



To study the association between plasma homocysteine and microvascular and macrovascular complications in type 1 Diabetes Mellitus.

Pharmacology

Sudhir Bhandari	Senior Professor, Department of Medicine, SMS, Jaipur
Prahlad Dhakar	Asst Professor, dept. of medicine, RUH College of medical sciences
Jaswant Goyal	Asst Professor, Department of Pharmacology, JNUIMSRC, Jaipur - Corresponding Author
Barkha Goyal	Tutor, Department of Biochemistry, JNUIMSRC, Jaipur
Purva Sharma	Junior Resident, JNUIMSRC, Jaipur

ABSTRACT

Background

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels¹.

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. 366 million people have diabetes in 2011; by 2030 this will have risen to 552 million. 183 million people (50%) with diabetes are undiagnosed. Diabetes caused 4.6 million deaths in 2011. 78,000 children develop type 1 diabetes every year².

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease.

In a large cross sectional study of type 1 diabetic subjects, a significant and independent relationship was found between increased total homocysteine concentrations and macroalbuminuria, renal function and hypertension which suggests that total homocysteine might play an important role in the pathogenesis of vascular complications in type 1 Diabetes³.

Materials & Methods

The study was conducted in out patient department of tertiary care hospital of north India. It was a hospital based Case-Control analytical type of observational study. This study was carried out From November 2011 to December 2012.

A total of 62 patients were enrolled in the study in which Cases include 31 patients of type 1 diabetes mellitus with One or more vascular complications and controls include 31 patients of type 1 diabetes Mellitus without any vascular complications.

They divide in 31 Cases (25 male and 6 female) and 31 controls (27 male and 4 female). Out of 31 cases 25 (80.65%) have retinopathy, 21 (67.74%) have nephropathy and 14 (45.16%) have neuropathy and 3 patients have coronary artery disease and 1 patient has peripheral artery disease. In 10 (32.26%) patients all 3 microvascular complications are present while in 9 (29.03%) patients two microvascular complications are present.

Plasma total homocysteine was measured by Chemiluminescence Microparticle Immunoassay. Complications were diagnosed as Retinopathy by Fundus examination, neuropathy by nerve conduction study, coronary heart disease by ECG and nephropathy by microalbuminuria (Random spot urine sample for albumin-creatinine ratio). Between case and control group no significant difference in mean age, duration of diabetes, HbA1c, and vitamin B12 and folate level.

Results

The mean plasma homocysteine was higher in case group as compared to control group. (14.27 ± 4.99 vs. 10.21 ± 3.0 , P-value < 0.01). Patients with non proliferative retinopathy have higher plasma homocysteine as compared to patients without retinopathy, but this is statistically non significant. (12.58 ± 3.92 vs. 11.22 ± 3.05 , P-value > 0.05). Patients with proliferative retinopathy have statistically significant higher plasma homocysteine as compared to patients without retinopathy (18.64 ± 4.41 vs. 11.22 ± 3.05 , P-value < 0.01). Patients with microalbuminuria have higher plasma homocysteine as compared to normoalbuminuric patients. (15.60 ± 4.61 vs. 11.50 ± 4.59 , P-value < 0.05). Patients with neuropathy have higher plasma homocysteine as compared to patients without neuropathy. (15.77 ± 5.09 vs. 13.05 ± 4.54 , P-value > 0.05).

Discussion

Above finding suggests that homocysteine might play an important role in the pathogenesis of vascular complications in type 1 diabetes. The biological mechanism for the interaction between diabetes and elevated plasma homocysteine on vascular complications is still not well understood, although proposals include oxidative stress, endothelial damage and decreased nitric oxide bioavailability.

Conclusion

In Type 1 Diabetes Mellitus: Plasma homocysteine level is higher in patients with vascular complications as compared to patients without these complications. Proliferative retinopathy and microalbuminuria significantly associated with Plasma homocysteine level, but nonproliferative retinopathy and neuropathy does not show significant association.

KEYWORDS

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels¹.

DM is classified on the basis of the pathogenic process that leads to hyperglycaemia. The Four broad categories of DM are designated (A) Type 1 DM is the result of complete or near-total insulin deficiency. (B) Type 2 DM is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. (C) Other specific types of DM include Genetic defects of β cell function and Genetic defects in insulin action etc. (D) Gestational diabetes mellitus (GDM)^{1,4}.

