

Research Article**Role of Various Biomarkers in Assessing Cardiovascular Status in Acute Myocardial Infarction (AMI) Patients****Qazi Najeeb^{*1}, Sajad Hamid², Ather Hafiz Khan³**¹Post M.D. Demonstrator, Department of Biochemistry, SKIMS Medical College, Bemina, Srinagar, India²Lecturer Anatomy, SKIMS Medical College, Bemina, Srinagar, India³Post M.D. Demonstrator, Department of Pathology, SKIMS Medical College, Bemina, Srinagar, India***Corresponding author**

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Abstract: The traditional risk factors for coronary heart disease do not account for the entirety of risk and there are many people who have events and do not fit into these traditional risk factors. The aim of the study was to assess multiple biomarkers representing in relation to cardiovascular events and to evaluate the levels of HbA1c, sialic acid, Homocysteine, high sensitive C-reactive protein in non-diabetic individual with acute myocardial infarction and possibility of their use as markers for coronary vascular events. Total 600 patients were included in the study and were divided into two groups: Group-I (cases): 300 clinically diagnosed non-diabetic myocardial infarction patients admitted in Emergency and ICU wards. Group-II (controls): age and sex matched 300 healthy subjects with no history of diabetes mellitus and/or myocardial infarction were included. The blood samples were collected from all the cases and the control and were immediately analyzed for biochemical parameters under the study. The result indicates that there is a statistically significant increase in Systolic-BP, Diastolic-BP, fasting blood glucose, HbA1c, LDL-C, sialic acid, homocysteine and Hs-CRP levels in cases as compared to control group (<0.001). Also, there is a direct and significant correlation between sialic acid with homocysteine, Hs-CRP and HbA1c respectively ($r = 0.24, 0.24, 0.30, p < 0.05$). HbA1c showed significantly positive correlation with homocysteine, Hs-CRP and Diastolic-BP ($r = 0.27, 0.24$ and 0.19) respectively and had negative correlation ($r = -0.20$) with HDL-C. Statistically, significant positive correlation of homocysteine and Hs-CRP was seen with each other ($r = 0.29, p = 0.00$) and with rest of the parameters, except with HDL-C which showed negative but significant correlation. As the concentrations of these cardiac biomarkers are increased in non-diabetic AMI subjects, it is recommended that HbA1c, sialic acid, homocysteine and high sensitive C-reactive protein should also be included in the growing armament of biochemical cardiovascular disease markers for a better, early and more objective assessment of the disease.

Keywords: Cardiovascular diseases, Myocardial infarction, HbA1c, Sialic acid, Homocysteine, High sensitive C-reactive protein.

INTRODUCTION

Cardiovascular disease is a universal health problem. Changing clinical definitions, criteria and biomarkers add new challenges to our understanding and ability to develop the health of the people [1]. Among various cardiovascular diseases, Myocardial infarction (MI) is one of the leading causes of morbidity and mortality worldwide [2]. In US alone, about 1.5 million people suffer from MI every year [3] with about 30% mortality and more than 50% of deaths occur on way to the hospital. In India, 31.7% of deaths occur due to MI which was about 7% in 1970 and had increased up to 32% in 2011[4]. Demographic data indicate that the heart disease rate among Indians is double that of the national averages of the western world. This may be attributed to an underlying genetic predisposition to metabolic deregulation as well as a recent shift of

modifiable risk factors [5]. Traditional risk factors such as hypercholesterolemia, hypertension and smoking do not account for the entirety of risk and there are many people who have events and do not fit into the traditional definition of "high risk" [6]. So, new biomarkers must be developed in order to find ways of identifying those individuals at risk in an attempt to alter their course and to enhance risk stratification in coronary artery disease [7].

HbA1c is an indicator of average glycemia over the previous 6 to 8 weeks. High levels of HbA1c were associated with raised atherosclerotic lesions and more extensive fatty streaks in the coronary artery [8]. Sialic acid (SA), an acetylated derivatives of neuraminic acid, is a terminal carbohydrate residue of the non-reducing end of the oligosaccharide chains of glycoproteins and

glycolipids in sera and tissues. Serum sialic acid levels are correlated with carotid atherosclerosis and is independent of major cardiovascular risk factors [9]. Homocysteine is a sulfur-containing amino acid absent in naturally occurring dietary sources [10]. Homocysteine exerts a detrimental effect on vascular walls and especially on endothelial cells by decreasing nitric oxide bioavailability, increasing intracellular oxidative stress and by triggering multiple proatherogenic mechanism. Epidemiological studies have clearly demonstrated that plasma homocysteine is an independent risk factor for atherosclerosis [11]. C-reactive protein, an acute-phase reactant, is synthesized in the liver in response to interleukin-6. High sensitive C-reactive protein is a strong independent predictor of future cardiovascular events, including myocardial infarction, ischemic stroke, peripheral vascular disease, and sudden cardiac death in individuals without cardiovascular diseases [12].

