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ORIGINAL ARTICLE

The Association between Follistatin like protein-1 Level and Severity of Asthma in Asthmatic Children in Zagazig University Hospitals

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Abstract

Background: Over 300 million individuals worldwide suffer from asthma. Furthermore, during the past three decades, western nations have demonstrated increased prevalence. 7.7% of people in Egypt's Nile Delta were found to have bronchial asthma. Follistatin-like 1 (FSTL1) may be crucial for the respiratory system, according to several studies. FSTL1 controls the maturation of alveoli, cartilage, and the development of the lungs. This study aimed to evaluate follistatin like protein 1 level in the serum of pediatric patients with asthma and determine its association to asthma severity. **Methods:** This case-control study was carried out on 60 children at pulmonology unit in pediatric department and clinical pathology department, Zagazig University Children Hospital in the period from May 2020 to June 2022. They were divided into group A: Consisted of 45 asthmatic children subdivided into 3 groups (mild – moderate – severe) and group B: consisted of 15 age and sex matched healthy children. **Results:** There was highly statistically significant increased FST level in severe group when compared with moderate group, mild group, and control group. **Conclusion:** In asthmatics, plasma FSTL1 levels were higher and positively correlated with severity of asthma.

Keywords: Follistatin like protein-1, Asthma, Children, Severity.

Introduction

Chronic airway inflammation is the hallmark of the diverse disease known as asthma. Respiratory symptoms such as wheezing, shortness of breath, chest tightness, and cough that fluctuate in duration and intensity, along with varying expiratory airflow limitation, provide a history of the condition **[1].** Over the past two decades, bronchial asthma prevalence among children has progressively increased worldwide. 8.2% of Egyptian children aged 3 to 15 were reported to have asthma **[2].**

Although the exact origin of childhood asthma is unknown, a mix of environmental exposures, innate biologic susceptibilities, and genetic predispositions have been suggested. Immune reactions to typical airway exposures (such as respiratory viruses, allergens, cigarette smoke, and air pollutants) can promote protracted, pathogenic inflammation and abnormal healing of damaged airway tissues in the vulnerable host. Airway remodeling and lung dysfunction ensue. These pathogenic processes in the developing lung have a negative impact on the development and differentiation of the airways, which results in altered airways as people age. Following the onset of asthma, persistent inflammatory exposures seem to make it worse, leading to illness persistence and raising the possibility of severe exacerbations. One of the most frequent reasons for children to visit the emergency room, be hospitalized, and miss school is asthma **[3].**

A secreted glycoprotein with 308 amino acids, follistatin-related protein (FRP) is also known as

transforming growth factor (TGF) 1-stimulated clone 36 (TSC-36) or follistatin-like protein 1 (FSTL1) **[4]**.

Increasing body of research has demonstrated that it promotes fibrosis, acts as a cardioprotective factor, and regulates tumor growth in both directions depending on the cell line that produces tumors. A novel proinflammatory molecule known as FSTL1 has also been shown to activate immune cells, boost gene expression, and release several proinflammatory cytokines and chemokines **[5]**.

According to reports, FSTL1 is involved in several biological processes, such as skeletal muscle growth, fibrosis, wound healing, and cell proliferation and differentiation **[6]**.

FSTL1 may be crucial for the respiratory system, according to several studies. FSTL1 controls alveolar maturation, cartilage growth, and lung development. Mice with FSTL1 knockouts are embryonally fatal, and their respiratory and skeletal systems exhibit a variety of developmental defects. Furthermore, FSTL1 contributes to lung cancer cell growth and death. Lung damage can increase FSTL1 production, which can encourage the formation of myofibroblasts and ultimately result in fibrosis. **Miller et al [7]** revealed that the FSTL1/oncostatin M pathway may promote airway remodeling in persons with severe asthma and that macrophages in the lungs of those with severe asthma express FSTL1 at high levels **[4].**

So, we aimed to evaluate follistatin like protein 1 level in the serum of pediatric patients with asthma and determine its association to asthma severity.

Patients and Methods

This case-control study was carried out on 60 children at pulmonology unit in pediatric department and clinical pathology department, Zagazig University Children Hospital in the period from May 2020 to June 2022. They were divided into group A: Consisted of 45 asthmatic children diagnosed through clinical examination and subdivided into 3 groups in each group 15 children (mild – moderate – severe) according to Classification of asthma severity. Group B: Consisted of 15 age and sex matched healthy children were recruited from the outpatient clinic presenting with minimal medical or surgical disorders, with no history of allergic disease or wheezing.

