Original Article

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Glycaemia Status, Lipid Profile and Renal Parameters in Progressive Diabetic Neuropathy

Physiology Section

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ABSTRACT

Introduction: Diabetic Peripheral Neuropathy (DPN) is a common complication of diabetes. Existence of systemic co-morbidities in DPN patients has not been studied much, especially in Indian population.

Aim: To evaluate glycaemic status, lipid profile, renal parameters and blood count to assess occurrence of co-morbidities as severity of DPN progresses.

Materials and Methods: A case control study involving 104 DPN patients and 43 controls of age 31-70years were selected. Patients were categorized into stage 0, 1, 2 and 3 of severity as per Dyck system of classification. Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Glycosylated haemoglobin (HbA1c), Lipid profile, Vitamin B12, Thyroid Stimulating Hormone, Urea, Creatinine and Complete blood counts were assessed along with baseline characteristics.

Results: Glycosylated haemoglobin was in uncontrolled range for DPN patients (9.03±2.09) FBS and PPBS were significantly more with progress of severity of DPN ($p<0.001$). HDL decreased ($p<0.001$) as severity progressed and Triglyceride increased in DPN cases. Mean urea values increased ($p=0.008$) while haemoglobin levels and RBC count decreased ($p<0.001$) as severity of DPN progressed.

Conclusion: Abnormal lipid profile, increased urea and decreased RBC levels point to co-existence of cardiovascular and renal comorbidities as severity of DPN progressed.

Keywords: Anaemia, Comorbidities, Glycosylated haemoglobin (HbA1c), Urea

INTRODUCTION

Diabetes Mellitus (DM) refers to a constellation of abnormalities mainly featuring hyperglycaemia along with disturbance in carbohydrate, fat and protein metabolism. Type II diabetes is chiefly due to resistance to insulin action and accounts for about 90 to 95% of all cases of diabetes mellitus. Diabetes mellitus is associated with increased risk of morbidity and premature mortality arising due to its various complications. Hyperglycaemia is associated with macrovascular complications like coronary artery disease, peripheral arterial disease and stroke and microvascular complications like neuropathy, retinopathy and nephropathy [1]. Microvascular complications are evident even at the time of diagnosis in many patients [2]. Uncontrolled hyperglycaemia for several years is the major factor in the development and progression of these complications [1]. One study has shown co-existence of retinopathy, neuropathy and cardiovascular disease along with diabetic nephropathy [3]. Prevalence of Diabetic Peripheral Neuropathy (DPN) in Type 2 diabetic subjects in an Indian study was shown to be 26.1% [4]. We did not find any Indian study regarding co-existence of multiple organ damage in Diabetic Neuropathy patients especially as the disease progressed. Aim of the study was to evaluate for the development of other complication as stages of severity of Diabetes Neuropathy progressed. In this study the different lipid, renal and, haematological and glycaemic parameters were explored in DPN patients in different stages of the disease. The aim of the current study was to evaluate the glycaemic status, LDL, HDL, VLDL, Total cholesterol, RBC, WBC, Haemoglobin, Urea and Creatinine to assess existence of comorbidities as severity of DPN progresses.

MATERIALS AND METHODS

The study was a case control clinical study. All the procedures followed were in accordance with the ethical standards of the committee on human experimentation of the institution. Informed consent was taken from all the participants before they were included in the study. A total of 104 diabetic patients and 43 control subjects attending the outpatient division of VIMS and RC were randomly selected for the study.

