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Evaluation of homocysteine levels in ischaemic heart disease proved by 2d- echocardiography

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Abstract
Background & Objectives: Homocysteine is a risk factor for atherosclerotic vascular disease, with adverse influence on endothelial cells, vascular smooth muscle cells, connective tissue, interactions with plasma lipoproteins and platelets. This study was conducted to study the association between levels of homocysteine and IHD, the clinical profile of subjects in relation to their lifestyle, and the socio-demographic profile of patients with established ischaemic heart disease.
Methods: The data for this study was collected from patients who presented to NRI Medical College& Hospital Chinakakani from August 2017 to August 2019. 30 cases and 30 age and sex matched controls were taken. All cases taken were proven cases of IHD proved by 2D echocardiography. Homocysteine levels were compared between the two groups and the results drawn.
Results: Homocysteine levels were found to be significantly higher in cases with IHD when compared to controls. Both cases and controls with dyslipidemia and other risk factors for IHD like age above 50 years, diabetes mellitus, hypertension and a history of CVA were found to have significantly higher homocysteine levels.
Interpretation & Conclusion: A significant association was found between elevated serum homocysteine levels and both IHD and risk factors for IHD even in the absence of IHD. This suggests that elevated homocysteine levels are an independent risk factor for developing IHD.

Keywords: IHD, homocysteine, stroke, dyslipidemia, hypertension, diabetes mellitus

Introduction
Atherosclerotic plaques are the most common cause of CAD [1]. More than 200 coronary risk factors have been reported. Recently homocysteine has been shown to be involved in the pathogenesis of CAD. Homocysteine is a sulfur containing amino acid produced by demethylation of the essential amino acid methionine [2]. Homozygotes with homocystinuria have high levels of circulating tHcy (> 100Gmol/l) are at high risk for premature arteriosclerotic vascular disease and venous thrombosis. If homocystinuria remains untreated, about 50% of patients may experience thromboembolic events and mortality could reach 20% before the age of 30 years. Although the exact mechanism of atherothrombosis associated with hyperhomocysteinemia is not clearly understood, in many of the reported effects of plasma homocysteine are thought to be mediated by its atherogenic effects, such as vascular smooth cell migration and proliferation [3] and prethrombotic properties, such as inhibition of thrombomodulin activity, reduction of protein C activation, increase of platelet aggregation and predisposition to endothelial cell injury [4].
Homocysteine has been under a lot of speculation since its discovery in 1932. Its chemical properties showed a similarity to cysteine, hence the name homocysteine. The heating of the amino acid methionine with sulphuric acid led to this amino acid of interest. The importance of this discovery cannot be emphasized on without alluding to the 1955 Nobel Prize in Chemistry, awarded to Vincent du Vigneaud "For his work on biochemically important sulphur compounds, especially for the first synthesis of a polypeptide hormone".

Aims and Objectives
To assess the association between levels of homocysteine, clinical profile, sociodemographics and IHD.

Materials and Methods

This is a study which has been carried out in the Department of General Medicine, NRI Medical College& Hospital, Chinakakani.

Source of data

The data for the study has been collected from the inpatients who fulfill the inclusion and exclusion criteria in the Department of General Medicine, NRI College & Hospital, Chinakakani who were proved as cases of IHD.

Study period

August 2017 to August 2019

Type of study

Case control / comparative study

Sample size

30 cases
30 controls

Collection of data

Data was collected by patient evaluation which was done by detailed history taking and clinical examination through a structured proforma specially designed for this study.

Inclusion Criteria

All cases of IHD – This includes all patients both sexes established cases of IHD, who give history of myocardial infarction and their echocardiography shows motion wall abnormalities.

Exclusion criteria

- Patients < 18 years
- Patients on Haemodialysis
- Patients with renal transplant
- Patients on drugs such as methotrexate, theophylline, metformin and niacin

Controls

Sex and age matched controls from general population without any evidence of IHD.

