(RESEARCH ARTICLE)

Assessment of neuron-specific enolase, glycated haemoglobin and fasting plasma glucose levels in patients with diabetes peripheral neuropathy in Calabar Metropolis, Nigeria: A case control study

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Abstract

Background: Persistent hyperglycemia has been implicated in the development of neuronal complications in diabetes patients. Nerve damage and metabolic abnormalities may cause the release of neuron specific enolase into circulation.

Aim: To investigate the relationship between serum neuron-specific enolase (NSE), fasting plasma glucose (FPG) and glycated haemoglobin (HbA1C) levels in diabetic patients with peripheral neuropathy.

Methods: one hundred and five patients with peripheral neuropathy and 60 apparently healthy non-diabetic controls aged 45-69 years attending Clinics in General Hospital Calabar and the University of Calabar Teaching Hospital, Calabar, between July 2021 and January 2022, were recruited into the study. Fasting plasma glucose was estimated using glucose oxidase peroxidase method. Glycated haemoglobin and NSE were determined using Boronate Affinity High Performance Liquid Chromatography and ELISA methods respectively. Height and weight were measured and BMI computed, and data analyzed using Student's t-test, ANOVA, post hoc analysis and Pearson's correlation at P<0.05.

Results: Body mass index, blood pressure, fasting plasma glucose, glycated haemoglobin and neuron specific enolase concentrations were significantly different (P=<0.05) between the diabetic patients and the controls. Neuron specific enolase vary significantly (P<0.05) among the diabetic patients with different forms of diabetes peripheral neuropathy. Fasting plasma glucose correlated positively with NSE (r=0.441, P=0.000) and HbA1C (r=0.328, P=0.001) respectively. Glycated haemoglobin correlated positively with BMI (r=0.412, P=0.000) and NSE (r=0.328, P=0.001) in that order.

Conclusion: This study has shown that glycated haemoglobin and fasting plasma glucose are related with neuron specific enolase levels in patients with diabetes peripheral neuropathy. Thus, rising levels of glycated haemoglobin and fasting plasma glucose (poor glycemic control) may be associated with progressive nerve damage in patients with diabetes peripheral neuropathy.

Keywords: Diabetes; Complication; Neuropathy; Neuron Specific enolase

1. Introduction

Diabetes mellitus is a potentially severe metabolic disorder that constitutes a major global health problem as a result of its high occurrence and associated complications, among which is diabetic peripheral neuropathy (DPN). Diabetes is also associated with speedy atherosclerotic disease affecting arteries that supply the lower extremities, brain and heart [1]. Diabetic peripheral neuropathy is the existence of signs indicative of peripheral nerve damage in patients with

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diabetes after other causes have been governed out. It is one of the most common complications in diabetic patients occurring in about 60% of individuals with longstanding type 1 or type 2 DM [2]. The development of diabetic neuropathy is controlled by both the blood arteries that nourish the peripheral nerves and diabetes related metabolic abnormalities [3]. Diabetic peripheral neuropathy causes diabetes-specific pathologies in a number of organs over the long term, specifically those that are insulin insensitive (eg vascular endothelial cells, neurons, kidneys, and retina), due to the failure of the cells of these organs to down-regulate the uptake of glucose when extracellular glucose concentrations are high [4, 5]. Glucose concentrations in these organs corresponds with the plasma levels in these patients. The long term complications of diabetes in certain parts of the body confers an increased overall risk of premature death [2]. The metabolic abnormalities of diabetes cause a single hyperglycemia-induced process, mitochondrial superoxide (the initial oxygen free radical formed by the mitochondria, which is then converted to other more reactive species) overproduction [6]. This hyperglycemia-induced increased superoxide production through mitochondrial dysfunction is the principal mediator of diabetes tissue damage through the activation of five major pathologic mechanisms. These pathways include (1) activation of the polyol pathway, (2) increased intracellular formation of advanced glycation end-products (AGEs), (3) increased expression of the receptor for advanced glycation end products and its activating ligands, (4) activation of protein kinase C (PKC) isoforms, (5) over-activity of the hexosamine pathway with the inactivation of two significant anti-atherogenic enzymes; prostacyclin synthase and endothelial nitric oxide synthase (eNOS). A number of evidence indicate that all five mechanisms are activated by a particular upstream event: mitochondrial overproduction of reactive oxygen species (oxidative stress) [7, 8]. Inactivation of the anti-atherogenic enzymes; prostacyclin synthase and endothelial nitric oxide synthase (eNOS) results in microvasculature alteration and reduced peripheral perfusion [9]. Consequently, nerve ischemia occurs, due to raised wall thickness and hyalinization of the basal lamina of blood vessels and basement membrane of the capillaries that provide nutrients and perfuse peripheral nerves [10, 11]. These events lead to peripheral nerve damage through mitochondrial overproduction of reactive oxygen species that cause damage to the myelin sheath (covering on nerves), weakens and thickens the wall of blood vessels that bring oxygen to the nerves leading to hypoxia and ischemic nerve damage [6]. Damaged nerves may either stop sending messages, send messages slowly or at the wrong times or uncoordinatedly called diabetic neuropathy.

