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FACTORS DETERMINING SERUM HOMOCYSTEINE LEVEL IN TYPE 2 DIABETIC PATIENTS.

Diabetology

KEYWORDS: Hyperhomocysteinemia, Microangiopathy, Vit B12deficiency, Folate deficiency

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ABSTRACT

Hyperhomocysteinemia is an independent risk factor for micro and macroangiopathy in diabetic patients. We designed this study to determine the factors which regulate homocysteine levels in patients with type 2 diabetes.

Methods: We conducted a cross sectional study during one month posting in the department of neurochemistry Christian medical college,Vellore as a part of fellowship training, which included 20 randomly selected Type 2 diabetic patients either on oral anti-diabetic agents or on insulin. The clinical profile of these patients was documented. Serum homocysteine, vitamin B12 &folate were analysed. Serum Homocysteine assay was done by the HPLC method.

Results: The mean serum homocysteine level was 23.01 µmol/l (normal<15µmol/l). Seventy percent of the patients had serum homocysteine level more than 15µmol/l. There was no correlation between serum homocysteine level and age, BMI, duration of diabetes, HbA1C, serum creatinine and urinary microalbumin. The median vitamin B12 was 275 pg/ml and mean serum folate level was 10.48 ng/ml. We found significant negative correlation between fasting serum homocysteine levels and vitamin B12 and folate. The median vitamin B12 levels in those with serum Homocysteine levels less than 15 and more than 15 were 592 and 210 pg/ml respectively. The mean folate in those with serum Homocysteine levels less than 15 and more than 15 were 15 and 8 ng/ml respectively. The difference in the vitamin B12 and folate levels between these two groups were statistically significant.

Conclusion: In our study hyperhomocysteinemia did not correlate with any of the microvascular complications of diabetes, but there was significant negative correlation with vitamin B12 and folate levels.This shows that hyperhomocysteinemia is an indirect marker of vitamin B12 and folate deficiency. It is important to assess vitamin B12 and folate levels in diabetic patients with elevated homocysteine levels.

Introduction

Diabetes mellitus is a major health burden in developing countries. Diabetes related complication is increased with the duration of diabetes and due to underlying hyperglycemic state of the subject. Though so many theories including advanced glycation end products, hyperglycemia, oxidative stress,explaining the microangiopathic complication in diabetes, serum homocysteine has demonstrated strong association with diabetes mellitus(1). Homocysteine is a sulphur containing amino acid abundant in animal protein, which is derived from methionine. High homocysteine has been postulated to be associated with occurance of coronary, cerebral and peripheral vascular disease. In diabetic patients hyperhomocysteinemia has been found to have a modest association with occurance of microangiopathy (2). Serum homocysteine level is varied by many factors in diabetic patients. We analysed the factors which determine the serum homocysteine level in Type 2 diabetic patients

Materials and Methods

Aim is to determine the factors which regulate the serum homocysteine levels in patients with type 2 diabetes mellitus. This was conducted during one month peripheral posting in the department of neurochemistry,Christian medical college, Vellore as a part of fellowship training programme in endocrinology.

It is a cross sectional study which included randomly selected 20 type 2 diabetic patients who attended our out patient diabetic opd. Subjects with either on oral anti-diabetic agents or on insulin were included in the study.

The clinical profile of these patients were documented. Informed consent was taken.All the patients height ,weight and BMI has been calculated. Dilated fundus examination of the patient was done by ophthalmologist. Serum sample has been collected for HbA1C, lipid profile, serum creatinine. HbA1c was done by HPLC-High performance Liquid Chromatography method. Urine sample analysed for microalbumin. Urine microalbumin has been graded as < 150 and >150 mg/ day. Along with this serum fasting homocysteine, vitamin B12 &folate were estimated. Serum homocysteine was done by HPLC method (High performance Liquid Chromatography). Patients with hypothyroidism, history of smoking , alcohol intake, other autoimmune diseases, chronic kidney disease stage 4 or 5, on any vitamin B supplements are excluded from the study.

Homocysteine assay

Homocysteine is measured by HPLC method (High performance Liquid Chromatography).

The sample brought to laboratory within 60 mins of collection, serum was separated by centrifugation at 450 g for 10 mins (25 degree Celsius).The sera was stored at -20° c until the assay is processed.