Investigation

All patients and controls included underwent detailed clinical examination and following investigations.

- RBS or FBS and PPBS
- Blood urea, S. creatinine
- Lipid profile
- Plasma homocysteine
- ECG
- 2D echocardiography

Method of detection of homocysteine

Homocysteine is present in plasma primarily bound as disulfides with itself, Cys and albumin. A moderate increase of homocysteine in these disorders justify the introduction of HCY assay in routine clinical chemistry laboratory.

The current assay used for detection of HCY in serum or plasma is luminescent oxygen channelling immunoassay (LOCITM) chemical reactions involved in the assay can be divided into 3 steps. [4]

- Serum disulfide bonds are reduced to release HSG as monothial.
- A derivative (HCY – acetyl benzoic acid (HCYABA) is formed by reacting with alkylating agent p-2 chloroacetyl benzoic acid phosphate (ABA).
- And the HCY– ABS concentration is measured in a competition assay using an anti HCY ABA.

A two-reagent assay protocol is used in this system.

The LOCI HCY assay is performed on an automated instrument (TECAN), and patients sample is incubated by mixing 5 NL of serum or EDTA– treated plasma with50 NL of first reagent nehich contains 2 mmol/l TCEP, 5 mmol/L, LABA phosphate and5 Ng of HCY – ABA coated sensitizer particles. After 7 min incubation at 37 ^{0}C and addition of 50 NL of the second reagent, which contains 50 Ng of alkaline Phosphatase and 12.5 Ng of anti HCY-ABA monoclonal antibody – coated chemiluminescent particles. After addition of 145 NL of 0.1 mmol/l borate buffer, pH 9.2, the mixture is incubated an additional 2.6 min. The chemiluminescent signal is then measured by repetitively irradiating at 680 nm for 1s and reading at 600-620 nm for 1 sec. The assay signal is inversely related to the amount of tHCY present in serum sample, concentrations are determined using pooled serum calibrates.

Statistical Methods

The Student t test has been used to find the significant difference of means of homocysteines between cases and controls with and without risk factors, normal/abnormal lipid profiles and the presence and absence of clinical features. Analysis of variance (ANOVA) has been used to find the significant difference of homocysteine levels between the changes in the ECG. Effect size has been used to find the effect of IHD on homocysteine.

Results

Study Design

A case-control study consisting of 30 IHD patients and 30 age and sex matched controls is undertaken to investigate the relationship of homocysteine with IHD, Clinical features, lipid profiles, risk factors.

Age distribution of cases and controls

Maximum numbers of cases are seen in the age group of 61 to 70 years. The youngest case being 30 and the oldest being 80 years.

Table 1: Age distribution

Age in years	IHD cases		Controls	
	No	%	No	%
≤ 30	1	3.33	1	3.33
31-40	5	16.6	5	16.6
41-50	6	20	7	23.3
51-60	7	23.3	9	30
61-70	9	30.0	4	13.3
>70	3	10.0	4	13.3
Total	30	100.0	30	100.0

Sex Distribution

Among the 30 cases, 20 are males and 10 are females. Among the 30 controls, 22 are males and 8 are females. The cases and controls are well matched according to sex.

Table 2: Sex distribution

Sex	IHD cases		Controls	
	No	%	No	%
Male	20	66.6	22	67.5
Female	10	33.3	8	32.5

Age distribution with sex

There is a predominance of males in cases and controls, and it was seen that more females affected with IHD were in the age group of 30 to 50 years.

Table 3: Age distribution with sex

Age in years	IHD cases		Controls	
	Male	Female	Male	Female
≤ 30	-	1(10)	-	1(12.5)
31-40	2(10)	3(30)	4(18.8)	2(25)
41-50	1(5)	2(20)	6(27.2)	2(25)
51-60	7(35)	1(10)	7(31.8)	1(12.5)
61-70	9(45)	2(20)	3(13.6)	1(12.5)
>70	1(5)	1(10)	2(9.09)	1(12.5)
Total	20(100.0)	10(100.0)	22(100.0)	8(100.0)

Lifestyle

Maximum cases and controls had sedentary lifestyle. However 1/5 of patients with active lifestyle also had IHD but these patients were also found to have other risk factors such as diabetes, hypertension and dyslipidemia.

