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Plasma Homocysteine Status in Patients with Ischemic Heart Disease

Zahra Rasheed[1], Mehreen Ijaz[2], Namood-e-Sahar[3], Zohra Tariq[4], Rafia Fatima[5], Madeeha Tariq[6]

[1]Lecturer, Department of Home Economics, Lahore College for Women University, Lahore, Pakistan.
[2]Associate Professor, Department of Home Economics, Lahore College for Women University, Lahore, Pakistan.
[3]Lecturer, Department of Home Economics, Lahore College for Women University, Lahore, Pakistan.
[4]Lecturer, Department of Home Economics, Lahore College for Women University, Lahore, Pakistan.
[5]Lecturer, Department of Home Economics, Lahore College for Women University, Lahore, Pakistan.
[6]Teaching Assistant, Department of Home Economics, Lahore College for Women University, Lahore, Pakistan.
Correspondence: zohra_tariq@hotmail.com[4]

ABSTRACT

Aim of the Study: The study aimed to evaluate total plasma homocysteine (tHcy) levels in patients with already developed Ischemic Heart Disease in the Pakistani population.

Methodology: The current study was a cross sectional study. A total of 120 patients undergoing angioplasty procedure were selected through convenient sampling from angiography ward of Punjab Institute of Cardiology (PIC), Lahore. Fasting blood samples were drawn and analyzed in laboratory for tHcy levels.

Results: Results indicated elevated homocysteine levels (16.58±7.73) Mean±Standard deviation (SD). The study found higher levels of plasma homocysteine in males as compared to females (17.66±7.97 vs 12.97± 5.64) Mean± SD. No significant relationship was found between age, Body Mass Index (BMI)-Obesity and fasting hours of patients with tHcy. Homocysteine levels t(42)= 2.56,p=0.014 were also found to be high in patients suffering from Hypertension in comparison to diabetes.

Conclusion: The study highlights the significance of homocystine to be recognized as an essential biomarker in assessment and therapeutic management of heart disease in developing countries like the Pakistan. It supports the claims of previous studies recognizing homocysteine as a significant and possibly independent risk factor for incidence and progression of IHD. It is suggested that plasma homocysteine should be identified as an important risk factor by the clinicians and dieticians. Managing homocysteine levels in the prevention and treatment of ischemic heart disease should be considered in the Pakistani population.

Keywords: Plasma, Homocysteine, Ischemic Heart Disease, Angioplasty, Developing Countries, Pakistan, BMI, Hypertension.

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Introduction

Inadequate myocardial perfusion, which is a symptom of ischemic heart disease (IHD), can be brought on by either a decreased blood supply or an increased need for oxygen in the heart is, by far, the most typical cause of myocardial ischemia, which is why IHD is also known as coronary heart disease (CHD) (de Louna & Fiol-Sala, 2008; Falk et al., 2007). According to World Health Organization (WHO) statistics, 80% of 17 million deaths all around the world occurred due to cardiovascular disease in developing countries (Aje, & Miller, 2009). Many studies state developing South Asian countries like India, Pakistan, Sri Lanka, Bangladesh and Nepal are found to have the highest proportion of cardiovascular disease (CVD) as compared to any other region globally (Ramaraj, & Chellappa, 2008). High disease burden of IHD have been concluded by researchers in the Pakistan (Abbas, Katchlew, & Abbas, 2009). As conventional risk factors have been unsuccessful in explaining part of the cases, homocysteine- a sulfur containing amino acid, an intermediate product i.e. produced during the metabolism of methionine, a newly identified risk factor is being viewed with escalating interest (Aje, & Miller, 2009; Stanger, et al., 2004; Wilson, 2004).

