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Serum Vascular Endothelial Growth Factor Levels in Asthmatic Children

Shimaa Moawad Hamed Elkholy[1], Ahmed Mohamed Abd-ElRazek[1], Amal Said ElBendary[2] and Ahmed Ibrahim Harkan[3].

Department of Pediatric Medicine[1] and Department of Clinical Pathology [2], Faculty of Medicine, Department of Applied Statistics, Faculty of Commerce[3] - Tanta University, Egypt

Corresponding author: Shimaa Moawad Hamed Elkholy; Mobile: 01122211709; Email: shimaakholy@yahoo.com

ABSTRACT

Background: Bronchial asthma is a heterogeneous disease, which is usually characterized by chronic airway inflammation. Asthma is a global problem with 300 million affected individuals. Angiogenesis has been recognized as an essential step in the development and maintenance of airway inflammation and tissue remodeling. Vascular endothelial growth factor plays a key role in both the physiological and pathophysiological forms of angiogenesis**. Aim of the present study was** to measure serum levels of vascular endothelial growth factor in asthmatic children to assess its relation with asthma severity. **Subjects and Methods:** This study was conducted on two groups, group A: 30 asthmatic children and group B: 20 healthy children of matched age and sex. Serum vascular endothelial growth factor (VEGF) levels were measured by ELISA. **Results:** There was no significant difference between asthmatic and control groups as regard age, sex and weight. There was significant increase in serum VEGF levels in asthmatic group than control group. There was negative correlation between VEGF serum levels and pulmonary function tests. There was positive correlation between serum VEGF levels and asthma severity. **Conclusion:** There were higher levels of serum VEGF in asthmatic patients than healthy control. Serum VEGF was higher in those with moderate and severe asthma than those with mild disease.

Keywords: Vascular Endothelial Growth Factor, Asthmatic Children.

INTRODUCTION

Bronchial asthma is a global problem with about 300 million people with asthma. It is considered as the most common chronic disease in children. Asthma is one of the top 20 chronic conditions in terms of the global ranking of disability in children (5–14 years of age). Death rates from asthma in children range from 0.0 to 0.7 per 100,000. There are significant global differences in the prevalence of asthma in children, with up to 13-fold differences between countries [(1)].

Vascular endothelial growth factor (VEGF) is a pluripotent growth factor that has a broad effect on endothelial cell function. VEGF is also one of the most potent angiogenic factors that stimulates endothelial cell proliferation and induces angiogenesis. VEGF is widely expressed in different vascularized organs, including the lung [(2)].

VEGF is a major regulator of blood vessels growth in the airways of patients with bronchial asthma, by promoting proliferation and differentiation of endothelial cells and also inducing vascular leakage and increased permeability. VEGF expression and its receptors are closely related to new blood vessels formation, suggesting that VEGF may also be involved in the remodeling of the airways [(3)]. The main sources of VEGF in the airways are alveolar epithelial cells, smooth muscle cells, bronchial epithelial cells, fibroblasts, and alveolar macrophages [(4)]. It has been suggested that eosinophils are the main sources of peripheral VEGF, but also other cellular components including platelets and neutrophils may be important compartments for circulating VEGF [(5)].

The aim of this study was to measure serum levels of vascular endothelial growth factor in asthmatic children in comparison with healthy children to detect the role of VEGF in the pathogenesis of bronchial asthma in pediatric age and to assess its relation with asthma severity.

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PATIENTS AND METHODS

This prospective case control study was performed in the Pediatric Intensive Care Unit (PICU) at Tanta University Hospital including patients attending the unit from October 2016 to September 2017. **The study was approved by the Ethics Board of Tanta and an informed written consent was taken from each participant in the study.**

A total of 50 subjects were included in this study and were categorized into 2 groups: **Group A: 30** asthmatic children aged 3-12 years diagnosed according to GINA guidelines 2017 [(6)] in chest unit of pediatric department of Tanta University Hospital (16 males and 14 female). **Group B: 20** apparently healthy children of matched age and sex coming for routine health care serving as a control group. Children with malignancy, thyroid dysfunction, rheumatoid arthritis, sepsis or treated with immunosuppressive drugs during the last 4 weeks were excluded.