Table 6: Life style

	IHD cases		Controls	
	No	%	No	%
Sedentary	18	60	20	66.6
Moderate	5	16.6	4	13.3
Heavy	7	23.3	6	20

Clinical features

Recurrent angina, raised JVP, pedal oedema, basal crepitations, tachypnea and PND, which are features of cardiac decompensation, were the most common clinical features present in the cases.

Table 7: Presentation of clinical features in IHD

Clinical features	Number(n=30)	%
Recurrent angina	12	40
JVP increased	12	40
Pedal edema	15	50
Basal crepitation	10	33.3
Tachypnea	12	40
PND	12	40

Homocysteine levels and lipid profile

Serum homocysteine levels were found to be significantly elevated in cases with dyslipidemia, when compared to cases without dyslipidemia. However, the homocysteine levels in cases without dyslipidemia was significantly higher than in controls.

Risk factors

Hypertension was the most prevalent risk factor among controls. Cerebrovascular accident was the most common associated condition among IHD cases. In our study incidence of diabetes and hypertension was also significantly increased among cases. Family history of IHD and smoking were relatively less frequent.

Table 4: Presentation of risk factors

Risk factors	IHD cases (n=30)		Controls (n=30)	
	No	%	No	%
DM (+)	18	60	10	33.3
HTN (+)	20	66.6	12	40
CVA (+)	22	73.3	3	10
Smoke (+)	15	50	12	40

Homocysteine and family history of IHD

Among 30 cases, 15 cases were found to have positive family history out of which 10 have high homocysteine levels. 5 were found to have normal homocysteine levels. Among the 30 controls, 4 had positive family history and none of them had high homocysteine levels.

Table 5: Family history of IHD and homocysteine level

Family History of IHD	IHD cases (n=30)		Controls (n=30)		P value
	No	%	No	%	
Normal Homocysteine (n=5)	5	16.6	4	13.3	*P*>0.05
Elevated Homocysteine (n=10)	10	33.3	-	-	0.010**
Over all (n=15)	15	50.0	4	13.3	0.030**

Table 8: Homocysteine levels and lipid profile

Lipid profiles	Homocysteine levels (Mean ± SD)	
	Cases (n=30)	Controls (n=30)
TC normal	20.97 ± 8.35	14.58 ± 6.77
TC Abnormal	27.26 ± 8.78	15.48 ± 6.07
TG Normal	23.50 ± 9.18	13.75 ± 6.24
TG Abnormal	29.70 ± 6.56	19.25 ± 6.83
HDL normal	24.10 ± 9.24	13.94 ± 6.07
HDL Abnormal	27.43 ± 7.49	20.16 ± 8.35

Homocysteine levels and clinical features

No significant association was found between clinical features and serum homocysteine levels.

Table 9: Homocysteine levels and clinical features

Clinical	Homocysteine levels		Significance	Effect size
	Absent	Present		
Recurrence	22.75 ± 8.61	27.24 ± 9.38	0.131NS	0.48
JVP	23.25 ± 7.84	26.41 ± 10.79	0.290NS	0.32
Pedal edema	23.54 ± 9.17	25.64 ± 9.02	0.720NS	0.23
Crepitation	22.55 ± 8.38	28.34 ± 9.46	1.964NS	0.23
Tachypnea	23.68 ± 9.08	25.82 ± 9.16	0.707NS	0.23
PND	23.21 ± 8.24	27.29 ± 10.83	0.195NS	0.45

Homocysteine levels and Risk factors

Homocysteine levels were compared with each risk factor for IHD, and it was found that homocysteine levels are elevated in cases without risk factors, but are even higher in cases with risk factors. This shows that elevated homocysteine levels are strongly associated with IHD and its various risk factors.