Higher plasma homocysteine concentrations are associated with an increased incidence of extracranial carotid artery stenosis of > or = 25% in both men and women thus leading to a high prevalence of IHD (Sydow, & Boger, 2001). In 1969 McCully first proposed the homocysteine "hypothesis of arteriosclerosis" when he observed premature coronary, peripheral and cerebral vasculature atherothrombosis in children suffering from homocystinuria. Wilcken and Wilcken provided the first evidence in general population in 1976 of a relationship between disorder in homocysteine metabolism and heart disease. Since then many epidemiological and clinical investigations have implied the role of homocysteine in CVD and suggest that it can be helpful in identifying individuals at risk (Ciaccio, et al., 2008; Refsum, et al., 2006; Smith, & Refsum, 2021). In addition to being the risk factor it may also contribute to aggravate the disease progression (Ajoston-Coldea, et al., 2010)

Usually two pathways are involved in the metabolism of homocysteine in the body: remethylation and transsulfuration. Remethylation takes place in non-hepatic cells during which homocysteine acquires a methyl group from methyltetrahydrofolate (MTHF) and in a vitamin B12-dependent reaction forms methionine (Loscalzo, 2006). Methyltetrahydrofolate (methyl donor) depends on availability of methylenetetrahydrofolate for its formation. Methylentetrahydrofolate is derived from dietary folate, and methylentetrahydrofolate reductase (MTHFR). Adenosine triphosphate (ATP) activates a considerable proportion of the methionine to form S-adenosylmethionine (SAM) which serves as a universal methyl donor to various acceptors like hormones, nucleic acids, phospholipids and neurotransmitters. Homocysteine is formed again as the by-product of methylation reactions S-adenosylhomocysteine (SAH) is hydrolyzed. This homocysteine again becomes available for initiation of another cycle of methyl-group transfer (Ciaccio, et al., 2008). In transsulfuration pathway, taking place in hepatic cells, condensation of Homocysteine with serine takes place to form cystathionine. This is an irreversible reaction which is catalyzed by the cystathionine β-synthase (CBS), a pyridoxal phosphate-containing enzyme. Next hydrolyzation of cystathionine to cysteine takes place, the excess of which is excreted through urine (Loscalzo, 2006).Thus, excess homocysteine is catabolyzed effectively through the pathway of transsulfuration. Plasma normally maintains homocysteine levels up to 10μmol/L with the help of cellular homocysteine export mechanism (Ciaccio, et al., 2008).

Severe hyperhomocysteinemia (>100μmol/L), a rare genetic defect is caused by the deficiency of methylenetetrahydrofolate reductase, cystathionine beta synthase or in enzymes involved in methyl-B_{12} synthesis and homocysteine methylation. Hyperhomocysteinemia that is mild (15-20 mol/L) or severe (20-50 mol/L) is typically caused by acquired disorders. Vitamins insufficiency especially those required as cofactors or substrate in Homocysteine metabolism has been found to be the most frequent cause. An inverse relation has been observed between serum homocysteine levels and serum vitamin B_{12}, B_6 and folate levels (Ciaccio, et al., 2008). Other causes of elevated total plasma homocysteine include lifestyle factors, such as smoking, heavy coffee consumption, and lack of exercise (Nurk, et al., 2004). Plasma

homocysteine can be raised by various drugs and conditions interfering with the metabolism of folate, vitamin B12 and vitamin B6 (Stanger, et al., 2004; Ciaccio, et al., 2008).

Majority of the known type of damage are associated with oxidative stresses mediated by Homocysteine (Stanger, et al., 2004). It has been still not clearly stated how elevated Homocysteine causes the impairment of vascular function. Laboratory studies have indicated a number of potential causes, such as endothelial function impairment, reactive oxygen species (ROS) production and low density lipid oxidation (Hayden, & Tyagi, 2004). As a consequence increased monocyte adhesion to the vessel wall , activation of the inflammatory pathway, increased lipid uptake and retention, stimulatory effects on smooth-muscle proliferation, hypofibrinolysis, platelet dysfunction and thrombotic tendency caused by coagulation factors activation (Sydow, & Boger, 2001; Ciaccio, et al., 2008).

Based on the everyday rise in the epidemiology of cardiovascular disease among the Pakistani population, the current study aimed to assess the status of this recently identified risk factor, i.e. homocysteine in patients who have already developed IHD. The focus of the study was to determine whether this risk factor is found in the Pakistani population who has developed heart disease. This would help create awareness related to this worldwide acknowledged risk factor amongst the patients as well as personnel related to the medical field, especially those involved in managing or handling such patients.

