

Hematological and Biochemical Profile of Patients Suffering from Non-Atopic Asthma

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Abstract

Background: The prevalence rate of asthma is increasing at an alarming rate worldwide and number of patients with asthma is projected to reach 100 million by 2025. Few tests are being used for diagnosis of asthma, but at present no established biomarker is available which may be used for diagnosis and prognosis of asthma. Main aim of this study was to investigate the potential of routine hematological and biochemical parameters as reliable diagnostic biomarker(s) of asthma.

Methods and findings: We performed hematological and biochemical analysis by following standard clinical methods using Sysmex and Spectra.

Results: Many hematological and biochemical parameters were either up-regulated (i.e., Hb, WBC, LYM#, MXD#, NEUT#, NEUT%, Albumin/Globulin ratio, cholesterol, creatinine, urea, blood urea nitrogen, Na⁺ and K⁺) or down-regulated (i.e., MPV, LYM%, total protein, globulin, bilirubin, AST and ALT) in both male and female asthma patients as compared to the gender specific normal controls. However, few parameters (RDW, HCT, MCH, MCV, RBCs, CK-NAC, CK-MB-NAC, albumin, triglycerides, and HDL-C) exhibited gender specific variations (p<0.05).

Conclusion: To our knowledge this study is the first to confirm the role of hematological and biochemical parameters in the pathogenesis of asthma. We suggest that combinatorial use of multiple parameters can be helpful for asthma diagnosis.

Keywords: Alanine transaminase; Bilirubin; Cardiac profile; Lipid profile; Kidney profile

Abbreviations: RBCs: Red Blood Cells; Hb: Hemoglobin; HCT: Hematocrit Blood Test; MCV: Mean Corpuscular Volume/Mean Cell Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: RBCs Distribution Width; PLT: Platelet; MPV: Mean Platelet Volume; WBCs: White Blood Cells; LYM: Lymphocytes; MXD: Mixed Cell Volume/Relative Content of Mixture (Monocytes; Basophils; and Eosinophils); NEUT: Neutrophils; ALT: Alanine Transaminase; AST: Aspartate Transaminase; CBC: Complete Blood Count; SGOT: Serum Glutamic Oxaloacetic Transaminase; SGPT: Serum Glutamic-Pyruvic Transaminase; HDLC: High-Density Lipoprotein Cholesterol; TG: Triglycerides; Na⁺¹: Sodium; K⁺¹: Potassium; Ca⁺²: Calcium

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Introduction

Asthma, an inflammation of the bronchial airways, is triggered or stimulated by a variety of factors provided there is genetic predisposition to develop asthma [1,2]. Prevalence of asthma is high (4.3%) in both children and adults and approximately 300

million asthma patients are present worldwide. Around 255,000 deaths occur per year due to asthma [3]. However, ratio of death due to asthma is higher (i.e., 80%) in low or middle-income countries. In Pakistan ~20 million adults (i.e., 12% of population) are asthma patients and incidence rate of asthma is increasing by 5% per year [1,4,5].

Any measurable cellular, biochemical or molecular alteration in the biological media that reflects either normal or pathogenic process is called biomarker [6,7]. Biomarkers facilitate early diagnosis, prognosis and identification and hence are potential therapeutic targets [8-11]. Serum IgE levels, CBC/blood eosinophils counts, Fractional Exhaled Nitric Oxide (FENO) levels, and sputum eosinophils counts are widely used to diagnose asthma [12-18]. None of these can be considered as established biomarker(s) of asthma [19]. It is essential to examine asthma associated cellular or molecular alterations which can be used for better diagnosis and management of asthma.

Urine, plasma and sera of the patients are analyzed in routine clinical practice for diagnosis. Analysis of biological fluids is considered as better tool to identify any change in the body physiology. Any change in body physiology is reflected by variation in the composition of body fluids [20]. There is little information available regarding hematological, biochemical and urine analysis of asthma patients. Considering this fact we hypothesized that as the physiology of asthma patients differs from normal healthy individuals hence the biological fluids of asthma patients will differ from normal healthy individuals. In an attempt to identify factors that may potentially contribute towards pathogenesis of asthma we initiated this study to explore diagnostic relevance of hematological, biochemical and urinary biomarkers for diagnosis of asthma.

