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Original Research Article

Is Homocysteine a silent marker for cardiovascular disease?Nithin Varghese Abraham¹, Priyanka S. Bannikoppa² and Pauline Anand^{*3}¹Father Muller Medical College, Mangalore 575002, Karnataka, India²Division of Nutrition, St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore 560034, Karnataka, India³Department of Physiology, Father Muller Medical College, Mangalore 575002, Karnataka, India

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Article History:*Received:** 01/12/2017**Revised:** 14/12/2017**Accepted:** 14/12/2017**DOI:** <https://doi.org/10.7439/ijbar.v8i12.4515>**Abstract**

Background: Cardiovascular disease (CVD) remains one of the leading causes of morbidity and mortality in India. Association between hyperhomocysteinemia and Metabolic Syndrome (MS) or its components in a population with or without cardiovascular disease is not well established. This study aims to check levels of homocysteine (Hcy) and its relationship with CVD.

Aims and Objectives: To check Hcy levels in healthy adults and MS patients to reflect its association with CVD.

Material and Methods: Case control study was done on 40 subjects patients with MS (n=20) and controls (n=20) of 30 - 60 years at Fr. Muller Medical College Hospital, Karnataka.

Results: Homocysteine levels were significantly higher in MS group when compared to controls (p = 0.04).

Conclusion: MS group had higher homocysteine levels along with blood glucose, triglycerides, total cholesterol when compared to controls. Elevated levels of Hcy can be a marker for cardiovascular disease. Furthermore, the results of this study suggest the overall role of homocysteine function on insulin resistance, and its association with cardiovascular parameters.

Keywords: Methionine, vitamin B12, cardiovascular disease, metabolic syndrome.

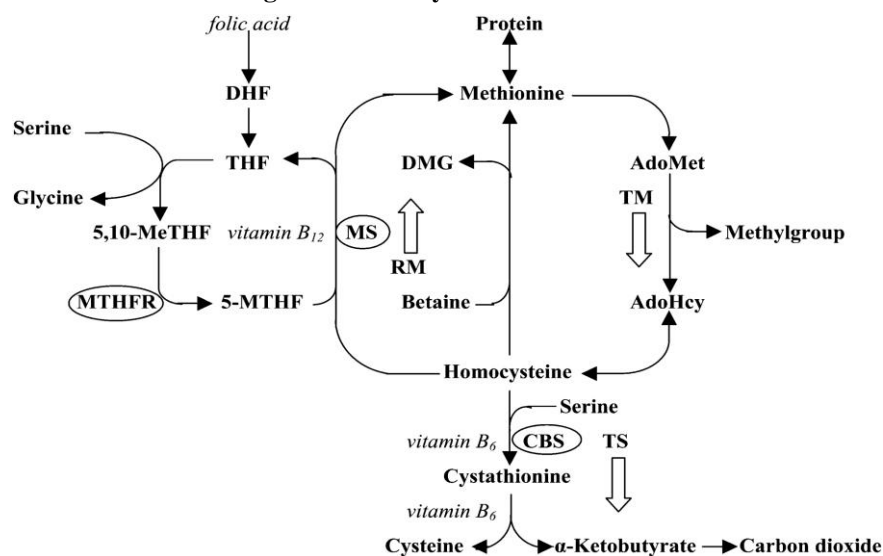
1. Introduction

Over the last few decades, there has been a drastic transition of disease pattern, where mortality due to non-communicable diseases like cardiovascular diseases (CVD) is increasing in comparison to communicable diseases [1]. By 2020, around 2.6 million Indians are likely to die due to CVD by 2020[2]. Thus there is an urgent need to identify and evaluate various risk factors responsible for this rapidly rising burden of CVD.

Individuals with Metabolic Syndrome (MS) have 30-40 % increased risk for developing CVD, depending on the number of dysfunctional components present such as

elevated glucose, dyslipidemia and/or elevated blood pressure[3]. The number of people affected by MS in South Asian countries, especially India is on a rise [4]. This has been attributed to distinct shifts in lifestyle, brought about by economic growth, urbanization, sedentary life habits and changes in dietary pattern.

Among various risk factors, homocysteine (Hcy) is increasingly drawing the research interests since elevated levels of plasma Hcy is directly linked with risk of CVD[5,6].

Figure 1: Homocysteine metabolism

TM: Transmethylation, **TS:** Transsulfuration, **RM:** Remethylation, **CBS:** Cystathionine β synthase, **MS:** Methionine synthase

Homocysteine is an intermediary product formed from methionine, an essential amino acid. It can be metabolized by remethylation (RM) pathway back to methionine with its cofactors, vitamin B₁₂ and folate or by transsulfuration (TS) pathway in which Hcy condenses with serine to form cystathionine which is further reduced to form cysteine. Transsulfuration to cysteine is catalyzed by vitamin B₆ along with the enzyme cystathionine-β-synthase (CBS)[7].

