ORIGINAL ARTICLE

COMPARISON OF SERUM HOMOCYSTEINE AND FOLIC ACID LEVELS IN ACUTE MYOCARDIAL INFARCTION AND NORMAL HEALTHY POPULATION

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Background: Acute Myocardial Infarction (AMI) is a leading cause of mortality worldwide. An emerging risk factor for AMI is raised levels of Homocysteine (Hcy). Deficient levels of folic acid are associated with Hyperhomocysteinemia (HHcy). Fortification of folic acid has been known to improve endothelial dysfunction. We aimed to determine the levels of Hcy and folic acid in patients with AMI and healthy individuals. **Methods:** This cross-sectional comparative study was conducted on 80 subjects, with 40 subjects in each group. Group A included individuals diagnosed with AMI and group B included healthy individuals. Serum Hcy and folic acid levels were measured by Enzyme Linked Immunosorbent Assay (ELISA). Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured, and Body Mass Index (BMI) was calculated. **Results:** Increased Hcy and lower serum folic acid levels were found in group A as compared to group B (p<0.001). The correlation between serum Hcy level and folic acid in group A was moderate and negative (r= -0.48, p=0.001); for group B it was also moderate and more negative (r= -0.66, p<0.001). **Conclusions:** There were high levels of Hcy and low levels of folic acid in diseased group as compared to healthy participants.

Keywords: Acute Myocardial Infarction, AMI, Myocardial Infarction, MI, Coronary Artery Disease, CAD, Folic acid, Homocysteine, Hcy

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INTRODUCTION

The major causes of mortality in both developed and developing nations are Coronary Artery Disease (CAD) and Acute Myocardial Infarction (AMI).[1] Thirty-seven percent of all cases of symptomatic heart failure were ascribed to AMI alone globally and the incidence is anticipated to be doubled in South Asia than in any other region worldwide over the next two centuries.[2] Persistent and severe post-sternal pain are typical clinical symptoms of AMI.[3] Prompt diagnosis of AMI is crucial for the timely application of therapy to preserve cardiac function and limit myocardial injury.[4] According to WHO criteria, history of chest pain, physical examination, estimation of cardiac troponins levels and electrocardiogram (ECG) are tools for diagnosis of AMI.[5]

Hypertension (HTN) and smoking are the two important risk factors in almost 90 percent of patients with AMI.[6] One of the risk factors for AMI is raised Hcy levels.[7] Hcy levels normally vary between 5–15 µmol/l.[8] An unusually higher blood level of Hcy (>15 µmol/l) is termed as Hyperhomocysteinemia (HHcy) and is considered mild if values are between 15.1 to 30 µmol/l, moderate if between 30.1 to 100 µmol/l, and severe if >100 µmol/l.[9] HHcy causes the proliferation and thickening of smooth muscle cell in the intima of the blood vessel and upsurge in the oxidation of low-density lipoproteins which then activates the coagulation system leading to thrombi and thus AMI.[10]

Certain vitamin deficiencies like folic acid and vitamin B_{12} or B_6 also contribute to raised plasma Hcy levels. The real determinant of serum Hcy level is folic acid and there exists an inverse relationship between the levels of these two in the patients suffering from AMI. Folic acid levels between 6 to 17 ng/ml are considered normal.[11] In the United States 17,000 deaths from cardiovascular events each year have been prevented by recommending food fortified with folic acid to these patients, thereby reducing their Hcy levels.[12]

The association between Hcy levels and AMI disease is still a matter of controversy and there is a debate going on in literature. One study undervalued the risk of having AMI in patients with high blood Hcy levels.[13] On the other hand in recent two large studies meta-analysis was done with a total of 6,814 patients, and demonstrated that there was a higher risk prediction of developing AMI when blood Hcy levels were raised.[14] It has been anticipated that metabolic dysfunction occurs in South Asians due to their genetic and lifestyle aspects, and they are susceptible to cardiometabolic ailments; yet it has not been proven.[15] A study from all the provinces of Pakistan found that around 28% of AMI patients were below 45 years of age.[16] In a study at Faisalabad, nearly one-third of patients suffering from AMI were of age <45 years.[17] Definitive data depicting association of raised serum Hcy and lowered folic acid levels with AMI is lacking in Asia in general and Pakistan in particular. The present study was planned to determine and compare the serum levels of Hcy and folic acid following AMI in the local population to compare them and to determine their importance, if any, as new emerging risk factors.

