STUDY OF BIOMARKERS ON ASTHMATIC PATIENTS WITH ASSOCIATED CO-MORBIDITIES

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ABSTRACT

Introduction and Aim: Asthma is a variable condition which gives load to patients, their families and community. Diabetes involved multiple factors and also cardiovascular risk factors are signs of pre-diabetic. Asthma is directly proportional to coronary artery disease.

Materials and methods: Blood sample for HbA1c and HCY was collected in EDTA vial. HbA1c determination was based on turbidimetric inhibition immunoassay (TINIA) on Roche cobas c311 auto-analyzer kit. HCY was measured by ELISA method on ELISA Reader (Tulip) by using Alpha diagnostic international kit. The subjects enrolled were 165 asthmatic patients and 165 healthy controls.

Results and conclusion: When comparing means of different biochemical parameters between controls and cases with student's t-test, HbA1c, Cholesterol, HDL, TG, LDL and HCY showed a highly significant ($p<0.0001$) difference. Adult with asthma, particularly those using multiple medications may be at increased risk of diabetes and CAD.

Keywords: Diabetes, coronary artery disease, inflammation, infection

INTRODUCTION

Asthma is a chronic disease characterized by recurrent attacks of breathlessness, chest tightness and wheezing which vary in severity and frequency from person to person. The prevalence of asthma in adults in India is 12%.[1] All asthma is bronchial asthma, one cause may be allergy. The non atopic persons also develop asthma mostly in adult life (Late onset asthma) through an unknown mechanism or by type III immunological reaction leading to airway hyper reactivity. Asthma also

causes loss of enzymes that normally breakdown inflammatory mediators. Allergy is related to the phenomenon of atopy which is an innate tendency to excessive IgE synthesis.

Diabetes is a metabolic disorder and hyperglycaemia is a characteristic feature of it. The metabolic dysregulation leads to secondary patho-physiological changes in multi-organ system. Several mechanisms involve for the risk of diabetes in asthma patients such as genetic, lung related inflammatory cytokines and their effect on insulin sensitivity and direct effect of hypoxia on glucose metabolism.[2]

CAD is a multi-factorial disease and these factors are called risk factors such as hyperhomocysteinemia, hyperlipidemia, diabetes mellitus and inflammatory markers. Some population with CAD may be on silent or pre-clinical stage.[3] CAD is a serious health problem in both developed and developing countries which are mainly caused by atherosclerosis with endothelial dysfunction.[4] Asthma is directly proportional to coronary artery disease. The aim of this study is to assess the risk of associated co-morbidities like DM and CAD in asthma patients.

In this study, the assessment of risk of diabetes was done by the estimation of glycated hemoglobin (HbA1c) and hyper-triglyceridemia is the commonest lipid abnormality occurred in diabetic patients and the risk of coronary artery disease was assessed by the estimation of total HCY in plasma because HCY is an independent risk factor for pre-mature cardiovascular disease.[5]

This study was conducted in Bhopal, Madhya Pradesh. The major effect of Bhopal gas tragedy comes under generation to generation and the lungs was most adversely affected as well as other organs too. This study was not conducted Bhopal area previously.

MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, People's College of Medical Science and People's University Bhopal. The study protocol was approved by the DDC, RAC, IEC and UDC committees of our institute/university.

Inclusion criteria

Patients having history of already physician diagnosed asthma (on the basis of severity of symptoms and frequency of symptoms) were selected for the study.

Exclusion criteria

Patients with other chronic respiratory (COPD) and non-respiratory (renal, cardiac, liver) diseases, Osteomalacia, Rickets, Smokers, Alcoholics, Pregnant women, Gestational Diabetes and patients who did not reported history of already physician diagnosed asthma.

Sample size was estimated according to the prevalence of asthma in adult in India that is 12%.[1] The exact prevalence of asthma in adult in Madhya Pradesh was not found. The sample size was found to be 162. So we had taken 165 cases.

Ratio of cases and controls were taken 1:1 i.e. 165 cases and 165 controls. Hence, total 330 blood samples of subjects were included in the study.

Patients were classified into mild and severe asthma because there is not much difference between moderate asthma cases and severe asthma cases on the basis of frequency of symptoms. Asthma symptoms included daily cough, chest tightness, wheeze and breathlessness and symptoms was stable from more than 3 months. According to GINA guidelines, the frequency of symptoms at day time in mild asthmatic patients are more than once per week but less than once per day and night

time symptoms is more than twice per month. Perhaps, in severe asthmatic patients the frequency of symptoms is daily and night time symptoms are also very frequent from the last 3 months.