Methods

Molecular biology grade chemicals/reagents (Merck/Fluka/BDH/Sigma) were used for analysis. The study was conducted after approval of appropriate institutional technical committees including departmental Ethics and Research Committee and by the Advanced Studies and Research Board (ASRB) of the University. Experimental work involving humans was in complete compliance with the Helsinki Declaration.

Collection of blood and urine samples

Patient group comprised of asthma patients (n=74) who visited outpatient department of a local hospital for their treatment during asthma attack and consented to participate in the study. Apparently healthy residents (n=99) of the region having either no health problem or not receiving any therapeutic treatment were used as the normal control group. At the time of sample collection information regarding demography, lifestyle and health/medical history was recorded in the predesigned questionnaire.

Blood and urine (not first urine of day) samples were collected from normal controls (n=99) and asthma patients (n=74) with due consent. Part of the collected blood (2 ml) was transferred into EDTA quoted vials for hematological analysis and remaining 3 ml blood collected in vials containing no anti-coagulant was processed to obtain serum. All sera were screened using immunochromatographic HBV/HCV test strips (CTI-Biotek). Following the exclusion criteria of study, asthma patients (n=23) found HCV/HBV positive were excluded from the patient group.

Hematological and biochemical analysis

Hematological analysis was done on automatic hematology analyzer sysmex (Sysmex KX-21N™, Germany) whereas biochemical analysis was carried out using commercial kits (Human, Germany) on Wiener Selectra E (Germany). Asthma linked variations in the studied parameters were identified through comparison of male (n=26) and female (n=25) asthmatic subjects with gender specific controls (female=48; male=51).

Urine analysis

Urine strips (Niles Biological Inc., USA) were used for the biochemical analysis of urine.

Statistical analysis

All tests were performed in triplicate and data was subjected to statistical analysis using SPSS 18.0, and confidence interval CI=0.95). D'Agostino-Pearson test helped to identify the normality distribution pattern of the data. Independent sample t test and Mann-Whitney U test were employed to calculate p value for normally distributed/Gaussian data and non-Gaussian data, respectively.

Results

Demographic information of asthma patients

Average age of asthma patients was 30.25 ± 16.36 years. Almost equal number of male (n=41) and female patients (n=33) was included in the study. Majority of the females were housewives and major proportion of the male patients was unskilled laborer. Most of the patients belonged to rural areas. Almost 51% of the patients reported having asthmatic family members whereas 43% reported that their relatives are suffering from asthma. History of previous medication was documented to exclude role of any medication on hematological and biochemical parameters. At the time of collection most of the patients denied use of any medication (data not shown).

Majority of the patients of had low socioeconomic status. Vegetables being the cheapest food were commonly used and regular exercise was not being practiced by the majority of the patients (i.e., 90%). Smoking is considered a risk factor for asthma [21] but majority of the patients denied smoking. Similarly night sleep pattern was normal (i.e., 5-8 hours) in most of the patients. Only a small fraction reported sleep apnea and restlessness and that was obviously associated with asthma attack (**Supplementary data Table 1**). Data were also obtained from normal controls (**Supplementary data Table 2**).

Hematological profile of asthma patients

Hemoglobin (Hb) levels were higher in both male and female asthmatics than the corresponding normal controls. However, RDW (red cell distribution width) and HCT (hematocrit) levels were significantly elevated ($p < 0.05$) in female asthma patients only. Similarly in case of male asthma patients MCHC (mean cell/corpuscular hemoglobin concentration) levels which provides information about the proportion of Hb in each red blood cell

[22] were higher than the normal controls ($p < 0.05$). Total number of RBCs was reduced along with volume of Hb in each red blood cell (MCV) in male asthmatics ($p < 0.05$) but female asthmatics showed no significant variation.

WBCs related hematological parameters exhibited no gender specific variation. Majority of the parameters including WBC ($p < 0.0005$), LYM# ($p < 0.05$), MXD# ($p < 0.005$), NEUT# ($p < 0.0005$) and NEUT% ($p < 0.005$) was up-regulated except LYM% level ($p < 0.005$) which was down-regulated in all patients with asthma. However, few parameters like PLT and MXD% did not significantly vary from the normal controls (**Tables 1 and 2**).

