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ESTIMATION OF SERUM HOMOCYSTEINE LEVELS AMONG TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT HYPERTENSION

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ABSTRACT:

Introduction: Homocysteine produces oxygen radicals (superoxide anions and hydrogen peroxide), which are known to cause vasoconstriction. Individuals with diabetes mellitus often develop hypertension. It is conceivable that diabetic individuals' hypertension is caused by elevated plasma homocysteine levels.The present study was conducted to estimate the serum Homocysteine levels in type 2 diabetic patients with and without Hypertension

Materials and Methods: This is a cross sectional study performed at Department of Biochemistry, Viswabharathi Medical College, Kurnool. The study consisted of 30 type 2 diabetic patients with Hypertension and 30 type 2 diabetic patients without Hypertension. Plasma homocysteine &serum malondialdehyde (a lipid peroxidation product)were measured in all the patients.

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Results: The mean age was 61.02±5.13 in case group, while in control group, the mean age was 54.36±6.51. in case group 20(67%) were male & 10 (33%)were female. In control group, 21(70%) were male & 9(30%) were females. The mean SBP in case group was 158.87 ± 13.64 and mean SBP in control group was 116.28 ± 6.25. the mean DBP in case group was 93.19± 7.12 and mean DBP in control group was 82.02 ± 6.75.The Homocysteine levels were significantly higher in case group compared to control group and Melondialdehyde levels were higher in case group compared to control group

Conclusion: Homocysteine levels were significantly higher in type II diabetes mellitus patients with hypertension than in type II diabetes mellitus patients without hypertension, indicating that hyperhomocysteinemia may be responsible for causing and maintaining hypertension in type II diabetes mellitus patients.

Keywords: Type 2 Diabetes Mellitus, hypertension, hyperhomocysteinemia, malondialdehyde,

Introduction:

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin which can be autoimmune type1 diabetes or to adult onset type 2 diabetes. Diabetes occurs worldwide and the incidences of both type 1 and type 2 diabetes are rising. Worldwide, approximately 200 million people currently have type II diabetes mellitus (DM), a prevalence that has been predicted to increase to 366 million by 2030. [1]

Homocysteine is a sulphur-containing non-protein amino acid, an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine [2] . It can be produced via demethylation of the terminal carbon of dietary methionine, which is abundant in animal protein [3] . This amino acid is present in plasma in different forms; 1% circulates as free thiol, while 70% - 80% is present as a disulphide bound to plasma proteins, mainly albumin and 20% - 30% combines with itself to form the dimer homocysteine [4] . Homocystéine has mol.wt of 135.18 g/mol, and it is a homologue of the amino acid cysteine, from where it derived its name because of its molecular similarities to cysteine. Homocysteine can also be produced by heating the amino acid methionine with sulphuric acid [2] [5] .

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Homocysteine is thought to help regulate glucose metabolism. [6] High homocysteine levels have been reported in diabetic patients. Homocysteine is known to produce oxygen radicals through various mechanisms. Homocysteine generates oxygen radicals spontaneously (7). Superoxide anion, hydrogen peroxide, and hydroxyl radical are produced during auto-oxidation of homocysteine (8,9). The sulfhydryl group of homocysteine acts catalytically with ferric or cupric ions to generate hydrogen peroxide, oxygen radicals, and homocysteine (10). Oxygen radicals are known to produce endothelial cell injury (11,12). Superoxide anion and hydrogen peroxide produce vasoconstriction (13,14).

Hyperhomocysteinemia could produce hypertension by causing endothelial cell damage and hence, unopposed action of vasoconstrictors, and/or vasoconstriction through oxygen radicals.Hypertension contributes to 75% of all strokes and heart attacks. [15] Several studies, some population-based, found plasma homocysteine levels linked to blood pressure, especially systolic pressure. Therefore, The present study was conducted to estimate the serum Homocysteine levels in type 2 diabetic patients with and without Hypertension.

Materials and Methods:

This observationalcase-control study was done on 60 randomly selected Type-2 DM in the Department of Biochemistry, Viswabharathi Medical College, Kurnool for a period of 6 months after taking informed consent. The approval of institutional ethics committee was taken prior to the commencement of this study. These diabetic mellitus patients are divided into 2 subgroups: case group (n = 30) included type 2 diabetes mellitus patients with Hypertension; control group (n = 30)includes type 2 diabetes mellitus patients without Hypertension.

Inclusion Criteria:

1. Age should be between 40 and 65 years.

2 type 2 diabetes mellitus patients with hypertension

3. type 2 diabetes mellitus patients without hypertension.