Serum homocysteine is analysed by pretreating the sample by reduction with 35 mM beta mercaptoethanol,50 mMTris HCL containing 0.34Mm EDTA PH 8.0 for 1 hr at 37°c followed by deproteinizing with TCA to 2.5%

The deproteinized supernatant was treated with 200mM iodoacetamide adjusted to ph 8.0 with 2M KOH-KHCO3 for 15 min at 25°c in the dark to alkylate reduced homocysteine.An aliquot of the reaction mixture was diluted 1.33 times with 335 mM sodium citrate ph 2.22, filtered through 0.22micro m filter and 10 micro l taken for HPLC analysis.Aminoacids are identified by retention times and quantified by the area under the peak read against standard.

Statistical analysis

Statistical analysis has been done by SPSS 16 Version

Results

Total number of patients studied are 20 . In which 40% are female and the remaining 60% of them are male. The mean age of the patient is 53.7 & the mean duration of diabetes is 7.2years.The mean weight and body mass index are 62&24 respectively. (Table-1).

Table-1- Basic demographic data

Variable	Mean	Std. Deviation	Range
Age(years)	57.7	6.55	42-65
Duration(years)	7.2	4.0	1-17
Height(cm)	159.6	7.41	145-173
Weight(kg)	62.03	10.16	42-77
BMI(kg/m^)	24.3	3.63	17-32

In total 20 patients 75% of the patient was on only oral diabetic drugs (glimeperide and glyciphage/glyciphage), 20% was on insulin and oral diabetic drug and remaining 5% was on only insulin . The mean HbA1c is 7.6%. Among the total no 25% of them had urine microalbumin more than 150mg/dl and 70% had less than 150mg/dl urine micro albumin. The median vitB12 is 275 pg/ml and mean folate is 10.48ng/ml (Table-2).

Table-2-Biochemical parameters of the subjects

Variable	Mean	Std. Deviation	Range
Sr. Creatinine (mg/dl)	1.04	0.32	0.6-2
HbA1c(%)	7.6	1.81	5.6-12.3
Urine protein (mg/gm)	20 ¥	458.59	5-2000
Vit.B12 (pg/ml)	275.65 ¥	352.28	93-1392
Folate (ng/ml)	10.48	5.51	4.16-20
Homocysteine (µmol/l)	23.02	9.39	9.8-39.4
GFR (ml/min/1.72sq m	77.78	26.46	32-146

The mean serum homocysteine is 23.02µmol/l. Six of them had normal serum homocysteine level (15 µmol/l), six had mild hyperhomocysteinemia (6-25µmol/l), eight had moderate (26-50 µmol/l) & none had severe hyperhomocysteinemia (>50 µmol/l). Among the total 20 patients six (30%) of them had serum homocysteine level less than 15 µmol/l. While the other 14(70%) had serum homocysteine level more than 15 µmol/l.

Homocysteine group has been divided in to 2groups ,with those having less than 15 µmol/l and more than 15µmol/l. Median serum B 12 in the < 15µmol/l and >15µmol/l homocysteine group is 591pg/dl & 210pg/dl respectively. Mean folate in the < 15µmol/l and > 15µmol/l homocysteine group is 15ng/dl & 8 ng/dl respectively. Mean difference in vit B12 and folate in the two groups are statistically significant(Table-3).

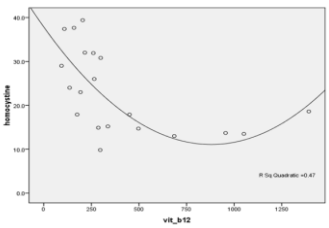
There is no correlation between the serum homocysteine level and the duration of diabetes, BMI, serum creatinine, HbA1C and urine protein.

Table- 3-Vitamin B12 and folate level in the two homocysteine groups

Serum Homocysteine		Vitamin B12 (pg/ml)	Folate (ng/ml)
<15	Number	6	6
	Mean	628.55	15.23
	Median	591.30	17.38
	Std. Deviation	326.49	5.69
>=15	Number	14	14
	Mean	306.20	8.44
	Median	210.05	7.42
	Std. Deviation	326.81	4.12
	P value	0.012	0.041

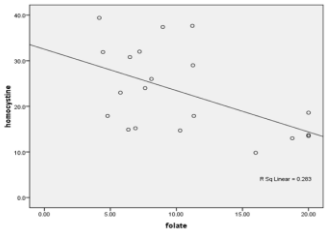
There was a significant negative correlation between vitamin B12(Fig-1), folate(Fig-2)and fasting serum homocysteine.This shows that hyper homocysteinemia is an indirect marker of vitamin B12 & folate deficiency.It is important to assess vitamin B12 and folate levels in diabetic patients with elevated homocysteine levels.