Biochemical profile of asthma patients

To identify any differentially expressed biochemical parameters in asthma patients, results were compared with biochemical profile of gender specific normal controls. A few parameters including alkaline phosphatase, amylase, uric acid, Ca^{+2} , and PO_4^{-3} exhibited no statistically significant variation compared to normal controls. However, other parameters varied significantly.

Total protein and globulin levels were decreased in both male and female asthma patients. Albumin levels were decreased in only female asthma patients. However, there was no statistically significant variation of albumin in case of male asthma patients as compared to normal male controls.

Total cholesterol levels were elevated in all asthma patients with no gender discrimination. Results were influenced in a gender specific manner for triglycerides and HDL-C levels. No statistically significant variation was observed in case of female asthma patients. While in male asthma patients triglyceride levels were decreased and HDL-C levels were increased compared to normal controls.

Table 1: Nature and strength of variation in the hematological profile of asthmatic patients.

S. No.	Parameter	Variation in female asthmatics (n=25)	Variation in male asthmatics (n=26)
1	RBC ($\times 10^6/\mu\text{L}$)	----	↓*
2	Hb (g/dL)	↑*	↑*
3	HCT (%)	↑*	----
4	MCV (fL)	----	↓***
5	MCH (pg)	----	----
6	MCHC (g/dL)	----	↑*
7	RDW (%)	↑*	----
8	PLT ($\times 10^3/\mu\text{L}$)	----	----
9	MPV (fL)	↓***	↓***
10	WBC ($\times 10^3/\mu\text{L}$)	↑***	↑***
11	LYM (%)	↓***	↓**
12	LYM ($\times 10^3/\mu\text{L}$)	↑*	↑***
13	MXD (%)	----	↑*
14	MXD ($\times 10^3/\mu\text{L}$)	↑**	----
15	NEUT (%)	↑**	↑**
16	NEUT ($\times 10^3/\mu\text{L}$)	↑***	↑***

↑=increased, ↓=decreased, ----=No statistically significant variation, *= $p < 0.05$, **= $p < 0.005$, ***= $p < 0.0005$, p value was calculated by comparison with gender specific controls. Details are available in supplementary data.

Table 2: Overall summary of the asthmatic patients' hematological profile.

Nature of variation (as compared to gender specific normal controls)	Varied hematological parameters in asthma patients		
	Both male and female	Female (n=25)	Male (n=26)
Up-regulation	Hb, WBC, LYM#, MXD#, NEUT# and NEUT%	RDW, HCT	MCHC
Down-regulation	MPV, LYM%	-----	MCV, RBCs
No change	MCH, PLT, MXD%	MCHC, MCV, RBCs	RDW, HCT

Normal female controls (n=48), Normal male controls (n=51), n=Total number of subjects in a particular group, Variation was documented by comparing results of asthma patients with gender specific normal controls. Details are available in supplementary data.

We observed elevation of CK-NAC and CK-MB levels in female asthma patients as compared to normal female controls ($p < 0.05$). However, in case of male asthma patients the levels of both enzymes were not significantly different from normal male controls. Most of the kidney function related parameters (i.e., creatinine, urea and blood urea nitrogen) were increased in all asthma patients.

We observed low levels of bilirubin in all patients with asthma regardless of gender. However, albumin was also significantly decreased in female asthma patients (as mentioned earlier in section 3.3.1).

We observed statistically significant reduction ($p < 0.0005$) in serum levels of AST and ALT in both male and female asthma patients (**Tables 3 and 4**). In current study asthma patients were found to have higher levels of Na^+ and K^+ as compared to the gender specific normal controls. In case of female asthma patients RBG was significantly elevated than normal controls. However, variation was non-significant in case of male asthma patients.

Urine analysis of asthma patients

To monitor any abnormality present in urine profile we performed urine biochemical (using urine strips) and microscopic analysis. No abnormality was found that can be identified as associated factor of asthma (**Supplementary data Tables 3-10**).

Discussion

Hematological profile of asthma patients

We propose that asthma related pulmonary hypoxia manages mild, but statistically significant elevation of Hb level. Our supposition is supported by the earlier report that arterial oxygen tension is greatly reduced in young patients of asthma after exposure to methacholine, a drug which causes mild obstruction of the airway. Another study has shown that chronic and acute fluctuations in alveolar oxygen tension promote up-regulation of Hb in alveolar epithelial cells. To the best of our knowledge there is no report about elevated Hb level in blood of asthma patients. So the present study is the first to report up-regulation of Hb in blood of asthma patients. RDW provides an account of the

Table 3: Nature and strength of variation in the biochemical profile of asthmatic patients.