Table 10: Homocysteine levels and Risk factors

Risk factors	Cases (n=30)	Controls (n=30)	Significance (Student t)
Age < 50	23.50 ± 10.53	12.61 ± 4.09	3.192**
Age >50 year	24.88 ±8.44	16.26 ± 7.69	3.744**
Male	24.20 ± 8.49	14.62 ± 5.74	4.764**
Female	24.67± 9.81	14.90 ± 8.37	2.875**
DM -	21.37 ± 9.82	12.96 ± 5.59	3.701**
DM+	26.94 ± 7.71	18.79 ± 7.22	3.008**
HTN-	22.45 ± 9.97	11.60 ± 3.97	5.006**
HTN +	26.42 ± 7.77	20.50 ± 6.70	2.342*
CVA-	19.61 ± 7.98	14.61 ± 6.89	2.923**
CVA+	27.99 ± 8.22	15.63 ± 3.44	1.629NS
Smoking -	22.20 ± 8.95	14.42 ± 6.22	3.449**
Smoking +	27.77 ± 8.39	15.12 ± 7.27	4.638**
Inference	The homocysteine levels significantly increased in IHD cases compared to controls when risk factors are absent (P<0.05). However the presence of risk factors further increases the homocysteine levels in both cases and controls		

Discussion

Over the last decade, convincing evidence has been gathered on the relation between moderate elevation of plasma tHcy and ischemic stroke. Several studies have reported that HHcy is associated with two to threefold increased risk of ischemic stroke

It was first reported in Singapore[5] in 1959 that people hailing from Indian sub-continent had a higher probability of dying from IHD. Studies in the past have shown that high rates of IHD in Indians are accompanied by paradoxically low prevalence of conventional risk factors such as

- HTN
- DM
- Dyslipidaemia
- Smoking
- Sedentary life style

The strong evidence for above statement comes from studies analyzing risk factors for CAD among Asian Indians Physicians in US [6], where the prevalence of CAD was four fold higher than the national average. The data suggested the presence of powerful risk factor that is unaffected by even maximal modifications of conventional risk factors.

The lack of epidemiological data in our population regarding homocysteine prompted us to undertake this study of defining their association to IHD.

In the present study 30 cases of IHD and 30 controls were taken.

It was found in this study, that IHD was higher in males 20/30 (66.6%) when compared to females – 10/30 (33.3%), and more commonly encountered in the sixth and seventh decade, which correlates well with many other studies done both within the country and abroad.

Table 11: Sex wise distribution of IHD cases

Studies	Year	Total cases	Male	Female
Robinson *et al*. [7]	1999	375	220(58%)	155(42%)
Present study	2017	30	20(66.6%)	10(33.3%)

In the present study, the youngest patient is a 30 year old female, and another four female patients were also in the age group of 31 to 40 years, all being in the reproductive age group. Though females are said to have hormonal protection during this age group from IHD, our study shows 5 females having loss of this hormonal protection. Among these five, three had CVA, with two to three other conventional risk factors. All of them had increased levels of homocysteine. Only one patient with proven IHD had CVA and no known risk factors, and normal levels of homocysteine. Though not investigated extensively, this patient could have developed IHD probably due to other undetected factors including anomalies of coronary vessels. Hence it may be concluded that higher homocysteine levels could be a risk factor for IHD in younger females.

Risk factors

American heart association in 1992 and Cooper *et al.* [8] in 1993 proposed the development of risk factors and their relationship to incidence of IHD.

In 1996 Pearson *et al.* [9] at the 27th Bathesda Conference categorized risk factors based on matching the intensity of risk factor management with hazard of IHD. The risk factors were categorized into four groups.

