



PERIPHERAL NERVE CONDUCTION STUDY IN PREDIABETES, A CROSS SECTIONAL STUDY

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ABSTRACT

Aim of the study is to compare the nerve conduction in subjects with deranged glucose tolerance i.e. prediabetics and normal glucose tolerance. In prediabetes, nerve conduction anomalies are documented in various research publications with procedures like Intra epidermal nerve fibre density assessment and doppler imaging. The study is a Cross sectional study, includes 50 subjects with prediabetes and 50 with normal glucose tolerance test. Median & ulnar nerve of both the upper extremity, sural & superficial peroneal nerve of both the lower extremity were tested. Glycated Haemoglobin (HbA1c) was quantitatively determined by ion exchange resin using fully automated analyser (CPC turbochem 100) & Nerve conduction was carried out using RMS Eleron machine. There was no statistically significant difference found between the two groups, for nerve conduction study parameters like amplitude, latency and nerve conduction velocity ($p > 0.05$). Nerve conduction studies usually detects the large fibre involvement, which may or may not be seen in prediabetes stage. It has been documented that neuropathy often is subclinical therefore; if a patient does not show signs of neuropathy on the clinical examination, nerve conduction studies which is cost effective and noninvasive are usually advised as an ancillary tool to detect incipient neuropathy.

KEY WORDS

HBA1c, Nerve conduction, Neuropathy, Prediabetes, Oral glucose tolerance test.

INTRODUCTION

Increasing obesity, unhealthy diets, and sedentary lifestyles have led to a global population that is more prone to diabetes mellitus and its complications [1,2]. Diabetic neuropathy is a common complication seen in routine health care and is the most common form of peripheral neuropathy in the developed world [3-5].

There is currently a debate as to whether peripheral neuropathy can occur before the onset of established diabetes mellitus, i.e. in the prediabetes stage [6-9]. Several randomized and controlled studies have shown that enhanced glucose control prevents the development of peripheral neuropathy in type 1 diabetes mellitus [10,11]. Prediabetes is an intermediate state of hyperglycemia with glycemic parameters above normal but below the diabetes threshold. The World Health Organization (WHO) has defined prediabetes as a state of intermediate hyperglycemia using two specific parameters, impaired fasting glucose (IFG) defined as fasting plasma glucose (FPG) of 6.1-6.9 mmol/L (110 to 125 mg/dL) and impaired glucose tolerance (IGT) defined as 2h plasma glucose of 7.8-11.0 mmol/L (140-200 mg/dL) after ingestion of 75 g of oral glucose load or a combination of the two based on a 2h oral glucose tolerance test (OGTT) [12].

The American Diabetes Association (ADA) on the other hand has the same cut off value for IGT (140-200 mg/dL) but has a lower cut-off value for IFG (100-125 mg/dL) and has additional hemoglobin A1c (HbA1c) based criteria of a level of 5.7% to 6.4% for the definition of prediabetes [13]. Both IGT and IFG are associated with insulin resistance and increased risk of cardiovascular disease [14,15]. IGT usually precedes type 2 diabetes mellitus, and individuals can remain in the IGT state for many years before progressing to overt type 2 diabetes mellitus [16]. There is also increasing evidence to demonstrate a higher frequency of idiopathic polyneuropathy, painful sensory neuropathy and small fiber neuropathy among prediabetic individuals with IGT [17,18].

MATERIAL AND METHODS

Study design: The present study is a Cross Sectional study.

Ethical statement: Approval was obtained prior to data collection from institutional ethics committee, Government Medical College Aurangabad.

Participants and eligibility criteria: According to ADA the hemoglobin A1c (HbA1c) based criteria of a level of 5.7% to 6.4% for the definition of prediabetes was used. The prediabetes subjects were selected from diabetes outpatient department (O.P.D.) of medicine from Government and pvt institutes and were reclassified and confirmed as prediabetics after HbA1c estimation. The healthy controls were selected from relatives of patients and staff members. The procedure was explained to the subjects in their mother tongue and informed consent was obtained.

Sample size: A total of 100 subjects were selected 50 in each group.

Study Subjects	Groups	No of subjects enrolled
Prediabetes	Group I	50
Nonidiabetes control	Group II	50

Inclusion criteria:

- 1) Subjects with prediabetes (i.e),
 - a) FPG: 5.6-6.9 mmol/L i.e (100-125 mg/dL).
 - b) 2hr PG: 7.8-11mmol/L i.e (140-199mg/dL).
 - c) HbA1c: 5.7 – 6.4 %.
- 2) Age: >25 Yrs.
- 3) Able to give informed & written consent.

