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BIOCHEMICAL AND MOLECULAR STUDIES ON SUBJECTS WITH DIABETES MELLITUS AND AUTONOMIC NEUROPATHY

Medical Science

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ABSTRACT

Diabetic autonomic neuropathy (DAN) is a serious and common complication of diabetes, often overlooked and misdiagnosed. It is a systemic-wide disorder that may be asymptomatic in the early stages. The pathogenesis is still unclear and probably multifactorial. The most and clinically important form of DAN in diabetes is genetic studies. Hence the aim of the present study is to evaluate biochemical and molecular studies on subjects with diabetes and autonomic neuropathy. Fifty five diabetic subjects as study subjects and twenty two healthy subjects without any chronic illness as control were selected for the present study. The extent of somatic DNA damage is quantified by Cytokinesis Block Micronuclei (CBMN) assay. Detailed demographic and clinical characteristics were recorded and compared. The present study demonstrated that micronuclei frequency was significantly elevated in the study subjects than control subjects. A positive correlation between micronuclei frequency and various risk factors showed increased genetic instabilities. Prevention strategies are based on strict glycemic control with intensive insulin treatment, multifactorial intervention and lifestyle modification.

KEYWORDS

Diabetes mellitus, Diabetic autonomic neuropathy, Cytokinesis block micronuclei assay and Cardiovascular autonomic neuropathy

INTRODUCTION

Diabetes mellitus is taking its place as one of the most important diseases in the world. It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic b-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia[1].

When diabetes remains undiagnosed, untreated, or inadequately treated, it can develop into serious and potentially fatal conditions, including kidney failure, heart attack, stroke, ulcers requiring limb amputation, erectile dysfunction, and neuropathy[2].

Diabetic autonomic neuropathy (DAN) is one of the major diabetic complications, and it increases morbidity and mortality in patients with diabetes mellitus (T2DM)[3]. It is a form of peripheral neuropathy, i.e. damage to parasympathetic and/or sympathetic nerves in people with diabetes[4]. Diabetic neuropathy is a 'length dependence' disease resulting in affection of the neurons with the longest axons at the beginning[5]. One of the most overlooked of all serious complications of diabetes is cardiovascular autonomic neuropathy (CAN)[6]. Cardiovascular autonomic neuropathy is defined as the impairment of autonomic control of the cardiovascular system. This encompasses damage to the autonomic nerve fibers that innervate the heart and blood vessels, resulting in abnormalities in heart rate control and vascular dynamics[7].

Now a day's number of diabetic patient's increases day by day. According to the international diabetic association (8), it was estimated that there are currently 382 million people with diabetes across the world and this figure is projected to rise to 592 million by 2035. Most of the persons suffering from diabetes mellitus have complications in nerve function especially in parasympathetic nervous system. Study about biochemical and molecular aspects of diabetes mellitus and autonomic neuropathy is helps to know more about the incidence and genetic factors of the disease. India is the second largest country in terms of population and persons suffering from diabetes and diabetic autonomic neuropathy. Hence this study was undertaken to provide proper awareness to the people about diabetes mellitus and autonomic neuropathy.

Materials and Methods

Fifty seven subjects suffering from diabetes mellitus were taken as study subjects. Twenty two control subjects were also selected for this study. All these subjects were referred from Hridayalaya institute of preventive cardiology, Thiruvanathapuram to Genetika, Centre for Advanced Genetic Studies, Trivandrum, Kerala for genetic testing. Detailed socio-economic, demographic and other relevant clinical information were recorded using proforma. 7 ml of venous blood was collected aseptically from all the subjects after overnight fasting. 3 ml of blood was collected in sodium heparinized vacutainers for Cytokinesis block micronuclei (CBMN) assay. The remaining 4 ml of blood was allowed to clot, serum separated and blood sugar, lipid profiles were estimated using semi analyzer.