S. No.	Parameter	Variation in female Asthmatics (n=25)	Variation in male asthmatics (n=26)
1	Total protein	↓***	↓***
2	Albumin	↓***	-----
3	Globulin	↓***	↓***
4	Albumin/ Globulin ratio	↑***	↑***
5	Total bilirubin	↓*	↓**
6	AST/SGOT	↓***	↓***
7	ALT/SGPT	↓***	↓***
8	Alkaline phosphatase	-----	-----
9	Total Cholesterol	↑***	↑***
10	Triglycerides	-----	↓***
11	HDL-C	-----	↑*
12	CK-NAC	↑*	-----
13	CK-MB-NAC	↑*	-----
14	Uric acid	-----	-----
15	Creatinine	↑*	↑**
16	Urea	↑***	↑***
17	Blood urea nitrogen	↑***	↑***
18	Na ⁺	↑**	↑***
19	K ⁺	↑*	↑***
20	Ca ⁺⁺	-----	-----
21	PO ₄ ⁻³	-----	-----
22	Glucose	↑***	-----
23	Amylase	-----	-----

↑=Increased, ↓=Decreased, -----=No statistically significant variation, *=p<0.05, **=p<0.005, ***=p<0.0005, p value was calculated by comparison with gender specific controls. Details are available in supplementary data.

Table 4: Asthmatic patient's biochemical profile summary.

Nature of variation (as compared to gender specific normal controls)	Varied biochemical parameters in asthma patients		
	Both male and female	Female (n=25)	Male (n=26)
Up-regulation	Albumin/ Globulin ratio, cholesterol, creatinine, urea, blood urea nitrogen, Na ⁺ , K ⁺	CK-NAC, CK-MB-NAC	HDL-C
Down-regulation	Total protein, globulin, bilirubin, AST, ALT	Albumin	Triglycerides
No change	Alkaline phosphatase, uric acid, Ca ²⁺ , PO ₄ ⁻³ , amylase	Triglycerides, HDL-C	Albumin, CK-NAC, CK-MB-NAC

Normal female controls (n=48), Normal male controls (n=51), n=total number of subjects in a particular group, Variation was documented by comparing results of asthma patients with gender specific normal controls. Details are available in supplementary data.

variation in size of RBCs population and a higher RDW correlates with higher rate of RBCs synthesis while HCT corresponds to the %age volume of RBCs in blood [22-24]. As hypoxia elevates HCT hence it is concluded that hypoxic conditions may provoke synthesis of more Hb and RBCs [25]. Variations in RBCs and indices are being reported for the first time. There is a need to extend the study for understanding the underlying mechanism and to explore basis of gender specific effects.

Results of the present study are in accordance with the earlier report of increased neutrophil contents observed in the airways and sputum of asthma patients during smoking and acute exacerbations [26]. Neutrophils or WBCs are major components of pus cells and might release large amounts of histamine in the lung. Histamine is the inflammatory compound that is responsible to cause runny/watery nose and wheezing during allergic reactions [27]. Higher levels of WBCs should be further investigated in association with histamine and other inflammatory markers.

Biochemical profile of asthma patients

In the present study lowered levels of total protein, globulin and albumin are observed in the sera of asthma patients. Serum proteins are mainly synthesized by liver. Normal serum levels are adjusted through a balanced rate of protein synthesis and degradation [28-31]. Albumin and globulin are the major blood proteins. Albumin levels are reduced because of malnutrition, inflammation, HIV infection, auto-immune diseases, liver and kidney diseases [32-37]. Asthma is characterized as a chronic airway inflammation and it is known that during inflammatory process Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) levels are raised in the cells. Cells are provided with enzymatic and non-enzymatic antioxidant systems to prevent cellular damage caused by ROS cells. Among other acute phase proteins albumin is the component of non-enzymatic antioxidant system. Albumin can bind with metals which might undergo oxidation reduction reactions and hence can regulate/reduce the production of free radicals through metal catalysis [38-41].

