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Serum Soluble CD93 as a Biomarker of Asthma Exacerbation in Children

Mohamed Osman Hafez[1], Sanaa Mahmoud Abdelsalam[2], Hanan Samir Ahmed[1], Thuraya Rafea Goda Rafea*[1]

Departments of [1]Pediatrics and [2]Clinical Pathology, Faculty of Medicine – Zagazig University, Egypt.

***Corresponding author:** Thuraya Rafea Goda Rafea, **Mobile:** (20)01129172494,

Email: thurayarafea2021@gmail.com

ABSTRACT

Background: Asthma is considered one of the most prevalent diseases affecting over than 300 million individuals worldwide**.** Soluble CD93 was normally detected in human plasma and induced by the inflammatory mediators TNF-α and LPS, suggesting that physiologic pathways trigger the cleavage event.

Objective: To evaluate the diagnostic value of serum soluble CD93 level in acute asthma exacerbation in children and to find if there is a relation between serum level of soluble CD93 and acute exacerbations of asthma among children.

Patients and Methods: Our study included 30 patients who were diagnosed as acute asthmatics with acute exacerbation (diagnosed and classified according to GINA 2018) as group I. Group Π, which included the same 30 patients after receiving treatment and relieve of symptoms by clinical examination as well as routine laboratory investigations that confirmed their healthy state. Plasma sCD93 concentration using ELISA (at the time of exacerbation and repeated on the follow up day) and spirometry were done.

Results: Regarding severity (after classification of cases into intermittent, mild and moderate), there was no statistical significance difference in severity either pre- or post-treatment. Regarding sCD93, there was statistical significance reduction in sCD 93 level post-treatment compared to pre-treatment in all cases. There were no statistical significance relation between gender, residence and family history and sCD 93 levels among the studied group. There were no statistical significance relation between WBCs and x-ray and sCD 93 levels among the studied group.

Conclusion: sCD93 was not affected by gender or age and did not affect by reliever or controller medications. sCD93 showed a modest decrease in the controlled stage of asthma, which allowed to interpret its role as inflammatory biomarker.

Keywords: Serum soluble CD93, Biomarker, Asthma exacerbation.

INTRODUCTION

Asthma is considered one of the most prevalent diseases affecting over than 300 million individuals worldwide **(1).** It is defined as a chronic immunological lung disorder causing reversible obstruction, inflammation of air ways and increases its hyper-responsiveness to various provocations **(2).**

The diagnosis and management of asthma is generally based on symptoms together with pulmonary function tests results. But these criteria may not reflect the underlying airway inflammation so asthma research is shifting to the narrow-focus cellular profiles, protein analysis, biomarkers, and genetic markers. Asthma biomarkers include biomolecules that undergo cellular, biochemical, or molecular alterations in asthmatic patients versus healthy subjects that can be measured in biological samples, such as lung tissue, bronchoalveolar lavage fluid, nasal fluid, or blood, but being these currently unavailable in asthma, reliable and noninvasive biomarkers would ideally be standard in the daily clinical routine **(3).**

Type 1 transmembraneglycoproteine CD93 (C1qRP) is encoded by the CD93 gene found on chromosome 20**.** It is expressed early on the surface of myeloid cells during B-cell differentiation in the bone marrow and on the surface of hematopoietic stem cells, natural killer cells, endothelial cells, platelets, and microglia **(4).** The predicted molecular weight of CD93 is 120 kD and consists of a c-type carbohydrate-recognition domain, a fine rationale epidermal growth factor-like domain, a single transmembrane domain, a main domain, and an intracellular domain **(5).** CD93 has been implicated in regulating adhesion and expression on the endothelial and circulating cells and cellular homing to the sites of inflammation. Also, CD93 could be involved in endothelial cell migration, angiogenesis, leukocytes extravasation, removal of immune complexes and pathogenic factors via the process of phagocytosis **(6).** Soluble CD93 was normally detected in human plasma and induced by the inflammatory mediators TNF-α and LPS, which suggest physiologic pathways that trigger the cleavage event **(7).**

The aim of this work was to evaluate diagnostic value of serum soluble CD93 level in acute asthma exacerbation in children.

PATIENTS AND METHODS

This study was carried out over 18 months from February 2017 to August 2018 in the Outpatient Clinic of Pediatric Department, Faculty of Medicine, Zagazig University Hospitals on 30 asthmatic patients (according to GINA guidelines 2018 **(8)**). The patients were classified into group I (patients group before treatment), which included 30 asthmatic patients with acute exacerbation (17 females and 13 males). Their ages ranged from 5-14 years with a mean of 9.7 ± 3.24

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years. Their weight ranged from 17.5-47 with a mean of 31.45 ± 9.04 kg. Group Π (patients group after treatment), which included the same 30 patients after receiving treatment and relieving of symptoms by clinical examination as well as routine laboratory investigations that confirmed their healthy state.

Inclusion criteria:

A history of physician-diagnosed asthma, patients may or may not take inhaled corticosteroids (ICSs), and age of patients is > 4 years.