Methodology

The current research had a quantitative approach and was mainly a cross-sectional study. Hyperhomocysteinemia is a recognised risk factor for coronary artery disease (CAD). The current study aimed to assess plasma homocysteine status in patients with developed IHD.

Subjects

Sample was collected from a total of 120 patients with an age range of 32-64 years including both males and females from the angiography ward of Punjab Institute of Cardiology (PIC), Lahore. Informed written consent was received from patients beforehand. Patients whose blood samples showed clotting were excluded. Therefore, a total of 100 patients were included in study. Only those patients were selected who had undergone angioplasty procedure and were admitted in hospital overnight for observation. Ischemic Heart disease was confirmed with evidence of electrocardiogram (ECG) report, angiography report and blood tests confirming their condition. Demographic characteristics, clinical history hours of fasting were also recorded using a self-structured questionnaire.

Blood Collection

Fasting (7-19 hrs) blood samples were collected from patients. After collection of blood samples in fasting state, the samples, stored at cool temperature were delivered to the laboratory for testing of plasma homocysteine levels and results were collected later. Data that was obtained was further statistically analyzed to get results. In order to draw blood in fasting state only those patients were selected who had angioplasty procedure done and were admitted overnight in hospital. Blood was drawn using syringes (5ml) and vacutainers containing clot activator and gel for serum separation (5ml) by Becton, Dickinson and Company (BD) for drawing blood samples.

Analysis of Homocysteine

Four forms of homocysteine are present in plasma: 1% circulating as free thiol; 70%–80% exists as disulfide-bonds to plasma proteins (albumin chiefly); whereas the rest 20–30% combines with itself forming homocysteine dimer or with other thiols (including cysteine thus forming homocysteine-cysteine mixed disulfide). The term “total plasma (or serum) homocysteine” (tHcy) involve all four forms of homocysteine (Ciaccio, et al., 2008). The ARCHITECT Utilizing CMIA technology and adaptable assay procedures known as Chemiflex, the homocysteine test, a one-step immunoassay, was used to quantify total L-homocysteine in human serum/plasma. Dithiothreitol (DTT) reduces bound or dimerized homocysteine (oxidised form) to free homocysteine, which is then transformed to S-adenoyl

homocysteine (SAH) by the recombinant enzyme S-adenosyl homocysteine hydrolase (rSAHHase) in the presence of too much adenosine. Then, for particle-bound monoclonal antibody, the SAH competes with acridinium-labeled S-adenosyl cysteine. After a wash phase and magnetic separation, pre-trigger and trigger solutions are added to the reaction mixture, and the ensuing chemiluminescence is quantified as relative light units (RLUs). The amount of homocysteine in the sample and the RLUs picked up by the ARCHITECT have an indirect relationship.

Statistical Method

Data of patients suffering from ischemic heart disease was analyzed using SPSS version 15. Descriptive statistics was used to analyze frequency and percentages for the demographic characteristics, clinical history. Mean, standard deviation and minimum as well as maximum values were analyzed for fasting plasma homocysteine levels and hours of fasting. To find the relationship of homocysteine with age, BMI, fasting hours, of patients with IHD pearson co-efficient of correlation was applied. Independent t-test was used to compare the effect of gender, obesity and effect of other accompanying diseases on plasma homocysteine levels in patients with IHD.

Results

Table 1: *General Characteristics of Patients with IHD*

Characteristics	**Levels**	**N**	**%**
Occupation			
	Householder	23	23
	Agriculturist	11	11
	Laborer	25	25
	Driver	7	7
	Tailor	3	3
	Salesman	6	6
	Business man	6	6
	Any other	16	16
	Police	3	3
Gender			
	Male	77	77
	Female	23	23
Education			
	Illiterate	27	27
	Just read and write	6	6
	Middle	27	27
	Matric	24	27
	Inter	6	6
	Graduate	7	7
	Highly qualified	3	3

Majority of patients suffering from ischemic heart disease (IHD) were laborers, fewer were householders and agriculturists. Majority of the patients with IHD were males whereas incidence of IHD in females was found to be relatively low. Most of the patients in current research were undergraduates.