NOTE: (For grouping study subjects as prediabetes and nondiabetes, in this study HbA1c values obtained from subjects were taken into consideration as per American Diabetes Association guidelines)

Exclusion criteria:

- 1) Diagnosed cases of type 1 and type 2 diabetes mellitus.
- 2) Past History of: HIV, regular alcohol consumption, liver diseases, thyroid disorders, rheumatoid arthritis and neurological disorders.
- 3) Sports person/athletes.

A detailed history was taken regarding complaints, personal history, past history, family history and treatment history if any.

General and systemic examination: General Examination along with systemic examination of the cardiovascular, respiratory, abdomen and central nervous system was done.

Basic data collection: Following parameters were used while collecting information and recording the observations:

Age: The present age of the subject in complete years, which was confirmed by their school leaving certificate or other documents mentioning their age.

Body Weight: Body weight was recorded in kilograms.

Height: Bare foot standing height was recorded using stadiometer in centimeter.

GLYCATED HAEMOGLOBIN ESTIMATION:

Glycated Haemoglobin (HbA1c) was quantitatively determined by ion exchange resin using fully automated analyser (CPC turbochem 100). The results were expressed in percentage (%).

Principle:

Whole blood is mixed with lysing reagent to prepare a hemolysate. This is then mixed with a weakly binding cation exchange resin. The non-glycosylated hemoglobin binds to the resin leaving GHb free in the supernatant. The GHb percentage is determined by measuring the absorbance of the GHb fraction and of the total Hb.

Assay procedure:

Assay temperature: $23 \pm 1^\circ\text{C}$ or $30 \pm 1^\circ\text{C}$, wave length: 415 nm.

Step I - Hemolysate preparation:

1. Pipette 0.25 ml of lysing reagent in a test tube.
2. Add to it 0.05 ml of well mixed whole Blood/control.
3. Mix well and allow to stand at room temperature for 5 minutes.

Step II - GHb separation and assay:

1. Bring resin tube to assay temperature by incubating the tube in a water bath.
2. Add to it 0.1 ml of hemolysate (from step1).
3. Position a resin separator in the tube, so that the rubber sleeve is approximately 3 cms above the resin level.
4. Mix the contents on vortex mixer continuously for 5 minutes.
5. Allow the resin to settle at assay temperature for 5 minutes, push down the resin separator in the tube until the resin is firmly packed.
6. Pour the supernatant directly into a cuvette and measure the absorbance against deionized water.

Step III - Total Hemoglobin (THb) assay:

1. Pipette 5.0 ml of deionized water into a test tube.
2. Add to it 0.02 ml of hemolysate (from step 1).
3. Mix and read absorbance against deionized water.

Interpretation: HbA1c <5.7%(normal),5.7-6.4(prediabetes)

NERVE CONDUCTION STUDY.

Nerve conduction study was done using the standard Recorders Medicare System (RMS) EMG EP MK II Machine. Recordings were taken using standard procedures, careful distance measurements and recording of well-defined and artifact free responses of, action potential amplitudes, latencies and conduction velocities of median, ulnar, sural and superficial peroneal nerve of both the extremities.

Before the procedure:

The procedure was explained to the patients and consent was taken. Generally, no fasting or sedation is required, so no such instructions were given. Normal body temperature must be maintained, as low body temperature slows nerve conduction. The subjects were grounded properly. Past history of HIV, regular alcohol consumption, liver diseases, thyroid disorders, rheumatoid arthritis and neurological disorders was taken. Sports person/athletes were excluded from the study. Patients were asked to dress clothes that permit access to the area to be tested and to stop using lotions or oils on skin for few days. Patients were asked to

remove any jewelry, hairpins, hearing aids or other metal objects that may interfere with the procedure.

During procedure:

Patients were asked to sit or lie down for the test and then the area overlying nerve was cleaned with saline at proximal and distal ends. The cup electrodes were fixed on the skin overlying muscle supplied by nerve only after application of electrode jelly. The electrodes were connected to the oscilloscope through the preamplifier. The sweep was kept at 5 ms / cm.

Procedure:

The Nerve conduction velocity test was done with surface patch electrodes (recording, reference, stimulating electrodes). The patch electrodes were placed on the skin over the nerve. One electrode stimulates the nerve with a very mild electrical impulse. The intensity and duration of this transcutaneous stimulus was gradually increased until all of the axons within that nerve get depolarized, sparking an action potential that travels down the nerve to the recording site. The resulting electrical activity was recorded by the electrode.

The distance between electrodes and the time it takes for electrical impulses to travel between electrodes were used to calculate the speed of impulse transmission. In case of sensory conduction study distance between active electrode and cathode of stimulator was divided by onset latency to give sensory conduction velocity.