Antioxidant defense mechanism is associated with pathogenesis of asthma because in asthma patients the levels of oxidant marker (i.e., lactate) is elevated and levels of certain antioxidants including uric acid, albumin, and bilirubin are reduced. It is known that status of oxidant and anti-oxidant serum markers correlates with the severity of asthma [42,43]. Decrease in albumin level might be the outcome of either the reduced rate of its synthesis or increased rate of degradation. It is known that catabolic activities are elevated by pro-inflammatory cytokines and growth factors during inflammation [35,40]. Decreased level of albumin if not compensated with increased synthesis of globulin, then it results in an overall reduced Total Serum Proteins (TSPs) and this is what we have observed [44,45]. Albumin is significantly reduced in female asthma patients included in our study, but in case of male asthma patients the variation was statistically non-significant. To the best of our knowledge no information is available regarding gender specific variations in patients with asthma. However, it is known that serum albumin levels are more significantly reduced in symptomatic asthmatic patients as compared to non-

symptomatic asthmatics [43,46]. In the present study we have observed that in case of both male and female asthma patient's globulin is significantly reduced and albumin/globulin ratio was enhanced. Globulin protein fraction is composed of four major sub-groups (i.e., gamma, beta, alpha-2 and alpha-1) and includes 60 different proteins of diverse nature (i.e., glycoproteins, antibodies, clotting factors and carrier/transport proteins). Low globulin levels are mostly considered an outcome of decreased antibodies (gamma globulin) synthesis and have been observed in many diseases particularly in chronic liver, kidney and chronic inflammatory diseases [47]. Elevated levels of serum IgE have been observed in asthma by many workers [48,49]. We have not performed the detailed study of globulin fraction hence there is a need to further extend the study and characterize the nature of variation.

In the available scientific literature there are contradicting reports about the association of cholesterol with asthma. According to some reports cholesterol has no association with the pathogenesis of asthma [50,51]. However, few workers have shown that cholesterol level is elevated in asthmatic patients and can help to evaluate the effect of treatment [52,53]. However, certain studies have shown that cholesterol levels are lowered in asthma and there is no change in HDL-C [54].

Asthma is an airway inflammatory disorder promoted by pro-inflammatory cytokines and chemokines [55]. Asthma and dyslipidemia have been identified as the factors that contribute to pathogenesis of asthma [56]. Dyslipidemia, obesity and hyperinsulinemia may promote vascular inflammation and activate both, innate and acquired immune systems in the respiratory tract. It is not still understood that how cholesterol is implicated in inflammation and pathogenesis of asthma [56]. Higher prevalence of asthma has been observed in children having elevated cholesterol levels in serum. Cholesterol metabolism and lung inflammation were noticed to be linked phenomena [57-59]. It is known that there is inverse correlation between cholesterol levels and lung function [43,60]. As far as the mechanism of action is concerned, it is known that high cholesterol level is responsible to promote TH2 inflammation in animal models of asthma [61,62]. It has also been suggested that pro-inflammatory cellular responses are triggered by cholesterol loading and depletion of leukocytes including neutrophils, macrophages and mast cells [63-65]. Contrary to these findings few studies have shown that inflammation leads to lower the cholesterol level [66]. On the basis of this information scientists have proposed that the bidirectional association of cholesterol with inflammation might affect asthma in differential manner [54]. At this moment although we have observed abnormal lipid profile in asthmatic patients, we cannot propose the lipid profile as biomarkers of asthma unless otherwise the question is addressed completely and role of any other contributing factor is excluded. There are certain drugs and hormones which modulate lipid metabolism. Catecholamines often incorporated in asthma treatment enhance lipolysis of triglyceride depots stored in adipose tissues [67]. Higher levels of triglycerides have been found in asthmatic children [68]. Similarly treatment with drugs containing prednisolone enhances the activity of lipoprotein lipase

enzyme and ultimately results in the elevation of cholesterol and HDL-C [69]. It is important to note that positive correlation exists between epinephrine level and HDL-C. However, epinephrine has negative impact on serum triglycerides level [70]. There is a need to design the study using appropriate controls and document the impartial behavior of lipid profile parameters.

Creatine Kinase (CK) is the intracellular enzyme present in the cytosol and mitochondria of muscle cells, responsible to catalyze transphosphorylation reaction between creatine and ATP and hence helps to regenerate ATP in the muscle cells from phosphocreatine and ADP [71,72]. Although CK is present in many organs, tissue specific isoforms with highest concentration in skeletal muscles (CK-MM), cardiac muscles (CK-MB) and brain cells (CK-BB) are known. The level of three isozymes contributes to total CK level [72].