Two ml blood was added to a culture tube containing 10 ml RPMI 1640 medium supplemented with 100units/mL penicillin, 100µg/mL streptomycin, 15% fetal bovine serum and 10µg/mL phytohaemagg lutinin. Cytochalasin B was added to the cultures at a final concentration of 4.5µg/mL (Sigma) after 44th hours. Cells were harvested after 72nd hr incubation, and they were treated with a hypotonic KCl solution (0.075M KCl) for 10 min and fixed in fresh fixative solution (methanol: acetic acid, 3:1). The cells were dropped onto slides and the slides were air dried and stained with 10% Giemsa. Micronucleated cells were analyzed under light microscopy at 100X magnification. The number of micronuclei is not less than 1000 binucleated cells were scored and the distribution of micronuclei among binucleated cells was recorded.

Observations and Results

The present study included 55 study subjects and 22 control subjects. Age of study subjects ranged from 33 to 60 years with a mean age of 52.16 years. Age of control subject ranged from 32 to 68 years with a mean age of 51.90 years. In the 55 study subjects, 35 of them were females and 22 of them are males. Control subjects with 14 males and 10 females. Among the subjects majority of them had a non -sedentary type of occupation with medium socio economic status. 10 individuals were smokers, 5 were chewing and 11 of them have drinking habit. All the study subjects are suffering from diabetes. 80% subjects had hypertension, 81.81% of the subjects had dyslipidemia. Majority of the study subjects (41.81%) having a poor physical activity.

Table 1: Distribution of mean CBMN frequency according to subjects

Category	Variables	Number (Percentage %)	Mean CBMN Frequency
H/o Hypertension	Yes	44 (80%)	12.15
	No	11 (20%)	12.09
H/o Dyslipidemia	Yes	45 (81.81 %)	12.11
	No	10 (18.18%)	12.1
Habit of smoking	Yes	10 (18.18%)	12.11
	No	45 (81.81%)	12.08
Habit of chewing	Yes	5 (9.09%)	12.11
	No	50(90.9%)	12.08
Habit of drinking	Yes	11 (20%)	12.15
	No	44 (80%)	11.91
Regular exercise	Yes	13 (23.63%)	12.07
	No	42 (76.36%)	12.21
Diet	Non vegetarian	45 (81.81%)	12.11
	Vegetarian	10 (18.18%)	12.07

Subjects	Number	Mean CBMN frequency
Study subjects	55	12.55
Control subjects	22	10.54

Distribution of mean CBMN frequency according to subjects were given in table 1. 55 study subjects were showed mean CBMN frequency of 12.55 and 22 control subjects were showed mean CBMN frequency of 10.54.

Table 2: Distribution of mean CBMN frequency according to demographic characters of subjects

Age of the subjects were grouped into <40, 40 to 50 and >50 years. Subjects with age >50 years were showed highest mean CBMN frequency of 12.66. Most of them were females and having highest mean CBMN frequency. Subjects with highest birth order (7 to 9) were showed highest mean CBMN frequency. Majority subjects were residing in rural residence; here increased micronuclei were showed by subjects residing in coastal region. Sedentary workers were showing mean CBMN frequency of 12.3. Majority of the study subjects have medium socioeconomic status. High mean CBMN frequency shown by low economic subjects. Major risk factor is BMI, in which 2 subjects were obese and 43 were overweight. These 2 groups have high mean CBMN frequency.

Table 2: Distribution of mean CBMN frequency according to clinical and lifestyle characters of subjects

Clinical and lifestyle characteristics of the subjects were given in table 2. 44 subjects have H/o hypertension and these have mean CBMN frequency of 12.15. Subjects having H/o dyslipidemia were showed highest mean CBMN frequency. 10 subjects were smokers, 5 were chewers and 11 have the habit of drinking. All these subjects were showed highest mean CBMN frequency. 13 subjects do regular exercise; these have lowest mean CBMN frequency. Most of them were non vegetarians and possessed mean CBMN frequency of 12.11.