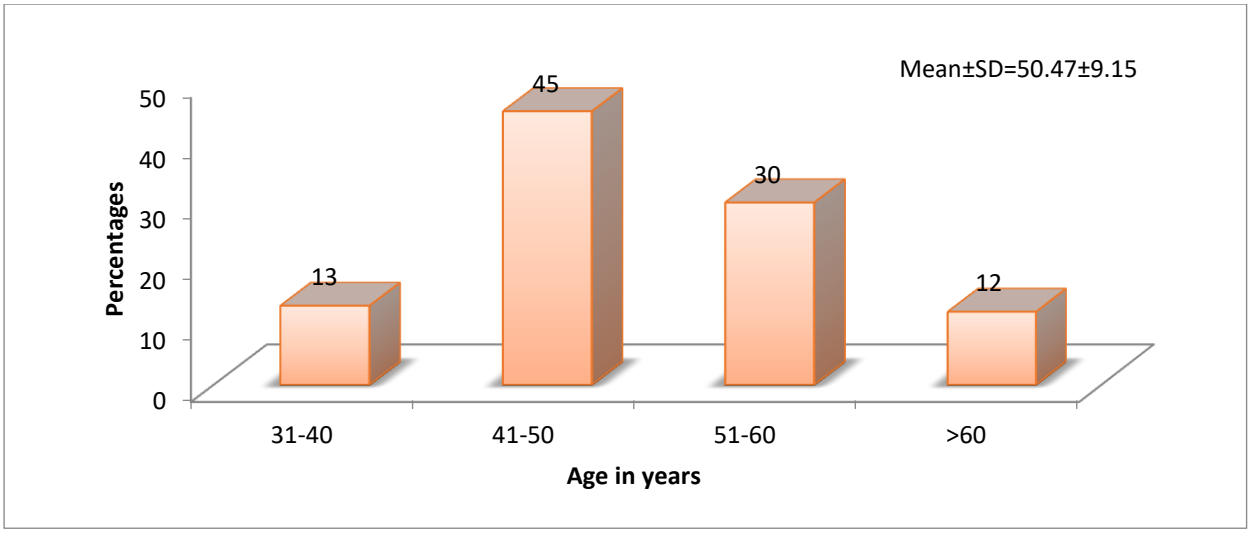

Figure 1: *Distribution of age in years*

The maximum number of patients lie in the age range of 41-50 years and lesser patients were within the age range of 51-60 years. The mean age was 50.47±9.15 standard deviation(SD). Therefore most of the patients in the current study were older adults suffering from ischemic heart disease.

Table 2: *Clinical History of Patients with IHD*

Characteristics	**Levels**	***N***	***%***
Family History			
	None	58	58.0
	Yes	42	42.0
IHD Illness Duration			
	1-4weeks	5	5.0
	>1-6months	41	41.0
	7-12months	22	22.0
	>1-5 year	22	22.0
	6-10 year	5	5.0
	>10 year	5	5.0
Patient Category			
	Stable IHD	38	38.0
	Unstable IHD	62	62.0
Patient Symptoms			
	Angina Pectoris	31	31.0
	Acute Chest Pain	69	69.0

The majority of patients (58%) had no family history of ischemic heart disease (IHD). Most of the patients (41%) were suffering from IHD since one to six months, whereas a relatively smaller (22%) number of patients were suffering from IHD since >1-5 years. Majority of patients (62%) had unstable ischemic heart disease (chest pain at rest). Maximum patients (69%) showed symptoms of acute chest pain (unstable angina or myocardial infarction, acute coronary syndrome) whereas other patients showed symptoms of angina pectoris (chest pain on exertion, in cold weather or emotional situation) on admission. Most of the patients as showed by data suffered from angina or heart attack in resting state without any exertion.