Mean Known Population size = 1.1±0.4%

Relative precision of 6%

α level of 5% (95% confidence interval)

Sample size=120

Formula:

$n = z^2(1- \alpha /2)\ \sigma^2/\varepsilon^2\mu^2$

Z =1.96 at 5% α level

σ = Standard deviation

ε = relative precision

μ =Mean

$(1- \alpha /2)$= Desired confidence interval

The inclusion criteria were: (a) DM type 2 patients with and without DPN; and (b) Age of subjects ranging from 31 to 70 years. Subjects with regular Alcohol Consumption, Hypertension, and Neuropathy other than DPN (Known B12 deficiency, Rheumatoid Arthritis, thyroid disorders, HIV patients) was excluded from the study in consultation with neurologist. Age and gender matched healthy controls were recruited for the study from master health check-up subjects, patient attendants and staff of VIMS and RC. In this study non diabetic controls were recruited for the study rather than stage 0 patients of DPN because most of the patients were already in later stages even at the time of diagnosis. Almost 1 in 3 subjects proposed to be in stage 0, when evaluated for nerve conduction was found to be stage 1 or 2. We could only evaluate a limited number of subjects due to economic and man power constraints. A prestructured proforma was used to collect the baseline data. All participants underwent general physical examination and systemic examination of cardiovascular, respiratory and nervous system.

Screening and Staging for Diabetic Neuropathy

Michigan Neuropathy Screening Instrument (MNSI) [4,5] was used for screening patients for DPN. Nerve Conduction Studies (NCS) was done using RMS EMG EP MARK-11. Dyck grading system

[6] was used for staging severity of diabetic peripheral neuropathy [Table/Fig-1]. Patients were divided into four stages based on the severity of DPN.

Stage	Evidence DPN
N0	N0 objective evidence of DN
N1	Asymptomatic polyneuropathy N1a: No symptoms or signs but neuropathic test abnormalities N1b: Test abnormalities* plus neuropathy impairment on neurological exam
N2	Symptomatic neuropathy N2a: Symptoms, signs, and test abnormality N2b: N2a plus significant ankle dorsiflexor weakness
N3	Disabling polyneuropathy

[Table/Fig-1]: Dyck's grading system used for staging severity of diabetic peripheral neuropathy.
* Electrophysiological Abnormalities

Method of Collection of Blood Sample

Five ml of venous blood sample was collected after 8-12 hours overnight fasting. Fasting blood sugar, Lipid profile, Thyroid stimulating hormone, Vitamin B12 and glycosylated haemoglobin, urea, creatinine and Complete Blood Count (CBC) were measured. HbAlc and CBC blood samples were collected in EDTA tube and rest were collected in plain tubes. Blood samples were collected after 2 hours post meal for post-prandial blood sugar.

System(s) with SYNCHRON CX MULTI™ calibrator were used with respective reagents and method for the different bio-chemical parameters. FBS and PPBS was measured GLU reagent in the GOD-POD method [7]. The HaemoglobinA1c (HbA1c) reagent kit was used for assessing glycosylated haemoglobin [8]. Lipid Profile; HDL was measured using by HDL reagent [7,9], LDL by LDL reagent [7,10]. Triglyceride by triglyceride GPO reagent [11,12] and Cholesterol reagent was used to measure cholesterol concentration by timed-end point method [13,14]. VLDL, LDL/HDL ratios was done by calculation . TSH was estimated using the Access HYPER sensitive hTSH assay and Vitamin B12 was by competitive binding immunoenzymatic assay[15,16]. Urea reagent was used to measure urea concentration by an enzymatic rate method [17,18]. Creatinine reagent was used to measure the creatinine concentration by a modified rate Jaffé method [19-21]. Complete Blood Count (CBC), Red Blood Cell (RBC) count was measured in Beckman Coulter Act 5 diff. Spectrophotometric method at 550 nm. Haemoglobin was measured by Automated cell counter -550, WBC by aperature method.

STATISTICAL ANALYSIS

Descriptive statistical analysis was carried out in the present study. Results on continuous measurements have been presented as Mean ± Standard Deviation (SD) and categorical measurements are presented as number. The Statistical software namely SPSS 15.0, was used for the analysis of the data and Microsoft word and Excel was used to generate graphs and tables. Significance was assessed at 5% level of significance. Analysis of variance (ANOVA) was used to find the significance of study parameters between three or more groups of patients.

RESULTS

This study had 104 cases of DPN and 43 controls. Patients were divided into four stages based on the severity of diabetic peripheral neuropathy (DPN). Stage 0 had 12 patients, Stage I had 31 patients, stage II had 31 patients and Stage III had 30 patients.

