ROLE OF SERUM CLUSTERIN IN BRONCHIAL ASTHMA

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Received: 17/4/2023
Accepted: 18/6/2023

Online ISSN: 2735-3540

ABSTRACT:

Background: *Bronchial asthma is a known chronic inflammatory disease. Asthma is categorized into atopic asthma and non-atopic asthma based on absence or presence of atopy which is a positive skin prick test 3 mm or Immunoglobulin E (IgE) specific antibodies that are produced against common allergens. Oxidative stress has a role in pathogenesis of bronchial asthma. Clusterin is a glycoprotein chemokine and has a role in recruitment of inflammatory cells.*

Aim of the Work: *Our aim was to measure serum level of Clusterin in bronchial asthma patients including both (atopic and non-atopic) and to correlate it with the severity of the disease.*

Materials and Methods: *Case control study was done on (96) adult patients diagnosed with bronchial asthma (64 atopic and 32 nonatopic), in addition to (32) sex and age matched healthy controls. Serum Clusterin & IgE levels were measured using enzyme linked immunoassay.*

Results: *Clusterin level was higher in atopic and non-atopic patients' groups when each was compared to control group ($p<0.01$, respectively). Clusterin was elevated in severe atopic asthma compared to mild and moderate atopic patients ($p=0.002$ and 0.003, respectively). Clusterin was elevated in moderate non atopic asthma compared to mild non atopic patients ($p=0.021$). Multi-ROC of IgE and Clusterin at 23 IU/mL (IgE) and 20 ng/mL (Clusterin) showed diagnostic performance efficacy (98.4%) to differentiate between control and non-atopic group. Clusterin 10 ng/mL & total IgE 125 IU/mL discriminate severe cases of bronchial asthma versus mild and moderate cases of bronchial asthma.*

Conclusion: *Our study revealed that serum Clusterin can be a promising marker in diagnosis of bronchial asthma, and detection of degree of severity.*

Keywords: *Clusterin, Bronchial asthma, Atopy.*

INTRODUCTION:

Bronchial asthma is known to be one of the most popular chronic inflammatory diseases affecting respiratory system around the world[(1)]. Based on presence or absence of atopy, which is a positive skin prick test 3 mm or Immunoglobulin E (IgE) specific antibodies that are produced against common allergens, bronchial asthma is classified into atopic and non atopic asthma[(2)].

Atopic asthma (Allergic asthma or Extrinsic asthma) is the major type of asthma in subjects between the age of 5-30 years. In this type of asthma, T-helper lymphocyte type 2 (TH2) drives the making of IgE at time of exposure to popular environmental allergens like dust mite, cat, dog and molds[(3-5)]. Non atopic asthma (Non-

allergic asthma, Idiopathic asthma, Intrinsic asthma or Infective asthma) is mainly an inflammatory reaction as inhaled pollutants and microbes lead to interleukin 5 (IL)-5 and IL-13 production by innate lymphoid cells (ILC2s) [(6)]. Epithelial cytokines IL-25 and IL-33 are participating in the primary stages of the inflammatory process. These cytokines are produced due to airway epithelial damage and exaggerate a T2 immune response even when allergy is not found [(7)].

As mentioned by the Global Initiative for Asthma (GINA) classification, individuals with bronchial asthma are classified in accordance with asthma severity into: severe, moderate and mild. In mild bronchial asthma, patients have symptoms that occur less than two times per month or **t**wo times per month or more but resolve within one day or patients who have no risk factors for asthma exacerbation include major environmental exposure, socioeconomic problem, or severely decreased lung function. In moderate bronchial asthma patients have symptoms present most days or are waking up due to asthma at least once a week or patients with uncontrolled symptoms on daily low-dose inhaled corticosteroids (ICS) or patients with persistent symptoms despite adherence to therapy. The worst degree of bronchial asthma is the severe bronchial asthma in which asthma symptoms remain, cannot be controlled in spite of high-dose of inhaled corticosteroids (ICS), leukotriene receptor antagonist (LTRA) treatment and long-acting beta agonist (LABA) [(8)].

Asthma is characterized by variability in airway obstruction which is assessed by lung function tests using spirometry. The latter assesses forced vital capacity (FVC %), peak expiratory flow rate (PEF %), and the forced expiratory volume in the first second (FEV1 %). The decrease of the forced expiratory volume in 1 second (FEV1 %) to forced vital capacity (FVC %) ratio (FEV1/FVC ratio and expressed as percentage) can be used to ensure that there is airway obstruction[(9)]. If there is clinical suspicion for the presence of bronchial asthma in the absence of a reduced FEV1/FVC ratio, the spirometry should be repeated on another time[(10)].

An imbalance between oxidants and antioxidants has been implicated in the pathogenesis of asthma. One of the antioxidants studied in the past few years is Clusterin. Clusterin is 80 kDa secreted glycoprotein also known as apolipoprotein J, consists of two chains–α-clusterin (α-Clu) and β-clusterin (β-Clu) that are linked by 5 disulfide bonds which are a covalent bond between two sulfur atoms which are unique to proteins synthesized in the endoplasmic reticulum (ER) and stabilize the central core region[(11)].