Gaps in knowledge still exist on the link between homocysteine levels and risk of CVD. Though the consequences of hyperhomocysteinemia have been reported, the mechanisms of action of Hcy mediated risk are not well understood especially among Indian population. In addition, one of the key pathophysiological features of MS is insulin resistance, as it is known to interfere in various metabolic pathways including methionine cycle of homocysteine metabolism [7,8].

Thus the aim of the present study is to understand the relationship between classical CVD risk factors, such as triglycerides, HDL-C, blood glucose with homocysteine levels in individuals with and without MS and to broaden the knowledge regarding the same.

2. Methods

A case control study was conducted and experimental protocol was approved by the Institutional Human Ethical Committee and written informed consent was obtained by study participants at enrollment. The study participants were recruited from the General Medicine outpatient department. Patients with known history of MS (n=20) aged between 30 - 60 years comprised the MS group whereas 20 apparently healthy, age matched adults comprised the control group.

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At recruitment, detailed information on the socio-demography, dietary habits and anthropometry of the study subjects were recorded. 4 ml of fasting blood sample was collected for Hcy analysis.

2.1 Biochemical analysis of Hcy

Fasting blood samples were collected into plain tubes and centrifuged at 4°C. The serum was separated and stored at -20°C until analysis. The measurements of total Hcy were done by chemiluminescent immunoassay (CLIA) method. Homocysteine concentration of <15 μmol.l⁻¹ was considered to be the normal range [9].

3. Statistical analysis

Descriptive analyses of all the parameters (mean, SD) were done. Independent 2-tailed, t-test was used to compare the parameters in control and MS groups. Pearson's correlation was done to check the relationship between Hcy and other associated parameters. Statistical significance was considered at p < 0.05. The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp Released 2011).

4. Results

Results are expressed as arithmetic mean ± SD. General Characteristics of the study subjects are represented in Table 1. The mean age of normal group was 44 years and MS was 46 years. BMI was significantly higher in MS group when compared to the normal group (p=0.01). Fasting blood sugar (FBS) was higher in MS group when compared to controls (p=0.01). HDL-C did not differ between the groups whereas triglycerides, total cholesterol and LDL-C were significantly higher in the MS group in comparison with the normal group (p=0.01). Hcy levels was significantly higher in MS group than in controls (p=0.04).

Table 1: General characteristics of the study participants at enrollment

Variables	Normal (n = 20)	Metabolic syndrome (n = 20)
Age (yrs)	44 ± 10	46 ± 9
Weight (Kgs)	61 ± 13	75 ± 15*
BMI (kg/m ²)	24.1 ± 4.20	28.5 ± 4.27*
Blood pressure-Systolic (mmHg)	123.2 ± 10.4	125.4 ± 15.6
Blood pressure-Diastolic (mmHg)	82.7 ± 5.4	86.0 ± 9.0
Fasting blood sugar (mg/dl)	94.7 ± 5.8	118.1 ± 23.0*
HDL-C (mg/dl)	43.9 ± 14.8	43.1 ± 11.2
Triglycerides (mg/dl)	100.6 ± 44.7	180.3 ± 58.2*
Total cholesterol (mg/dl)	169.6 ± 37.4	206.0 ± 46.8*
LDL-C (mg/dl)	107.6 ± 22.8	142.3 ± 43.8*
VLDL (mg/dl)	21.5 ± 9.8	35.8 ± 13.2*
Hcy (µmol/l)	18.1 ± 7.6	21.1 ± 6.4**

Values are expressed in mean and SD. *p=0.01. **p= 0.04

Table 2 represents the correlation between Hcy and other cardio metabolic factors in normal group. Significant negative correlation between Hcy and BMI was seen ($r = -0.31$, $p=0.05$), whereas it correlated positively with HDL-C ($r=0.38$, $p<0.05$).

Table 2: Represents the correlation between Hcy and other variables in normal group

	Age	BMI*	FBS	HDL	TG	TC	LDL	VLDL
Hcy levels	-0.19	-0.31*	0.17	0.38*	-0.07	0.27	0.16	-0.22

*p=0.05

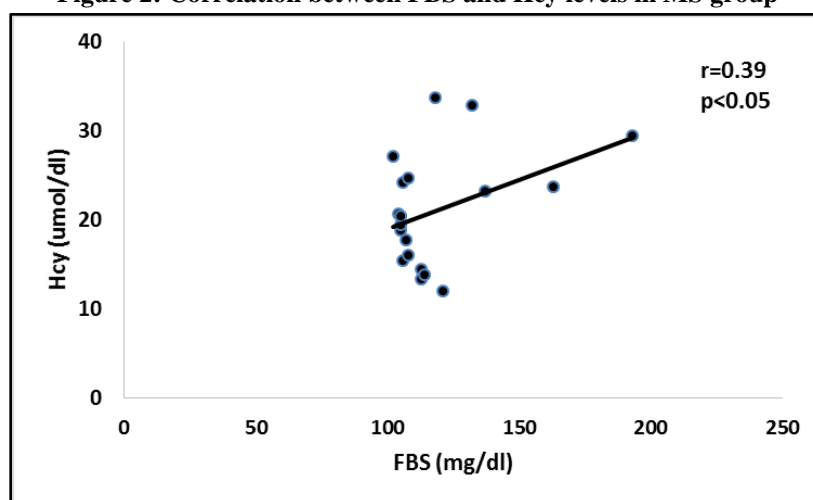
Table 3 represents the correlation between Hcy and other cardio metabolic factors in MS group. A significant negative correlation was seen between Hcy levels and BMI of the subjects which was significant ($r = -0.42$, $p=0.01$).

