Section: Medicine

Relationship between Peripherial Neuropathy and Serum Homocysteine Level in Type 2 Diabetic Patients.

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ABSTRACT

Background: Diabetes is worldwide one of most common cause of peripheral neuropathy(PN).Diabetes is associated with high level of serum homocysteine level and prevalence of hyperhomocysteinem was strongly associated with diabetic peripheral neuropathy in multiple logistic regression model that adjusted for factors that may influence homocysteine level and neuropathy. **Methods:** This study was conducted in department of medicine GMC Srinagar. 207 type 2 diabetic patients with clinical symptoms of peripheral neuropathy were enrolled in across sectional study after detail history and relevant clinical examination. All base line biochemical investigation along with serum total homocysteine level were done after confirmation of peripheral neuropathy by electro diagnostic test (NCS) nerve conduction study. **Results:** 207 consecutive patients were enrolled with. Mean age of patients was 60.92±6.01SD (41-80 years). Females 70%, NCV was abnormal in 64 patients (30.9%), 55 axonopathy (85.9 %) and 9 with Demyelination (14.1%). Motor abnormalities n10 (15.6%), sensory n 38(59.3%), Mixed n 16 (25%). Mean age of patients with abnormal NCV was 61.86±8.03 years significant (p value=0.0034). Female sex, urban population, and increased homocysteine levels was associated with increased risk of NCS abnormalities p value of 0.001, 0.0001and 0.001 respectively the mean homocysteine level with abnormal NCS was 23.22µmol/l and 13.28µmol/l in normal NCS. A higher homocysteine levels was associated with increased risk of abnormal NCS (p value .000001). **Conclusion:** Elevated Homocysteine concentration was major risk factor for development of neuropathy in diabetics though our study being a small scale hospital based study.

Keywords: Homocysteine, Nerve condition velocity, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by increase blood glucose level resulting from defects in insulin secretion, insulin action, or both.[1] The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.[1] The number of people with diabetes is increasing due to population growth, ageing, urbanization and increased prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important and allow rational planning and allocation of resources.[2] There are an estimated 23.6 million people in the U.S. (7.8% of the population) with diabetes and 17.9 million being diagonised4 90% of whom are type 2 diabetics.[3]

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In 2000, In India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. According to Wild et al.[4] the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease.[4,5] India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country. Homocysteine (Hcy) is an amino acid, is a homologue of the amino acid cysteine differing by an additional methylene (CH_2) group. It is biosynthesized from methionine by the removal of its terminal methyl group and can be recycled into methionine or converted into cysteine with the aid of B-vitamins.[6] Increased circulating levels of homocysteine are seen in patients with homocysteinuria.

Patients with homozygosity for the thermolabile variant of methylenetetrahydro-folate reductase or

individuals with dietary deficiency of folate and or cynocobalamin.[7] Homocysteine levels and the prevalence of hyper homocysteinemia were strongly associated with diabetic peripheral neuropathy (DPN). There were significant differences in the prevalence of hyperhomocysteinemia and homocysteine levels between patients with neuropathy and those without neuropathy. Hyperhomocysteinemia was significantly associated with DPN in multiple logistic regression models that adjusted for factors that may influence homocysteine levels and neuropathy. Recent data published by Ambroscht et al[8] and Li et al[9] suggest that high homocysteine levels could be an independent risk factor for DPN in patients with either type 1 or type 2 diabetes after adjustment for related variables. Data from animal models and in vitro experiments have linked homocysteine to neuropathy, as suggested by Sheng et al[10], Hofmann et al[11], and El Boghdady et al[12]. DPN was defined using the criteria proposed by the Diabetic Neuropathy Expert Panel Meeting.[13] High levels of plasma homocysteine are toxic to vascular endothelium via the formation of free radicals. These free radicals cause direct injury to endothelium by disrupting its integrity and exposing the underlying vascular matrix and smooth muscle thus promoting a hypercoagulability state by activation of platelets and thrombus formation.[14-18]