An informed consent were taken from all patients who were comes under inclusion criteria. Blood sample for HbA1c and HCY was collected in EDTA vial. HbA1c determination was based on turbidimetric inhibition immunoassay (TINIA) method on Roche cobas c311 auto-analyzer instrument by using kit. HCY was measured by ELISA method on ELISA Reader (Tulip) by using Alpha diagnostic international kit. Data entry has been done in the form of MS Excel. Data were expressed as mean±SD. ANOVA test was performed for the comparison among more than two groups. A p-value of <0.05 was taken as statistically significant using a two-tailed distribution. Statistical analysis was performed using SPSS version 20.

RESULTS

The subjects enrolled were 165 asthmatic patients and 165 healthy controls. Study population were divided into three age groups: 1) 0-18 years (9%) (Children cum teenagers) 2) 19-45 years (52%) (Adults) 3) more than 45 years (39%) (Old age people). Out of 165, 8 asthmatic patients were pre-diabetic (5%), 5 asthmatic patients were on the risk of CAD (3%) and 2 asthmatic patients were on risk of both diabetes and CAD (1%) (Figure I). Female were taken 58% (n = 96) and 42% (n = 69) male in patients population (Table I). Patients with severe asthma showed presence of co-morbidity in 14 (8.48%) out of 93 (56.36 %) patients, while only one (0.61 %) patient of mild asthma showed presence of co-morbidity.
On statistical analysis with chi-square test, co-morbidities were showed significant association with severity of asthma when compared to those with mild asthma cases.

Table I General characteristic of asthma patients

Distribution of asthma patients (n = 165)		
Characteristics		**N (%)**
Age (Years)	0-18	15 (9.09)
	19-45	86 (52.12)
	>45	64 (38.79)
Gender	M	69 (41.82)
	F	96 (58.18)
Type of asthma	Mild	72 (43.64)
	Severe	93 (56.36)
Duration of asthma	≤10 Y	118 (71.52)
	>10 Y	47 (28.48)
Co-morbidity	DM	8 (4.8)
	CAD	5 (3.03)
	DM+CAD	2 (1.2)

Figure I: Showing distribution of cases according to associated co-morbidity

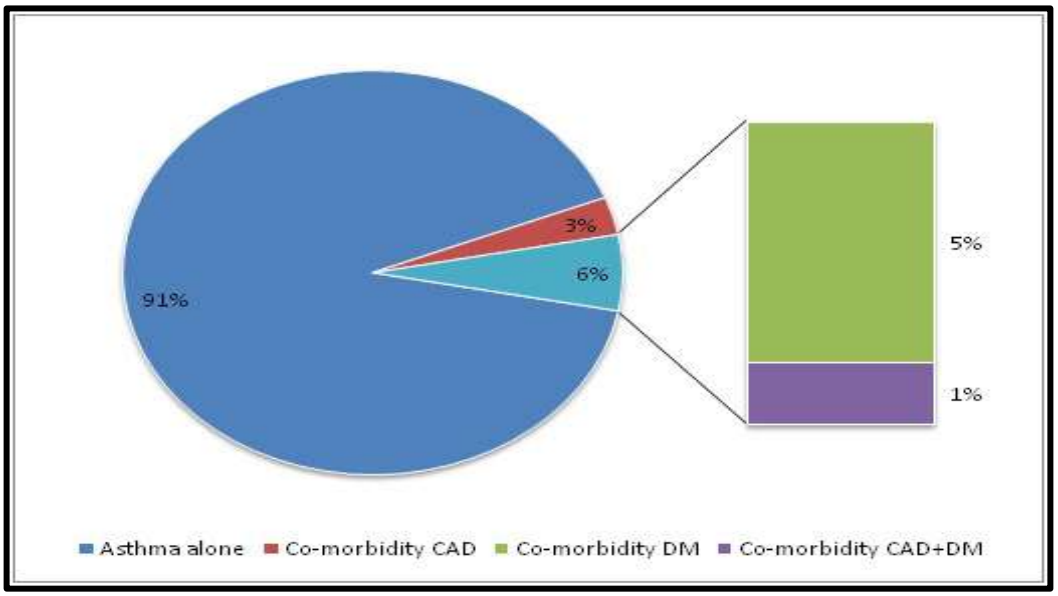

Table II. Comparison between asthma patients and healthy controls

Parameters	Healthy controls Mean±SD (n = 165)	Asthma patients Mean±SD (n = 165)	p-value
HbA1c	4.69 ± 0.44	4.96 ± 1.22	0.007
HCY	6.25 ± 1	12.62 ± 4.22	0.0001
Total cholesterol	136.82 ± 39.29	170.98 ± 17.63	0.0001
TG	98.43 ± 33	127.09 ± 23.3	0.0001
HDL-C	49.45 ± 7.37	30.11 ± 9.19	0.0001
LDL-C	93.46 ± 25.39	105.26 ± 19.69	0.0001