Several changes in serum biochemical factors occur in acute myocardial infarction (AMI) and from a clinical perspective; this is a need of the hour to further explore new biomarkers by using newer analytical methods. In this study, we assessed multiple biomarkers representing distinct pathophysiological pathways in relation to cardiovascular events and proposed to evaluate the levels of HbA1c, sialic acid, homocysteine, high sensitive C-reactive protein (HsCRP) in non-diabetic individual with acute myocardial infarction and to assess the possibility of using these markers for coronary vascular events.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry in a tertiary care hospital in Srinagar for the period of one year. Total 600 patients were included in the study and were divided into two groups: Group-I (cases): 300 clinically diagnosed non-diabetic myocardial infarction patients admitted in Emergency and ICCU wards. Group-II (controls): age and sex matched 300 healthy subjects with no history of diabetes mellitus and/or myocardial infarction were included.

The present study was approved by the ethical committee of the hospital and after having the study explained, verbal informed consent was taken from the patients/attendants of the patients.

Inclusion criteria

All non-diabetic myocardial infarction patients of either sex with age more than 35 years diagnosed was based on ECG findings and cardiac enzymes (TROP -T / CPK - MB etc).

Exclusion criteria

Patients with diabetes mellitus having myocardial infarction, recent history of surgery and trauma within the preceding 2 months, renal insufficiency (serum

creatinine >1.5), Patients with cerebrovascular accidents or previous history of cerebrovascular accidents, patients having evidence of infections, inflammatory disease, malignancy, patient taking drugs like vitamin B-complex or folic acid, statins, hormone replacement therapy etc.

Sample Collection and Analysis

The initial evaluation of patients with myocardial infarction consisted of history, physical examination, ECG changes and laboratory investigations. The blood samples were collected from all the cases and the control and were immediately analyzed for biochemical parameters under the study.

Methods

Under all aseptic precautions, about 5 mL of venous blood samples were collected, and about 3 mL of collected blood was put in gel tubes, allowed to clot and it was centrifuged at 5000 rpm for 5 minutes. The serum thus separated was used for the estimation. Fasting blood glucose (FBG) was estimated by glucoseoxidase-peroxidase (GOD-POD) method. The remaining 2 ml of venous blood, collected in vacutainers containing EDTA was used for the estimation of glycated hemoglobin. HbA1c was estimated by Latex agglutination inhibition method, serum HDL-cholesterol, serum LDL-cholesterol was estimated by enzymatic end point method using in the Abbott auto analyzer. Serum homocysteine concentration was measured by competitive chemiluminescent enzyme immunoassay method. Serum sialic acid was estimated manually using thiobarbituric acid reagent as chromophore and estimation of High Sensitivity C-reactive protein by immune-zymometric chemiluminescence immunoassay.

Statistical analysis

The data obtained was compiled and analyzed using SPSS 11.5 for Windows version. Mean \pm standard deviation were calculated and student t-test was applied to find out significance level. Statistical significance was defined as two-tailed $p < 0.05$ for all tests unless otherwise specified. Pearson correlation test was used to find the correlation.

RESULTS

The study group included 300 cases (234 males, 66 females) having acute myocardial infarction without diabetes and 300 controls (192 males, 108 females). The mean age in cases and controls were 58.06 ± 11.80 and 48.70 ± 10.44 years respectively. Among cases, mean \pm SD of Systolic-BP, Diastolic-BP, fasting blood glucose, HbA1c, HDL-C, LDL-C, sialic acid, homocysteine and Hs-CRP were 133.76 ± 19.82 mm Hg, 84.20 ± 14.36 mm Hg, 106.74 ± 15.57 mg/dL, $6.27 \pm 0.81\%$, 27.80 ± 6.16 mg/dL, 165.62 ± 35.10 mg/dL, 81.16 ± 3.66 mg/dL, 14.0 ± 1.02 μ mol/L and 17.02 ± 3.93 mg/L respectively. In controls mean \pm SD of Systolic-BP, Diastolic-BP, fasting blood glucose,

HbA1c, HDL-C, LDL-C, sialic acid, homocysteine and Hs-CRP were 129.52 ± 6.80 mm Hg, 85.00 ± 5.02 mm Hg, 100.16 ± 6.80 mg/dL, $3.95 \pm 0.45\%$, 35.24 ± 7.18 mg/dL, 110.76 ± 14.99 mg/dL, 58.76 ± 6.54 mg/dL, 8.81 ± 1.35 μ mol/L and 1.80 ± 0.21 mg/L respectively. The result indicates that there is a statistically significant increase in Systolic-BP, Diastolic-BP, fasting blood glucose, HbA1c, LDL-C, sialic acid, homocysteine and Hs-CRP levels in cases as compared to control group (<0.001) (Table-1). However, HDL-C levels were significantly low in cases as compared to controls.