MATERIAL AND METHODS

In this cross-sectional comparative study, 40 patients (group A) admitted to Cardiac Care Unit of Pakistan Institute of Cardiology (PIC), Lahore with documented AMI showing characteristic ECG signs and rise in troponin I concentration were recruited. From local community 40 healthy volunteers were included as a control group (group B) after considering the inclusion and exclusion criteria. Approval of the study was taken from ethical review board of University of Health Sciences (UHS), Lahore. Convenient sampling technique was used for the collection of samples. Individuals suffering from chronic systemic diseases, pregnant women, smokers, and individuals taking folic acid and/or vitamin B_{12} were excluded.

All the experimental work was carried out in the Department of Physiology and Cell Biology, UHS, Lahore. Data was collected in October 2018, after obtaining written informed consent from each subject. Information regarding demographic data was recorded. Every individual was assessed clinically by taking history and performing general physical and systemic examination. Systolic and diastolic blood pressure was recorded. Weight in Kg and height in Cm were recorded for calculation of BMI. Under aseptic conditions, 3–5 ml blood was drawn from ante cubital vein. Serum was obtained through centrifugation technique and stored at -70 °C. Serum Hcy and folic acid levels were measured with ELISA kits (Biolab, England). HHcy was considered if Hcy levels were >15 µmol/l. Folic acid levels were taken as deficient if <6 ng/ml.

SPSS-23 was used for data analysis. Mean±SD and median with inter-quartile range was given for quantitative variables. Frequency and percentage were given for gender. Normality of data was checked by Shapiro-Wilk test. For normally distributed data, independent student *t*-test was used and in case of not normally distributed data and for comparison of the mean difference in quantitative variables between groups, Mann Whitney U test was applied. Chi-square-test was applied to determine the gender difference between groups. Pearson correlation coefficients were used for correlation analysis between serum Hcy levels and folic acid levels, and $p \leq 0.05$ was considered statistically significant.

RESULTS

Sixty-two (77.5%) participants out of 80 were male. In group A of AMI patients, 34 (85.0%) participants were male, whereas in group B (healthy individuals), 28 (70.0%) participants were female. Chi-square test revealed no differences in gender distribution between groups (p=0.11). Mann Whitney U test showed no significant differences between the median age of AMI patients (46.0 years) compared to healthy controls (45.0 years; p=0.19). There were no significant differences between the median pulse rate of AMI patients. Group A and B had mean BMI 25.3±3.6 and 23.9±3.4 respectively. No statistically significant differences were noted in mean BMI among both the groups (p=0.08). Median SBP and DBP of group A was 110 (110–120) mmHg and 80 (70–90) mmHg respectively and median SBP and DBP of group B was 110 (110–120) mmHg and 80 (70–80) mmHg, respectively. No statistically significant differences were noted in SBP (p=0.75) and DBP (p=0.98) between the groups (Table-1).

There were significantly raised levels of mean serum Hcy of AMI patients (22.9±6.0 µmol/l) in comparison to healthy controls (12.1±3.1 µmol/l, p<0.001). There were significantly lower levels of mean serum folic acid in AMI patients (6.29±1.50 ng/ml) in comparison to healthy controls (9.09±2.14 ng/ml, p<0.001) (Table-2).

The correlation between serum Hcy level and folic acid in group A and group B was moderate and negative (r= -0.48, and -0.66 respectively; p<0.001).

Table-1: Comparison of study parameters between groups A and B (n=40)

Parameters	Group	Mean±SD	Median (IQR)	Min	Max	*p*
Age	A	46.8±8.8	46 (41–54)	28	66	0.19
	B	43.9±5.2	45 (40–47.5)	28	50	
Pulse rate per minute	A	78.4±10.7	80 (70–90)	60	98	0.05
	B	74.1±8.7	75 (70–80)	60	90	
Body Mass Index	A	25.3±3.6	24.2 (23–28.3)	16.5	31.6	0.08
	B	23.9±3.4	24.1 (21.3–26)	18.5	33.2	
Systolic BP	A	115.9±12.2	110 (110–120)	90	160	0.75
	B	114.1±6.9	110 (110–120)	90	125	
Diastolic BP	A	76.5±8.9	80 (70–90)	60	100	0.98
	B	76.0±7.1	80 (70–80)	60	90	