Exclusion Criteria: Pregnancy, Severe renal impairment, Severe hepaticimpairment, Cancer, Thyroid disease, Patient on lipidloweringdrugs, Patient on vitamin B supplements, Patientaged less than 40 and more than 65 years.

Measurement of Blood Pressure: Blood pressure was assessed with a standard mercury sphygmomanometer. After 5 minutes of rest in a sitting position, blood pressure was measured three times at 5-minute intervals in the right upper arm with the appropriate size

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cuff. The average of the three measurements was used for analysis. Hypertension was defined as systolic and diastolic pressure greater than 120 mm Hg and 90 mm Hg, respectively.

Biochemical analysis: Hettich Universal 32 Centrifuge (Germany) was used to spin the blood specimens.Serum Homocysteine concentration was assayed using a commercially ELISA Kit (Nelsin Medical Co., Limited, China). Serum homocysteine concentrations were read on a micro-plate reader (RT-6000 Rayto China).The homocysteine content in the serum was expressed as umol/L.MDA levels in serum were measured as thiobarbituric acid reactive substance. Thiobarbituric acid reactive substances were extracted in a mixture of butanol and pyridine, which was separated by centrifugation. The fluorescence intensity of butanol pyridine mixture was measured at 553 nm with excitation at 513 nm. The MDA content in the serum was expressed as umol/L.

Statistical analysis: SPSS software version 23 was used to perform statistical analyses. Descriptive statistics for quantitative variables was represented as mean +/- SD. Qualitative variables was represented as frequency and percentages. using two-tailed student's t-test. $P < 0.05$ was considered statistically significant.

Results:

The study was composed of a total of 60 subjects comprising 30 type II diabetic mellitus and hypertensive patients (Case group)and 30 type II diabetic mellitus and normotensive patients (control group). In our study, the mean age was 61.02±5.13 in case group, while in control group, the mean age was 54.36±6.51. in case group 20(67%) were male & 10 (33%)were female. In control group, 21(70%) were male & 9(30%) were females. The mean SBP in case group was 158.87 ± 13.64 and mean SBP in control group was 116.28 ± 6.25. the mean DBP in case group was 93.19± 7.12 and mean DBP in control group was 82.02 ± 6.75 as shown in Table 1

Table 1: Demographic &Clinical characteristics of the study groups.

	Case group	Control group
Number of Subjects (n)	30	30
Age (yrs) mean ± SD	61.02±5.13	54.63±5.47
Sex (m/f)	20/10	21/9

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SBP (mm Hg) mean ± SD	158.87 ± 13.64	116.28 ± 6.25
DBP (mm Hg) mean ± SD	93.19± 7.12	82.02 ± 6.75

The Homocysteine levels were significantly higher in case group compared to control group and Melondialdehyde levels were higher in case group compared to control group as shown in Table 2, Fig. 2 & Fig. 3

Table 2: Comparison of Homocysteine and Melondialdehyde levels in case & control group

	Case group	Control group	P value
Homocysteine (µmol/L)	20.47 ± 6.12	14.86 ± 6.86	0.003*
Melondialdehyde (µmol/L)	1.83 ± 1.14	1.12±0.54	0.02*