In cases, results showed that there is a direct and significant correlation between sialic acid with homocysteine, Hs-CRP and HbA1c respectively ($r = 0.24, 0.24, 0.30, p < 0.05$) (Fig. 1-3) whereas no

correlation was seen with Systolic-BP, Diastolic-BP, LDL-C and HDL-C. HbA1c showed significantly positive correlation with homocysteine, Hs-CRP (Fig. 4, 5) and Diastolic-BP ($r = 0.27, 0.24$ and 0.19) respectively and had negative correlation ($r = -0.20$) with HDL-C. Statistically, significant positive correlation of homocysteine and Hs-CRP was seen with each other ($r = 0.29, p = 0.00$) (Fig. 6) and with rest of the parameters, except with HDL-C which showed negative but significant correlation. Systolic-BP showed significant positive correlation with Diastolic-BP, LDL-C and homocysteine whereas there was a significant negative correlation with HDL-C levels. Diastolic-BP also showed the statistically significant correlation with HbA1c ($r = 0.19, p < 0.05$). Fasting blood sugar didn't showed correlation with any parameters (Table 2).

Table 1: showing Mean \pm SD of different parameters, their significance in non-diabetic acute myocardial infarction patients and controls

Parameters	Cases (n=300)	Controls (n=300)	p-value
Age (in years)	58.06 ± 11.80	48.70 ± 10.44	--
Sex (M/F)	234/66	192/108	--
SBP (mm Hg)	133.76 ± 19.82	129.52 ± 6.80	<0.001
DBP (mm Hg)	84.20 ± 14.36	85.00 ± 5.02	<0.001
FBG (mg/dL)	106.74 ± 15.57	100.16 ± 6.80	<0.001
HbA1c (%)	6.27 ± 0.81	3.95 ± 0.45	<0.001
HDL (mg/dL)	27.80 ± 6.16	35.24 ± 7.18	<0.001
LDL (mg/dL)	165.62 ± 35.10	110.76 ± 14.99	<0.001
Sialic acid (mg/dL)	81.16 ± 3.66	58.76 ± 6.54	<0.001
Homocysteine (μ mol/L)	14.0 ± 1.02	8.81 ± 1.35	<0.001
Hs-CRP (mg/L)	17.02 ± 3.93	1.80 ± 0.21	<0.001

Table 2: Correlation of different parameters and their significance

Parameters	SBP	DBP	HbA1c	LDL-C	HDL-C	Hcy	Hs-CRP	SA	
SBP	Pearson	--	0.82	0.47	0.22	-0.27	0.33	0.13	0.04
	Sig. (2-tailed)	--	0.00**	0.64	0.02*	0.01**	0.00**	0.18	0.63
DBP	Pearson	0.82	--	0.19	0.33	-0.22	0.35	0.17	0.09
	Sig. (2-tailed)	0.00**	--	0.04*	0.00**	0.02*	0.00**	0.09	0.33
HbA1c	Pearson	0.47	0.19	--	0.12	-0.20	0.27	0.24	0.30
	Sig. (2-tailed)	0.64	0.04*	--	0.21	0.03*	0.00**	0.01**	0.00**
LDL-C	Pearson	0.22	0.33	0.12	--	-0.11	0.32	0.33	0.05
	Sig. (2-tailed)	0.02*	0.00**	0.21	--	0.25	0.00**	0.00**	0.61
HDL-C	Pearson	-0.27	-0.22	-0.20	-0.11	--	-0.21	-0.19	-0.16
	Sig. (2-tailed)	0.01**	0.02*	0.03*	0.25	--	0.03*	0.05*	0.09
Homocysteine	Pearson	0.33	0.35	0.27	0.32	-0.21	--	0.29	0.24
	Sig. (2-tailed)	0.00**	0.00**	0.00**	0.00**	0.03*	--	0.00**	0.01**
Hs-CRP	Pearson	0.13	0.17	0.24	0.33	-0.19	0.29	--	0.67
	Sig. (2-tailed)	0.18	0.09	0.01**	0.00**	0.05*	0.00**	--	0.00**
Sialic Acid	Pearson	0.04	0.09	0.30	0.05	-0.16	0.24	0.67	--
	Sig. (2-tailed)	0.63	0.33	0.00**	0.61	0.09	0.01**	0.00**	--

* Correlation is significant at the 0.05 level (2-tailed), ** Correlation is highly significant at the 0.01 level (2-tailed).