The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. 366 million people have diabetes in 2011; by 2030 this will have risen to 552 million. 183 million people (50%) with diabetes are undiagnosed. Diabetes caused 4.6 million deaths in 2011. 78,000 children develop type 1 diabetes every year².

The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications [coronary heart disease (CHD), peripheral arterial disease (PAD), cerebrovascular disease]. Nonvascular complications include problems such as

gastroparesis, infections, and skin changes⁴.

In type 1 diabetes, the major risk is microvascular complications, although macrovascular complications are also increased. The primary risk factor is hyperglycaemia, although other risk factors, such as hypertension and dyslipidaemia, may occur secondary to uncontrolled hyperglycaemia or renal disease. Complications are therefore usually acquired after diagnosis⁵.

Elevated total plasma homocysteine levels are associated with cardiovascular disease (CVD) risk in the general population, independent of classical risk factors^{6,7}. People with type 1 and 2 diabetes are particularly at high risk of CVD, as well as microvascular complications, and these risks cannot be completely accounted for by conventional risk factors.

There is considerable evidence for a relationship between elevated total homocysteine and CVD^{8,9} and all-cause mortality^{10,11} from several prospective studies in patients with type 2 diabetes mellitus. Elevated total homocysteine levels were associated with nephropathy^{12,13}, and retinopathy^{14,15} in patients with type 2 diabetes mellitus. Given the clear vascular nature of microvascular complications, it is reasonable to hypothesize that total homocysteine may also be involved in the genesis of these complications in type 1 diabetes.

Homocysteine (Hcy) is a nonessential sulfur-containing amino acid and an intermediary metabolic product derived from the demethylated essential amino acid methionine.

Most previous cross-sectional studies in patients with type 1 diabetes reported positive associations between total plasma homocysteine and nephropathy^{16,17}, but not for retinopathy^{18,19}, although some did²⁰

In a large cross sectional study of type 1 diabetic subjects, a significant and independent relationship was found between increased total homocysteine concentrations and macroalbuminuria, renal function and hypertension which suggests that total homocysteine might play an important role in the pathogenesis of vascular complications in type 1 Diabetes²¹.

A possible explanation for the hypothetically increased susceptibility of diabetic subjects to raised plasma homocysteine concentrations may be an acceleration of glucose-induced oxidative stress on endothelial cells. This hypothesis was confirmed in an animal model, showing homocysteine induced endothelial dysfunction much more readily in the presence of diabetes than in its absence²².

Materials & Methods

The study was conducted in out patient department of tertiary care hospital of north India . It was a hospital based Case-Control analytical, randomized type of observational study. This study was carried out From November 2011 to December 2012.

A total of 62 patients were enrolled in the study after obtaining written informed consent in which Cases include 31 patients of type1 diabetes mellitus with One or more vascular complications and controls include 31 patients of type1 diabetes Mellitus without any vascular complications.

They divide in 31Cases (25male and 6 female) and 31 controls (27 male and 4 female). Out of 31 cases 25 (80.65%) have retinopathy, 21(67.74%) have nephropathy and 14 (45.16%) have neuropathy and 3 patients have coronary artery disease and 1patient has peripheral artery disease. In 10 (32.26%) patients all 3 microvascular complications are present while in 9(29.03%) patients two microvascular complications are present.

Patients with age>50 years , seriously ill and patients on life supporting measures were not included in the study.

A thorough History and physical examination was done of enrolled cases and controls. Anthropometric parameters such as weight, height, body mass index (BMI) were measured.

All following routine and special investigations were done:

- Blood sugar: fasting and post prandial.
- Renal function test: S. urea, S. creatinine, S. electrolytes.
- Liver function test: S. bilirubin, SGOT, SGPT.

- Glycated Haemoglobin(HbA1c),
- Total lipid profile.
- Serum Vitamin B12 and folate
- Plasma total homocysteine (Fasting) [By Chemiluminescence Microparticle Immune Assay:CMIA. Normal range:3.7to13.9 micromol/L.]
- Urine examination: routine complete examination, for microalbuminuria (Random spot urine sample for albumin – creatinine ratio).
- Fundus examination.
- Various other routine investigations such as Complete blood count, ESR, ECG, X-ray chest & USG abdomen.

Plasma total homocysteine was measured by Chemiluminescence Microparticle Immunoassay.