Asthmatic children in the current study were classified into 4 groups according to the severity, as follows:

1- **Intermittent asthma:** They were 10 children (6 males,

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4 females) Characterized clinically by:
- Days with symptoms< 1 time per week.
- Nights with symptoms ≤ 2 times per month.
- Lung function: FEV1 or PEF ≥80% of predicted; PEF variability < 20%.

2- **Mild persistent asthma:** They were 11 children (7 males, 4 females) Characterized clinically by:
- Days with symptoms >1 attack per week but <1 time a day.
- Nights with symptoms > 2 times per month.
- Lung function: FEV1 or PEF ≥ 80% of predicted, PEF variability 20-30%.

3- **Moderate persistent asthma:** They were 8 children (3 males, 5 females) Characterized clinically by:
- Daily symptoms with daily use of treatment.
- Nights with symptoms more than 1 attack per week
- Lung function FEV1 or PEF 60 to 80% of predicted; PEF variability > 30%.

4- **Severe persistent asthma:** It was one female child. Characterized clinically by:
- Continuous daily symptoms.
- Nights with symptoms are frequent.
- Lung function FEV1 or PEF ≤ 60% of predicted; PEF variability more than 30%.

All children in this study were subjected to the following:

1- Complete medical history including: Family history of asthma, past history of asthmatic attacks and symptoms such as episodic breathlessness, wheezing, cough, and chest tightness.

2- Physical examination: The most useful physical finding is wheezing on auscultation. Other findings such as tachypnea, hyper inflated chest, use of accessory muscles and intercostal retraction were noted.

3- Investigations:

Blood sampling: Collection and preparation of samples:

5 milliliter of venous blood was collected. Each sample was fractioned as follows:

a) 3 ml blood was allowed to clot for half an hour in water path at 37 °c then it was centrifuged for 15 minutes at 3000 rpm for separation of serum for determination of VEGF level by enzyme linked immuno-sorbent assay (ELIZA).

b) 2 ml blood on EDTA was used to perform complete blood picture (CBC).

All patients and controls were subjected to the following investigations:

- ***Routine Laboratory investigations*** including: Complete blood count (CBC), Erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), thyroid stimulating hormone (TSH), T3 and T4, lactic acid dehydrogenase (LDH), stool analysis and pulmonary function test (FEV1 and PEF): it was performed using spirometer (Spirostik Geratherm). The patient placed a clip over the nose and breathed through the mouth into a tube connected to a spirometer. First the patient breathed in deeply, and then exhaled as quickly and forcefully as possible into the tube. After training of the child about the maneuver, three accepted maneuvers were performed and the highest value was recorded and the best of the three results as regard FEV1 and PEF were recorded. They are done for those > 6 years [7].
- ***Specific Laboratory investigations***: Serum levels of vascular endothelial growth factor by ELISA (Enzyme-linked immuno-sorbent assay) technique in both patients and control. In asthmatic patients VEGF levels were sampled in between asthma attacks.

Statistical analysis

In addition to the descriptive data, statistical analysis was done using IBM SPSS program version 22. Data were expressed as frequency and percentage; and mean ± SD and analyzed using the **Chi square (x^2) test** and the independent **Student's t-test** to assess the significance of difference in the levels between different parameters. P < 0.05 was accepted as significant. Coefficient (r) of two variables was also done by using **Pearson correlation coefficient (r)** with **P Value** calculation. Receiver operating characteristic (ROC) curve was plotted and sensitivity, specificity, PPV, NPV, accuracy and cutoff point were calculated.

RESULTS

Assessments of the measured parameters in the different submitted groups are presented in the following table and figures:

Table (1): Demographic data and body weight of asthmatic group and control group

		Asthmatic	Control	Test	p. value
Age	Mean ± SD	4.98 ± 2.36	5.04 ± 1.72	T: 0.098	0.923
Sex	Male (%)	16 (53.3%)	10 (50%)	X^2: 0.053	0.817
	Female (%)	14 (46.7%)	10 (50%)		
Weight	Mean ± SD	20.22 ± 7.94	23.05 ± 6.92	T: 1.298	0.200

Table (1) showed the following:

- As regard age, sex and weight there was no statistically significant difference between the two groups.

