DISCUSSION

The ongoing battle to reduce the incidence of cardiovascular disease has led to the discovery of various clinical markers. Although a list of traditional modifiable and non-modifiable risk factors are available, in the absence of them in some cases, a focus on other predisposing factors, which may contribute to myocardial infarction or predispose a person to the high risk of MI has led us to conduct the research to study an association between various cardiac biomarkers and AMI.

The present study was conducted in the department of biochemistry in Santosh Medical college Hospital &RC Ghaziabad and Combined District Hospital Sanjay Nagar Ghaziabad. A total of 172 patients were included in the study. The study patients were divided into two groups. Group I included 100 Diagnosed case of non-diabetic Myocardial infarction admitted in emergency & ICUs and Group II consisted of 72 Healthy subjects with no history of diabetes and/or myocardial infarction as controls. Inclusion and exclusion criteria were followed. All patients more than 35 years of age (male and female) of confirmed non diabetic Myocardial infarction based on ECG findings & Cardiac enzymes (Trop – T / CPK- MB) were included in the study. 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 pervious history of cerebrovascular accidents, patients having evidence of infections, inflammatory disease, malignancy, patient taking drugs like vitamin B-complex or folic acid, hormone replacement therapy and those who were not willing to participate were excluded from the study. In a cross sectional study by Ashraf MU[98] in Aligarh, 200 patients admitted with acute coronary syndrome in the coronary care unit of J N Medical College, AMU, Aligarh, India, were included in the study and Patients with cardiomyopathy, congenital heart disease, valvular heart disease; patients with severe comorbid conditions;

patients already taking folic acid, vit. B6, or vit. B12 supplementation were excluded from the study as seen in our study. In our study, we have tried to find the possible association between plasma homocysteine levels and CK-MB, LDH and CRP in Troponin positive(cases) and Troponin negative patients(controls). Informed consent was taken from all patients. Information extraction forms were used to collect data from patient files.

In our study, Troponin-T was taken as a standard positive marker for AMI and all correlations of the cardiac biomarkers were done on basis of the groups defined as Troponin T positive (cases) and Troponin-T negative (controls). It has been shown that Troponin-T serves as one of the most sensitive markers for myocardial injury. In Shah et al study, it was seen that Cardiac Troponin T was 20 times higher among patients than normal controls. Although both cardiac and skeletal muscles contains TnT like other cardiac markers, the amino acid sequence of the protein in the two types of muscles differs, thus making it possible to raise antisera against cardiac-specific TnT and detect it more efficiently. The high specificity and sensitivity of cardiac TnT in diagnosing and monitoring AMI, the undetectable values of cardiac troponin T in healthy individuals, has made its measurement a powerful tool in the diagnosis of acute myocardial infarction.[90]

In our study, the mean age of the cases were 62.15+-7.75 years and the controls were 61.49+-8.35 years. There was no statistical difference between the ages of two groups (p-value>0.05) Troponin T positive group had 29% females and 71% males and the control group had 29.17% females and 70.83% males, which was comparable (p-value>0.05). In a similar study, mean age was 56.3±13.2 years, 145 (72.5%) were males and 55 (27.5%) females.[98]In a study by Sorathia P[97] the mean age of the 30 cases were 48.5+-10.3 years and the 20 controls were 44.5+-12.8

years which was comparable. In another study by Prajapati P(2016)[100] there were around 85% males and 15% females with maximum patients between 36-50 years of age. In a latest study by Halder et al (2017),[102] the mean age of the cases were 55.66± 6.86 years in males, and 56.23±5.42 years in females) and in controls were 54.97±6.45 in males and 57.30±4.14 years in females which was comparable but slightly less than our study.

CK-MB is one of the most important myocardial markers, and it is an established marker in confirmation of AMI. In AMI, plasma concentration of CK-MB increases within 4-9 hours of onset of chest pain, the peak is reached within 9-30 hours and return to baseline levels after 48 - 72 hours.[40]

In the present study, the mean values of CK MB in the Troponin T positive group was 111.94 +- 29.59 and in the Troponin T negative group was 16.36 +- 3.77. The difference was statistically significant (P-value < 0.0001) CK MB was deranged in 100% Troponin T positive group, and in only 2.78% in Troponin T negative group. The difference was statistically significant (P-value < 0.0001). Shah H et al[90] showed that CK-MB values were better than CK and the mean values of CK-MB after 6 hours of chest pain were 98.8 +- 72.7 and was 8.5 to 14.9 among controls in Troponin-T negative patients which corroborated with our study. Aseri ZA[95] showed a significant difference between CK-MB values of STEMI and NSTEMI patients. (p=0.034) Thereby proving the relevance of CK-MB as a cardiac marker. Shah PK[99] also assessed various cardiac markers in 50 AMI cases and 20 controls. The mean values of Total CPK was 148 ± 279.1 in control and 648 ± 806.7 in MI cases. CPK-MB was 20.9 ±9.58 in control and 89.4 ± 118.07 in MI cases. The difference was statistically significant.(P=0.002) and it was comparable to our study.