Clusterin is present in almost all cells and body fluids including plasma, urine, semen, cerebrospinal fluid, and breastmilk[(12)]. It plays an important role in regulation of apoptosis, immune regulation, cell adhesion, membrane recycling, epithelial cell differentiation, tumorigenesis and transformation[(13)], tissue remodeling, lipid transport, complement regulation, and a very sensitive cellular biosensor of oxidative stress protecting cells from oxidative injury by recruitment of inflammatory cells by binding to chemokine receptor type 6 (CCR6) expressed on dendritic cells (DCs), neutrophils, and memory T lymphocytes[(14)]. The concentration of clusterin is elevated in many diseases, including atherosclerosis, diabetes, some malignancies, and Alzheimer disease, in which the pathogenesis of the disease involve oxidative injury[(15&16)]**.**

Clusterin is abundant in the submucosa of the airway of smokers and its function is to protect the fibroblasts of the airway from oxidative stress that is produced by the extract of cigarette smoke outside the body[(17)]. Oxidative stress is the cornerstone in the asthma pathogenesis, either atopic or non-atopic type. It leads to several

pathophysiologic changes such as the increased release of chemoattractants, increased vascular permeability and airway remodeling. Thus, serum level of clusterin is important to be assessed in bronchial asthma[18].

AIM OF THE WORK:

The aim of our study was to measure serum level of Clusterin in bronchial asthma patients including both types (atopic and non-atopic) and to correlate it with the disease severity.

PATIENTS AND METHODS:

We conducted this case-control study from January 2021 till June 2021 on 96 bronchial asthma patients. They were recruited from Allergy Clinic at Ain Shams University Hospital. In addition, 32 sex and age matched seemingly healthy subjects were included as healthy controls. The study was done according to the Declaration of Helsinki (2013). The participants wrote consent to join our study. The study has been approved by Faculty of Medicine, Ain Shams University Ethical Committee.

Subjects were classified into three groups:

Non-atopic bronchial asthma group which included 32 patients [Median age 30.5 years (23.5-45)]. They were 20 females and 12 males, classified according to GINA classification which uses the frequency of symptoms to classify patients according to severity into mild (n=24) and moderate (n=8) subgroups. Their diagnosis was based on negative skin prick test, serum IgE level, chest X-ray and spirometry findings.

Atopic bronchial asthma group which included 64 patients [Median age 34 years (26.25-44.75)]. They were 32 females and 32 males, classified according to GINA classification into mild (n=20), moderate (n=34) and severe (n=10) subgroups. Their diagnosis was based on positive skin prick test, serum IgE level, chest x ray and spirometry findings.

Control group included 32 participants apparently healthy age and sex matched subjects [median age 35 years (26.5-47)] they were 14 females and 18 males.

Subjects with any other lung lesions detected by X-ray as lung collapse, fibrosis or malignancy were excluded from our study. Written informed consent was taken from all participants before any intervention.

All patients in this study were subjected to full history taking with emphasis on, age, tobacco smoking and family history of bronchial asthma. Thorough clinical examination and Spirometry were performed for patients only. A decrease of the forced expiratory volume in 1 second (FEV1 %) to forced vital capacity (FVC %) ratio (FEV1/FVC ratio %) was used to prove bronchial obstruction. The definition of bronchial obstruction is the reduction in the FEV1/FVC ratio %, an FEV1/FVC ratio of less than 70% is diagnostic for obstruction [12]. Other investigations include radiological studies (as X ray of chest) and skin allergy testing for antigen detection (positive skin prick test 3 mm or more were done for cases only considered as sign of atopy.

Six milliliters (6mL) of venous blood were withdrawn under complete aseptic conditions from all subjects. Blood was divided into three tubes: Two mL of blood were put in a sterile K3 EDTA vacutainer for CBC with differential count using hematology analyzers Sysmex (XN-1000) to evaluate eosinophil level. Four mL of blood were put in a two sterile plain vacutainers (2 mL in each vacutainer) for assessment of Clusterin level and IgE level. The separated serum was stored at negative 20℃ till analysis to measure Clusterin level and IgE level using enzyme linked immunoassay kit (from DRG International, lnc., USA for IgE

and Human CLU (Clusterin) ELISA kit from Elabscience® Biotechnology (USA) for Clusterin.

Analytical method of measurement of Clusterin and IgE:

This assay employs the quantitative sandwich immunoassay technique. These kits contain a micro-ELISA plate with an antibody specific to Human Clusterin (CLU) (or IgE). Samples, standards and controls were added to the micro-ELISA plate wells and combined with the antibody. A biotinylated detection antibody specific for Human CLU (or IgE) and an Avidin-Horseradish Peroxidase (HRP) conjugate were then added to each microplate well and incubated. The free components were washed away. Each well was filled with substrate solution. The reaction of the enzyme-substrate was stopped by putting stop solution. Optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was directly proportional to the concentration of Human CLU (or IgE). A standard curve was constructed where concentration of Human CLU (or IgE) in the samples and controls was deduced.

Statistical methods:

IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2019) was used for analysis of data. Date was presented as median and percentiles for quantitative non-parametric data and percentage for categorized data. As some of the data showed skewedness, non-parametric methods for statistical analysis were used.

1. Descriptive data was expressed as median and inter quartile range (IQR) and percentage.
2. Kruskal–Wallis test (H test) was applied for the statistical comparison between three or more sets of data if at least one of them has a skewed distribution.
3. Wilcoxon's Rank Sum test was used to compare subgroups showing statistically significant difference by Kruskal–Wallis test.
4. The Chi-Square test had been used to study associations between two qualitative independent variables.
5. Spearman's rank correlation coefficient (rs) was performed in correlation statistics to measure the degree of association between 2 sets of variables if either or both express a skewed distribution.