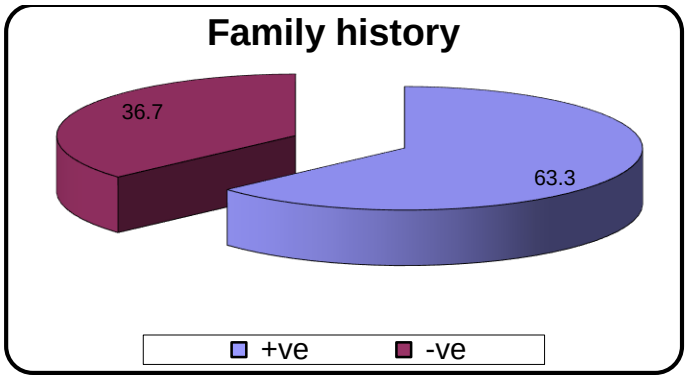

Figure (1): Percentage of positive and negative family history of allergy among asthmatic group.

- Figure (1) showed family history of allergy among asthmatic group with 19 patients (63.3%) had positive family history and 11 patients (36.7%) had negative family history of allergy.

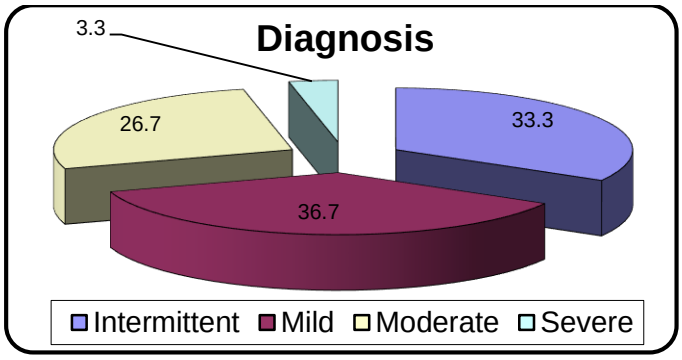

Figure (2): Distribution of patients among four asthma subgroups (intermittent, mild, moderate and severe persistent asthma).

- Figure (2) showed the distribution of patients among four asthma subgroups with 10 patients had intermittent asthma (33.3%), 11 patients with mild persistent asthma (36.7%), 8 patients with moderate persistent asthma (26.7%) and 1 patient with severe persistent asthma (3.3%).

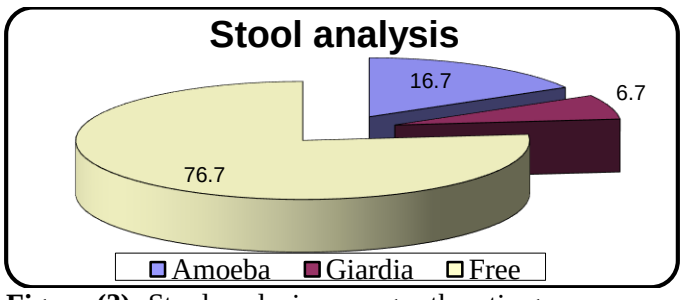

Figure (3): Stool analysis among asthmatic group.

- Figure (3) showed the stool analysis among asthmatic group with 5 cases had amoeba (16.7%), 2 cases had giardia (6.7%) and 23 cases with free stool analysis (76.7).

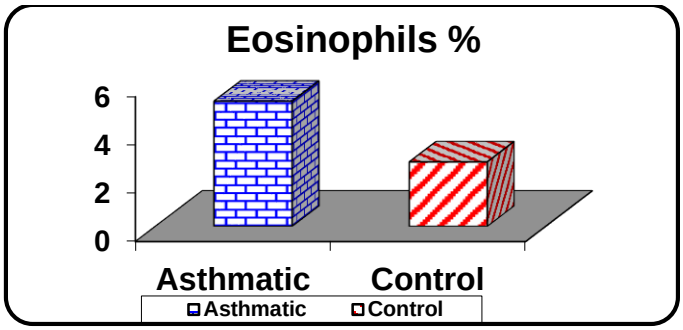

Figure (4): Comparison between asthmatic group and control group as regard eosinophilic count.