Inclusion criteria

Both genders included, their ages ranged between 5-15 years; a minimum of one of the following qualifications: a favorable bronchodilator response of 12% and a 200mL rise in forced expiratory volume in one second (FEV1), as well as day to day airflow variability, diagnosis of asthma according to GINA **[1]**, guidelines and classification of asthma exacerbation severity according to EPR–3 **[8]**. With no personal history of asthma or other atopic manifestation and not suffered from allergic diseases by clinical examination.

Exclusion criteria

History of coronary artery therapy, cardiac surgery, primary cardiomyopathy, secondary cardiomyopathy, congenital heart disease, renal failure, autoimmune disorders, pulmonary fibrosis, and renal failure patients who within the past two weeks experienced any respiratory infection symptoms.

Methods

All patients included in the study were given a thorough clinical evaluation, which included history, clinical assessment of the severity of their asthma, and physical examination. Laboratory examinations including serum levels of FSTL1 using ELISA Kits (SunRed, China) and pulmonary function tests. All asthmatic patients underwent spirometry assessment to determine the health of their lungs. To diagnose and treat asthma, precise lung function testing is required **[9]**.

A good indicator of how rapidly full lungs may be emptied is the FEV1 (forced expired volume in one second), which measures the volume exhaled in the first second of maximal expiration following a maximal inspiration. The maximum expiratory flow rate, or PEF (peak expiratory flow), is reached very early in the forced expiratory maneuver.

The Technique - How to do it and common pitfalls and problems.

The FVC maneuver was carried out with maximal effort right after a maximum inspiration to achieve an appropriate outcome; it should have a quick start, and the spirogram and flow-volume curve should be a smooth continuous curve. The method was thoroughly described to the patient to get good results, making sure that he or she is sitting

upright with feet planted firmly on the floor (the most comfortable position in children the vital capacity is often greater in the standing position). The patient's nose was clipped, and instructions were given to: Inhale deeply (must be completely full); Lips should be sealed around the mouthpiece. As soon as possible, force all the air out of the lungs until they are entirely empty. Breath in once again, as forcefully and fully as you can (if inspiratory curve is required and the spirometer is able to measure inspiration) **[10]**.

Rules of the test were to take a deep breath (must be full). a mouthpiece with a good seal. Extremely intense effort from the beginning of the maneuver until there is no more breath that can be expelled. No forward slanting when taking the test. Obtain a minimum of three repeatable tests that are acceptable. Three technically sound procedures were accomplished, with the optimum FEV1 variability being less than 0.15 L between the highest and second-highest result. If a test satisfies the following acceptability and repeatability requirements, it is acceptable.

Acceptability Criteria

Throughout the test, a continuous maximal expiratory maneuver that started with a full inspiration was accomplished. Throughout the test, there was no indication of reluctance. A quick start was used during the test. The PEF is rising rapidly (flow-volume). No premature termination, i.e., expiration continued until there was no change in volume and the patient had blown for approximately 3 seconds (for patients under the age of 10) or for approximately 6 seconds (for patients over the age of 10). If the patient cannot or should not continue, the patient or practitioner may stop the blow. No leaks were present. No sneeze (note FEV1 may be valid if cough occurs after the first second). Glottis not closed (Valsalva). There is no blockage of the mouthpiece (e.g., by the tongue or teeth). There is no proof that the patient breathed in more during the expiratory maneuver.

Repeatability Criteria

Get three tests that are satisfactory; each test should satisfy the indicated acceptability requirements. FEV1 and the two greatest results for FVC should be within 0.15 L of one another **[10]**.

Ethical approval

Parents or legal guardians of study participants provided their informed consent. The research and ethics committees of the participating hospitals gave their approval to the study. The study was conducted in accordance with the Declaration of Helsinki and the Code of Ethics of the World Medical Association (IRB # 4942/4-11-2018).

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS version 20.0) program was then used to import the data and perform analysis. The following statistical tests were applied: Mann-Whitney U test, Chi-Square test, Fischer Exact test, student t-test, one-way ANOVA test, Kruskal Wallis test, and Pearson's correlation coefficient.

Results

Table 1; showed there were no statistically significant differences in age or sex between the study groups (p-value > 0.05). Table 2; showed that there were highly statistically significant (p-value < 0.001) increased day time symptoms, increased night awakenings, interference with activity and increased SABA use in severe group (median = 7, IQR = 7 - 7) more than moderate group (median = 7, IQR = 7 - 7) and mild group (median = 2, IQR = 1 - 2).
Table 3; showed that there was highly statistically significant (p-value < 0.001) decreased FEV1 predicted and FEV1/FVC in severe group when compared with moderate group, mild group, and control group. Table 4; showed that there was highly statistically significant (p-value < 0.001) increased FST level in severe group when compared with moderate group, mild group, and control group.
Table 5 using roc curve, it was shown that: Serum FST can be used to discriminate between patients' group and control group at a cut off level of > 2302, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). Serum FST can be used to discriminate between control group and moderate group at a cut off level of > 2476, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). Serum FST can be used to discriminate between control group and severe group at a cut off level of > 2634, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). Serum FST can be used to discriminate between mild group and severe

group at a cut off level of > 3122, with 100% sensitivity, 100% specificity, 100% PPV and 100% NPV (AUC = 1.0 & p-value < 0.001). Serum FST can be used to discriminate between moderate group and severe group at a cut off level of > 3048, with 86.7% sensitivity, 93.3% specificity, 92.8% PPV and 87.5% NPV (AUC = 0.98 & p-value < 0.001).