It is known that CK level is elevated in response to any tissue injury or change in cellular permeability. It has been proposed that any strenuous activity which can disturb the integrity of muscle cell membrane releases muscle cell constituents into circulation and serum levels of the muscle enzymes are increased [73-76]. Many workers have noted elevations of CK in asthmatic patient's sera during the episode of acute severe asthma [77-79]. Various independent studies have proposed asthma associated vigorous and repeated muscular contractions as strenuous activity and to justify the elevated serum CK levels. Scientists have speculated that increased respiratory efforts and hypoxemia might cause the skeletal muscle injury. A case of patient who developed rhabdomyolysis (diagnosed muscle damage) and acute renal failure after status asthmaticus (severe acute asthma) has been reported [75,78]. There are contradictory reports about the severity of asthma and CK-levels. After finding significant association of the admission respiratory rates with raised CK levels, a group of workers has suggested that although elevated CK level is associated with the severity of asthma exacerbation, but it is not the reliable predictor of the severity of asthma exacerbation [78]. However, a recent study has shown higher CK levels in patients who had more intubation, ICU days and hospital days. Besides muscular damage CK levels are also elevated in response to inflammation, necrosis and treatment using various drugs like theophylline, IV infusions of aminophylline and salbutamol [79,80-84]. Few workers have concluded that it is not of significance to project further investigations of elevated CK levels unless otherwise there is myocardial, CNS or skeletal muscle injury [78]. Elevated CK level can be due to the enhanced level of either one or more of the three known isozymes (i.e., CK-MM, CK-MB and CK-BB) hence estimating levels of individual isoenzymes is helpful to identify the site of origin and pinpoint the target for therapeutic treatment [85]. Considering this we attempted to estimate the levels of CK-MB levels in asthma patients and observed statistically significant elevation in female asthma patients. It is known that besides extreme physical activity, aggressive muscle regeneration, and left ventricular hypertrophy CK-MB level is also enhanced in response to the continuous albuterol and other β 2-adrenergic agonists (i.e., terbutaline and salbutamol) nebulization that is considered an effective treatment of severe asthma [86-89]. High doses of

albuterol are known to cause transient elevation of CK-MB level in asthmatic patient's sera and it has been proposed that repeated nebulization events and associated adrenergic stimulation might elevate CK-MB levels through their effects on cardiac muscles [90,91]. It is required to further characterize the variations of CK and CK-MB along with other isozymes of CK levels and conduct study with appropriate controls to exclude the influence of drugs and any other contributing parameter.

Higher serum levels of creatinine, urea and blood urea nitrogen are the indicators of renal insufficiency [92]. For a long time it is known that there is relationship between asthma and renal diseases and term 'renal asthma' has been used commonly [93]. A few years ago a case of 25 years old man has been reported who developed acute renal failure and myoglobinuria followed by status asthmaticus (acute asthma). During acute asthma burden on airway muscles is increased, respiratory efforts are enhanced and hypoxia is resulted which might cause myoglobinuria, dehydration and renal failure [75]. It is also known that increased muscular contractions which are characteristic feature of acute asthma are correlated with rhabdomyolysis and rhabdomyolysis is further linked with acute renal failure [78,94,95]. In the present study, however, none of the patients was identified to develop acute renal failure, but after observing significantly higher levels of creatinine, urea and BUN levels in asthma patients as compared to gender specific normal controls we conclude as that there might be a link between the pathogenesis of asthma and renal insufficiency. It is further proposed that future studies should be initiated to address the question and understand the basis of asthma associated renal insufficiency.

Oxidative stress due to airway inflammation, lowered antioxidant and Reactive Oxygen Species (ROS) levels are implicated in the pathogenesis of asthma [41,43]. Damaging effects of oxidative stress and ROS are limited or minimized by antioxidants which are capable to scavenge ROS present in the fluid lining the respiratory tract [96]. Antioxidants are categorized as dietary (e.g. vitamins) as well as non-dietary (e.g. albumin, bilirubin, uric acid, and glutathione peroxidase) and both groups have been implicated in the pathogenesis of asthma and contribute to the severity of asthma. Reduced intake of dietary antioxidants and decreased synthesis of non-dietary antioxidants result in oxidant-antioxidant imbalance which further promotes or exacerbates chronic inflammation and tissue damage associated with severe asthma [41,43].