- Figure (4) showed that there was statistically significant difference between asthmatic group and control group as regard eosinophilic count as it was higher in asthmatics than controls.

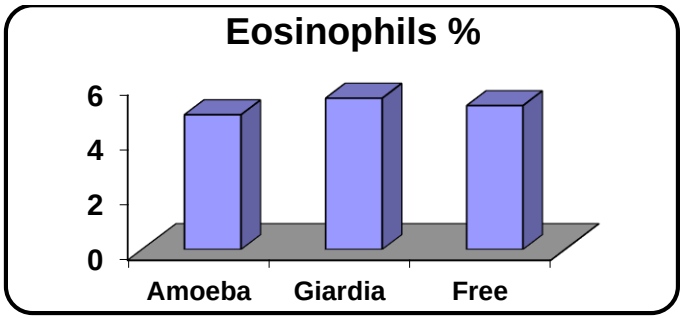

Figure (5): Association between eosinophilic count and stool analysis in asthmatic group. Figure (5) showed that there was no significant association between eosinophilic count and stool analysis among asthmatic group.

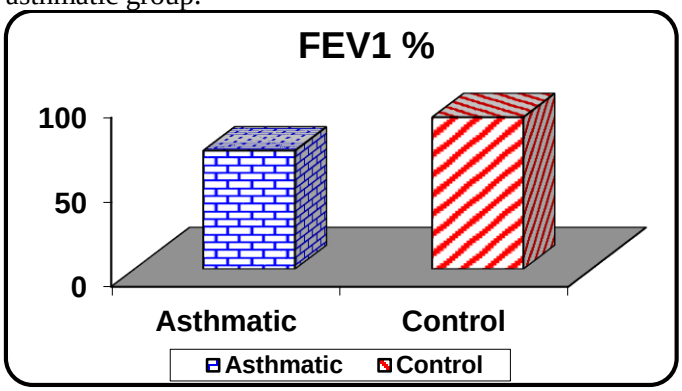

Figure (6): Comparison between asthmatic group and control group as regard forced expiratory volume in the first second (FEV1 %).

- Figure (6) showed that there was statistically significant difference between asthmatic group and control group as regard FEV1 as it was lower in asthmatics than controls.

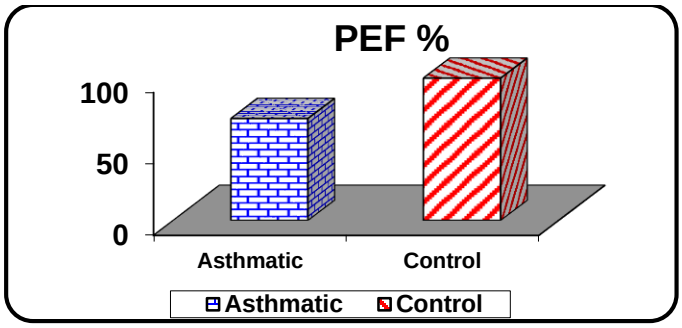

Figure (7): Comparison between asthmatic group and control group as regard peak expiratory flow (PEF %).

- Figure (7) showed that there was statistically significant difference between asthmatic group and control group as regards (PEF %) as it was lower in asthmatic group than control group.