P values >0.05 were statistically non-significant, P values <0.05 were considered statistically significant and P values <0.01 were considered highly significant.

For evaluating diagnostic performance characteristic of Clusterin, diagnostic sensitivity, diagnostic specificity, diagnostic efficacy, negative predictive value (NPV) and positive predictive value (PPV) were calculated. Receiver operating characteristic (ROC) and multi ROC curves were used to get the most specific and sensitive cutoff for each and combined test parameters. AUC was also calculated to evaluate the most discriminating markers between the compared groups.

Ethical Considerations

This research followed the tents of the Declaration of Helsinki. The Institutional Ethical Committee at Ain Shams University approved all study protocols. Accordingly, written informed consent was taken from all participants before any intervention. Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

RESULTS

Results of the present study are illustrated in tables (1-13) and in figures (1-6). Table (1) represents descriptive and

comparative analysis of all studied parameters between all groups using the Kruskal Wallis test. Atopic group had the highest levels of WBCs, eosinophils, Clusterin and total IgE (Table 1). Comparing non atopic and control groups using Wilcoxon Rank sum test (Table 2), non-atopic patients showed significant higher eosinophils, total IgE and Clusterin when compared to control (p = 0.045, 0 and 0.0015, respectively). Comparison between the atopic group and control group showed that the atopic group had significantly higher WBCs, esoinophils, total IgE, and Clusterin when compared to control group (p = 0.001, 0, 0 and 0.0012, respectively) (Table 3). The atopic group also showed higher WBCs, eosinophils, and total IgE compared to the non-atopic group (p = 0.026, 0.007 and 0, respectively) (Table 4).

Table 1: Descriptive and comparative analysis of all studied parameters between all groups using Kruskall-Wallis test and Chi-square test

Group parameter	non atopic asthma (N=32) median (IQR)	atopic asthma (N=64) median (IQR)	Control (N=32) median (IQR)	H	P	sig
WBCs ($X10^9$ /L)	6.45 (5.45-8.9)	7.85 (6.75-10.325)	6.1 (5-7.675)	12.086	0.002	S
Eosinophils ($X10^9$ /L)	0.145 (0.0925-0.26)	0.32 (0.1525-0.6575)	0.095 (0.07475-0.1175)	24.804	<0.001	HS
Total IgE (IU/mL)	51.95 (30.75-73.875)	201 (127-409.425)	12.5 (5.75-20.75)	49.719	<0.001	HS
Clusterin (ng/mL)	41.5 (22.5-51.5)	44 (24-65)	21.5 (14.5-42)	12.54	<0.002	HS
FEV1 (%)	81.25 (65.75-84.5)	80 (67.25-87.375)	N/A	N/A	N/A	N/A
FVC (%)	85.35 (80.375-87.9)	85.5 (78.5-95.275)	N/A	N/A	N/A	N/A
FEV1/FVC ratio (%)	81.9 (74.49-88)	77.55 (70.975-86.41)	N/A	N/A	N/A	N/A
Family history	No = 18 (56.3%)	No =34 (53.1%)		0.042*	0.838*	NS
	Yes = 14 (43.8%)	Yes = 30 (46.9%)				

P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference. H: Kruskall-Wallis test for skewed data and * for Chi-square test value

Table 2: Descriptive and comparative analysis of all studied parameters between non atopic and control groups using Wilcoxn Rank Sum test:

Parameters	Non atopic group (N=32) median (IQR)	Control group (N=32) median (IQR)	Z	P	Sig
WBCs ($X10^9$ /L)	6.45 (5.45-8.9)	6.1 (5-7.675)	1.093	0.274	NS
Eosinophils ($X10^9$/L)	0.145 (0.0925-0.26)	0.095 (0.07475-0.1175)	2.002	0.045	S
Total IgE (IU/mL)	51.95 (30.75-73.875)	12.5 (5.75-20.75)	4.416	<0.001	HS
Clusterin (ng/mL)	41.5 (22.5-51.5)	21.5 (14.5-42)	3.165	<0.002	HS

Median & interquartile range (IQR) for skewed data P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference Z: Wilcoxcon rank sum test for skewed data

Table 3: Descriptive and comparative analysis of all studied parameters between atopic and control groups using Wilcoxn Rank Sum test:

Parameters	Atopic group(N=64) median (IQR)	Control group(N=32) median (IQR)	Z	P	Sig
WBCs ($X10^9$ /L)	7.85 (6.75-10.325)	6.1 (5-7.675)	3.025	0.001	HS
Eosinophils ($X10^9$/L)	0.32 (0.1525-0.6575)	0.095 (0.07475-0.1175)	4.792	<0.001	HS
Total IgE (IU/mL)	201 (127-409.425)	12.5 (5.75-20.75)	5.58	<0.001	HS
Clusterin (ng/mL)	44 (24-65)	21.5 (14.5-42)	3.287	0.0012	HS

Median & interquartile range (IQR) for skewed data P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference Z: Wilcoxcon rank sum test for skewed data

Table 4: Descriptive and comparative analysis of all studied parameters between non-atopic and atopic groups using Wilcoxn Rank Sum test:

Parameters	Non atopic group (N=32) median (IQR)	Atopic group (N=64) median (IQR)	Z	P	Sig
WBCs ($X10^9$ /L)	6.45 (5.45-8.9)	7.85 (6.75-10.325)	-2.231	0.026	S
Eosinophils ($X10^9$ /L)	0.145 (0.0925-0.26)	0.32 (0.1525-0.6575)	-2.692	0.007	HS
Total IgE (IU/mL)	51.95 (30.75-73.875)	201 (127-409.425)	-5.239	<0.001	HS
Clusterin (ng/mL)	41.5 (22.5-51.5)	44 (24-65)	-0.449	0.654	NS
FEV1 (%)	81.25 (65.75-84.5)	80 (67.25-87.375)	0.131	0.896	NS
FVC (%)	85.35 (80.375-87.9)	85.5 (78.5-95.275)	-0.766	0.444	NS
FEV1/FVC ratio (%)	81.9 (74.49-88)	77.55 (70.975-86.41)	0.613	0.54	NS

Median & interquartile range (IQR) for skewed data P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference Z: Wilcoxcon rank sum test for skewed data

Descriptive and comparative analysis of studied parameters in all subgroups using Wilcoxn Rank Sum test showed that Clusterin was significantly higher in patients associated with moderate non-atopic asthma compared to patients associated with mild non-atopic asthma (p=0.021) (Table 5). Similarly, Clusterin and total IgE were significantly elevated in patients with severe atopic asthma when compared to mild and moderate atopic asthma patients (p=0.002 and 0.003, respectively) (Table 6,7) (Figure 1).

Table 5: Descriptive and comparative analysis of studied parameters using Wilcoxon Rank Sum test between non-atopic subgroups (mild and moderate):

Parameters	Non atopic mild (N=24) Median (IQR)	Non atopic moderate (N=8) Median (IQR)	Z	P	Sig
WBCs ($X10^9$ /L)	6.22 (5.45-9.275)	6.75 (5.6-8.5)	-0.121	0.903	NS
Eosinophils ($X10^9$ /L)	0.182 (0.1075-0.3365)	0.095 (0.09-0.1075)	1.762	0.078	NS
Total IgE (IU/mL)	44.65 (30-65.25)	74.5 (51.425-104.025)	-1.699	0.089	NS
FEV 1 (%)	81.25 (55.075-82.75)	82.95 (73.475-88)	-0.789	0.43	NS
FVC (%)	85.35 (80.375-87.9)	85 (77.75-91.5)	0.061	0.951	NS
FEV1/FVC ratio (%)	79.345 (55.745-86.54)	88.5 (81.25-94.25)	-1.881	0.06	NS
Clusterin (ng/mL)	36.5 (16.25-48)	65 (43.75-79.5)	-2.306	0.021	S

Median & interquartile range (IQR) for skewed data P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference Z: Wilcoxcon rank sum test for skewed data

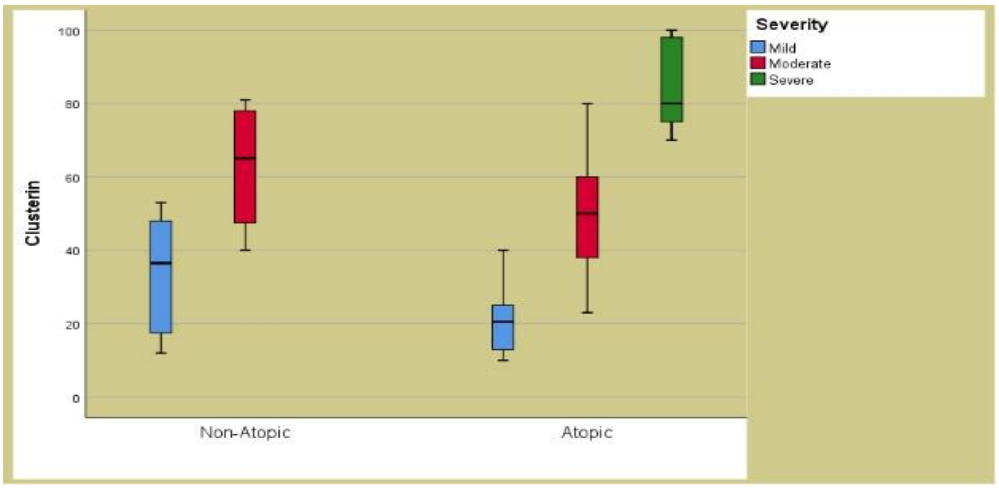


Fig (1) Clusterin levels in non-atopic and atopic groups.

Table 6: Descriptive and comparative analysis using Kruskall-Wallis Test between atopic subgroups (mild, moderate and severe):

Parameters	Atopic mild (N=20) Median (IQR)	Atopic moderate (N=34) Median (IQR)	Atopic severe (N=10) Median (IQR)	H	P	Sig
WBCs ($X10^9$ /L)	7.4 (6.275-8.525)	8.25 (7.2-10.7)	8 (6.46-13.45)	2.3	0.317	NS
Eosinophils ($X10^9$ /L)	0.28 (0.13-0.825)	0.39 (0.146-0.63)	0.34 (0.1625-0.77)	0.141	0.932	NS
Total IgE (IU/mL)	120.5 (110.25-164.25)	208 (146.5-349.1)	956 (781-1798)	17.859	<0.001	HS
FEV 1 (%)	80.55 (70.55-87.875)	83 (67.5-88.8)	66.9 (59.15-89.3)	1.926	0.382	NS
FVC (%)	84.5 (77.25-94.65)	85 (80-100.5)	87.9 (67-94.35)	0.274	0.872	NS
FEV1/FVC ratio (%)	73.84 (69.25-86.9675)	80 (72.545-88.205)	74 (67.95-81.65)	1.202	0.548	NS
Clusterin (ng/mL)	20.5 (12.75-27.5)	50 (38-60)	80 (72.5-99)	21.501	<0.001	HS