* significance

Fig 1: Comparison of Homocysteine in case & control group

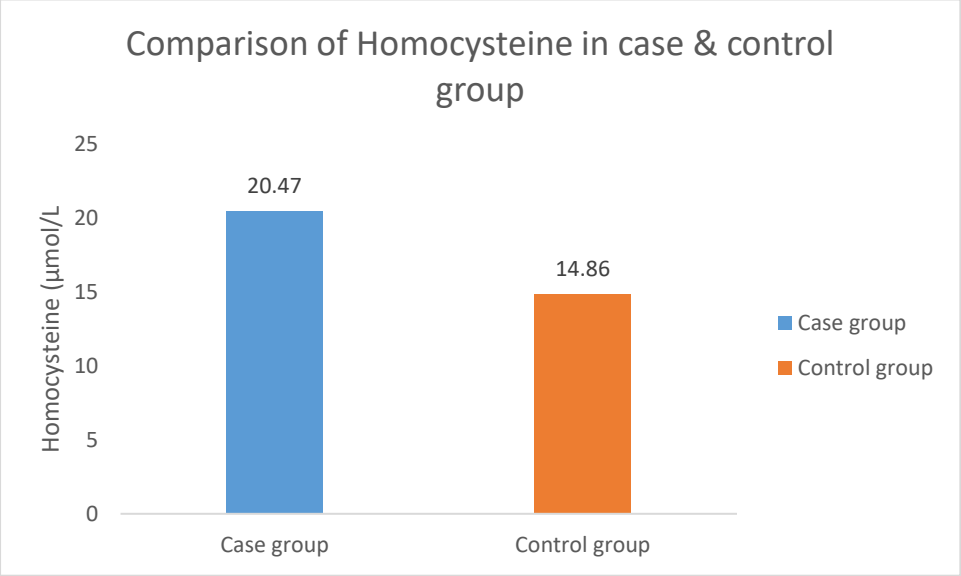

Fig 2: Comparison of Melondialdehyde in case & control group

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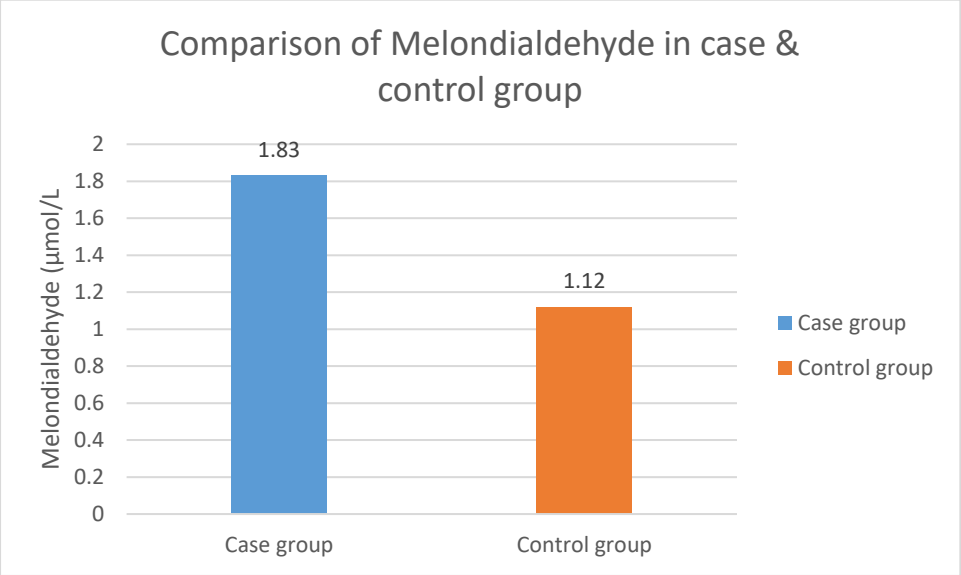

Discussion:

Our study found that the average age in cases and controls was 61.02±5.13 and 54.36±6.51, respectively. Kangabam et al. [16] found that patients in cases and controls had mean ages of 64.53±7.70 and 57.63±9.64, respectively. In a study done by Ashok Kumar Behera et al. [17], the average age of patients in cases and controls was 64.0±5.14 and 58.0±6.24, respectively.

In our study, we found that there were 67% men and 33% females in cases, and 70% males and 30% females in controls. Males predominated in our stud. In a comparable study conducted by Kangabam et al. [16], there were 77% men and 23% females, indicating male preponderance.

Our research found that the average Homocysteine levels in patients and controls were 24.14±9.51 and 15.17±7.96, respectively. Kangabam et al [16] found that patients with diabetes and cardiovascular complications had significantly higher Homocystein levels than those with only diabetes.In a comparable study done by Ashok Kumar Behera et al [17], the mean homocysteine levels in patients and controls were 19.4±7.5 and 12.75±4.2, respectively. Another study conducted by Jayakrishnan et al [18] found that homocysteine increases diabetes complications and the advancement of cardiovascular illnesses.

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These findings indicate that homocysteine levels are higher in diabetic patients with hypertension. MDA levels in hypertensive diabetic individuals are likewise increased, indicating high quantities of oxygen radicals. Homocysteine may cause hypertension by generating oxygen radicals [19,20], which cause vasoconstriction. [21,22] Homocysteine may potentially cause hypertension by damaging the vascular endothelium. Oxygen radicals are known to cause endothelial cell damage [23,24], which reduces or eliminates the synthesis of vasodilators like nitric oxide. Because of the injury, circulating vasoconstrictors (norepinephrine, epinephrine, and angiotensin II) will have unopposed effect on the vasculature, resulting in contraction and hypertension. These findings indicate that hyperhomocysteinemia may be responsible for initiating and maintaining hypertension in diabetic individuals.levels than those with only diabetes.

Conclusion:Homocysteine levels were significantly higher in type IIdiabetes mellitus patients with hypertension than in type II diabetes mellitus patients without hypertension, indicating that hyperhomocysteinemia may be responsible for causing and maintaining hypertension in type II diabetes mellitus patients.

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