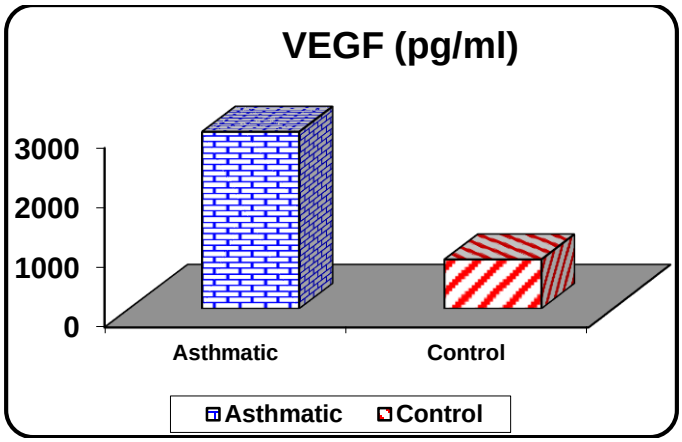

Figure (8): Comparison between asthmatic group and control group as regard serum vascular endothelial growth factor levels (VEGF pg/ml).

Figure (8) showed that there was statistically significant difference between asthmatic group and control group as regard (VEGF pg/ml) as it was higher in asthmatic group than control group.

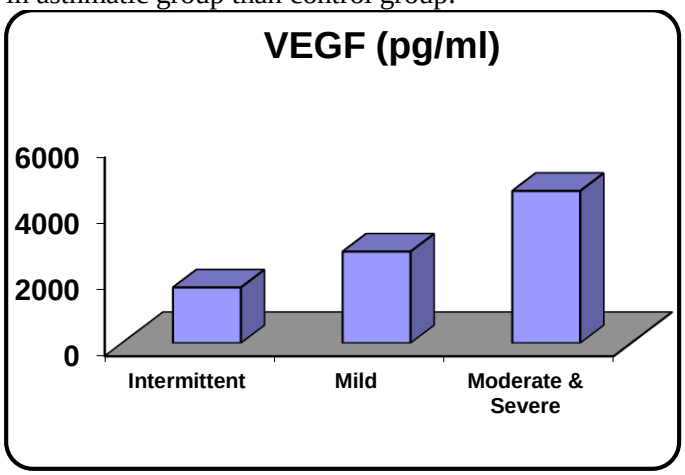

Figure (9): Correlation between serum vascular endothelial growth factor levels (VEGF pg/ml) and severity of asthma.

Figure (9) show that there was significant positive correlation between serum VEGF levels and severity of asthma as VEGF levels were higher in moderate and severe persistent than intermittent and mild persistent asthmatic patients.

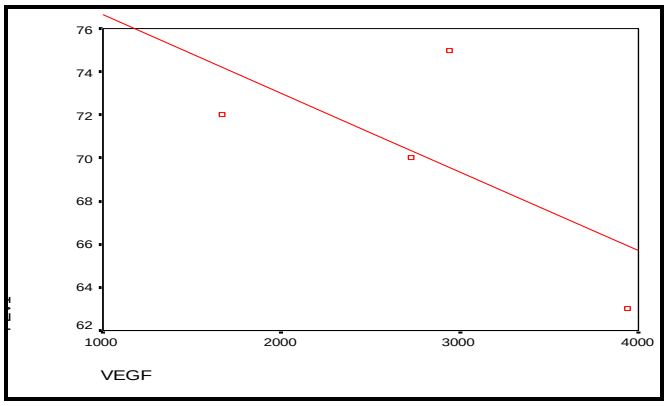

Figure (10): Correlation between vascular endothelial growth factor (pg/ml) and forced expiratory volume in the first second (FEV1 %).

- Figure (10) show that there was negative correlation between vascular endothelial growth factor (VEGF) and pulmonary function tests (FEV1).

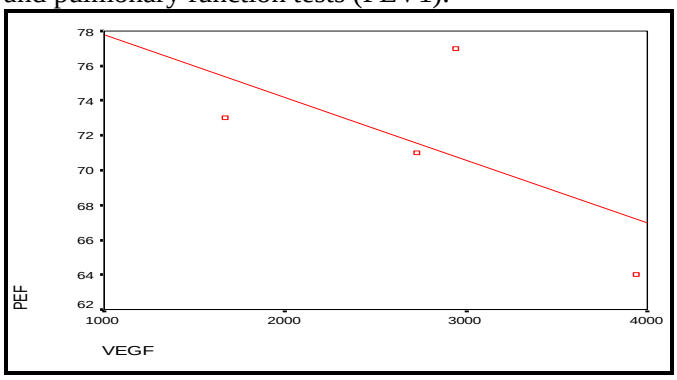

Figure (11): Correlation between vascular endothelial growth factor (VEGF pg/ml) and peak expiratory flow (PEF %). Figure (11) show that there was negative correlation between vascular endothelial growth factor (VEGF) and peak expiratory flow (PEF %).