Oxidative stress plays a central role in the development of diabetes complications. Chronic exposure to hyperglycemic and ischemic/hypoxic environments induce oxidative stress in the nervous system [12]. The physiopathology of DPN involves hypoxia of the peripheral nerves. It has been postulated that hypoxia may be the cause of diabetic polyneuropathy due to alteration in the wall of the arteries that supply the nerves.

Oxidation of free fatty acids in diabetics inactivates several glycolytic enzymes, including enolase, in neurons [13]. The relatively high-energy demand under such conditions causes an upregulation in the glycolytic enzymes in compensatory response to increase the survival of neurons with subsequently leakage of neuron specific enolase through damaged neuron into circulation [14]. Glycemic variability has been documented as the most significant risk factor for DPN development [15-17].

Early detection and good glycemic control have been demonstrated to avert adverse consequences related with DPN, thus, crucial to slowing the rate of progression of disability and death [18]. Timely detection of diabetic neurological complications may be facilitated through the evaluation of nerve damage via the quantification of neuronal derived proteins such as neuron-specific enolase (NSE). Neuron-specific enolase is a glycolytic enzyme, located in the neurons and neuroendocrine cells, its role is to regulate the growth and development of nerve cells.

Studies on assessment of NSE in patients with diabetic neuropathy are relatively extensive, however studies correlating the level of NSE with indices of glycemic control are scanty in our locality. Also, currently, effective treatment available for diabetic neuropathy or to reverse progression of neuropathy is still indescribable beyond stringent control of blood glucose level even though efforts can be made on early diagnosis and to terminate the progression of diabetic neuropathy. Moreover, early detection and good glycemic control can prevent diabetes related neuropathy adverse outcomes, but there is a lack of consensus report regarding glycemic variability and a biomarker specific to diabetic related neuronal damage. The pathophysiologic mechanisms of diabetic neuropathy has not been fully elucidated, hence, the reason why some patients develop neuropathy and others do not is not yet fully understood. This study assessed NSE level in relation to indices of glycemic control in patients with diabetes mellitus.

2. Material and methods

2.1 Study design

This case-control study was conducted in Calabar Metropolis, among patients with diabetes peripheral neuropathy attending clinics in General Hospital Calabar, and the University of Calabar Teaching Hospital (UCTH), Calabar, Southern Nigeria.

The diabetic patients were further subcategorized based on the dominant sign of peripheral neuropathy expressed as confirmed by the attending physician into those with unsteady gait (n=16), burning sensation (n=22), hypersensitivity (n=12), numbness (n=26), spontaneous response (n=20), tingling (n=9). Socio-demographic data, family history, medical history and anthropometric data were obtained from each participant using a well-structured questionnaire.

2.2 Study setting

The population consists of patients with diabetes neuropathy in Calabar Metropolis, comprising of residents of Calabar South and Municipal Local Government Areas, Southern, Nigeria. Diabetic patients aged 48-69 years attending clinics in General Hospital Calabar and the University of Calabar Teaching Hospital (UCTH), Calabar between July 2021 and March 2022 and apparently healthy non-diabetic controls residing in the same geographic location were recruited into the study.

2.3 Participants and inclusion criteria

The study participants included 105 type 2 diabetes mellitus patients with confirmed diabetic peripheral neuropathy attending Clinics in General Hospital Calabar and the University of Calabar Teaching Hospital (UCTH), living in Calabar Metropolis and 60 apparently healthy non-diabetic controls residing in the same geographic location. Subjects for the study were chosen from diabetic patients with persistent diabetes and no open lower-extremity ulcers, diagnosed with DPN and not on high doses of opioid and other pain relievers in the last 24 hours. And having usual or maximum tingling, numbness, and burning pains during the day or at night, who gave written informed consent were recruited into the study. The patients were confirmed with diabetic peripheral neuropathy using a combination of Michigan Neuropathy Screening Instrument (MNSI) and physical examination (score >2.5) [19]. Diabetic patients on opioids and those not willing to participate in the study were excluded.