Creatinine kinase catalyses the transfer of phosphate group from ATP to creatine to form creatine phosphate . CK, which is also known as CPK has three isoenzymes each having two subunits M(muscles) and B(brain) and exist as CPK-BB,CPK- MB,CPK-MM. Increase in the total plasma CK activity signifies skeletal muscle damage and it is not AMI-specific. However CPK-MB fraction (also called CK-2) comprises about 40% of the CK activity in cardiac muscle and 2% or less of the activity in most muscle groups and other tissues is the first enzyme to be released in to circulation and serves as a more sensitive and specific marker for MI.[99] Due to the newer methods of detection and sensitive CK-MB quantitative assays, its role has increased in the early diagnosis of AMI.

Serum lactate dehydrogenase (LDH) is a known pathologic marker for a diversity of diseases, including myocardial ischemia. Its activity rises in serum within 12-24 hrs after AMI, reaches to peak level after 48-72 hrs after AMI and comes back to normal in 10-14 days. That's why considered as late marker.[14,99]

In the present study, LDH was deranged in 100% Troponin T positive group, and in only 16.67% in Troponin T negative group. The difference was statistically significant (P-value < 0.0001). The mean value of LDH in the Troponin T positive group was 564.43 +- 110.99 and in the Troponin T negative group was 223.68 +- 36.23. The difference was statistically significant (P-value < 0.0001). Shah PK(2016)[99] results were slightly higher than ours for LDH values. The mean value of LDH was 510 ± 199.4 in control and 836.6 ± 553.1 in MI cases.(P=0.002) Aseri ZA[95] showed a significant difference between LDH values of STEMI and NSTEMI patients. (p=0.047) Thereby proving the relevance of LDH as a cardiac marker. Another study showed that the LDH values in males were 442±3.02 whereas in females was 458±3.82 showing a

significant raise when compared with the controls 286±3.02 (P<0.001)[1] which was matching our study.

Lactate dehydrogenase (LDH or LD) is an enzyme with five isoenzymes LDH 1 to 5, and is tetrameric having two types of subunits namely M (for muscles) and H (for Heart) whose most important role is catalyzing the interconversion of pyruvic acid and lactic acid by NAD+ dependent reaction. LDH-1 isozyme is normally found in the heart muscle and LDH-2 is found mainly in the blood serum. In case of myocardial infarction the activity of LDH1 is much more greater. Thus a high ratio of LDH-1 level to LDH-2 is able to suggest MI. Normal level of LDH is usually less than 250 units/liter. However, the exact range of normal value varies between labs and patients.[99] Thus it should be interpreted carefully and is not a very specific cardiac marker as it stays high for a longer time and thus cannot identify a reinfarct.

Inflammation plays an important role in the pathogenesis of atherosclerosis. The chronic inflammatory process develops to an acute clinical event by the induction of plaque rupture and, therefore, cause acute coronary syndromes.[95]

In the present study, the mean values of CRP in the Troponin T positive group was 15.69 +- 4.04 and in the Troponin T negative group was 6.08 +- 2.02. The difference was statistically significant (P-value < 0.0001). Aseri ZA[95] showed a significant difference between hsCRP values of STEMI and NSTEMI patients. (p=0.023) This observational study reports that the inflammatory markers measurement in ACS, prior to the tissue necrosis, is significantly related with classical cardiac enzyme markers and may be of prognostic significance. CRP has been studied mostly in acute coronary syndromes as a marker of proinflammatory state and plaque instability. Few studies also found that there was no significant correlation between hsCRP levels

and Gensini score index and no relationship between hsCRP levels and the presence and severity of CAD in patients with stable angina.[95]

In our study, the mean value of Homocysteine in the Troponin T positive group was 30.56 +- 19.79 micromol/L and in the Troponin T negative group was 10.28 +- 4.03 micromol/L. The difference was statistically significant (P-value < 0.0001) Homocysteine was deranged in 98% Troponin T positive group, and in only 18.06% in Troponin T negative group. The difference was statistically significant (P-value < 0.0001). In other similar studies, the mean value of Homocysteine in the cases was 25.89 +- 6.18 micromol/L,[97] 24.59 +- 6.14 micromol/L[91] and in the controls was 13.83 +- 3.51 micromol/L,[97]and 13.73 +- 3.54 micromol/L.[91] The difference was statistically significant (P-value < 0.01) as seen in our study. In another study on 100 patients by Prajapati P, Homocysteine values were below 13 in 13% patients and high in 87% patients. It was >50 in 17 % patients and 13-50 in 70% patients. They also concluded that hyperhomocysteinemia was more common in vegetarians(78.16%) as they lack vitamin B12 in their diet, than non vegetarians(21.83%).[100]In another study by Halder et al(2017),[102]the mean plasma level of homocysteine in patients (16.88 ± 10.84μmol/ lit) was slightly higher than the control group (14.18 ± 4.19 μmol/lit).His findings were in contrast to our study as the mean Homocysteine values were not significantly different among cases and controls. Their results do not support the hypothesis that coronary heart disease is related to high serum homocysteine concentration. The results are not conclusive due to inability to adequately control for potential confounders as well as inadequate sample size.[102]

A meta analysis conducted by Boushey et al, study conducted by Stampfer et aland Chamber et al, all showed that homocysteine was an independent, risk factor for coronary heart disease , independent of conventional risk factors.[91] Ashraf MU[98]showed Homocysteine association with

Troponin-I. An association between homocysteine concentrations and plasma markers of thrombosis activation in patients admitted for acute coronary syndrome was shown in the study by Al-Obaid et al, 2000.[87]

All these studies signify the importance of hyperhomocysteinemia as an independent risk factor in acute Myocardial infarction and thusserum homocysteine should be evaluated in all MI patients in collaboration with other cardiac biomarkers.