Category I (Risk factors for Neluch Intervention have proven to lower IHD)

- Smoking
- LDL Cholesterol
- HTN
- LVH

Category II (Risk factors for which intervention are likely to lower IHD)

- DM
- Sedentary life style
- HDL
- Obesity
- Postmenopausal

Category III (Risk factors associated with IHD that if modified might lower the risk)

- Psychosocial factors
- Lipoprotein (a)
- Homocysteine
- Oxidative stress
- Alcohol abstinence

Category IV (Non modifiable risk factors)

- Age
- Male gender
- Family history

Hypertension was one of the most prevalent risk factors among cases and controls. Many studies done in India and abroad show that hypertension does bring an increase in the

homocysteine levels. It was seen that homocysteine levels in hypertensive cases and controls was much higher when compared to non hypertensives.

Table 12: Homocysteine levels and hypertension

Mac Mohan1990 [16] (Srilanka)	70% patients with hypertension had higher homocysteine levels
Present Study	66.6% of cases and HTN

Correlation of homocysteine levels with diabetes

Diabetes mellitus was present in 60% of our cases and 33.3% controls. Diabetic patients were also found to have higher homocysteine levels when compared to non-diabetics. Cannon *et al.*, in 1991 [11], conducted a meta-analysis and found the incidence of diabetes in IHD to be 32 to 67%.

Correlation of homocysteine levels with physical activity

In the present study, most of the cases and controls were found to be sedentary workers. However, 20% of the patients, despite an active lifestyle, were found to have IHD. These cases had other conventional risk factors.

Clinical profile of cases with IHD

In the present study, it was found that most of our cases (22 out of 30) had features of congestive cardiac failure. This could be explained on the basis of the presence of cardiac decompensation along with the presence of other risk factors such as diabetes, hypertension and dyslipidemia, which per se are also known to affect the vasculature of the heart.

Homocysteine levels

In our study, the mean homocysteine levels above which an increased risk of developing IHD was found was 13.5 µmol/l.

Table 13: Levels of HCY

Studies	Year	Mean HCY levels
Taylor *et al*. [12]	1991	12.5 µmol/l
Present study	2017	13.5 µmol/l

In the present study, homocysteine levels in cases were found to be almost twice to that of controls.

Graham *et al.* 1998 [13] in large European study ECAP showed that HCY levels were significantly higher in patients than controls.

Gills *et al.* [14] in 2000 (3rd NHANES Study) also concluded that a 2 fold increase in MI occurred in patients with a mean concentration of HCY > 15 mmol/m^2.

Correlation of homocysteine levels with sex

No significant difference between homocysteine levels was found in males and females. Some studies done in India and abroad show that males have higher Hcy levels as compared to females the reason being more muscle mass in males. However patients coming to our hospital mostly belong to the lower socioeconomic strata and have poor nutritional status and therefore the comparable values of Hcy in males and females can be explained on that basis, there was an increase in homocysteine levels with increasing age. According to other studies also conducted by Lussier *et al.* (1992) [8] and Bree *et al.* (2001) [15] showed that homocysteine levels increase with age.

Limitations of study

Our study though validating the view that increased HCY levels are associated with IHD needs further verification in large prospective case control studies.

The mean HCY levels were significantly higher than those reported in other studies. This can be attributed to the inclusion of patients with severe IHD in our study.

Conclusion

In the case control study with 30 cases of IHD and 30 age and sex matched controls conducted at NRI Medical College& Hospital, Chinakakani the following conclusions were drawn.

- Homocysteine levels are comparable in cases and controls.
- With advancing age, there is an increase in level of HCY.
- Lifestyle and personal habits were not significantly associated with HCY levels.
- Hypertension was found to bring an increase in homocysteine levels.
- Absence of hypertension in IHD cases was also associated with increased HCY levels hence proving the association between IHD and HCY.
- Similarly diabetes, stroke and dyslipidemia were found to increase HCY levels marginally but their absence was also associated with increased levels of homocysteine in IHD cases, hence proving the co-relation between HCY and IHD.
- No clinical feature was found to be statistically significant with level of homocysteine.

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