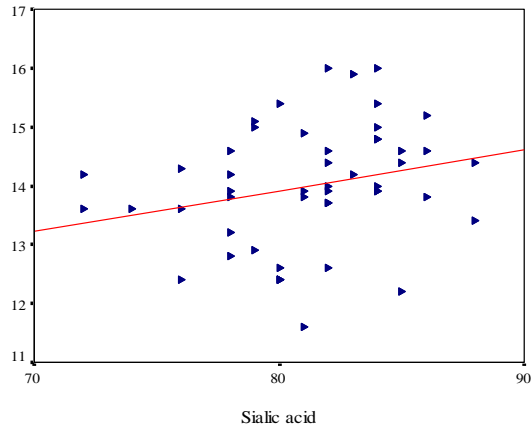


Fig. 1: Showing correlation between Homocysteine & Sialic acid

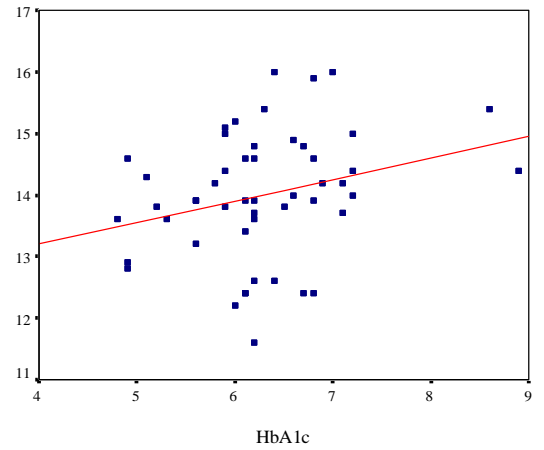


Fig. 4: Showing correlation between Homocysteine & HbA1c

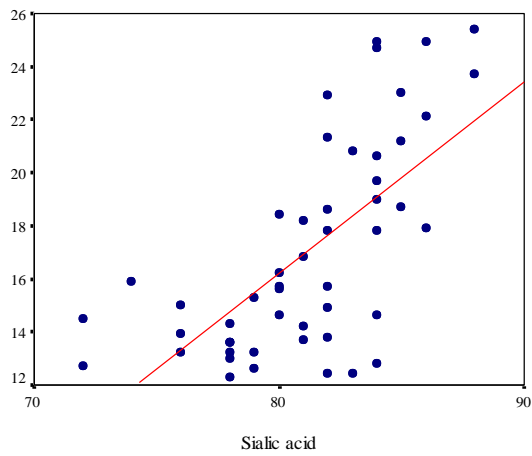


Fig. 2: Showing correlation between HsCRP & Sialic acid

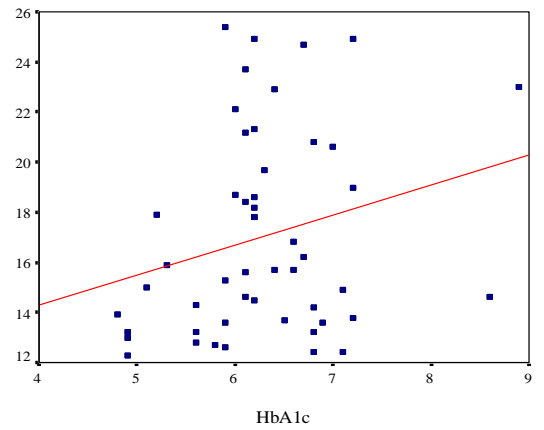


Fig. 5: Showing correlation between HsCRP & HbA1c

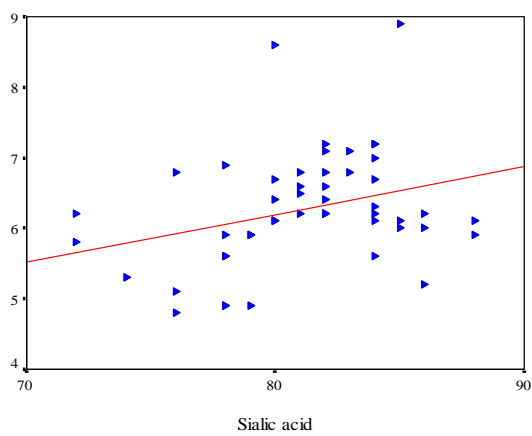


Fig. 3: Showing correlation between HbA1c & Sialic acid

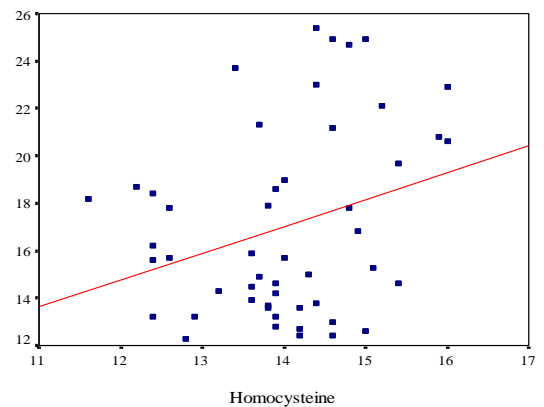


Fig. 6: Showing correlation between HsCRP & Homocysteine

DISCUSSION

Despite recent advances in the management of AMI, it remains one of the leading cause of death worldwide. There is a substantial interest in the use of newer biomarkers to identify persons who are at risk for the development of CVD. In this study, we observed that patients who had suffered an acute attack of MI had statistically significant increase of sialic acid with respect to controls, this is in concordance with the study conducted by Allain *et al.* which showed elevated level of sialic acid in patients with coronary heart disease [13]. Also sialic acid had a positive correlation with HbA1c, homocysteine and Hs-CRP it had a negative correlation with serum HDL-C supporting the observations by Rastam *et al.* [14]. Our finding showed that the mean serum sialic acid in patients with AMI was higher than those of control group, thus suggesting the role of sialic acid in AMI.

HbA1c concentration is an indicator of average blood glucose concentration over last 3 months and recently has been used as a diagnostic test for detecting diabetes. In EPIC – NORFOLK study showed possibility of HbA1c being a predictor of death from CVD among non-diabetic patients [15] and in our study there is a statistical significant increase in HbA1c levels in non-diabetic patients with MI with respect to controls which supported the results with that study. We found that HbA1c correlated significantly with homocysteine, Hs-CRP and sialic acid but showed negative correlation with HDL-C. Stakos *et al.* had demonstrated that HbA1c even in concentration on the higher side of normal range was independently related to left ventricular mass and aortic function. Higher HbA1c was associated with greater left ventricular mass and pulse wave velocity and lower aortic distensibility in asymptomatic non-diabetic individuals with normal glucose tolerance [16]. James *et al.* observed that HbA1c plays a contributory role in atherosclerosis even in non-diabetic subjects with CAD. Several literatures have claimed that HbA1c is a new cardiovascular risk factor [17-19].