Complications were diagnosed as Retinopathy by Fundus examination, neuropathy by nerve conduction study, coronary heart disease by ECG and nephropathy by microalbuminuria (Random spot urine sample for albumin–creatinine ratio).Between case and control group no significant difference in mean age, duration of diabetes, HbA1c, and vitamin B12 and folate level.

All information was collected on pre-designed Performa. Data thus collected was entered in MS Excel 2010 worksheet in the form of master chart.

This data was analysed as per the results of the study and observations were made.

Outcome variables

Microvascular complications:

1. Diabetic Nephropathy – Microalbuminuria.
2. Diabetic Retinopathy – Fundus examination.
3. Diabetic Neuropathy – Nerve conduction study.

Macrovascular complications:

1. Coronary heart disease – ECG.
2. Peripheral vascular disease (PVD) - Doppler study of peripheral arteries.
3. Cerebrovascular disease- neurological deficit→ CT/MRI of Brain and Carotid Doppler.

Results

Table No.-1

Distribution of cases & controls according to age & sex

Age group (In yrs)	Case			Control		
	Male	Female	Total	Male	Female	Total
< 25	10 (32.26)	3 (9.68)	13 (41.94)	18 (28.06)	1 (3.23)	19 (61.29)
> 25	15 (48.39)	3 (9.68)	18 (58.06)	9 (29.03)	3 (9.68)	12 (38.71)
Total	25 (80.64)	6 (19.35)	31 (100.00)	27 (87.10)	4 (12.90)	31 (100.00)

Mean + SD = 26.45 + 5.19 Mean + SD = 23.87 + 5.39 P>0.05

Table no.1 shows, that males are 25(80.64%) in cases and 27(87.10%) in controls. In cases mean age is 26.45 + 5.19 and controls mean age is 23.87 + 5.39. The difference of age and sex between cases and controls is not significant.

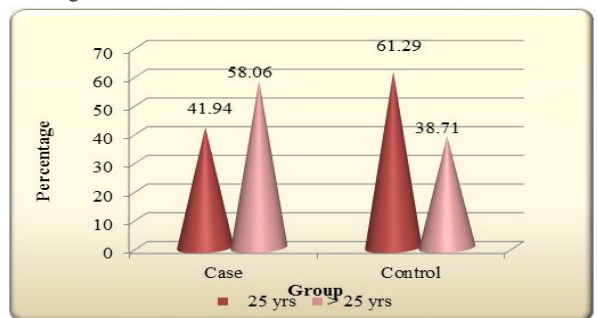
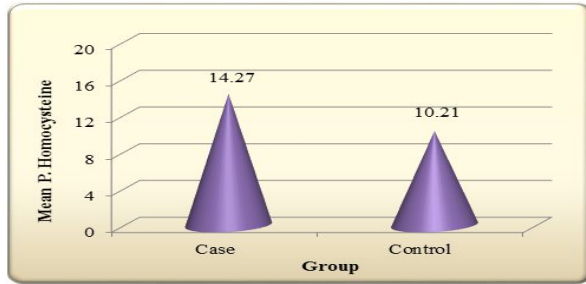


Table No.-2

Mean + SD of Plasma Homocysteine of case & control patients

P.Homocysteine	Mean + SD		P-value	Significance
	Case	Control		
	14.27 + 4.99	10.21 + 3.07	< 0.01	Sig

Table no.2 shows mean plasma homocysteine in both groups. In cases mean plasma homocysteine is higher as compare to controls (14.27±4.99 vs.10.21±3.07). This is statistically significant (P-value <0.01).



Graph showing : Mean of Plasma Homocysteine of case & control patients

Table No-3
Mean + SD of P.Homocysteine according to duration of DM of case & control subjects

	Duration (In yrs)		P-value	Significance
	< 5	> 5		
Case	11.25 + 3.99 (n=13)	16.46 + 4.47 (n=18)	< 0.01	Sig.
Control	9.51 + 2.87 (n=17)	11.08 + 3.10 (n=14)	>0.05	NS

Table no.3 shows, that in both case and control group plasma homocysteine is higher in patients with duration > 5 years as compare to patient with duration < 5years. This is significant in cases not in controls. (Cases: 16.46 + 4.47 vs.11.25 + 3.99, p<0.01, controls: 11.08 + 3.10, 9.51 + 2.87, P>0.05)