Table 3: Represents the correlation between Hcy with variables in metabolic syndrome group.

	Age	BMI*	FBS	HDL	TG	TC	LDL	VLDL
Hcy levels	0.08	-0.42*	0.13	0.09	-0.05	-0.22	-0.14	0.09

*p=0.01

A significant positive correlation was seen between FBS and Hcy levels in MS group ($r=0.39$, $p<0.05$) (Figure 2).

Figure 2: Correlation between FBS and Hcy levels in MS group

5. Discussion

This study was aimed at investigating the relationship between plasma Hcy levels and the risk of CVD in patients with MS. Metabolic syndrome is defined as the constellation of interrelated metabolic abnormalities which includes insulin resistance, abdominal obesity,

atherogenic dyslipidemia and hypertension [10,11]. In our study, we found that Hcy levels were significantly higher in MS group along with other cardio metabolic risk factors viz., blood glucose, triglycerides and total cholesterol as compared to controls.

Optimal methylation removes homocysteine. Excess Hcy, which is produced during the metabolism of methionine, has to be metabolized through its transsulfuration pathway (Figure 1), if not, will lead to hyperhomocysteinemia.

This study showed that the presence of MS was associated with elevated levels of Hcy, while Hcy levels were normal in the control group. Mild Hcy elevation ($>15\mu\text{mol/l}$) which occurs in 20–30% of patients with atherosclerotic disease, can be normalized with oral folates[12] but in our study, the MS group had Hcy level was as high as $>21.14\mu\text{mol/l}$ which can be fatal. Hence, it can be a marker for risk estimation of CVD.

Several mechanisms may explain the association between elevated Hcy levels and MS. Epidemiological literature suggests that hyperhomocysteinemia is associated with insulin resistance, a chronic inflammatory condition [13]. In mouse model, Hcy has shown to induce insulin resistance through adipose endoplasmic reticulum stress and inflammation [14]. Thus the current study corroborates the role of hyperhomocysteinemia in diabetes.

On the other hand, we observed a significant, positive association between impaired fasting glucose and Hcy levels (Figure 2). This can be explained on basis of transsulfuration pathway of the methionine cycle. Cystathionine- β -synthase (CBS), a key enzyme of the transsulfuration pathway could be possibly down regulated in the insulin resistant state [12]. This impairment can cause hyperhomocysteinemia which induces the expression and secretion of proinflammatory factors [14] which can impair insulin signaling mechanisms [15].

Homocysteine levels are also influenced by folate and B_{12} vitamins levels which in turn depend on the diet [16]. Hyperhomocysteinemia is associated with low levels of vitamin B_{12} , B_6 and folate. Worldwide studies of vegetarians and vegans show that the less animal food they eat, the higher are their blood concentrations of Hcy[17,18]. It is interesting to note that in our study, overall in both the groups, 95% of the populations were non-vegetarians (data not shown). In spite of consuming a non-vegetarian diet, which is rich in vitamin B_{12} , the Hcy levels were still shown to be higher among the MS patients.

This infers that the insulin resistant state impedes the methionine cycle independently by down regulating the transsulfuration pathway and as a consequence, the intermediary metabolite Hcy levels could have plausibly increased. It could also be noted that plasma concentrations of vitamins B_6 , B_{12} and folate may provide additional information on the relationship between the MS and CVD since they play key role in driving the methionine cycle. However, this finding needs to be explored further especially in relation to the body mass index of the person.

6. Conclusion

The present study helped to identify the levels of homocysteine as biomarker of CVD in MS patients. This finding would help the health care professionals to develop an insight into the importance of checking the homocysteine levels during the routine checkup of the patients. It is worthwhile to study a larger population to create an awareness regarding the levels of homocysteine as one of the marker to minimize the risk of heart diseases in Indians. Currently, there is no universal recommendation for checking homocysteine levels in the clinical practice. Since detection of high levels of homocysteine has been linked to cardiovascular disease, lowering homocysteine levels may improve outcomes. Additionally, improving insulin sensitivity is key to improve the optimal methylation which can prevent the accumulation of homocysteine in the cells. Lifestyle modification such as monitoring the dietary pattern and by increasing the physical activity can have long term benefits to prevent or delay the occurrence of CVD events.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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