Median & interquartile range (IQR) for skewed data P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference H: Kruskall-Wallis test for skewed data

Table 7: Comparative analysis of different studied parameters between atopic subgroups using Wilcoxn Rank Sum Test:

Parameters	Mild VS Moderate			Mild VS Severe			Moderate VS Severe		
	Z	P	sig	Z	P	sig	Z	P	sig
WBCs ($X10^9$ /L)	-1.533	0.125	NS	-0.797	0.426	NS	0.118	0.906	NS
Eosinophils ($X10^9$ /L)	-0.176	0.86	NS	-0.245	0.806	NS	0.353	0.724	NS
Total IgE (IU/mL)	-2.863	0.004	HS	-3.065	0.002	HS	-3.252	0.001	HS
FEV 1 (%)	-0.276	0.782	NS	1.347	0.178	NS	1.215	0.224	NS
FVC (%)	-0.452	0.651	NS	-0.245	0.806	NS	-0.353	0.724	NS
FEV1/FVC ratio (%)	-0.603	0.547	NS	-0.245	0.806	NS	1.136	0.256	NS
Clusterin (ng/mL)	-3.798	0	HS	-3.062	0.002	HS	-2.99	0.003	HS

P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference Z: Wilcoxcon rank sum test for skewed data

Correlation study between Clusterin and other measured parameters in all groups was done and there was a significant positive correlation with FVC.Perc in non atopic group (r = 0.726, p = 0.001) in addition to total IgE in atopic group (r= 0.6, p=0). Moreover, it was found a negative correlation with total IgE in control group (r=-0.525, p= 0.037) (table 8).

Table 8: Ranked Spearman Correlation Test between Clusterin and other measured parameters in all groups:

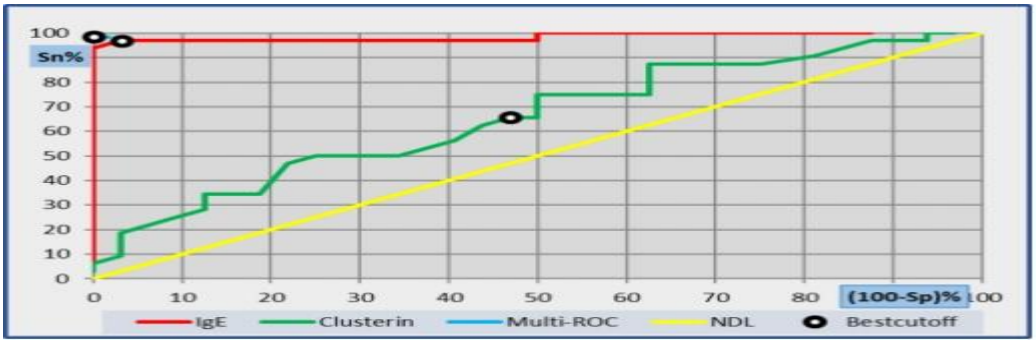
Group	Non atopic group (n=32)			Atopic group (n=64)			Control group (n=32)		
parameter	r	p	sig	r	p	sig	r	p	sig
Age (years)	0.272	0.307	NS	0.317	0.077	NS	-0.252	0.347	NS
WBCs ($X10^9$ /L)	-0.004	0.987	NS	0.147	0.426	NS	0.196	0.467	NS
Eosinophils ($X10^9$ /L)	-0.485	0.057	NS	-0.031	0.867	NS	0.162	0.549	NS
Platelets ($X\ 10^9$/L)	-0.003	0.991	NS	-0.045	0.808	NS	0.106	0.696	NS
Total IgE (IU/mL)	-0.009	0.974	NS	0.6	0	HS	-0.525	0.037	S
FEV 1 (%)	0.228	0.395	NS	-0.13	0.477	NS	N/A	N/A	N/A
FVC (%)	0.025	0.926	NS	-0.054	0.77	NS	N/A	N/A	N/A
FEV1/FVC ratio (%)	0.726	0.001	HS	0.038	0.836	NS	N/A	N/A	N/A

P > 0.05: no significant difference; P < 0.05: significant difference P = or < 0.01: highly significant difference R: Ranked Sperman Correlation test for non-parametric data

While comparing the diagnostic performance of IgE and Clusterin in atopic patients versus non-atopic and control patients, IgE showed high diagnostic performance efficacy (96.9%) at a cut off of 96 IU/mL, while Clusterin showed diagnostic performance efficacy (59.4%) at a cut off 35 ng/mL. But Multi-ROC of IgE and Clusterin at 96 IU/mL (IgE) and 40 ng/mL (Clusterin) showed increase of diagnostic performance efficacy (99.2%) across all parameters compared to IgE alone (Table 9) (Figure 2).