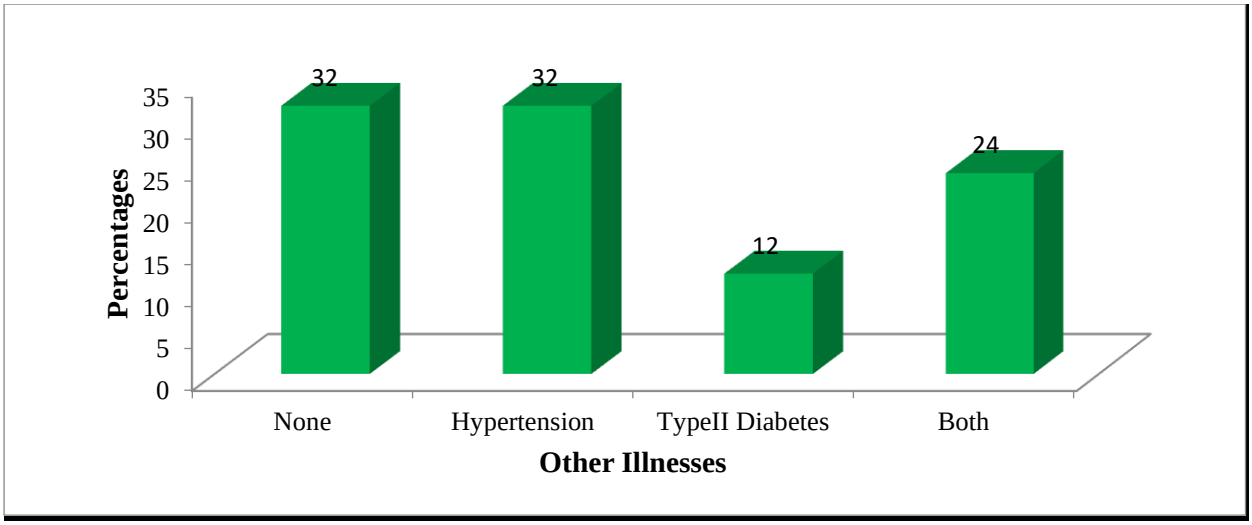

Figure 2: *Other Illnesses amongst Patients with IHD*

Most patients recorded hypertension as an accompanying ischemic heart disease. The second prevalent and accompanying disease was Type II diabetes.

Homocysteine Levels

The Homocysteine levels of the subjects and fasting hours are indicated in Table 3.Whereas Correlation of Homocysteine with Age, BMI, Fasting Hours of Patients with IHD is mentioned in Table 4.

Table 3: *Homocysteine Plasma Levels and Fasting Hours in Patients with IHD*

	N	**Minimum**	**Maximum**	**Mean**	**SD**
Homocysteine	100	3.20	51.00	16.58	7.73
Fasting hours	100	7	19	10.24	2.071

The Mean±Standard deviation (SD) of plasma homocysteine levels are significantly high in patients with ischemic heart disease(IHD). The Mean ± Standard deviation fasting hours of patients before the collection of blood sample was 10.58±2.071 SD.

Table 4: *Correlation of Homocysteine with Age, of Patients with IHD*

Correlations	**R**	**p-value**	**N**
Age vs Homocysteine	0.066	0.512	100

The Pearson co-efficient of correlation calculated indicated no statically significant correlation between age and Homocysteine levels.

Table 5: *Comparison of Gender, Weight and Other Illnesses Regarding Plasma Homocysteine Levels of Patients with IHD*

	Male N=77		**Female N=23**		**t-value**	**df**	**p-value**
	Mean	SD	Mean	SD			
Homocysteine	17.6651	7.972	12.9652	5.639	2.633	98	.010
	Normal and Over weight N=57	Obese N=43	t-value	Df	p-value		

	Mean	SD	Mean	SD			
Homocysteine	17.4467	8.67	15.4407	6.19	1.288	98	0.201
	Hypertension N=32	Type II Diabetes N=12	t-value	Df	p-value		
	Mean	SD	Mean	SD			
Homocysteine	17.6250	8.57	12.9883	3.43	2.562	42	0.014

Independent t-test indicates no statistical significance in the mean score of IHD patients who were of normal weight (BMI=19-24.5) or were overweight (BMI=25-29.9), from obese IHD patients at α level of 0.05. Thus obesity has no effect on total plasma homocysteine status in patients with IHD. Although the mean scores from the gender indicate higher levels of plasma homocysteine in males as compared to females. Also, the mean score of hypertensive patients was found to be statistically significant in comparison to IHD patients suffering from type II diabetes. Thus hypertension possibly affects the plasma homocysteine status.