Baseline characteristics of the participants were studied for Age, Gender, Duration, Vitamin B12 and TSH. Age and Gender distribution as shown in [Table/Fig-2] were statistically similar in all the groups with p=0.221 and p=0.129. Duration of the disease was significantly less in stage 0 participants with p <0.001 compared to other groups. Vitamin B12 was within normal range in all the groups but was significantly higher range of normal in later stage of disease. One reason was that most of these patients were on vitamin B 12 supplements. The mean TSH µIU/mL was within normal range in all the groups studied.

Lowered LDL in cases was because of hypolipidemic drugs. CVS involvement like blood pressure or autonomic changes were ruled out in exclusion criteria while screening patients. Thus lipid profile findings were not linked with CVS co-morbidities.

Glycaemic parameters such as Fasting Blood sugar (FBS), Post Prandial blood sugar (PPBS) and glycosylated haemoglobin [Table/Fig-3] showed significant increase in DPN cases than the control group. [Table/Fig-4] shows FBS, PPBS, HbA1c across different stages of DPN. Significant increase in FBS, PPBS and HbA1c was seen across Controls and Stages 0,1,2,3 with a p-value of <0.001.

Lipid profile included parameters like Total Cholesterol (TC), Triglyceride (TG), High density lipoproteins (HDL), Low density lipoproteins (LDL), Very low density lipoproteins (VLDL) in mg/dl and Low density lipoproteins/ High density lipoproteins (LDL/HDL ratio), were compared across five groups of patients as presented in [Table/Fig-4]. The HDL showed significant decrease as severity of DPN increased. Triglyceride increased in cases than controls (p= 0.090), HDL levels decreased in cases than controls (p<0.001), LDL/HDL ratio increased significantly in cases than controls (p=0.018). The LDL showed a significant reduction in controls as well as in patients with DPN through stage 0 to stage 3

Clinical Variables	Control(n=43) Mean ± SD	DPN patients (n=104) Mean ± SD	p-value
FBS mg/dl	94.47±7.59	205.58±99.79	<0.001
PPBS mg/dl	103.42±14.14	270.67±88.65	<0.001
HbA1c	5.52±0.40	9.03±2.09	<0.001
Cholesterol mg/dl	181.35±36.18	172.15±43.01	0.219
Triglyceride mg/dl	129.09±68.84	150.05±67.29	0.090
HDL mg/dl	43.33±10.37	32.68±16.59	<0.001
LDL mg/dl	124.33±39.36	101.73±34.87	0.001
LDL/HDL	3.72±1.18	4.20±1.09	0.018
VLDL mg/dl	26.08±13.55	29.41±12.44	0.156

[Table/Fig-3]: Glycaemic status and lipid profile between normal and DPN patients.

Clinical Characteristics with Normal Reference Interval	Control's (N=43) Mean ± SD	Stage 0 (N=12) Mean ± SD	Stage 1 (N=31) Mean ± SD	Stage 2 (N=31) Mean ± SD	Stage 3 (N=30) Mean ± SD	p-value
Age in years Mean ± SD	49.62±9.45	49.41±8.19	54.58±7.82	51.87±9.27	55.5±7.10	0.221
Gender (Male/Female)	31:12	8:4	23:8	25:6	28:2	0.129
Duration of disease(yrs)	0.00	2.42±2.71	6.58±4.40	6.65±3.73	7.90±5.54	<0.001
Vitamin -B12 (180-914 pg/ml)	302.1±208.4	311.5±214.3	496.6±346.9	571.4±450	694.1±393.2	<0.001
TSH (0.34-5.6 µIU/ml)	2.37±1.73	2.63±1.4	1.83±0.9	1.83±0.97	2.57±1.51	0.078

[Table/Fig-2]: Baseline characteristics of participants across stages of severity of DPN.