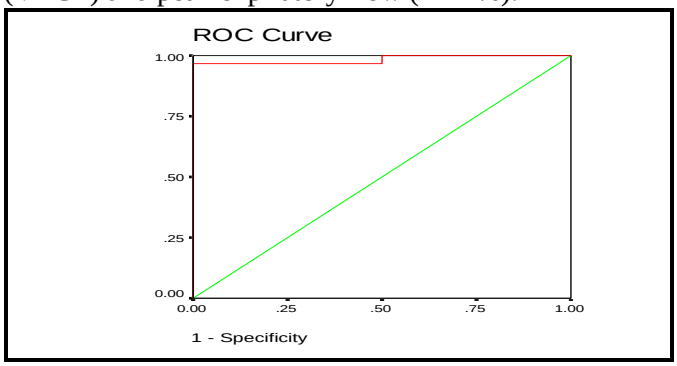

Figure (12): Receiver operating characteristic (ROC) curve of serum levels of vascular endothelial growth factor (VEGF pg/ml) as a biomarker of asthma in children.

- Figure (12) show that the sensitivity of VEGF as a biomarker of asthma was 96%, the specificity was

90%, PPV was 93%, NPV was 95% and the accuracy was 94%.

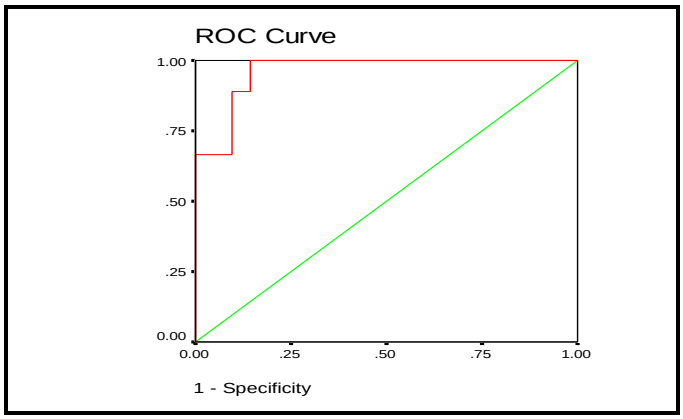

Figure (13): Receiver operating characteristic (ROC) curve of serum level of vascular endothelial growth factor (VEGF) as an indicator of asthma severity in children.

- Figure (13) show that the sensitivity of VEGF as an indicator of asthma severity was 95%, the specificity was 89%, PPV was 92%, NPV was 90%, accuracy was 92% and cutoff point was 3990.

DISCUSSION

Over years, the proportion of children with asthma has increased significantly. The goals of developing effective asthma treatments for children are to improve the quality of life of asthmatic children by reducing symptoms and frequency of exacerbation, to provide undisturbed sleep and performance of daily activities, and also to ensure a more healthy adult future. Despite modern advances in the medical care and the introduction of effective therapies, as inhaled corticosteroids, still poor asthma control exists in clinical practice [(8)].

Due to its various properties in airway inflammation, the involvement of VEGF in asthmatic patients has been investigated on a large scale. **Asai *et al.*** [(9)] showed that increased levels of VEGF in induced sputum of asthmatic subjects were associated with airway vascular permeability. **Feltis *et al.*** [(10)] examined VEGF in bronchoalveolar lavage fluid from adult asthmatic patients and normal controls, and showed that level of VEGF was increased proportional to the vessels number and density in asthmatic airway.

In addition, **Hoshino *et al.*** [(11)] found more VEGF-positive cells in bronchial biopsy samples from the airways of asthmatic patients and concluded that this was linked to the degree of airway vascularity.