Increased plasma levels of homocysteine are found in any enzyme deficiency in the remethylation[19] or transsulphuration[20] process of methionine, and in thermolabile variant of the enzyme MTHFR[21,22]. It was found to be associated with age (probably due to decreased intake of folic acid and vitamin B12[23,24]), renal failure,[25-27] and medications[28-32]. Hyperhomocysteinemia and non-insulin-dependent diabetes mellitus (NIDDM) are both associated with premature vascular disease. Data indicate that homocysteine is independently associated with the prevalence of peripheral sensorimotor and autonomic neuropathy in type 2 diabetic patients. According to 2005US Congress report there are twenty million Americans suffering from peripheral neuropathy (PN).[33] PN may be caused by numerous aetiologies, such as metabolic, infectious, inflammatory, and toxic (including adverse effects of certain drugs and radiation), malnutrition, inherited or auto immune-mediated mechanisms. However a large percentage (32-70%) of all neuropathies remains "idiopathic" after a routine clinical investigation. Patients who were diagnosed with idiopathic neuropathy may have a heretofore unidentified aetiology, which is less than optimally investigated.[34] Recent clinical studies disclosed that elevated plasma level of homocysteine (eHcy) exaggerates the prevalence of PN in diabetics and exacerbates the pre-existing diabetic neuropathy.[8.35.36] The European Union Concerted Action Project, "homocysteinaemia and vascular disease", indicated that a plasma homocysteine level above 0.162 mg% accelerates the risk of myocardial infarction, cerebral or peripheral vascular disease in both men and women. There are studies suggesting that an elevated level of homocysteine in poorly controlled type-2 diabetes mellitus is related to increased risk of atherosclerosis and cardiovascular disease.[37]

MATERIALS AND METHODS

The present study was conducted in Postgraduate Department of Medicine, Government Medical College, Srinagar which is a tertiary care hospital catering to a population of about 60000 after obtaining the ethical clearance from the institutional ethical committee. The study was conducted over a period of one and a half year.

Patients with type 2 diabetic mellitus with features of peripheral neuropathy like tingling, numbness, paraesthesia's were enrolled in a cross-sectional hospital based study. A detailed history and relevant clinical examination was performed in patients enrolled for the study. It was a prospective hospital based study.

Diabetic neuropathy status was documented by presence of clinical signs and confirmed by electrodiagonastic tests like nerve conduction studies (NCS). All the base line investigation including lipid profile, HbA1C, creatinine clearance, nerve conduction studies, relevant radiographs and electrocardiography were performed to evaluate the micro and macro vascular complications of diabetes mellitus.

Plasma total homocysteine concentration was measured using florescence polarization immunoassay. The HbA1C estimation was carried out by a modified calorimetric method. Traditional risk factors for diabetic neuropathy was obtained from fasting blood samples and interviewer-Questionnaire. A written and informed consent was taken from the patients for participation in the study.

Inclusion Criteria
Following group of patients were included for the study.

- Known cases of type 2 diabetes mellitus of either gender.
- Patients who agreed ready to give consent for participation in the study.

Exclusion Criteria
Patients with acute pancreatitis, pernicious anaemia, severe hepatic impairment, psoriasis, hypothydorism, systemic lupus erythematosus (SLE), anorexia nervosa, organ transplantation, malignancies of breast, ovary, pancreas and acute lymphoblastic leukaemia, renal failure, severe skeletal muscle damage or trauma, the patients

already on folic acid,pyroxidine and vitamin B12 therapy or those who refused to give written consent for participation in the study.

All such patients who met the inclusion criteria were evaluated for their blood glucose level and plasma homocysteine level by taking 3cc fasting venous blood sample in a disposable syringe and sent to laboratory for analysis.

The normal plasma homocystiene level is between 5 to <15µmol/l. The result of plasma homocysteine level were interpreted as normal and increased.

Statistical Analysis

Individual Data were expressed in mean and standard deviation (SD). The students t- test was used to test the significance of differences between the two means. The correlation coefficient and the chi – square tests were used to measure the relationship between two quantitative and qualitative variables.

RESULTS

A total of 207 consecutive patients were enrolled in the study. Mean age of study patients was 60.92±6.01SD with a range of 41-80 years. Females 70% (n=144) outnumbered males (n=63). Majority of study patients 174.9% (n=155) belonged to rural areas and 52 patients (25.1%) belonged to urban areas. 134 patients were on OHA's and 73 were on insulin therapy for the treatment of Diabetes.

In current study, NCV was abnormal in 64 patients (30.9%) and Normal in 143 patients (69.1%). 55 patients had axonopathy (85.9%) and 9 had demyelination pattern (14.1%). Motor abnormalities were diagnosed in10 (15.6%), sensory in 38(59.3%), Mixed in16 (25%).

Mean age of patients with abnormal NCV was 61.86±8.03 years and mean age of patients with normal NCV was 59.99±4.82 years, which was statistically significant (p value=0.0034) .Female sex, urban population, and increased homocysteine levels was associated with increased risk of NCS abnormalities in diabetic patients with a p value of 0.001, 0.0001and 0.001 respectively [Table 1 and 2].

Table 1: Demographic and clinical parameters of study patients.