Homocysteine is known to induce atherothrombosis in many ways: like homocysteine thiolactate, a by product of oxidation of homocysteine which combines with LDL to form foam cells. The LDL rich foam cells then get embedded in the vascular endothelium and become fatty streak, which is the starting of an atherosclerotic plaque. Homocysteine thiolactate has also been suggested to impair oxidative phosphorylation and enhance proliferation and fibrosis of smooth muscle cells in the vessels.[91]

One mechanism is that the homocysteine reduces the concentration of HDL cholesterol in plasma by inhibiting the hepatic synthesis of apoA-I, the main HDL apolipoprotein. HDL which is a good cholesterol and maintains the reverse cholesterol transport is decreased and chances of atherosis starts increasing. Elevated homocysteine also cause endothelial cell injury which is followed by platelet aggregation and thrombus formation and lastly atherosclerosis.[100]

Homocysteine enhances lipid peroxidation which may decrease the expression of endothelial NO synthase and directly degrade NO. Auto oxidation of homocysteine results in oxidation of LDL through generation of the superoxide anion radical. Homocysteine may also reduce the antioxidant status which could injure endothelial cells. Homocysteine stimulates platelet generation of thromboxaneA2, which is a vasoconstrictor and proaggregant.[91]

Studies on the association of hyperhomocysteinemia with coronary artery disease in different populations have yielded different results with some studies providing evidence for an association while others have not (Deepa et al, 2001; Chacko 1998). This may be due to the ethnic or geographical differences or due to differences in the case selection. As genetic background and nutritional intake vary in different populations, the homocysteine level varies in different ethnic groups and this may be due to the polymorphism seen in the genes encoding enzymes involved in the metabolism of homocysteine.[91]

Our study results should be interpreted within the context of several limitations. First, the study design prevents inference of causal relationships among variables. Further, serum troponin and ejection fraction correlate with the extent of myocardial necrosis in patients of acute coronary syndrome was not done in our study as both these parameters have been shown earlier to be associated with higher cardiovascular morbidity and mortality in patients admitted with acute coronary syndrome.[98] Our findings of strong association of serum homocysteine with the cardiac biomarkers and Troponin T positivity indirectly highlights the significance of levels of serum homocysteine in the extent of myocardial damage and the immediate outcomes of patients of acute coronary syndrome. Finally, it is unclear whether the results of the study would generalize to other ethnicities and geography. Further research with a larger sample size must be conducted in major areas to confirm the validity of our results.

Summary and Conclusion

To summarise, the present study was conducted in the department of biochemistry in Santosh Medical college Hospital & RC Ghaziabad and Combined District Hospital Sanjay Nagar Ghaziabad. A total of 172 patients were studied in 2 groups. Group I included 100 Diagnosed case of non-diabetic Myocardial infarction and Group II consisted of 72 Healthy subjects as controls (Troponin T negative). All patients were more than 35 years of age (male and female).

Confirmed non diabetic Myocardial infarction cases based on ECG findings & Cardiac enzymes (Trop – T) were included in the study. 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 pervious history of cerebrovascular accidents, patients having evidence of infections, inflammatory disease, malignancy, patient taking drugs like vitamin B-complex or folic acid, hormone replacement therapy and those who were not willing to participate were excluded from the study.

The socio-demographic details of the study were comparable. The mean age in the cases were 62.15+-7.75 years and in the controls were 61.49+-8.35 years. Troponin T positive group had 29% females and 71% males whereas the control group had 29.17% females and 70.83% males.

CK-MB, CRP, LDH and Homocysteine values individually showed significant difference among Troponin T positive and negative patients. Overall Homocysteine showed strong correlation with CK-MB, CRP and LDH.

On performing univariate logistic regression, CRP and homocysteine levels were the significant risk factors of Troponin T positive patients and CK-MB and LDH showed high Standard error and thus did not show correlation.

After analysing the results we recommend that a panel of biomarkers shall be tested for acute MI patients with homocysteine being a part of it.

In conclusion, the current study highlights the fact that elevated serum homocysteine level has a strong correlation with and cardiac troponin-T in AMI patients. Therefore, it may be concluded that CRP, LDH, CK-MB and serum homocysteine are an independent and associated risk factor for AMI and thus shall be tested for all patients of AMI. Each cardiac marker rises and falls at a particular time and thus all values shall be noted to ascertain a fresh MI or recurrent MI. As Hyperhomocysteinemia can be treated easily, further studies may be required to ascertain the effect of homocysteine lowering treatment on the extent of myocardial injury during admission for acute MI.