Table 9: Diagnostic performance of IgE and Clusterin in atopic Vs non-atopic and control to discriminate atopic cases (ROC curve):

Parameters	Cut off value	Sensitivity (%)	Specificity (%)	-ve predictive value (%)	+ve predictive value	Efficacy (%)
Total IgE (IU/mL)	96	96.9	96.9	96.9	96.9	96.9
Clusterin (ng/mL)	35	65.6	53.1	60.7	58.3	59.4
Muli-ROC of IgE and clusterin	Clusterin 40 ng/mL & total IgE 96 IU/mL	98.4	100	98.4	100	99.2



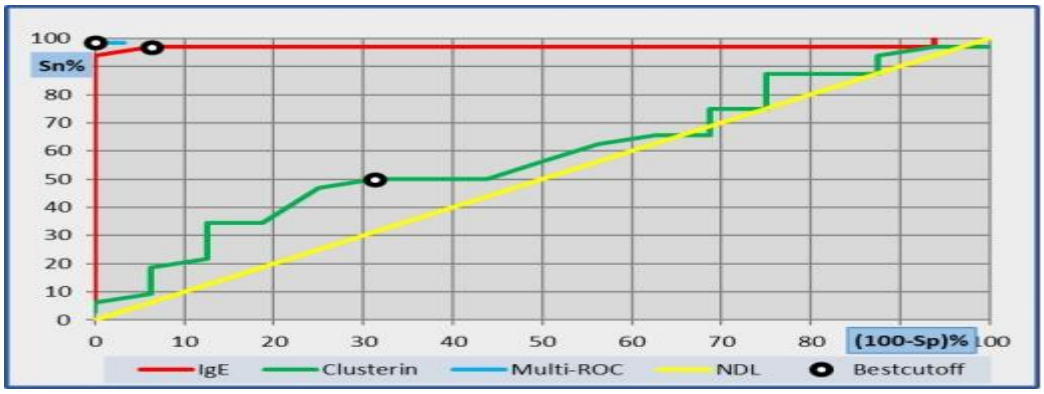
	AUC	SE	95CI	
IgE	0.859	0.048	0.766	0.952
Clusterin	0.622	0.070	0.485	0.759
Multi-ROC	0.905	0.039	0.828	0.982

Fig. (2): ROC curve analysis showing diagnostic performance of IgE and Clusterin and their combination for discriminating atopic cases from those non-atopic and control (AUC: area under curve, SE:sensitivity, CI:confidence interval)

When comparing the diagnostic performance of IgE and Clusterin in atopic patients versus non-atopic patients, IgE showed very high diagnostic performance efficacy (98.5%) at a cut off of 96 IU/mL, while Clusterin showed diagnostic performance efficacy (56.3%) at a cut off 48 ng/mL. Howevr, multi-ROC of IgE and Clusterin at 96 IU/mL (IgE) and 40 ng/mL (Clusterin) showed higher diagnostic performance efficacy (98.9%) across all parameters compared to IgE alone (Table 10) (Figure 3).

Table 10: Diagnostic performance of IgE and Clusterin in non-atopic cases Vs atopic cases (ROC curve):

Parameters	Cut off value	Sensitivity (%)	Specificity (%)	-ve predictive value (%)	+ve predictive value	Efficacy (%)
Total IgE (IU/mL)	96	96.9	93.8	93.8	96.9	98.5
Clusterin (ng/mL)	48	50	68.8	40.7	76.2	56.3
Muli-ROC of IgE and Clusterin	Clusterin 40 ng/mL & total IgE 96 IU/mL	98.4	100	96.9	100	98.9



	AUC	SE	95CI	
IgE	0.907	0.039	0.831	0.983
Clusterin	0.585	0.071	0.445	0.725
Multi-ROC	0.924	0.035	0.855	0.993

Fig. (3): ROC curve analysis showing the diagnostic performance of IgE and Clusterin and their combination for discriminating atopic cases from those non atopic (AUC: area under curve, SE:sensitivity, CI:confidence interval)

Also, by comparing the diagnostic performance of IgE and Clusterin in non-atopic patients compared to control patients, IgE showed high diagnostic performance efficacy (93.8%) at a cut off of 23 IU/mL, while Clusterin showed diagnostic performance efficacy (71.9%) at a cut off 25 ng/mL. But multi-ROC of IgE and Clusterin at 23 IU/mL (IgE) and 20 ng/mL (Clusterin) showed higher diagnostic performance efficacy (98.4%) across all parameters compared to IgE alone (Table 11) (Figure 4).

Table 11: Diagnostic performance of IgE and Clusterin in non-atopic Vs control to discriminate non atopic cases (ROC curve):

Parameters	Cut off value	Sensitivity (%)	Specificity (%)	-ve predictive value (%)	+ve predictive value	Efficacy (%)
Total IgE (IU/mL)	23	93.8	93.8	93.8	93.8	93.8
Clusterin (ng/mL)	25	75	68.8	73.3	70.6	71.9
Muli-ROC of IgE and clusterin	Clusterin 20 ng/mL & 23 IU/mL total IgE	96.8	100	100	96.9	98.4

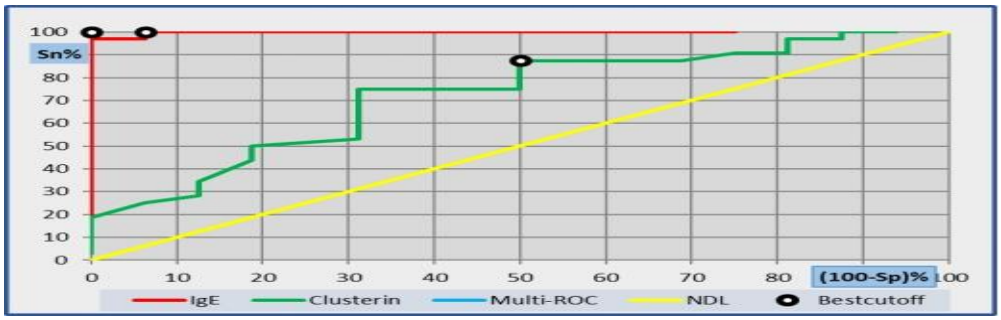
	AUC	SE	95CI	
IgE	0.709	0.065	0.582	0.836
Clusterin	0.621	0.070	0.484	0.759
Multi-ROC	0.884	0.043	0.799	0.969