OBSERVATIONS AND RESULTS:

Parameters like latency, amplitude and nerve conduction velocity of median nerve, ulnar nerve, sural nerve and superficial peroneal nerve of prediabetes and nondiabetes subjects were studied and compared. Glycated Haemoglobin (HbA1c%) was quantitatively determined by ion exchange resin using fully automated analyser (CPC turbochem 100). Statistical analysis was done by using unpaired 't' test to test whether the differences in means were statistically significant.

All the calculations and statistics were done using MedCalc statistical software version (14.8.1). A 'p' value of less than 0.05 ($p < 0.05$) was considered to be statistically significant. A 'p' value of less than 0.001 ($p < 0.001$) was considered to be statistically highly significant. A 'p' value greater than 0.05 ($p > 0.05$) was considered to be not significant.

Table no 1: showing number of subjects and their groups.

Study Subjects	Groups	No of subjects enrolled
Prediabetics	Group I	50
Non-diabetic Control	Group II	50

Table no 2: showing glycated haemoglobin (HbA1c%) levels in study groups

Study subjects	HbA1c% (Mean \pm SD)	P Value	Remarks
Group I	6.03 \pm 0.20	P<0.0001	HS
Group II	4.84 \pm 0.46		

HS-Highly significant.

Table no 3: showing baseline characteristics of study groups.

	Group I (Mean \pm SD)	Group II (Mean \pm SD)	P Value	Remarks
AGE (years)	46.10 \pm 5.72	44.74 \pm 6.62	P >0.05	(NS)
HEIGHT (cm)	169.32 \pm 3.27	168.58 \pm 3.13	P >0.05	(NS)
WEIGHT (kg)	68.92 \pm 8.31	68.90 \pm 5.89	P >0.05	(NS)
BMI (kg/m ²)	24.06 \pm 3.05	24.27 \pm 2.38	P >0.05	(NS)

NS-Not significant.

Table no 4: showing comparison of parameters measured for median (motor) nerve in study groups.

	Right limb.		Left limb		P value	Remark
	Group I (Mean \pm SD)	Group II (Mean \pm SD)	Group I (Mean \pm SD)	Group II (Mean \pm SD)		
Latency (ms)	2.51 \pm 0.36	2.52 \pm 0.33	2.59 \pm 0.40	2.59 \pm 0.40	P >0.05	(NS)
Amplitude (μ V)	12.98 \pm 3.83	13.10 \pm 3.16	13.39 \pm 2.90	13.39 \pm 2.90	P >0.05	(NS)
Velocity (m/s)	60.15 \pm 9.66	61.94 \pm 11.37	61.37 \pm 7.41	61.37 \pm 7.41	P >0.05	(NS)

 ms-Millisecond, μ v=Microvolt, m/s-meter per second, SD-Standard deviation, NS-Not significant.

Table no 5: showing comparison of parameters measured for median (sensory) nerve in study groups.

	Right limb.		Left limb		P value	Remark
	Group I (Mean \pm SD)	Group II (Mean \pm SD)	Group I (Mean \pm SD)	Group II (Mean \pm SD)		
Latency (ms)	2.57 \pm 0.24	2.58 \pm 0.25	2.63 \pm 0.15	2.63 \pm 0.11	P >0.05	(NS)
Amplitude (μ V)	26.05 \pm 5.69	26.50 \pm 5.32	24.38 \pm 3.28	23.31 \pm 3.94	P >0.05	(NS)
Velocity (m/s)	54.96 \pm 5.70	54.67 \pm 5.61	53.33 \pm 3.14	53.16 \pm 2.36	P >0.05	(NS)

 ms-Millisecond, μ v=Microvolt, m/s-meter per second, SD-Standard deviation, NS-Not significant.

Table no 6: showing comparison of parameters measured for ulnar (motor) nerve in study groups.

	Right limb.		Left limb		P value	Remark
	Group I (Mean ± SD)	Group II (Mean ± SD)	Group I (Mean ± SD)	Group II (Mean ± SD)		
Latency (ms)	2.26 ± 0.17	2.32 ± 0.18	2.27 ± 0.22	2.25 ± 0.23	P >0.05	(NS)
Amplitude (μV)	10.56 ± 1.63	10.79 ± 1.37	9.34 ± 0.81	9.59 ± 0.96	P >0.05	(NS)
Velocity (m/s)	65.15 ± 9.28	63.83 ± 7.80	67.24 ± 7.51	67.17 ± 7.37	P >0.05	(NS)

ms-Millisecond, μv=Microvolt, m/s-meter per second, SD-Standard deviation, NS-Not significant.

Table no 7: showing comparison of parameters measured for ulnar (sensory) nerve in study groups.