Table III. Comparison among asthma patients and associated co-morbidities

Parameters	Asthma associated Co-morbidities			Asthma only	p-value
	DM	CAD	DM+CAD		
HbA1c	8.27 ± 0.25	8.27 ± 0.09	9.4 ± 0.28	4.65 ± 0.56	0.0001
HCY	17.14 ± 1.98	17.75 ± 0.97	24.84 ± 1.2	12.05 ± 3.88	0.0001
Total cholesterol	165.2 ± 12.72	228.94 ± 12.92	218.09 ± 10.49	168.73 ± 13.41	0.0001
TG	125.22 ± 12.8	218.26 ± 9.17	217.35 ± 0.86	122.95 ± 13.48	0.0001
HDL-C	34.01 ± 8.97	22.45 ± 5.63	24.55 ± 3.88	30.23 ± 9.22	0.1279
LDL-C	99.45 ± 16.2	170.24 ± 8.95	169.62 ± 7.54	102.55 ± 14.32	0.0001

DISCUSSION

When comparing means of different biochemical parameters between controls and cases with student's t-test, HbA1c, Cholesterol, HDL, TG, LDL and HCY showed a highly significant ($p<0.0001$) difference (Table II). All co-morbid cases showed significant difference for HbA1c and HCY compared to asthma only cases. In lipid profile serum total cholesterol, triglycerides and LDL were significantly higher in CAD co-morbidity and CAD+DM co-morbidity cases compared to asthma only cases. Serum total Cholesterol, triglycerides and LDL were significantly different in CAD co-morbidity Vs DM co-morbidity and DM Vs CAD+DM co-morbidities cases. Hence, asthma is associated with CVD and DM co-morbidities.

Risk factors of asthma involves exacerbations, decrease lung activity (increases heart load) and adverse effect of medication (disturbance in metabolism of glucose) used for the treatment of asthma. These medications helps to reduce airway inflammation and hyper-responsiveness.[6] National / international guidelines recommend low dose as first line preventer therapy for mild asthma and medium dose for moderate asthma.[7] Receptors presents on the surface of lungs can absorbed drugs systemically (gluco-corticosteroids) very well and accounts pulmonary bioavailability of drugs. Potency and dose of drugs (gluco-corticosteiroid) determine its adverse outcome. The drug taken orally by mouth to reaches systemic regulation and first passes through lumen of GI tract then passing to the liver. Liver is the main organ to metabolize drugs. Much of the drugs which have been taken orally is destroyed by the liver.[8] Corticosteroids obstruct immunological reactions such as late-phase reaction to allergens, airway hyper-responssiveness and activation of inflammatory cells. ICS helps to minimize asthma symptoms, improve quality of live, reduces frequency and severity of exacerbations. Long term systemic steroid therapy causes hyper-glycemia and impact on their adverse effect of hyper-glycemia.[9] Glucocorticoids causes hyper-glycemia by increased binding.[10] Corticosteroids metabolically decreased insulin sensitivity in several tissues. The main function of corticosteroids is to inhibit the insulin secretion by pancreatic β-cells.[11]

The level of increased glycosylated hemoglobin might accelerate atherosclerotic processes which are supported by several mechanisms such as oxidative stress and protein glycation of vessel walls.[12]

Kaarthikeyani Sankaravadivelu et al (2019), Wasim A Wani et al (2019) observed significantly higher HbA1c level in children on inhaled corticosteroids for more than six months.[9, 13]

The phenomena of cholesterol metabolism and lung inflammation were reported interconnected.[14] Cholesterol enhances inflammation and IgE and Th2 cytokine production by mononuclear cells.[15] Hypercholesterolemia promotes pro-inflammatory mechanisms included increased levels of pro-inflammatory cytokines, cellular adhesion molecules and inflammation-sensitive plasma proteins.[16] Asthma is characterized by chronic airway inflammation. Meanwhile hypercholesterolemia plays a pro-inflammatory role, inducing release of inflammatory cytokines.[17]