Fig-1 Correlation between Vitamin B12 and Serum Homocysteine level



There is no significant association between serum homocysteine and diabetic microvascular complications such as nephropathy (P-0.603), neuropathy (P-0.303) and retinopathy (P-0.245).

Fig-2 Correlation betweem serum folate and serum homocysteine level



Discussion

Homocysteine is formed by demethylation of an essential aminoacid methionine. Hyperhomocysteinaemia is a high risk factor for endothelial dysfunction and artherothrombotic events. Endothelial damage is the earliest change in atherosclerosis. In diabetic patients it is mediated by multiple factors, such as advanced glycation end products and hyperhomocysteinaemia. Previous studies have proposed that greater association between increased plasma homocysteine level with greater occurrences of both microvascular and macrovascular complications leading to nephropathy and coronary artery disease. Homocysteine produces both endothelial injury and altered hypercoagulant state by various mechanism. Endothelial injury induced by production of free radicals such as superoxide and hydrogen peroxidase during autooxidation metabolism of homocystine. It also enhances the hyper coagulability by different mechanisms such as modulating the expression of nitricoxide synthase and glutathione peroxidase which facilitate the binding affinity of lipoprotein (a) to fibrin, it antagonizes the antithrombin III by reducing protein C activation, enhancing the platelet activation and also by inhibitng the synthesis of anticoagulant heparin sulphate(3). Several studies were done in homocysteine and vascular disease, they assessed various parameters but biochemical parameters like vit B12 and folate levels were not evaluated in relationship with homocysteine hence this study was designed.

In our study, we have assessed both clinical and laboratory parameters in all diabetic patients and the outcome of our study showed strong negative correlation between fasting serum homocysteine and serum vitB 12 level with highly significant P valve < 0.01 and there is also strong negative correlation between fasting serum homocysteine and serum folate with significant P value < 0.04. The above two comparison indicates that lower serum level of both B12 and folate with hyperhomocysteinaemia predominantly suggest that Vit B12 and folate deficiency can lead to hyper homocysteineamia. So hyperhomocystenemia and its relation with cardiovascular diseases which was proven in previous studies (4,5).Similarly vit B12 deficiency also has indirect impact on cardiovascular diseases by altering the serum homocysteine level(6).

Studies have demonstrated that there was no correlation between serum homocysteine , age, duration of diabetes, lipids level and HbA1c (3). Nabila Abdella et al found correlation between serum homocysteine with age, BMI and GFR(5,7). In our study such correlation was not elicited. Lakshman Ramachandran et al, found significant positive correlation between HbA1c and serum homocysteine(2).

İlhanTarkun et al found that there was a significant correlation between serum homocysteine and creatinine, urinary microalbumin(3). In our study there is no significant association between homocysteine level and different range of proteinuria, creatinine and GFR. So our study parameters are coinciding with the previous study done by N.A. Abdella et al(5) . In our study, serum folate levels and Vit B12 levels had negative correlation with serum homocysteine level which is similar to L.-K. Yang, et al(9) and the study done by Shargorodsky et al(10)

Serum homocysteine levels are high in patients with diabetic

retinopathy than in a patient without retinopathy(11). It is a novel risk factor for diabetic retinopathy. Among the retinopathy high serum homocysteine level has been found in proliferative retinopathy compared to non proliferative and without retinopathy (12). In our study there was no correlation between serum homocysteine and retinopathy.

High serum homocysteine level has been found in diabetic patients with peripheral neuropathy(13,14). It is one of the modifiable factor in diabetic neuropathy. However, we didnot find any correlation between homocysteine and neuropathy.

The major limitation in our study was small sample size, this was due to laboratory constraints. The overall outcome from our study is deficiency of both vit B12 and folate can accentuate hyper homocysteinemia which is a high risk factor for artherothrombotic events. Our study strongly suggest to measure serum level of both B12 and folate along with serum homocysteine to prevent vascular complications associated with hyperhomocysteinemia in diabetes mellitus. It is an ideal target to conduct further study in a larger diabetic population.

Conclusion

In our study serum folate level and vit B12 levels are indirect proportionate with serum homocysteine level strongly emphasises that deficiency of both B12 and folate can cause hyper homocysteinemia which is one of the high risk factor for vascular complication in type 2 diabetic patients. So we conclude that serum vit B12 and serum folate are the add on essential parameter should be investigated in diabetes patient while evaluating them for hyperhomocysteinemia induced vascular complication.

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