Statistically significant (p-value = 0.04**2)** Positive correlation (r = 0.305) were found between serum FST level and weight, BMI, daytime symptoms, awaking night, and SABA use in patients' group. Highly statistically significant (p-value < 0.001) Negative correlation (r = - 0.526) between serum FST level and FEV1 and FEV1/FVC in patients group as shown table 6.

Table (1): Comparisons between studied groups as regard demographic data.

		Groups								Test p-value
		Control (n = 15)		Mild (n = 15)		Moderate (n = 15)		Severe (n = 15)		
Sex	Male	10	66.7%	11	73.3%	9	60%	9	60%	$X^2 = 0.8$ p = 0.848 NS
	Female	5	33.3%	4	26.7%	6	40%	6	40%	
Age (years)	Median	9		7		6		8		KW = 1.79 p = 0.158 NS
	IQR	6 - 10		7 - 9		5 - 8		5 - 11		

Table (2): Comparisons between studied groups as regard clinical data.

		Groups						Test p-value
		Mild (n = 15)		Moderate (n = 15)		Severe (n = 15)		
BA	No	0	0%	0	0%	0	0%	**$X^2 = 60$ p < 0.001 HS**
	Yes	15	100%	15	100%	15	100%	
Daytime symptoms	Median	2		7		7		**KW = 38.2 p < 0.001 HS**
	IQR	1 - 2		7 - 7		7 - 7		
Night awakenings	Median	3		4		7		**KW = 28.9 p < 0.001 HS**
	IQR	2 - 3		3 - 5		6 - 8		
Interference with activity	No	3	20%	0	0%	0	0%	**$X^2 = 134.7$ p < 0.001 HS**
	Some	4	26.7%	15	100%	0	0%	
	Minor	8	53.3%	0	0%	0	0%	
	Extreme	0	0%	0	0%	15	100%	
SABA use	Median	2		7		7		**KW = 32.5 p < 0.001 HS**
	IQR	1 - 3		7 - 7		7 - 7		

Table (3): Comparisons between studied groups as regard spirometry.

		Groups				Test p-value
		Control (n = 15)	Mild (n = 15)	Moderate (n = 15)	Severe (n = 15)	
FEV1 predicted	Mean	93.7	86.3	75.5	56.2	**F = 217.8 p < 0.001 HS**
	±SD	2.3	5.8	5.1	2.9	
FEV1/FVC	Mean	94.1	87.0	76.2	57.1	**F = 210.4 p < 0.001 HS**
	±SD	2.5	5.9	5.0	2.8	

Table (4): Comparisons between studied groups as regard FST level.

		Groups				Test p-value
		Control (n = 15)	Mild (n = 15)	Moderate (n = 15)	Severe (n = 15)	
FST level	Median	2163	2405	2799	3312	KW = 55.1
	IQR	2103 - 2203	2335 - 2517	2716 - 2882	3097 - 3909	p < 0.001 HS

Table (5): Diagnostic performance of serum FST level in discrimination of studied groups.

	FST level	AUC	Sensitivity	Specificity	PPV	NPV	p-value
Patients vs Control	> 2302	1.0	100%	100%	100%	100%	< 0.001
Control vs moderate	> 2476	1.0	100%	100%	100%	100%	< 0.001
Control vs severe	> 2634	1.0	100%	100%	100%	100%	< 0.001
Mild vs severe	> 3122	1.0	100%	100%	100%	100%	< 0.001
Moderate vs severe	> 3048	0.98	86.7%	93.3%	92.8%	87.5%	< 0.001

Table (6): Correlation study between serum FST level and other studied data in all patients' groups.

Variables	Patients group	
	r	p-value
age	0.09	0.557
Weight	**0.305**	**0.042***
Height	0.218	0.15
BMI	**0.318**	**0.033***
Day time Symptoms	**0.425**	**0.004***
Awaking night	**0.458**	**0.002***
SABA Use	**0.374**	**0.011***
FEV1	**-0.526**	**< 0.001****
FEV1/FVC	**-0.527**	**< 0.001****
Hb	-0.091	0.556
WBCs	-0.054	0.73
PLTs	0.165	0.284

Discussion

This result demonstrated that, no statistical substantial age and sex differences across the study groups.