Al-Shawwa B (2006) and Moini A et al (2012) were found that higher cholesterol level in asthmatic patients which can help to evaluate the effect of treatment.[18, 19]

The adverse effect of hyper-homocysteinemia is hypertension, lipid and lipoprotein metabolism as well as development of inflammation. HCY causes remodelling of arterial wall leading to vascular damage. Raised HCY level may promote oxidative stress, inflammation of vascular endothelial cells and reduced the availability of nitric oxide (a strong relaxing factor).[20]

Another reason behind hyper-homocysteinemia may be genetic defects of enzymes such as 5, 10-methylene tetrahydrofolate reductase, methionine synthase and cystathionine-β-synthase participate in homocysteine metabolism.[21]

Various drugs are also responsible for increased homocysteine concentration which interfering folate, vitamin B_6 and B_{12} metabolism because vitamin B_6, B_{12} and folic acid are essential co-factors in HCY-methionine metabolism.[22]

Serum HCY levels are elevated in both type 2 diabetic patients as well as pre-diabetic individuals. Impairment in endothelium has significant role in development of diabetic complications.[23] Hyperglycemia, oxidative stress, genetic factors, disturbance of lipid metabolism and various growth factors are major causes of chronic diabetic complications. Insulin resistance is the main cause of oxidative stress. Reactive oxygen species interfere with insulin signaling at various levels and inhibit translocation of GLUT4 in plasma membrane leading to insulin resistance.[24] In this mechanism, an increase in insulin or glucose levels further increases the production of reactive oxygen species and oxidative stress, impairing both insulin action and secretion and accelerating the progression of DM.

Endothelial dysfunction of blood vessels initiated the pathogenesis of CAD.[25] Hyper-homocysteinemia causes endothelial dysfunction which leads to oxidative stress and inflammation.[26] Researches on HCY suggest that increased HCY approximately 3μmol/L will raise about 10% chances of CAD events. Humphrey et al found that elevated HCY 5μmol/L will increase approximately 20% risk of CAD events.[27]

Hyperhomocysteinemia accelerate proliferation of vascular smooth muscle cells which leads to narrowing of arterial lumen and creating its harmful effect. It is enhancing the activity of HMG CoA reductase and increases cholesterol synthesis resulting atherosclerosis and promoting risk factor for CAD.[28]

HCY metabolized by two pathways such as trans-sulfuration and remethylation. Excess amount of HCY form major by-product named homocysteine thiolactone which further reacts with LDL and form LDL-HCY thiolactone aggregates. These are taken up by macrophages and incorporated into foam cells in early atherosclerotic plaques. Homocysteine thiolactone acylates protein present inside the plaques which modifies the oxidative process of vessel and promoting athero-thrombosis. In addition, auto-oxidation of homocysteine resulting production of superoxide and hydrogen peroxide. These molecules initiate oxidation of LDL which causes endothelial dysfunction and promote proliferation of vascular smooth muscle cells.[29]

Evidence supported that oxidative mechanism attributed to increased HCY and atherosclerosis.[30] HCY can alter the surface properties of endothelial cells by changing their phenotype from anticoagulant to pro-coagulant.[31]

Liao et al (2006) found that HCY reduces concentration of HDL in plasma by inhibiting hepatic synthesis of apoA-I, the main HDL apolipoprotein.[32] Mikael et al (2006) supported the Liao's study.[33] High serum HCY level indicated the association with increased lipid peroxidation.[34]

In this patient population, health professionals should closely scrutinize known diabetes and CVD risk factors in asthma. Study result suggests that need to monitored lipids profile in individuals with asthma.

CONCLUSION

The rate of developing co-morbidities in poorly controlled asthmatic patients must be slowly progressive in nature. Mostly, adult on-set poorly controlled asthmatic patients may have co-morbidities due to hormonal imbalance, effect of medication etc. Cardiac events particularly stroke in adult-onset asthma was not found. Finally, because this was an observational study, so unmeasured confounding factors or residual confounding can't ignore because asthma was related to many known CVD and DM risk factors. First time in Madhya Pradesh was observed the asthma, DM and CAD association of children, men and women. This study provides evidence for prospective relationship between asthma and incidence of newly identified pre-diabetic and CAD patients.

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Conflict of Interest

There is no conflict of interest in this study.

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