Parameter	Normal NCV	Abnormal NCV	P value
Mean Age (years)	59.99	61.86	0.00034[*]
Males	55 (87.3%)	8 (12.6%)	0.000175[*]
Females	88 (61.1%)	56 (38.8%)	
Rural	135 (87.1%)	20 (12.9%)	0.000001[*]
Urban	81 (5.3%)	44 (84.6%)	
Mean-duration of disease (years)	2.97	4.97	0.62
Mean SBP	140	148	0.757
Mean DBP	92	82	0.757
Using OHA	96	38	0.2816
Using Insulin	47	26	0.2816

Table 2: Distribution of laboratory parameters.

Parameter	Normal NCV	Abnormal NCV	P value
MeanHbA1C %	6.88	7.98	0.69
Mean Serum homocysteine	13.28	23.22	0.001*
Mean B12	678	560	0.9044
Mean Folate	8.9	7.8	0.9044
Mean LDL	96	100	0.9416
Mean HDL	42	38	0.8351
Cholesterol	172	180	0.9414
Triglycerides	110	116	0.9313
Bloodsugar (F)	110	168	0.4524
Mean blood sugar (pp)	126	218	0.4524

Risk of abnormal NCS increased with age. All of the 8 patients in the age group of 71-80- years had abnormal nerve conduction velocity(ncv) while as none of the patients (n=16) in age group of 41-50 years had NCS abnormalties.Among patients in the age groups of 50-70 years (n=183) , 54(30.6%) had abnormal NCS. Female sex was associated with increased risk of abnormal NCS (p value Out of 63 males 8 (12.5%) had abnormal NCS and 55(87.3%) had normal NCS. Among females (n=144), 56 (38.8%) had abnormal NCS while as 88 (61.1%) had normal.

20 (12.9%) patients from the rural background (n=155) had abnormal NCS and 135 (87.0%) had normal NCS. 44 (84.6%) patients from the urban background (n=52) had abnormal NCS and 8 (15.3%) patients belonging to urban background had normal NCS.urban population had statistically significant increased risk of abnormal NCS (p vaue=0.001). Mean Fasting and Postprandial Blood sugar in Patients with abnormal NCS was168mg/dl and 218mg/dl while as it was 110mg/dl and 126mg/dl in Patients with normal NCS.
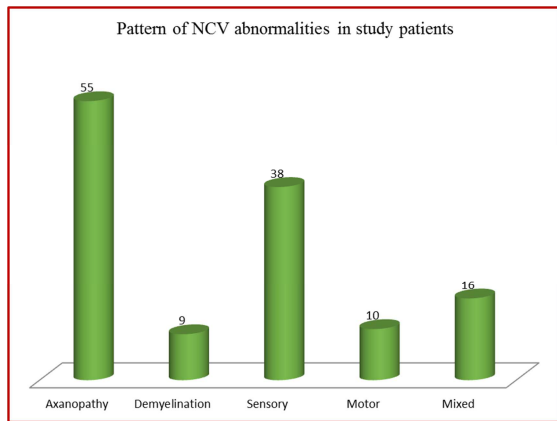
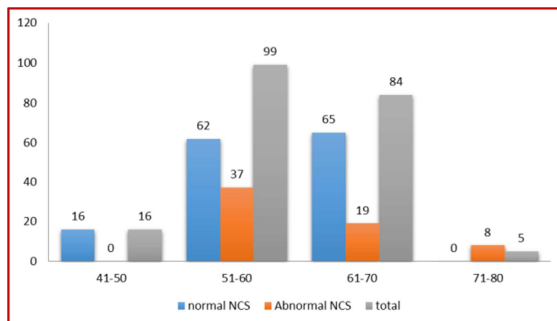**Figure 1: Pattern of NCV abnormalities in study patients.**

Figure 2: Risk of NCS with age.

The mean homocysteine level in patients with abnormal NCS was 23.22µmol/l and 13.28µmol/l in patients with normal NCS. A higher homocysteine levels was associated with increased risk of abnormal NCS (pvalue0.00). The mean duration of diabetes in patients with abnormal NCS was 4.97 years as compared to 2.97 years in patients with normal NCS. The mean HbA1c in patients with abnormal NCS was7.98µmol/l as compared to 6.88µmol/l in patients with normal NCS. 134 patients were on Oral hypoglycemic agents (OHAS) and 73 were on Insulin therapy for the control of Diabetes. Among patients on OHA, 38 (28.3%) had abnormal NCS and among patients on insulin 26(35.6%) had abnormal NCS. The Mean Systolic blood pressure and diastolic blood pressure in patients with abnormal NCS was 148mmhg and 92mmHg respectively while as it was 140mmHg and 82mmHg in those with normal NCS.