Discussion

The mean plasma homocysteine levels (16.58±7.73) were elevated in the current study. These results are supported by previous studies that observed level of 16.6 micromol/l in patients with cardiovascular disease (Geisel, et al., 2003). Additionally, homocysteine is known to significantly impact cardiovascular endothelium and smooth muscle cells, changing the underlying composition and functionality of the arteries (Ganguly, & Alam, 2015). Homocysteine levels have been found to be high (15.38+/-7.28 micro mol/L & >17.4 micromol/L) in patients with advanced coronary artery disease (Tanne, et al., 2003). High homocysteine level (11.6 ± 4.4) has been supported as an independent predictor of multiple vessel disease (Smith, & Refsum., 2021).

Researchers analyzing relationship between homocysteine concentrations and other risk factors into coronary artery disease (CAD) progression in patients have found elevated homocysteine levels (18.98 +/-4.72)in their study (n=208) of prior myocardial infarction. The plasma homocysteine levels in these studies are closer to the levels assessed in current study. Therefore the results of plasma homocysteine levels in current study are in concurrence with previous studies. Researchers have found that elevated Homocysteine level is not only a risk factor but may also be responsible to aggravate the progression of ischemic heart disease (Ajoston-Coldea, et al., 2010). A previous study concludes a 20% elevated risk of IHD with every 5 micromol/L rise in homocysteine level independent of traditional IHD risk factors (Humphrey, et al., 2008). These studies support the hypothesis that plasma homocysteine might be a significant independent risk factor for IHD.

The current study found no association between obesity or BMI and homocysteine. This is in consistence with the results of previous studies in which no association of homocysteine was seen with BMI (Lin, et al., 2008). In current study male gender was seen to have a significant effect on tHcy in which the plasma homocysteine of males was significantly higher than that of females (Refsum, et al., 2006; Chen, et. al., 2005; Lim, & Heo, 2002).

Amongst the accompanying diseases hyperytension had a significant association with homocysteine. This has been observed in previous studies as well (Sun, et al., 2019; Skeete, & Dipette, 2017; Wang, et al., 2014). Lowering in homocysteine levels through folic acid and B12 supplements has led to decrease in blood pressure levels. Possible mechanism could include increased arterial stiffness, endothelial dysfunction with decreased availability of nitric oxide, low folate status, and insulin resistance (Skeete, & Dipette, 2017). Researchers believe that these risk factors in synergy with tHcy increase the overall risk of IHD or contribute to the progression of diseases**.** There was no association of tHcy with age, sedentary

lifestyle and exercise (Wilson, 2004). A few limitations to the study included access to higher income group patients and control group for better comparison.

Conclusion

The tHcy levels (16.58±7.73) Mean± SD were found elevated in patients with IHD. Therefore, concluded that plasma homocysteine can be a significant predictor in Ischemic heart disease occurrence and progression. Therefore, homocystine measurement value should be considered when assessing the patients for heart disease and its importance as a precursor for morbidity in cardiac patients should not be ignored. Also appropriate therapeutic action/measures should be done to improve the biomarker.

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Conflict of Interest

Authors declared NO conflict of interest.

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ORCID iDs

Zahra Rasheed [1] https://orcid.org/0009-0001-8616-1192
Mehreen Ijaz [2] https://orcid.org/0000-0002-8717-2264
Namood-e-Sahar [3] https://orcid.org/0009-0009-2462-8285
Zohra Tariq [4] https://orcid.org/0000-0001-5577-8192
Rafia Fatima [5] https://orcid.org/0009-0009-5464-1560
Madeeha Tariq [6] https://orcid.org/0009-0000-6107-671X

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