Fig. (4): ROC curve analysis showing the diagnostic performance of IgE and Clusterin and their combination for discriminating non-atopic cases from control group (AUC: area under curve, SE:sensitivity, CI: confidence interval)

While comparing the diagnostic performance of IgE and clusterin in atopic patients versus control patients IgE showed very high diagnostic performance efficacy (97.9%) at a cut off of 23 IU/mL, while Clusterin showed diagnostic performance efficacy (75%) at a cut off 19 ng/mL. But multi-ROC of IgE and Clusterin at 23 IU/mL (IgE) and 12 ng/mL (Clusterin) showed higher diagnostic performance efficacy (100%) across all parameters compared to IgE alone (Table 12) (Figure 5).

Table 12: Diagnostic performance of IgE and Clusterin in atopic cases Vs control (ROC curve):

Parameters	Cut off value	Sensitivity (%)	Specificity (%)	-ve predictive value (%)	+ve predictive value	Efficacy (%)
Total IgE (IU/mL)	23	100	93.8	100	97	97.9
Clusterin (ng/mL)	19	87.5	50	66.7	77.8	75
Muli-ROC of IgE and clusterin	Clusterin 12 IU/mL & total IgE 23 IU/mL	100	100	100	100	100



	AUC	SE	95CI	
IgE	0.748	0.061	0.628	0.868
Clusterin	0.659	0.068	0.526	0.793
Multi-ROC	1.000	0.000	1.000	1.000

Fig. (5): The ROC curve analysis showing the diagnostic performance of IgE and Clusterin and their combination for discriminating atopic cases from those control group (AUC: area under curve, SE:sensitivity, CI:confidence interval)

While comparing the diagnostic performance of IgE and clusterin in mild patients versus moderate and severe patients IgE showed diagnostic performance efficacy (81.3%) at a cut off of 125 IU/mL, while Clusterin also showed diagnostic performance efficacy (81.3%) at a cut off 38 ng/mL. But multi-ROC of IgE and Clusterin at 125 IU/mL (IgE) and 10 ng/mL (Clusterin) showed higher diagnostic performance efficacy (94.3%) across all parameters compared to IgE alone (Table 13) (Figure 6).

Table 13: Diagnostic performance of IgE and Clusterin in mild cases Vs moderate and severe cases of bronchial asthma (ROC curve):

Parameters	Cut off value	Sensitivity (%)	Specificity (%)	-ve predictive value (%)	+ve predictive value	Efficacy (%)
Total IgE (IU/mL)	125	80.8	81.8	78.3	84	81.3
Clusterin (ng/mL)	38	88.5	72.7	84.2	79.3	81.3
Muli-ROC of IgE and clusterin	Clusterin 10 ng/mL & total IgE 125 IU/mL	97.9	90	97.3	92	94.3

	AUC	SE	95CI	
Clusterin	0.863	0.047	0.771	0.955
IgE	0.830	0.052	0.728	0.931
Multi-ROC	0.935	0.033	0.871	0.999

Fig. (6): ROC curve analysis showing the diagnostic performance of IgE and Clusterin and their combination for discriminating mild patients from moderate and severe bronchial asthma (AUC: area under curve, SE:sensitivity, CI: confidence interval)

DISCUSSION:

In this study, eosinophils were significantly higher in the atopic and non atopic groups when each group was compared to the control group. Moreover, atopic patients had significantly higher eosinophilic count when compared to non atopic ones. Eosinophils may play a role in airway hyper-responsiveness in asthma by the effects of granular proteins derived from eosinophil on the bronchial tree. It has been assumed that a defect in eosinophil programmed cell death would have role in occurrence and persistence of allergic airway inflammation that occur in asthma. Diagnosis of eosinophilic airway inflammation can be supported by the presence of blood eosinophilia [19&20].

When IgE level was assessed in the subjects included in our study, significantly high levels were found in both bronchial asthma groups as compared to control group. Furthermore, level of IgE was significantly high in atopic patients than non atopic ones. In bronchial asthma, eosinophils and mast cell activation via IgE-bound allergens causes dual action as this leads to their degranulation and release of several toxic

proteins including reactive oxygen species leading to airway destruction and, on the other hand, conduct its repair**.** The chronic activation of these processes surely leads to the signs of airway remodeling such as subepithelial fibrosis, epithelial desquamation and increased smooth muscle mass. These changes ultimately produce clinical symptoms and pathological changes characteristic for asthma [21-22].

Furthermore, Clusterin level in non-atopic patients were significantly higher than control group subjects. The same results were also obtained when comparing atopic and control groups. Our results came in accordance with the results of *Sobeih et al*., *Sol et al.* and *Dombai et al*. who declared that the level of serum Clusterin is higher in the asthmatics than in controls and confirms a previous study that showed high Clusterin levels in the serum and the sputum from asthmatic adults as it directly related to severity of asthma as a marker of oxidative stress [18,23,24]. In our study, on comparing Clusterin levels in the non-atopic and the atopic patient groups, serum level of Clusterin was higher in atopic group than non atopic group but it did not reach statistically significant value. This may be attributed to the fact that Clusterin production is induced by stress and is mostly sensitive to oxidative stress which is found in both atopic and non atopic asthma. The absence of statistical significance may also be due to small sample size. In the study by *Sobeih et al.,* the researchers found statistically significant difference in serum levels of Clusterin which was higher in atopic group than non atopic group[18].