In our study, we observed that patients with abnormal NCS had mean cholesterol, triglycerides, LDL, HDL levels as 180, 116, 100, 38 respectively, while as in patients with normal NCS the mean cholesterol, triglycerides, LDL, HDL levels were 172, 110, 92, 42, respectively (in mg/dl). The mean B12 levels in with abnormal NCS was 560pg/ml and 678pg/ml in patients with normal NCS. The mean Folate level in patients with abnormal NCS was 7.8ng/ml and 8.9ng/ml in patients with normal NCS. In conclusion, the current study; found that risk of abnormal NCS is associated with increased age,

female sex, increased duration of disease and increased homocysteine levels.

DISCUSSION

Plasma homocysteine levels are elevated in patients with diabetes particularly in patients with type 2-diabetes mellitus as well as in individuals in pre-diabetic states who exhibit insulin resistance. The levels of homocysteine in such individuals are also influenced by their insulin concentrations, therapy with insulin and medications such as metformin and glitazones that can either raise or lower homocysteine levels.[41]

Elevated plasma levels of homocysteine is accused for increased risk of atherosclerosis, stroke, peripheral neuropathy, cognitive impairment in elderly, possibly Alzheimer's disease and neural tube defects in pregnant women[8,39.40] independent of long recognized factors such as hyperlipidemia, hypertension, DM and smoking[39.42] as well as restenosis after coronary angioplasty.[43-45]
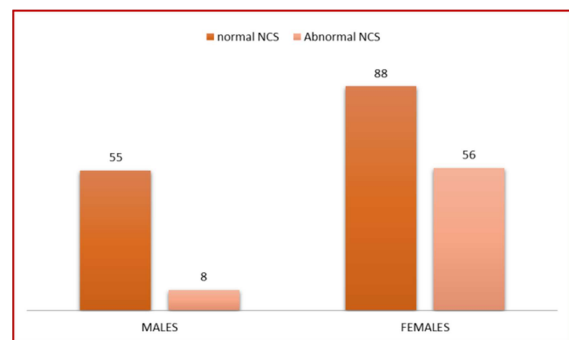

Figure 3: Male and Female Distribution.

The present study evaluated the association of hyperhomocysteinemia and diabetic peripheral neuropathy. In our study a total of 207 patients with diabetes were enrolled in which 69.6% were females and the mean age of patients was 61.85 years who had neuropathy. Homocysteine levels were found to be higher in patients with neuropathy then those without neuropathy which was consistent with study conducted by Ambroch[8] and collegues (2001) and Ebtesam Fahmy et. Al[38] (2010). Ambroch and colleagues estimated homocysteine levels in 65 patients with diabetes mellitus in which 43 have diabetic neuropathy. They found that the frequency of hyperhomocystenemia was significantly increased in neuropathic patients without neuropathy which concluded that homocysteine is independently associated with the prevalence of diabetic neuropathy in a collective of Type 2 diabetic patients. While as Ebtesam fahmy et.al[38] 2010 found 26 diabetic patients with neuropathy and 16 healthy control subjects.

In our study we found that diabetic peripheral neuropathy was more prevalent in the upper middle class (46.9%) as compared to the other socio

economic classes because of the prevalence of diabetes being more common in higher socio economic class. It was also found that diabetic neuropathy was more prevalent in urban population (68.8%) as compared to the patients from rural background because diabetes is more common in urban population as compared to rural population because of change in their life style and physical inactivity.

Since elevated HBA1C and serum triglyceride levels are associated with poor glycaemic control in diabetics, their correlation with elevated homocysteine levels suggest that homocysteine levels could be another marker of poor glycaemic control and a predictor of complications in diabetic patients. In our study we could not found a positive correlation between serum triglyceride and homocysteine levels in patients with neuropathy as compared to those without neuropathy, which was incosistent with a study conducted by Lakshman Ramachandran et al, 2012 who found positive correlation between serum triglyceride and homnocysteine levels

Our study though confirmed the correlation between hyperhomo-cysteinemia and diabetic peripheral neuropathy, the duration of Type 2 diabetes mellitus and HBA1c have been found affect homocysteine level and neuropathy which was consistent with study by Ebtesam Fahmy et al[38] (2010) and Drzewoski, Czupryniak and colleagues (2000). The type of neuropathy found in our study was predominantly axonal 85.6% which were in consistent with the study conducted by Ebtesam Fahmy et al[38] (2010) in which they found demyelinating neuropathy more prevalent than axonal variant. Although our study correlated with the findings of Ebtesam Fahmy at.al[38] 2010 in which they found that sensory neuropathy was more common than motor neuropathy.

CONCLUSION

Elevated Plasma total homocysteine concentration was a major risk factor for the development of neuropathy in diabetics though our study being a small scale hospital based study, large community based studies are probably needed to clarify the role of hyperhomocystenemia a clinically significant modifiable risk factor for the development of neuropathy in patients with type 2 diabetes mellitus.

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