Exclusion criteria:

Children with history of chronic inflammatory or autoimmune disorders, and children with lung diseases other than asthma.

Ethical approval:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee.

Every patient signed an informed written consent for acceptance of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Patients were subjected to the following:

Full history taking: including name, age, sex, weight, height, residence, disease duration and current medication. With special stress on history of physician-diagnosed asthma (age of onset), history of taking inhaled corticosteroids (duration and dose), history of having any autoimmune disease, and history of having any other lung disease.

Examination for all subjects was done including:

a) General examination: Including pulse, blood pressure, temperature, respiratory rate, chest, heart and abdominal examination.

b) Laboratory investigation: Measurement of Plasma sCD93 concentration using ELISA [at the time of examination and repeated on follow up day (7^{th} day)].

c) Radiological investigation: Chest X-ray.

d) Specific investigation: Spirometry measurement.

Measurement of Plasma sCD93 concentration using ELISA:

Principle of the test:

Kits were supplied by SunRed Biotechnology Company, Shanghai. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Complement component C1q receptor (C1Q R) in samples.

Statistical methods

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) was used to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value ≤ 0.05 was considered significant.

RESULTS

Table (1) showed that the age of the studied group ranged from 5 to 14 years with a mean of 9.07 years, weight ranged from 17.5 to 47 kg with a mean of 31.45 Kg and height ranged from 100 to 143 cm with a mean of 130.67 cm. 56.7% were females and 86.7% were from rural area.

Table (2) showed that 23.3% of the studied group had intermittent asthma, 46.7% had mild persistent and 30% had moderate persistent asthma.

Table (3) showed that there were no statistical significance differences between different severity cases in CD 93 either pre- or post-treatment. Regarding difference between pre- and post-treatment, there was statistical significance reduction in sCD 93 level post-treatment compared to pre-treatment in all cases because of its reduction in intermittent cases.

Table (4) showed that there were no statistical significance relation between WBCs, X-ray or controller medication and CD 93 level among the studied group.

Table (5) showed that there were statistical significance positive correlation between Hb level and CD 93 before treatment and significant –ve correlation between Cd 93 and CRP before treatment.

Table (1): Demographic data of the studied group

Variable	(n=30)	
Age : (year) Mean ± SD Median (Range)	9.07 ± 3.24 9 (5 – 14)	
Weight : (Kg) Mean ± SD Median (Range)	31.45 ± 9.04 29 (17.5 – 47)	
Height : (cm) Mean ± SD Median (Range)	130.67 ± 8.87 133 (100 – 143)	
Variable	**No**	**%**
Sex: Female Male	 17 13	 56.7 43.3
Residence: Urban Rural	 4 26	 13.3 86.7

Sd: Standard deviation

Table (2): GINA classification of disease severity among the studied group

Variable	(n=30)	
	No	**%**
Severity: Intermittent Mild persistent Moderate persistent Severe	 7 14 9 0	 23.3 46.7 30 0

Table (3): CD93 level among the studied group before and after ttt

Time	Variable	Total (n=30)	Intermittent (n=7)	Mild persistent (n=14)	Moderate persistent (n=9)	K	P
Pre	**CD 93:(ng/ml)** Mean ± SD Median	 19.01 ± 4.37 16.24	 18.86 ± 1.69 13.50	 21.76 ± 5.41 17.59	 14.85 ±3.91 12.95	1.39	0.50 NS
Post	**CD 93:(ng/ml)** Mean ± SD Median	 13.07 ± 3.35 12.20	 13.59 ± 4.72 11.29	 13.51 ± 4.22 12.76	 13.76 ± 7302 12.84	0.02	0.98 NS
Paired W		**2.36**	2.20	1.79	0.30		
P		**0.02***	0.30*	0.07 NS	0.77 NS		

SD: Standard deviation F: ANOVA test K: Kruskal Wallis test Paired W: Paired Wilcoxon test NS: Non significant (P>0.05) *: Significant (< 0.05)

Table (4): Relation between CD93 and WBCs, x ray and controller medication among the studied group

Variable	Normal (n=1)	Eosinophilia (n=22)	Neutrophillia (n=7)	K	P
CD 93					
Mean ± SD	11.06 ± 0.01	19.04 ± 1.01	20.05 ± 4.47	1.35	0.51
Median	11.06	15.57	14.220.90		NS
Variable	**Normal X-ray (n=17)**	**Abnormal (n=13)**		**MW**	**P**
CD 93					
Mean ± SD	14.67 ± 3.20	24.70 ± 1.19		1.74	0.08
Median	16.1	17.98			NS
Variable	**No controller (n=7)**	**Controller (n=23)**		**MW**	**P**
CD 93					
Mean ± SD	18.86 ± 1.69	19.06 ± 1.33		10.03	0.98
Median	13.50	16.37			NS

SD: Standard deviation K:Kruskal Wallis test MW: Mann Whitney test NS: Non-significant (P >0.05)

Table (5): Correlation between age, wt, laboratory findings and PFT of the studied group and CD 93 level before and after treatment