Our study also aimed to investigate the clinical utility of the serum Clusterin in different degrees of severity of bronchial asthma. Our study revealed that in non-atopic patients had higher levels of Clusterin in moderate degree of asthma than the mild forms. Moreover, in atopic group Clusterin was found to be higher among the more severe cases. Our results support the previous work that done by *Kwon et al. and Sobeih et al*. who declared that Clusterin could be a biomarker of the degree of oxidative stress and asthma severity in asthmatic individuals [18,25].

In this study correlation study between Clusterin and other measured parameters in all groups was done and there was a negative correlation with total IgE in control group, but a positive correlation with total IgE in atopic group. A positive correlation was found between Clusterin and FEV1/FVC ratio % in non atopic group. This may be attributed to the inhibiting action of Clusterin in the inflammatory response, differentiation, collagen matrix deposition, and apoptosis of normal human lung fibroblasts (NHLFs) that causes improvement in FEV1/FVC ratio % with Clusterin increase in non atopic group[26]. Although *Sobeih et al*. reported that no significant correlations with pulmonary function parameters (FEV1%, FVC% and FEV1/FVC ratio %) in asthmatic patients[18], *Lee et al.* reported that serum Clusterin levels were increased in severe asthma patients who show lower pulmonary functions tests as it increased with asthma severity [27].

In our study, multi receiver operating characteristics (Multi-ROC) curve was used to show the diagnostic performance of Ig E and Clusterin to discriminate between different groups. To discriminate between atopic patients

from non atopic together with control group, IgE and Clusterin at 96 IU/mL (IgE) and 40 ng/mL (Clusterin) showed diagnostic performance efficacy (99.2%). When comparing the diagnostic performance of IgE and Clusterin in atopic patients versus non-atopic patients, IgE and Clusterin at 96 IU/mL (IgE) and 40 ng/mL (Clusterin) showed diagnostic performance efficacy (98.9%). To compare the diagnostic performance of IgE and Clusterin in non-atopic patients with control patients, IgE and Clusterin at 23 IU/mL (IgE) and 20 ng/mL (Clusterin) showed diagnostic performance efficacy (98.4%). To discriminate between atopic patients from control group, IgE and Clusterin at 23 IU/mL (IgE) and 12 ng/mL (Clusterin) showed diagnostic performance efficacy (100%).

Multi receiver operating characteristics (Multi-ROC) was also used to show the diagnostic performance of Clusterin to discriminate mild group of patients from moderate and severe patients. At cut off 10 ng/mL, Clusterin together with IgE (125IU/mL) showed sensitivity, specificity and efficacy of (97.9 %, 90% and 94.3%) respectively. As regards total IgE *Al Obaidi et al.* study was conducted on 562 asthmatic patients and he reported that the cut off to differentiate asthma from healthy controls was IgE level of 200 IU/mL with sensitivity 93% and specificity 91%[28].

Finally, Clusterin is considered to be a sensitive cellular biosensor of oxidative stress which protects the cells from harmful free radicals' effects together with their byproducts, and it inhibited the toxic oxidants effects in numerous studies. Based upon this, the levels of Clusterin increase with asthma severity is more likely related to the oxidative stress in asthmatic individuals [29].

Conclusion

In a conclusion an increased level in clusterin is a promising marker to correlate with asthma severity and disease progression.

Authors' Contribution:

HAZ and MF supervised, reviewed, and validated the final manuscript and was responsible for conceptualization. MSA contributed to the writing and editing of the manuscript and contributed to setting the research methodology. Finally, SAA contributed to the manuscript review, sample collection, original draft preparation and writing, and research methodology. All authors read, revised, and approved the final manuscript.

Conflict Of Interest:

The authors declare that they have no conflicting interests.

Funding/Suppo:rt

No sources of funding were obtained to support the study.

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دور مصل الكلاسترين فى الربو القصبى

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من المعروف أن الربو القصبي هو أحد الأمراض الالتهابية المزمنة الشائعة. يصنف الربو إلى الربو التأتبي والربو غير التأتبي على أساس غياب أو وجود التأتب الذي هو اختبار وخز الجلد الإيجابي ٣ مم أو الأجسام المضادة الخاصة بالغلوبولين المناعي E (IgE)التي يتم إنتاجها ضد مسببات الحساسية الشائعة. ثبت أن الإجهاد التأكسدي مع اختلال التوازن المؤكسد ومضادات الأكسدة لها دور مهم في التسبب في الربو القصبي. الكلاسترين هو بروتين سكري معروف وله دور في تجنيد الخلايا الالتهابية. من المعروف أن جهاز الاستشعار الحيوي الخلوي الحساس للإجهاد التأكسدي يحمي الخلايا من الإصابة التأكسدية للجذور الحرة ومنتجاتها الثانوية عن طريق تثبيط آثارها السامة. كان الهدف من دراستنا هو قياس مستوى الكلاسترين في مرضى الربو القصبي بما في ذلك (التأتبي وغير التأتبي) وربطه بشدة المرض.