Clinical Characteristics & Normal Reference Interval	Control (N=43) Mean ± SD	Stage 0 (N=12) Mean ± SD	Stage 1 (N=31) Mean ± SD	Stage 2 (N=31) Mean ± SD	Stage 3 (N=30) Mean ± SD	p-Value
FBS 60-100 mg/dl	94.5±7.6	142.3±50.4	185.4±77.3	213.5±107.2	243.6±112	<0.001
PPBS 110-140mg/dl	103.4±14.1	202.2±72.8	268.2±73.8	299.2±106.1	271.1±76.2	<0.001
HbA1c 4.6-6.2	5.5±0.40	8.23±0.63	8.32±1.63	8.75±2.34	10.38±2.02	<0.001
Cholesterol 150-200mg/dl	181.4±36.2	192.7±37.8	182.4±40.5	173.1±44.9	152.2±39.4	0.008
Triglyceride 40-160 mg/dl	129.1±68.8	161.7±54.2	144.9±40.3	166.9±102.8	133.1±40.8	0.127
HDL 40-65 mg/dl	43.3±10.4	32.1±4.9	43.5±23.6	30.7±10.8	23.8±7.7	<0.001
LDL 60-130 mg/dl	124.3±39.4	121.3±37.8	107.0±34.6	105.88±3	84.1±28.6	<0.001
LDL/HDL <4	3.7±1.2	3.8±1.1	4.1±0.8	4.2±1.3	4.5±1.1	0.036
VLDL 0-40 mg/dl	26.1±13.5	26.3±5.9	28.8±5.9	33.9±20.2	26.6±6.5	0.086
Urea 16-40 mg/dl	23.4±4.6	21.5±3.12	28.8±9.2	27.9±12.3	30.2±13.3	0.008
Creatinine 0.4-1mg/dl	0.81±0.20	0.86±0.40	1.01±0.50	0.95±0.59	0.95±0.59	0.265
Total Leucocyte count (4000-11000cell /µl)	7.83±2.34	7.75±1.36	7.66±1.9	7.99±1.54	11.46±4.02	<0.001
RBC 4 - 6.2cells x10/µl	4.67±0.52	4.6±0.58	4.69±0.75	4.34±0.56	3.88±0.52	<0.001
Haemoglobin 11-18.8g/dl	13.31±2.44	13.35±1.6	11.64±2.31	12.69±1.59	11.32±1.48	<0.001

[Table/Fig-4]: Clinical characteristics of patients studied across stages of severity.

with p<0.001. LDL/HDL ratio however showed a significant increase in Stage 3 compared to all the previous groups.

Renal parameter i.e., serum urea increased significantly (p<0.008), while haemoglobin and RBC Count decreased from initial to later stages of severity of DPN with p<0.001. Total Leucocyte Count (TLC) increased in later stages of severity.

Nerve Conduction Studies (NCS) indicated that patients in Stage 0 were found to have normal values of latency, amplitude, velocity and F wave minimal latency values. Patients in stage 1 and 2 had marked decrease in velocity of motor and sensory nerves of upper and lower limbs. Patients in Stage 3 were overtly found to be neuropathic on clinical examination and MNSI screening and had swellings on lower limb for which NCS was not done on them.

DISCUSSION

DPN causes nerve damage and consequently leads to weakness. As DPN progresses it causes foot ulcers and consequently may lead to amputation. The focus of this study was to evaluate the biochemical manifestation of other complications in patients with DPN as the severity progressed. While it was easy to recruit patients at all stages of disease, it was particularly difficult to get patients from stage 0 DPN i.e. asymptomatic patients with no electrophysiological abnormality. Most of the asymptomatic patients screened were already in stage 1 showing electrophysiological abnormality. Stage 0 diabetic patients were mostly newly diagnosed or had shorter duration of diabetes. Even then the number of participants recruited was less in this stage 0. One of the reasons may be the duration is calculated only from the day of diagnosis of DM. But the patients may have developed DPN asymptomatically (stage 1) even before diagnosis. Studies have shown that onset of NIDDM can occur at least 4-7 years before initial diagnosis [22]. In fact, the American Diabetic Association in its guideline has also identified this and recommends that screening for peripheral neuropathy should be done along with the initial diagnosis of diabetes [23,24].