Albumin is known as the most important extracellular antioxidant which regulates the levels of glutathione in epithelial cells of lungs [38,97]. Bilirubin plays an important in protecting albumin from oxidative damage and along with vitamin E can also inhibit the low-density lipoprotein oxidation [98]. It is known that decreased plasma albumin and bilirubin levels adversely influence the severity of asthma. Plasma albumin is positively correlated with lung function whereas plasma bilirubin has been found to be inversely correlated with severe asthma [97]. Knowing this information our results have prompted us to conclude that in asthma patients included in study lowered bilirubin levels suggest severe asthmatic condition. There is a need to project study on

large scale and investigate the role of other antioxidant systems involved in asthma pathogenesis and severity.

ALT (alanine transaminase) and AST (aspartate transaminase) are the liver enzymes which play an important role in protein metabolism and are markers of liver function [99,100]. Hepatocellular damage and inflammation have been identified as factors responsible for elevated ALT and AST levels, but sometimes levels of both enzymes are falsely lowered [101]. Rhabdomyolysis that is considered a factor associated with asthma is accompanied by the elevated serum levels of AST and other muscle enzymes including Lactate Dehydrogenase (LDH) and aldolase [102]. Contrary to this, we have observed statistically significant reduction in serum levels of AST and ALT in both male and female asthma patients (**Table 2 and supplementary data Table 7**). To the best of our knowledge at present no clinical explanation is available for the low serum levels of ALT and AST in asthma. For reduced levels of ALT it is known that decreased ALT level along with the increased cholesterol level (similar to our findings) is the indicator of congestive liver [103]. Congestive liver is the condition when liver is overloaded with toxins to detoxify or carbohydrates rich diet is being used [104]. Considering these facts and figures we speculate that this might be due to less efficient working of liver, low protein synthesis and malnutrition. It is important to conduct study on large scale for characterization of the factors contributing to the lowered levels of ALT and AST in the sera of patients with asthma.

Although Na^+ and K^+ levels are not elevated to the extent that can be referred as hypernatremia and hyperkalemia, respectively we conclude that renal insufficiency might account for the mild up-regulation of Na^+ and K^+ . It is also known that Na^+/K^+ imbalance is the characteristic feature of rhabdomyolysis [105]. Our results are in accordance with the earlier study which has shown elevation of K^+ in the sera of patients suffering from bronchial asthma [106]. High Na^+ diet is also known to promote bronchial reactivity [107]. However, the majority of studies have showed that serum Na^+ and K^+ levels are decreased in asthma patients during attacks as compared to normal controls and asthma patients not suffering from asthma attack [108-110]. It will be beneficial to study the Na^+ , K^+ and other electrolyte imbalances for proper management of asthma.

It is established now that the drugs which are used to treat acute asthma induce hyperglycemia in asthma patients. Significant increase in RBG level has been noticed in patients being treated either with salbutamol nebulizer alone or in combination with hydrocortisone injections. However, long-term anti-asthma medicines do not cause hyperglycemia [111].

To the best of our knowledge there is no information available regarding the combinatorial effects of HBV/HCV infection and asthma. Our observations are important and present study may serve as pilot study for future studies to explore the combinatorial effects of asthma HBV/HCV infection. Although differences do exist between asthma patients having no HBV/HCV infection and asthma patients having HBV/HCV infection, it is necessary to extend the study to draw more reliable conclusions and suggest recommendations for treatment.

Conclusion

Variation of multiple hematological and biochemical parameters in asthma patients has indicated that multiple pathways and components are associated with the pathogenesis of asthma. Differential expression of biochemical and hematological parameters is not asthma-specific because these parameters also vary in other diseases or pathological conditions. It is, therefore, concluded that due to the lack of specificity these parameters lack the potential to be good future biomarkers of asthma. However, multiple parameters in combination can be helpful for better diagnosis of asthma and research must be continued to find better and more specific biomarkers of asthma.

Conflict of Interest

None of the authors have any conflict of interest. Authors do not

have any financial and personal relationships with other people and organizations that may inappropriately influence or bias their results.

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