Table-2: Serum folic acid and Hcy level among groups (Independent Student's *t*-test) (n=40)

Groups	Mean±SD	Median (IQR)	Min	Max	*p*
Serum Folic Acid Level					
A	6.29±1.50	6.15 (5.10–7.55)	3.8	9.3	<0.001
B	9.09±2.14	9.15 (7.73–10.88)	4.9	13.5	
Serum Hcy Level					
A	22.9±6.0	22.2 (19.2–27.3)	10.8	37.0	<0.001
B	12.1±3.1	11.4 (9.4–14.5)	7.5	19.1	

DISCUSSION

In the present study it was observed that the ages of AMI patients and the healthy participants were not statistically significantly different. Our results agree with a recent study conducted in Israel[18] and Pakistan[10] which showed no statistically significant difference among different age groups in AMI. However, our results differ from another recent study[19] which showed a significant difference between age groups. Similarly, the gender distribution between AMI patients and healthy participants did not show any statistically significant difference. These findings are similar with a recent study which also showed no significant difference.[20] However, women are comparatively at a higher risk of having AMI and thus fatality due to infarction as compared to men.[21]

Overweight class has been defined as BMI ≥25 Kg/m^2 according to revised criteria for inhabitants of Asia.[22] There were no statistically significant differences between both groups which is similar to a study, in which no statistically significant difference was found between both groups.[23] However, these results are in contrast to the results of a study conducted in Pakistan where significant statistical differences were found among both the groups.[6] Results of our study could be possibly due to limited sample size and unmatched gender between both the groups.

The difference in pulse rate was found to be statistically insignificant between the diseased and healthy participants. These results are comparable to a study in which there was no significant differences in heart rates among AMI patients and healthy individuals (p=0.26).[24] One possible explanation could be the physiological changes undergoing in the body's vascular system, for example, vasodilation and vasoconstriction of vessels which tries to maintain homeostasis.[25] In the current study, no statistically significant differences were observed in mean SBP and DBP between diseased and healthy study participants. However, our results contrast with a previous study in which patients with HTN were at more risk for AMI (p<0.05).[4] That study had a larger sample and it had 251 AMI cases and 464 age-matched and sex-matched non-AMI controls. The insignificant results in our study could be explained for not having a large sample size.

The diseased group in our study had statistically significant higher serum Hcy level as compared to healthier group. Our results are in agreement with the previous work in which Hcy levels were found raised in diseased group compared to healthy group.[24] The possible reason for increased Hcy levels in our study could be explained with the increased demands for DNA synthesis when the damaged tissue is undergoing repair which require the methylation of DNA, RNA and proteins. These reactions lead to production of Hcy as the end point in the methylation pathway.

Group A had mean serum folic acid level of (6.29±1.50) ng/ml and group B (9.09±2.14) ng/ml. Our results showed a statistically significant difference (p<0.001) in average serum folic acid levels between the diseased and healthy participants. The AMI patients had lower serum folic acid level as compared to healthy individuals. One possible explanation could be an unhealthy lifestyle with unbalanced diet especially lacking in fruits and vegetables leading to decreased folic acid and increased Hcy levels in our population. Our results are similar to a study which showed inverse relationship between the Hcy and folic acid levels in AMI patients.[26] These results are in contrast with a study conducted in India.[27] The results of our study showed a significant correlation between serum Hcy and folic acid levels, i.e., increased Hcy levels are associated with decreased folic acid levels in both AMI and healthier group, but the correlation was more negative in later. One possible explanation could be that diet of healthier group could have been more fortified with folic acid as compared to diseased group. Another reason could be that healthier participants may be using folic acid supplements. These results are in accordance with a study which showed significant correlation (inverse/ negative) between folic acid and Hcy in AMI group.[28] Our findings are in contrast with the results obtained in a study which showed no significant correlation between Hcy and folic-acid levels in both groups.[29]

There were certain limitations of our study. Being a single centre cross-sectional-study, the sample size was small and geographically limited. Further work with better matched sampling and accountability for influencing factors, is required for a better acquaintance of the role of Hcy and folic acid in the pathophysiology of AMI.

CONCLUSION

Hcy levels were higher and folic acid levels were lower in subjects with AMI than the healthy individuals. The results indicate the possibility that decreased folic acid levels due to improper diet as well as lack of supplementation may contribute to the progression of AMI and thus maintaining these levels in normal range may contribute to a better management of AMI.

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