Statistical analysis of data collected in this study showed that serum level of homocysteine in non-diabetes patients with AMI was significantly higher than those of control ($p < 0.001$). Humphrey *et al.* conducted a meta-analysis which included 24 cohort studies. They found that each increase of 5 $\mu\text{mol/L}$ in homocysteine level increases the risk of CHD events by approximately 20% [20]. Also, in two meta-analytical studies examining homocysteine and CVD showed a 25% lower homocysteine level reduced the risk of IHD by 11% and the risk of stroke by 19% [21, 22] There was significant correlation between homocysteine and Hs-CRP, HbA1c and sialic acid.

Duygu *et al.* suggested that Hs-CRP can be applied as an indicator for the development of atherosclerosis

[23]. Pearson *et al.* also suggested that Hs-CRP can be an independent and valid indicator for MI [24]. Ridker and Rafia showed that Hs-CRP levels can be used as the markers of atherosclerosis [25, 26]. In this study, we found significantly high levels of Hs-CRP (17.02 ± 3.93 mg/L) in the non-diabetic MI subjects. Soinio *et al.* also showed that Hs-CRP was independently associated with stroke or other vascular events over a 7 year follow up which is in concordance with our study [27]. Hs-CRP was significantly correlated with sialic acid, HbA1c, LDL-C and homocysteine but had negative correlation between HDL-C levels.

CONCLUSION

There is abundant evidence from the current study that novel biomarkers of atherosclerosis exist which exerts their effects on the arteries either in combination with or above and beyond the classical biomarkers. Thus, we recommend that HbA1c, sialic acid, homocysteine and high sensitive C-reactive protein should also be included in the growing armament of biochemical cardiovascular disease markers for a better, early and more objective assessment of the disease. As the concentrations of these cardiac biomarkers are increased in non-diabetic AMI subjects, therefore we suggest use of these biomarkers as a diagnostic tool for cardio-vascular patients.

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