important also for the onset of inflammation, particularly for allergic inflammation. Periostin is deposited in inflamed sites showing fibrosis, whereas it activates immune and nonimmune cells as a matricellular protein, further augmenting inflammation. The epithelial/mesenchymal interaction and/or the immune cell/non-immune cell interaction are important for periostin to exert its effects in the setting of inflammation.

The current work shows that there was statistically significant positive correlation between periostin and IgE, CRP and AEC.

This agreed with Inoue et al., (4) who found Periostin was slightly correlated with the eosinophil count, CRP and total IgE level.

Our study showing that there was statistically significant negative correlation between periostin with FVC1, FEV and PEF (increased periostin level is associated with decreased FVC1, FEV and PEF).

This agreed with Marwa Elhady et al., (13) who reviled a significant negative correlation between serum periostin level and FEV1 in asthmatic children. FEV1 is an independent predictor of asthma exacerbations; asthmatic children with a baseline FEV1 <60% predicted have a doubled risk for asthma exacerbations in the subsequent year as compared with children with an FEV1 >80% predicted. Fuhlbrigge AL et al., (14). Kanemitsu et al., (15) reported that high serum periostin concentration (≥95 ng/ml) is the unique biomarker, among several serum markers, associated with the greater annual decline in FEV1 (at least 30 ml/year).
On the other hand, Inoue et al. (4) demonstrated that serum periostin have not correlated either with PEFR or FEV1 in children with asthma. However, the previous study did not include children with severe or uncontrolled asthma.

The current work revealed that Periostin levels as a biomarker for asthma diagnosis (PVP=85.5%, PVN=86.5%, sensitivity=89%, specificity=86.5%, Accuracy=87%).

This agreed with Marwa Elhady et al. (13) who reported that (PVP=72.2, PVN=88.1, sensitivity=72.22, specificity=88.10).

Limitation

One of the limitations of the current study is the small number of included patients. Our study was based on cross-sectional analysis, so we could not monitor the changes in serum periostin level through the course of the disease or its response to therapeutic interventions. We included children with bronchial asthma mainly depending on their level of control on treatment over the previous 6 months. Due to the explorative nature of this study, results need to be confirmed in a larger sample of patients including follow up. Another limitation is that serum periostin level could be elevated in many allergic disorders. However if we strictly exclude all children with coincident atopy, this limits the number of available asthmatic children to be included.

V. CONCLUSION

It could be concluded that Periostin level was higher in asthmatic children than controls and correlated with asthma severity. Therefore, serum Periostin can be considered as a possible biomarker for diagnosis, grading of asthma severity and the degree of airflow inflammation.

REFERENCES