Distribution of mean CBMN frequency according to biochemical characters of subjects Table 4:

Category	Variables	Number (Percentage %)	Mean CBMN Frequency
FBS (mg/dl)	<110	1 (1.81%)	11.52
	≥ 110	54 (98.18%)	12.11
Total cholesterol (mg/dl)	<200	17 (30.9%)	12.02
	200-240	17 (30.9%)	12.07
	>240	21 (38.1%)	12.2
HDL (mg/dl)	<40	30 (54.54%)	12.12
	40-60	20 (36.36%)	12.11
	>60	5 (9.09%)	11.98
LDL (mg/dl)	<100	5 (9.09%)	11.98
	100-160	33 (60%)	12.02
	>160	17 (30.90%)	12.3
TG (mg/dl)	<150	32 (58.18%)	11.9
	≥ 150	23 (41.81%)	12.19
HbA1c (%)	7 to10	33 (60%)	12.06
	>10	22 (40%)	12.17

In table 4, biochemical characteristics of subjects with above the normal level possessed highest mean CBMN frequency than normal level. Subjects with FBS above 110 mg/dl were showed highest mean CBMN frequency.

Discussion

According to Ramachandran, (9) in developing countries, the majority of diabetes patients are in the age range of 45-64 years whereas in the developed countries are aged >65 years. From the study, it was clear that subjects with age range of 40-50 years showed a mean CBMN frequency of 12.05. Subjects aged above 50 years were showed a CBMN frequency of 12.66. So the present study suggests that there is a significant relationship between age and CBMN frequency. Mean CBMN frequency of the subject were increases with increase in age.

Belue et al., (10) reported that in many countries of sub-Saharan Africa, women are more likely to be obese or overweight than men and might therefore be expected to have higher prevalence of diabetes mellitus. The present study contained 34 females and 21 males. Mean CBMN frequency of the females are comparatively higher than that of the males.

According to Proper et al., (11) found that a strong evidence for sedentary behaviors and cardiovascular disease. Present study also showed an association between sedentary occupational life and mean CBMN frequency. The study includes, mean CBMN frequency higher in individuals with sedentary mode of work than individuals with non sedentary mode of work.

Cohen et al., (12) that the increased diabetes risk seen in low income groups is related to the increased prevalence of obesity within this group. Present study found out those subjects with low socio economic status showed higher CBMN frequency of 12.69.

Julie et al., (13) found that, a dose response relationship between cigarettes smoked per day and incidence of diabetes mellitus for both men and women. They were also found that women who had quit for >5 years and men who had quit for > 10 years had essentially the same incidence of diabetes as those who had never smoked diabetes. Present study indicates that CBMN frequency of individuals with habit of smoking are higher than the CBMN frequency of individuals without the habit of smoking. Present study reveals that mean CBMN frequency was higher in subjects with smoking habit.

According to Hendriks, (14) reported that the alcohol consumption is an influencing factor for diabetes. The biological mechanism is uncertain, but there are several factors that may explain the relationship, including increases in insulin sensitivity after moderate alcohol consumption.

According to Haffner, Lehto and Laakso, (15) hypertension develops in people with T2DM at the rate twice than those who are non-diabetic. In the present study also showed CBMN frequency of subjects having hypertension is higher than that of subjects without hypertension.

According to Parvez et al., (16), the increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity. In the present study it was observed that obese subject showed a mean CBMN frequency higher than non-obese subjects.

Supriya et al., (18) reported that, the incidence of autonomic neuropathy is associated with body mass index, smoking, weight and lower HDL cholesterol; high levels of triglyceride level are associated with diabetes and macro vascular disease. In the present study distribution of mean CBMN frequency of triglycerides showed that CBMN frequency is higher in subjects with high amount of triglycerides.

CONCLUSION

From this study, it can be concluded that the mean CBMN frequency was found higher in those individuals with increased age, increased weight, increased BMI, increased abdominal circumference and

individuals with history of diseases. Diabetic autonomic neuropathy is the most common and troublesome complication of diabetes mellitus. Symptoms may be confined to a target organ or organ system. Complications of autonomic neuropathy contribute greatly to the morbidity, mortality and reduce the quality of life. Controlling blood sugar level is the only preventive measure for diabetes and autonomic neuropathy. Proper diet, regular exercise and physical activity are the major way to reduce abdominal obesity. Avoid bad habits such as smoking, drinking and chewing helps to reducing the risk. Yoga based interventions can have positive influence on mind it will helps to reduce hypertension, depression and improve compliances to lifestyle changes.

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