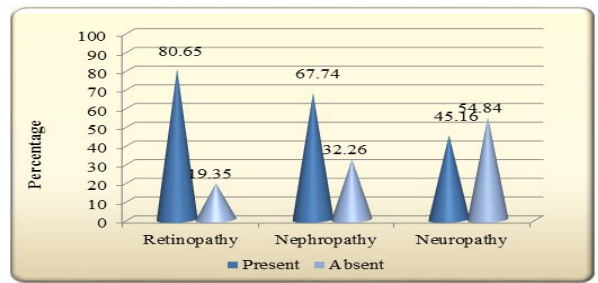


Graph showing : Mean of P.Homocysteine according to duration of DM of case & control subjects

Table No.-4
Distribution of micro-vascular complications in cases

	Retinopathy	Nephropathy	Neuropathy
Present	25 (80.65)	21 (67.74)	14 (45.16)
Absent	6 (19.35)	10 (32.26)	17 (54.84)
Total	31 (100.00)	31 (100.00)	31 (100.00)

Table no.4 shows that in cases retinopathy is present in 25(80.65%) patients, nephropathy in 21(67.74%) and neuropathy in 14(45.16%) patients.

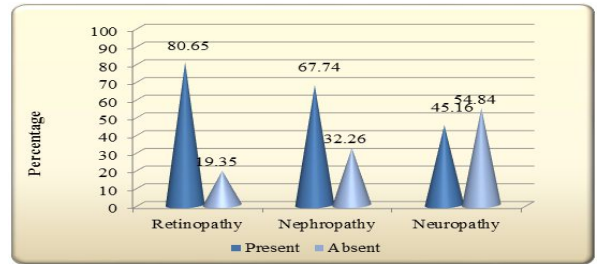


Graph showing : Distribution of micro-vascular complications in cases

Table No.-5
Distribution of micro-vascular complications in cases

	Retinopathy	Nephropathy	Neuropathy
Present	25 (80.65)	21 (67.74)	14 (45.16)
Absent	6 (19.35)	10 (32.26)	17 (54.84)
Total	31 (100.00)	31 (100.00)	31 (100.00)

Table no. 5 shows that in cases retinopathy is present in 25(80.65%) patients, nephropathy in 21(67.74%) and neuropathy in 14(45.16%) patients.



Graph showing : Distribution of micro-vascular complications in cases

Table No.-6
Distribution of macro vascular complications in cases

	Coronary artery disease	Peripheral arterial disease	Cerebro vascular disease
Present	3 (9.68)	1 (3.25)	0
Absent	28 (90.32)	30 (96.77)	31 (100.00)
Total	31 (100.00)	31 (100.00)	31 (100.00)

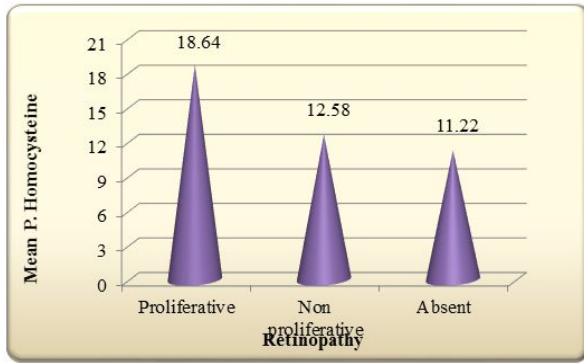
Table no.6 shows that only 3 patients have Coronary artery disease, 1 patient has peripheral artery disease and nobody have cerebrovascular disease.

Table No.-7
Mean + SD of P.Homocysteine according to Retinopathy of case group patients

Retinopathy	No.	Mean + SD
Nonproliferative	15	12.58 + 3.92
Proliferative	10	18.64 + 4.41
Absent	6	11.22 + 3.05

Non-proliferative vs. absent p > 0.05 Non Sig, Proliferative vs. absent P < 0.01. significant

Table no.7 shows in patients with proliferative retinopathy, mean plasma homocysteine is statistically significant higher as compare to patient without retinopathy. (18.64 + 4.41 vs. 11.22 + 3.05, p < 0.01). But nonproliferative retinopathy patients have no significant difference from patient without retinopathy. (12.58 + 3.92 vs. 11.22 + 3.05, p > 0.05).