Liu et al **[4]** and Wang et al **[5]** also found that there was no difference in age or sex across the groups, supporting the findings of our study. There were 32 asthmatic patients in Liu et al study's, of whom 11 (34.4%) were men. 10 (40%) of the 25 controls were men.

In the current study, there was highly statistically significant increased daytime symptoms, night awakenings, interference with activity in severe group and moderate group when compared with mild group.

This came in agreement with Peters et al. **[11]** and Nordon et al. **[12]** who discovered that people with severe asthma have symptoms (daytime symptoms and nocturnal awakenings) that interfere with their daily life and cause them to miss work or school.

Because of the obstructive pattern brought on by asthma, there is less expiratory airflow and more airway resistance. The forced vital capacity (FVC), forced expiratory volume in one second (FEV1), and the FEV1/FVC% are all normal or lowered in an obstructive pattern **[13].**

We showed that highly statistically significant (p-value < 0.001) decreased FEV1 predicted in severe group (56.2 ± 2.9) when compared with moderate group (75.5±5.1), mild group (86.3±5.8) and control group (93.7±2.3). Also, highly statistically significant decreased FEV1/FVC in severe group (57.1 ± 2.8) when compared with moderate group (76.2±5.0), mild group (87.0 ± 5.9) and control group (94.1 ± 2.5).

In agreement with our study, Liu et al. **[4]** and Kang et al. **[14]** showed that controls had higher forced expiratory volume in one second (FEV1) and FEV1/forced vital capacity (FVC) than asthmatics did (p <0 001 and p <0 001, resp.).

In addition, Firoozi et al. **[15]** found that the range from controlled to uncontrolled asthma was 89.5% to 67.3%, whereas the range from moderate to severe asthma was 89.8% to 61.5% for the mean projected value of the forced expiratory volume in 1 second (FEV 1). In 56 patients, the FEV 1 / FVC ratio was assessed. The range from mild to severe asthma was 75.8% to 61.8% (p = 0.030), whereas the range from controlled to uncontrolled asthma was 75.3% to 65.7%. (p < 0.001).

An essential measure of airway function is FEV1% predicted, and the more FEV1% predicted decline there is, the more severe the asthma is Kang et al. **[14].**

We showed that highly statistically significant increased FST level in patients' group when compared with control group. There was highly statistically significant increased FST level in severe group when compared with moderate group, mild group, and control group.

We demonstrated that neither the "control group and mild group" nor the "mild group and moderate group" had statistically significant differences. Between "control group and moderate group" and "moderate group and severe group," there was a statistically significant difference. Between the "control group and severe group" and the "mild group and severe group," there was a very statistically significant difference".

In agreement with our study, Liu et al. **[4],** Wang et al. **[5],** Liu et al. **[16]** showed that the asthma group's plasma concentrations of FSTL1 were considerably higher than those of the controls.

Also, Miller et al. **[7]** demonstrated that people with severe asthma have high levels of Fstl1 expression. Fstl1 was shown to be significantly expressed in the lungs of human asthmatics, and many of the Fstl1+ cells also coexpressed the macrophage marker CD 68. Also, they measured the levels of Fstl1 expression in bronchial samples taken from the lungs of severe asthmatics and control participants using immunohistochemistry. These findings showed that there were considerably more Fstl1+ cells in the lungs of severe asthmatics than there were in the lungs of healthy control patients (p < 0.005).

Murphy et al. **[17]** idiopathic pulmonary fibrosis and severe asthma patients had their Fstl1 expression upregulated in the lungs.

We demonstrated a statistically significant positive association between the serum FST level and the patient group's weight, BMI, daytime symptoms, nighttime awakenings, and use of SABA. Also, in the patient group, there was a highly statistically significant negative connection between the serum FST level and each of the FEV1 and FEV1/FVC.

In agreement with our study, Liu et al. **[4]** showed that the plasma levels of FSTL1 were positively correlated. FEV1 levels and plasma FSTL1 levels were inversely correlated. (rs = −0 459, *p* =0 008) and FEV1/FVC (rs = −0 351, *p* =0 049).

In addition, Mattiotti et al. **[18]** reported that the level of FSTL1 production correlated positively with indicators of airway remodeling and adversely with lung function metrics.

According to studies, FSTL1 is crucial for lung organogenesis and development as well as for lung fibrosis and airway remodeling **[4].**

Conclusion

Our study demonstrated that the levels of plasma FSTL1 were elevated in asthmatics and positively correlated with severity of asthma. This study and our continuing efforts may provide a novel treatment strategy and/or a diagnostic biomarker for asthma.

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