	Right limb		Left limb		P value	Remark
	Group I (Mean ± SD)	Group II (Mean ± SD)	Group I (Mean ± SD)	Group II (Mean ± SD)		
Latency (ms)	2.31 ± 0.18	2.38 ± 0.20	2.30 ± 0.19	2.24 ± 0.24	P >0.05	(NS)
Amplitude (μV)	19.81 ± 1.25	19.69 ± 0.98	19.71 ± 0.94	19.84 ± 0.72	P >0.05	(NS)
Velocity (m/s)	60.87 ± 4.71	59.21 ± 4.84	61.06 ± 5.02	62.92 ± 6.53	P >0.05	(NS)

ms-Millisecond, μv=Microvolt, m/s-meter per second, SD-Standard deviation, NS-Not significant.

Table no 8: showing comparison of parameters measured for sural (sensory) nerve in study groups.

	Right limb.		Left limb		P value	Remark
	Group I (Mean ± SD)	Group II (Mean ± SD)	Group I (Mean ± SD)	Group II (Mean ± SD)		
Latency (ms)	2.52 ± 0.33	2.47 ± 0.24	2.61 ± 0.33	2.53 ± 0.25	P >0.05	(NS)
Amplitude (μV)	22.03 ± 2.45	21.85 ± 1.98	21.76 ± 1.85	22.34 ± 1.79	P >0.05	(NS)
Velocity (m/s)	56.45 ± 7.19	57.08 ± 5.92	54.27 ± 6.65	55.75 ± 5.77	P >0.05	(NS)

ms-Millisecond, μv=Microvolt, m/s-meter per second, SD-Standard deviation, NS-Not significant.

Table no 9: showing comparison of parameters measured for superficial peroneal (sensory) nerve in study groups.

	Right limb.		Left limb		P value	Remark
	Group I (Mean ± SD)	Group II (Mean ± SD)	Group I (Mean ± SD)	Group II (Mean ± SD)		
Latency (ms)	2.47 ± 0.17	2.42 ± 0.15	2.37 ± 0.19	2.44 ± 0.22	P >0.05	(NS)
Amplitude (μV)	21.15 ± 2.84	21.10 ± 2.40	20.97 ± 2.39	21.44 ± 2.05	P >0.05	(NS)
Velocity (m/s)	56.91 ± 3.96	58.03 ± 3.58	59.35 ± 4.71	57.82 ± 5.33	P >0.05	(NS)

ms-Millisecond, μv=Microvolt, m/s-meter per second, SD-Standard deviation, NS-Not significant.

DISCUSSION

There was no significant difference in the base line characteristics like age, height and weight of the subjects in two groups as shown in table no 2. The mean glycated hemoglobin levels in group I and group II were 6.03 ± 0.20 and 4.84 ± 0.46 respectively, so there was a statistically highly significant difference in glycated hemoglobin levels between the two groups ($p < 0.0001$) as shown in table no 3.

There was no statistically significant difference found between the two groups, for nerve conduction study parameters like amplitude, latency and nerve conduction velocity measured for median, ulnar, sural and superficial peroneal nerves ($p > 0.05$) as shown in table no 4 to 9. Also, there was no abnormality in the parameters of nerve conduction of prediabetes subjects when compared with normative data. Our study does not support the occurrence of neuropathy in prediabetic stage.

In the study done by Dyck P J et al. (2012) it was concluded that though neuropathy may be present in IGT, its prevalence is very less. The frequency of neuropathy in IGT narrowly defined is only 3%. This also must be the factor responsible for negative findings in our study [19].

A similar study was done by Pourhamidi K et al. (2013) assessing small and large nerve fiber function in people with NGT, IGT, and type 2 diabetes. Like in our study, Pourhamidi also concluded that neuropathy does not occur in prediabetes stage [20].

In one of the latest Indian studies on nerve conduction, it was concluded that neuropathy sets in early in prediabetes. Devi MS (2015) studied for changes in nerve conduction velocity in prediabetes and diabetes. The nerve conduction velocity of peroneal and sural nerve was measured. The data obtained from this study showed that the conduction velocity of peroneal nerve in prediabetes was decreased when compared with controls and the sural nerve conduction velocity was significantly decreased in prediabetes (p value of 0.047) and diabetes (p value of < 0.001) when compared with the controls indicating early onset of neuropathy even in prediabetes [21].

CONCLUSION:

Nerve conduction parameters latency, amplitude and velocity measured in 50 prediabetes subjects when compared with 50 nondiabetes subjects show no

statistically significant difference ($p > 0.05$). A decrease in amplitude or nerve conduction velocity would have suggested axonal loss or neuropathy respectively.

It has been documented that neuropathy often is subclinical therefore; if a patient does not show signs of neuropathy on the clinical examination, nerve conduction studies which is cost effective and noninvasive are usually advised as an ancillary tool to detect incipient neuropathy. The early detection of abnormal glucose metabolism and neuropathy is particularly important as treatment will probably be most effective if administered early while neuropathy, when abnormalities of peripheral nerves are more likely to be reversible.

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