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# HOMOCYSTEINE LEVELS IN DIABETIC PATIENTS WITH AND WITHOUT CARDIOVASCULAR DISEASE: A CROSS-SECTIONAL ANALYTICAL STUDY.

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## ABSTRACT

Total Homocysteine (tHcy) levels are found to be significantly raised in patients suffering from cardiovascular diseases. In Non insulin dependent diabetes mellitus (NIDDM) cardiovascular disease (CVD) is one of the major macrovascular complications. The present study was conducted on a Goan population with an objective to evaluate the relation between the plasma tHcy in diabetic individuals with and without CVD. The one year cross sectional study comprised of 180 subjects, including 60 NIDDM individuals, 60 NIDDM individuals with CVD and 60 healthy controls. Plasma concentrations of tHcy levels were measured by High performance liquid chromatography (HPLC) in all study subjects. The data obtained was statistically analysed using student unpaired't' test and comparison between the groups was done by analysis of variance. The tHcy levels were found to be significantly elevated in NIDDM and also in NIDDM with CVD in Goan Population.

**KEYWORDS**: **Diabetes mellitus; Cardiovascular diseases; Total homocysteine; HPLC**.

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## INTRODUCTION

Diabetes mainly Non insulin dependent diabetes mellitus (NIDDM) affects nearly 150 million people worldwide and 30% rise is predicted by 2025 due to increased rate of obesity and the ageing population living in industrial countries[1, 2]. Due to the excessive cardiovascular mortality and morbidity associated with diabetes, it can be redefined as a state of premature cardiovascular death, associated with chronic hyperglycemia and sometimes with blindness and renal failure[3]. Homocysteine a non-protein-forming, sulfur amino acid, is a minor byproduct of methionine metabolism[4]. The term "total homocystine" (tHcy) is used to define the combined pool of hcy, homocystine, mixed disulfides involving hcy, and homocysteine thiolactone[5]. The studies have demonstrated a positive correlation between the thcy and cardiovascular injury[6, 7, 8, 9]. The potential regulation of homocysteine metabolism by insulin, through regulation of the enzyme cystathionine ß-synthase, provides a mechanistic link between hyperhomocysteinemia and diabetes[5]. In NIDDM, association between hyperhomocysteinemia and atherosclerotic vascular disease was found to be stronger when compared with nondiabetic subjects[5, 10]. In this study we aim to compare the levels of thcy in normal subjects, NIDDM patients and NIDDM patients with cardiovascular disease (CVD) in the Goan Population.

## MATERIALS AND METHODS

A one year cross sectional study was conducted in the Department of Biochemistry, at a Medical College & Hospital, after obtaining ethical clearance from The Hospital Ethical Committee. 180 study subjects were selected based on purposive sampling method, among them 120 were NIDDM patient, in the age group of 40 to 70years, attending the medicine and cardiac out-patient departments of a Medical College & Hospital in Goa. They were grouped into two groups. Group 1 (n=60, with men=38, women=22) patients of NIDDM without complications, group 2 (n=60, with men=30, women=30) patients of NIDDM with cardiovascular complications. Sixty, age and sex matched healthy controls were also included in the study. Patients with history of smoking, chronic alcoholism, rheumatoid arthritis, hepatic, renal, neurological, gastrointestinal disorders, tuberculosis and neoplasia were excluded from the study. Written informed consent was obtained from the participants of this study. Under aseptic precautions, fasting plasma samples from the study groups were obtained from whole blood collected into EDTA Vacutainer tubes and stored for a maximum of 3 months at 70°C before assay. High performance liquid chromatography (HPLC) was carried out by an isocratic system with fluorescence detection (SFM 25 spectrofluorometer), autosampler (SA 360), and HPLC pump supplied by SHIMADZU. Homocysteine calibrators (DL form) were prepared in borate buffer (0.1 mol/L, pH 9.5, with and without 2 mmol/L EDTA). Internal standard was added to the plasma and the homocysteine calibrator to achieve a final concentration of 10.0 µmol/L (30 µL of 50.0 µmol/L cysteamine + 120-µL sample).The calibration slopes were calculated with homocysteine/ cysteamine peak area ratios. The data obtained was tabulated and statistical analysis was done using student unpaired't' test and comparison between the groups was done by analysis of variance.