Variable	CD 93 pre (n=30)		CD 93 post (n=30)	
	r	P		
Age (Years)	-0.09	0.62 NS	-0.21	0.28 NS
Weight (Kg)	0.02	0.91 NS	-0.20	0.29 NS
Hb (gm/dl)	**0.58**	**0.001****	-0.09	0.62 NS
CRP (mg/dl)	**-0.37**	**0.04***	-0.22	0.25 NS
RBCs (mcL)	0.01	0.97 NS	0.12	0.54 NS
FEV1 (%)	-0.25	0.19 NS	-0.35	0.06 NS
FVC (L)	0.19	0.31 NS	0.01	0.98 NS
FVE1/FVC	-0.14	0.45 NS	-0.18	0.33 NS
CD 93 post (ng/ml)	0.29	0.13 NS	---	---

r: Spearman correlation coefficient NS: Non-significant (P>0.05) *:Significant (P<0.05) **:Highly significant (P<0.01)

DISCUSSION

In our study regarding sex 56.7% were females and 43.3% were males. On the contrary, **Al Ghobain *et al.*** [9] found that, the prevalence of asthma and asthma-related symptoms is high among 16 to 18 year old adolescents in Saudi Arabia, and the symptoms are more common in boys than in girls. **Yousry *et al.*** [10] also found that bronchial asthma (BA) is more common in males.

In our study, weight ranged from 17.5 to 47 kg with a mean of 31.45 and BMI ranged from 13 to 25.2 with a mean of 18.1 kg/m^2. **Borgmeyer *et al.*** [11] studied 510 children aged 3 to 17 years admitted to a pediatric hospital in the Midwest with a primary diagnosis of asthma and concluded that weight and chronic asthma severity were related in older children. The results support the importance of weight recognition and management in the care of children with asthma.

We found that 86.7% were from rural area and 13.3% from urban areas. While **Hijazi *et al.*** [12] investigated the prevalence of asthma in 1.020 urban and 424 rural children and found that the prevalence of asthma was 13.9% and 8%, respectively.

In our study, there were no statistical significance differences between different severity cases in FVC but there were statistical significance decrease in FEV1 and FEV1/FVC with increase of the severity. Peak flow meters were not included as they are designed as monitoring, not as diagnostic, tools in the office [13]. According to **Sigari *et al.*** [14] the mean of PEF on the first day of admission was 261.7 ± 90.3 L/min and increased to 316.1 ± 87.7 L/min on the last day of hospital stay (P < 0.001). The mean length of hospital stay was 4.7 ± 1.97 days. The association between sCD93 and PEF was not significant, but the mean value of FEV1 showed a modest decrease following ICS treatment, which agrees with our study. Only very few previous studies assessed sCD93 levels in asthmatic patients. **Sigari *et al.*** [14] showed the role of sCD93 as a novel biomarker in asthma exacerbation, however, the effects of anti-inflammatory drugs were not considered. **Park *et al.*** [15] suggested that serum sCD93 levels in patients with exacerbated allergic diseases (Allergic rhinitis, chronic systemic urticaria, and bronchial asthma) are higher than in those with stable diseases. Furthermore, they confirmed that inhaled corticosteroids (ICS) used in patients with bronchial asthma were inversely correlated with serum sCD93

levels in their cross sectional study. In a cohort study, also done by **Park *et al.* [15]** to confirm this hypothesis, they found that sCD93 levels were significantly decreased after ICS treatment, which suggested that serum sCD93 might worth further studies as a potential novel diagnostic and therapeutic marker for allergic disease, especially asthma. Also, they found that steroid-naïve patients who were diagnosed with BA for the first time at this visit showed higher serum sCD93 levels than healthy controls who were never diagnosed with BA, which increased suspicion that the medications, including not only steroids but also anti-histamines, that the patients took to control their symptoms affected serum sCD93 levels. **Baines *et al.* [16]** showed that CD93 mRNA levels were correlated with neutrophilia in sputum samples of asthma patients on a low antioxidant diet. However, CD93 was evaluated in sputum rather than in serum in this previous study, the number of patients was small (n=10), and asthma severity and other clinical conditions were not evaluated. In experimental studies of animals, **Liu *et al.* [17]** showed alterations in expression, localization, and involvement of CD93 in central nervous system inflammation.

Previous investigations demonstrated a potential clinical implication of CD93. **Malarstig *et al.* [18]** demonstrated a significant association between plasma sCD93 levels, premature MI, and the incidence of coronary artery disease.

The baseline serum sCD93 levels (ng/mL) in steroid-naïve BA patients (n=14) significantly decreased after 4 weeks of ICS use (n=12) and 8 weeks of ICS use (n=6) ($p < 0.05$). **Sigari *et al.* [14]** stated that the serum levels of sCD93 in asthmatics decreased in response to therapy. The difference in sCD93 between the first and last day of admission was significant.

CONCLUSION

sCD93 was not affected by gender or age and was not affected by reliever or controller medications. sCD93 showed a modest decrease in the controlled stage of asthma, which allowed to interpret its role as inflammatory biomarker. sCD93 may not be a specific biomarker for asthma and could be a surrogate predictor of treatment response.

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