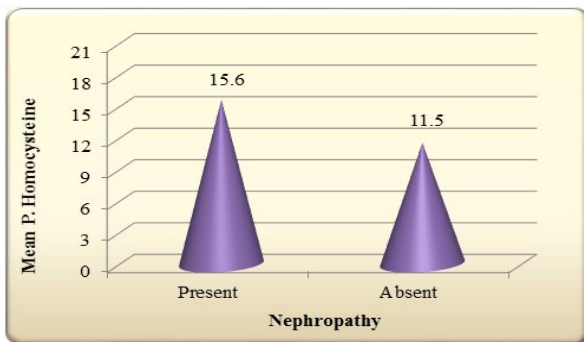


Graph showing : Mean of P.Homocysteine according to Retinopathy of case group patients

Table No.- 8 Mean + SD of P.Homocysteine according to Nephropathy of Case group patients

Mean + SD	Nephropathy		P-value	Significance
	Present	Absent		
	15.60 + 4.61 (n=21)	11.50 + 4.59 (n=10)		

Table no. 8 shows that patients with nephropathy have higher mean plasma homocysteine as compare to patients without nephropathy, this difference is statistically significant. (15.60 + 4.61 vs. 11.50 + 4.59, p< 0.05)

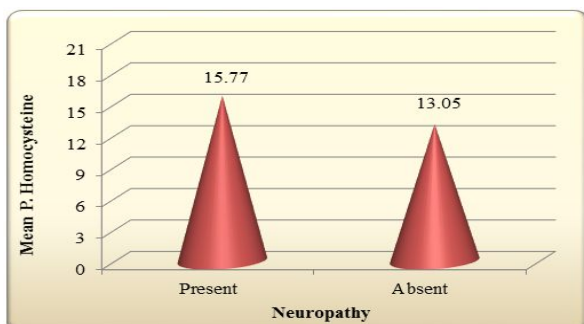


Graph showing : Mean of P.Homocysteine according to Nephropathy of Case group patients

Table No.-9 Mean + SD of P. Homocysteine according to Neuropathy of case group patients

Mean + SD	Neuropathy		P-value	Significance
	Present	Absent		
	15.77 + 5.09 (n=14)	13.05 + 4.54 (n=17)		

Table no.9 shows, Patients with neuropathy have higher mean plasma homocysteine as compare to patients without neuropathy but this difference is statistically non significant. (15.77 + 5.09 vs. 13.05 + 4.54, P>0.05)



Graph showing : Mean of P. Homocysteine according to Neuropathy of case group patients

Observations

1. The mean plasma homocysteine is higher in case group as compared to control group. This difference is statistically significant. (14.27 ± 4.99 vs. 10.21 ± 3.0, P-value < 0.01).
2. The plasma homocysteine has positive correlation with duration of diabetes in both cases and controls, but this is statistically significant in only cases, not in controls (cases: r-value + 0.447, P-value < 0.05, controls: r-value + 0.127, P-value > 0.05).
3. Patients with non proliferative retinopathy have higher plasma homocysteine as compared to patients without retinopathy, but this is statistically non significant (12.58 ± 3.92 vs. 11.22 ± 3.05, P-value > 0.05).
4. Patients with proliferative retinopathy have statistically significant higher plasma homocysteine as compared to patients without retinopathy (18.64 ± 4.41 vs. 11.22 ± 3.05, P-value < 0.01).
5. Patients with microalbuminuria have higher plasma homocysteine as compared to normoalbuminuric patients; this is statistically significant (15.60 ± 4.61 vs. 11.50 ± 4.59, P-value < 0.05).
6. Patients with neuropathy have higher plasma homocysteine as compared to patients without neuropathy, but this is statistically non significant. (15.77 ± 5.09 vs. 13.05 ± 4.54, P-value > 0.05).

Conclusions

In Type I Diabetes Mellitus:

- Plasma homocysteine level is higher in patients with vascular complications as compared to patients without these complications.
- Proliferative retinopathy is significantly associated with Plasma homocysteine level, but nonproliferative retinopathy does not show significant association.
- Microalbuminuria is significantly associated with plasma homocysteine level.
- Neuropathy does not show significant association with plasma homocysteine level.
- Above finding suggests that homocysteine might play an important role in the pathogenesis of vascular complications in type 1 diabetes, but this needs to be investigated further. A prospective study is needed to determine causal relationships and elucidate the mechanisms.

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