## RESULTS

The Coefficient of variation with internal standard for low control plasma (6.2 µmol/L) was 3.2%(n = 10), and for high control plasma (22.8 µmol/L) was 2.8% (n = 10).In group I, majority (50%) of the patients were in the age group of 51 to 60 yrs and in group II 62 % of the patients were in the 50 to 60 yrs age group. No significant difference was observed in distribution of cases among males and females (Table 1). The mean plasma level of tHcy in the control group, group I and group II was observed to be 9.8 µmol/L, 11.02µmol/L and 11.79µmol/L respectively. The p values of both group I and group II are <0.003 which indicates that the result is significant (Table 2).

**Table 1**
***Distribution of study subjects according to age***

| Age (years) | Controls | | GroupI | | GroupII | |
|---|---|---|---|---|---|---|
| | (n=60) | % | n=60) | % | (n=60) | % |
| 41- 50 | 36 | 60 | 25 | 42 | 20 | 33 |
| 51- 60 | 22 | 47 | 30 | 50 | 37 | 62 |
| >60 | 2 | 3 | 5 | 8 | 3 | 5 |

**Table 2**
***Values of plasma total homocysteine levels***

| | Healthy Controls | Group I | Group II |
|---|---|---|---|
| No. of study subjects | 60 | 60 | 60 |
| Mean plasma total homocysteine levels (µmol/L) | 9.8 | 11.02 | 11.79 |
| S.D | ±0.64 | ±1.66 | ±1.22 |
| p value | | 0.002 | 0.002 |

# DISCUSSION

The present study indicates association of hyperhomocysteinemia in NIIDM individuals with and without CVD in Goan population. The process of identifying homocysteine as a possible risk factor for CVD started in 1964[11]. In 1969, McCully made an observation linking elevated plasma homocysteine concentrations with vascular disease[12]. In 1976, Wilcken and Wilcken showed that hcy-cysteine mixed disulfide was slightly higher in coronary heart disease (CHD) patients than in controls, thus providing the first evidence of an association between mild hyperhomocysteinemia and vascular disease[13]. Many previous prospective studies have found high plasma or serum tHcy to increase the risk of cardiovascular disease or mortality[6, 7, 14]. The study at Harvard medical school demonstrated the association of hyperhomocysteinemia and abnormal urinary albumin excretion with hyperinsulinemia, suggesting the risk of CVD in insulin resistance patients[15]. Recent studies have demonstrated that is an important risk factor in patients who are already at high risk, such as those with NIDDM[5, 10]. Total homocysteine is frequently elevated in NIDDM patients who have coexistent CVD[16]. In contrast, patients with diabetes without vascular disease may have low thcy, but thcy is still a predictor of mortality[5]. Such an interaction between hyperhomocysteinemia and NIDDM with regard to cardiovascular risk may be clinically important, as it implies that homocysteine lowering treatment may be especially effective in NIDDM patients. A prospective, population based study with 5 year follow up indicates that hyperhomocysteinemia is a risk factor for overall mortality in NIDDM patients, independent of major cardiovascular risk factors. Moreover, hyperhomocysteinemia appeared to be a stronger (1.9 fold) risk factor for death in diabetic than in non diabetic subjects. For each 5-µmol/L increment of serum tHcy, the risk of 5-year mortality rose by 17% in the non diabetic and by 60% in the diabetic subjects[17]. A meta-analysis showed that folic acid treatment can lower serum tHcy by 15% to 40% within 6 weeks[18]. In addition, it has been estimated that lowering thcy by 5 µmol/L) may reduce the risk of cardiovascular death by approximately10 %[19]. On the contrary there are studies which did not find any association between plasma or serum tHcy with the risk of CVD [20, 22]. Preliminary data of an Indian study indicated that Plasma tHcy levels are not elevated in subjects with CVD and probably there is no association between total homocysteine and CHD in Indians. They found median total homocysteine and the percentage of abnormal values to be similar in CHD and non CHD groups and high homocysteine values in non diabetic non CHD groups[23.] Thus predictive value of hyperhomocysteinemia may vary from one population group to other.

## CONCLUSION

Homocysteine levels are elevated significantly in NIDDM individuals with or without CVD as compared to healthy controls in Goan population. A follow up study of thcy levels in NIDDM patients is required to see whether homocysteine can be used as an independent risk factor of CVD.

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