Most of the DPN patients in this study had uncontrolled hyperglycaemia. Patients in stage 3 had significant longer duration of uncontrolled hyperglycaemia and mean glycosylated haemoglobin values. The HbA1c levels in most DPN patients in this study were indicative of uncontrolled DM. Prolonged uncontrolled hyperglycaemia has been associated with DPN and other microvascular and macrovascular complications in several studies [25]. A longitudinal follow up study of 3 years showed that age, diabetes duration, HbA1c, height, body mass index and ankle-arm index together best predicted diabetic peripheral neuropathy score during follow-up [26].

Abnormal lipid profile is one of the main risk factor for cardiovascular diseases even in DM. Important findings seen in this study regarding lipid parameters was lower HDL levels, and increased levels of TG and increased LDL/HDL ratio in DPN patients compared to controls. Importantly, LDL and TC decreased significantly with increasing severity between the stages of DPN. One of the reasons that LDL was decreased in Stage 3 DPN may be that these patients were taking hypolipidemic drugs. Hypertensive patients or patients with autonomic insufficiencies were excluded from the study. Hence, cardiovascular autonomic complications were commented because of abnormal lipid profile and cannot be commented as such, as we do not have direct evidence.

Other studies have also found similar results [27]. A similar study done to identify and quantify risk factors for lower extremity amputation in diabetic persons identified that low levels of HDL sub-fraction was a statistically significant factor [28]. Another similar study concluded that elevated TG correlated with progression of DN independent of duration, age, diabetes control, or other variables [29]. Elevated TG and decreased HDL are considered as the best predictors of cardiovascular disease in patients with type 2 diabetes [27].

Serum urea and creatinine are commonly used parameters to indicate renal dysfunction. A comparison of kidney parameters i.e., urea and creatinine across different stages of DPN patients and controls showed a significant increase in urea across stages reaching maximum in stage 3, indicative of increasing renal abnormality across stages. An Indian cross-sectional study also concluded that Diabetes Nephropathy was found to be associated with proliferative diabetic retinopathy, neuropathy and cardiovascular disease by univariate analysis [3].

A decrease in RBC count and haemoglobin values are considered to be indicative of renal pathology in DM patients [30,31]. In this study RBC count and Haemoglobin levels were significantly reduced in the later stages of the disease than earlier stages, confirming the increasingly worsening renal function as evidenced by increased urea levels especially in later stages. This is consistent with other studies reporting development of anaemia in diabetic nephropathy patients. Similar results was seen in other studies [30,31] in that the presence of anaemia has been more in diabetic patients with renal involvement than patients with comparable non-diabetic renal impairment. Anaemia in the later stages indicates co-existing renal impairment. Another study has concluded that diabetic patients with anaemia may be at increased risk of adverse outcomes from diabetic retinopathy, nephropathy, neuropathy and cardiovascular disease. This and another study commented that treatment of anaemia will slow the progression of microvascular and macrovascular complications [32].

Comparing haematological parameters in DPN patients studied the TLC "(Mean ±SD") values showed a significant increase in stage 3 across groups with a significant p-value <0.001. This significant increase in TLC in Stage 3 could be because of the diabetic foot ulcers with infections seen in stage 3 of the disease, while the TLC of the other 3 stages was comparable.

Strengths of the study include stage-wise analysis of co-morbidities and ruling out other causes of DPN using biochemical test. Limitations of the study is that this was a cross-sectional study and has a smaller sample size.

CONCLUSION

It is evident from this study that DPN patients had uncontrolled hyperglycaemia, more so in later stages of the disease. These DPN patients showed a tendency towards developing co-morbidities like diabetic nephropathy and an increasing predisposition towards cardiovascular dysfunctions especially in later stages of the DPN. These co-morbidities especially in presence of uncontrolled diabetes increase the need for proper control of hyperglycaemia to protect susceptible organs from ongoing microvascular damage.

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