As regards family history in the current study, in asthmatic patients there were 19 children with positive family history of allergy and in control group there were no family history of allergy. The results of the present study showed no statistically difference between asthmatic and control groups as regards age, sex and weight. **Lee *et al.*** [(12)] agree with us as their study reveal that there were no significant differences regarding age and weight between asthmatics and healthy group. In the current study there was significant difference between two groups as regard eosinophil count between asthmatic and control groups. This is in agreement with **Williams *et al.*** [(13)] who reported that blood eosinophil counts increased in the airways of most persons who have asthma.

For exclusion of possible affection of parasitic infestation on eosinophilic count stool analysis was done [(14)]. The present study showed that there was no significant correlation between eosinophilic count and stool analysis among asthmatic group.

The current study showed significant difference between the studied groups as regards pulmonary function tests (FEV1, PEF). The mean values of FEV1 are lower in asthmatic than healthy children. This is in agreement with **Fuhlbrigge *et al.*** [(15)] who demonstrated a strong association between decreased FEV1 and risk of asthma severity.

The present study showed significant difference between asthmatic and control group as regard serum vascular endothelial growth factor level that reflect higher levels of serum vascular endothelial growth factor in asthmatic patients than healthy control. This is in agreement with **Lee *et al.*** [(12)] who reported that serum levels of VEGF were significantly increased in asthmatic patients compared with VEGF levels in healthy control subjects. This is also in agreement with **Chetta *et al.*** [(16)] who reported that in patients suffering with mild to moderate asthma, a significant increase in the number of vessels and percent of vascular area, as well as an increase in average capillary dimension, can be noticed in comparison to healthy controls.

The present study showed negative correlation between VEGF serum level and pulmonary function tests. This is in agreement with **Zou *et al.*** [(17)] who reported that the concentrations of VEGF in the serum of asthmatic patients with an FEV1/FVC value of <70% were higher than those with an FEV1/FVC value of >70%. This can be explained as VEGF may lead to an increase in both mucosal and submucosal vascular bed permeability, causing airway wall thickening. Therefore, mucosal edema can significantly affect airway function as recorded by **Yoo *et al***. [(18)].

The current study subdivided asthmatic group according to asthma severity into sub-groups: intermittent, mild, moderate and severe persistent asthmatic patients. The present study results revealed that there was significant difference between asthma sub-groups as regard serum VEGF levels. This is in

agreement with **Zou *et al.* [17] who** found that serum VEGF levels were higher in patients suffering severe asthma compared to those with mild asthma, indicating that VEGF may be associated with asthma severity and airflow restriction. VEGF may cause airway mucosal edema with thickening of the airway wall, resulting in remodeling that can cause irreversible limitation of airway function and also exacerbation of asthma severity via airway inflammation, vasopermeability and angiogenesis. This is also in agreement with **Hashimoto *et al*. [19]** whose study found a relationship between increased bronchial micro vascularity and the severity of asthmatic disease.

The present study revealed that serum VEGF can be used as indicator of asthma severity with high sensitivity (95%), specificity (89%) and accuracy (92%). The cutoff value was 3990 pg/ml as detected by ROC curve analysis, above which the case is suspected to have moderate to severe asthma.

Some efforts were made to inhibit VEGF or block its receptors, but benefits have only been demonstrated in animal experiments [20]. The present study results suggest that serum VEGF is a good biomarker in asthma, it also correlate with the severity of asthma and this is supported by other studies performed by **Zou *et al.* [17]** and **Lee *et al.* [12]** where in their studies they found correlation between serum VEGF and asthma. However, further prospective and larger studies are required to support the use of the serum VEGF as a marker for asthma severity in clinical practice.

In conclusion; the current study showed that there were higher levels of serum VEGF in asthmatic patients than healthy control with sensitivity of 96% and specificity of 90%. Serum VEGF levels were higher in patients with moderate and severe asthma than those with mild asthma. So serum levels of VEGF have positive correlation with asthma severity.

Sensitivity of VEGF as an indicator of asthma severity was 95%, the specificity was 89% and cutoff point was 3990.

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