2.4 Data collection

After an overnight fast, a standard venepuncture method was used to obtain 9 mL of venous blood from all the participants. Four milliliters of blood was dispensed into K_2 EDTA samples bottle for glycated hemoglobin estimation, 2mls was transferred into fluoride oxalate containers for fasting plasma glucose estimation, and 3mls into plain bottles, allowed to clot and then centrifuged at 3 000 rpm for 5 minutes at room temperature. The sera were separated immediately into aliquots using sterile Pasteur pipettes and stored at −20 °C in the UCTH laboratory until analysis. Patients' information on general health, history of past diseases and addictions were collected using an interviewer questionnaire method. The information collected from the patients included socio-demographic characteristics (such as age, gender, marital status, education, habits such as smoking, consumption of alcohol, medication) and bio-clinical information (such as disease duration, pain level across the different types of pain (tingling, numbness and burning pain, hypersensitivity, unsteady gait, spontaneous response). Systolic and diastolic blood pressures, BMI). Blood pressure was measured using a digital blood pressure machine from Omron Health limited, China. A medical weighing scale was used to measure the weight of each participant to the nearest 0.1 kg. Height was measured using a measuring tape on a vertical rod to the nearest 0.1 cm. Body mass index was computed as the ratio of weight (kg) to height (m²). Body mass index less than 18.5kg/m² was considered underweight. Normal weight was a BMI of 18.50-24.99 kg/m2, the overweight had a BMI of 25.00-29.99 kg/m2, and the obese had a BMI ≥30.00 kg/m2 [20].

2.5 Data sources/Laboratory measurement

2.5.1 Estimation of fasting plasma glucose by glucose oxidase peroxidase method obtained from Megazyme, Wicklow, IRELAND

Glucose in the presence of atmospheric oxygen is being oxidized by the enzyme glucose oxidase to gluconic acid and hydrogen peroxide. Hydrogen peroxide is further broken down by the enzyme peroxidase which catalysis its breakdown to water and nascent oxygen which then reacts with 4-aminophenazone in the presence of phenol to give a pink color. The color absorbance is directly proportional to the concentration of glucose present in the sample, read at 510nm using a spectrophotometer [21].

$$\text{D} - \text{Glucose} = \frac{\text{Change in Absorbance of Sample}}{\text{Change in Absorbance of D} - \text{Glucose standard}} \times 100$$

2.5.2   Estimation of glycated hemoglobin (HbA1C) was done using Boronate Affinity High Performance Liquid Chromatography, from Trinity Biotech Plc, Wicklow, Ireland

Glycated hemoglobin was determined by Boronate Affinity High Performance Liquid Chromatography method from Trinity Biotech Plc, Wicklow, Ireland. Trinity Biotech's (Premier Hb9210), instruments employ the principles of boronate affinity and high-performance liquid chromatography (HPLC). Glycated haemoglobins (HbA1C) and glycated plasma proteins (GPP) differ from non-glycated proteins by having a sugar moiety(s) attachment at various binding sites by means of a ketoamine bond. Glycated haemoglobins and GPP thus contain 1,2-cis-diol groups not found on non-glycated proteins, which provide the basis for separation of glycated and non-glycated components by boronate affinity chromatography. In this analytical technique, a boronate such as phenylboronic acid is bonded to the surface of the column support. When a solution of proteins (hemolysate or diluted plasma) is passed through the column, the glycated component is retained by the complexing of its diol groups with the boronate. After the unretained non-glycated component elutes from the column, the glycated component is eluted from the column with a reagent that displaces it from the boronate. The analytical column contains aminophenylboronic acid bonded to a porous polymer support (gel) and pumps transfer reagents and patient samples through the analytical column. Hemolyzed samples for HbA1C are automatically injected onto the column during the flow of elution Reagent #1 (Buffer 2A). The glycated component binds to the boronate, while the non-glycated component passes through the column to the spectrophotometric detector, where it is detected at 413 + 2 nm. After the elution of the non-glycated component, the instrument pumps elution Reagent #2 (Buffer 2A), which displaces the glycated component from the column. The glycated component then passes through the detector. The compositions of elution Reagents #1 and #2 are designed to exhibit almost identical absorption in the 413 + 2nm range to ensure a stable baseline. The detector signal is also referenced by the split-beam technique. In the final stage of the cycle, the column is re-equilibrated with elution Reagent #1. All reagent selection occurs in a timed series designed to allow complete elution of non-glycated and glycated components. All functions are controlled by the computer, which processes the signal from the spectrophotometric detector and calculates the concentration of glycated hemoglobin or plasma protein as a percentage of the total detected. Integration is by peak area in Absorbance Units (AU)/seconds. The computer produces printed reports by analyzing signal as it is received by the detector. A Batch Summary Report is printed at the end of the run. Calculation of the percentage of HbA1C in the sample is by the following formula, with peak area in AU/seconds [22, 23].

$$\frac{\text{AreaPeak2}}{(\text{AreaPeak1} + \text{AreaPeak2})} \times 100$$

The final result is obtained by comparison to reference samples, traceable to both National Glycohemoglobin Standardization Program and International Federation of Clinical Chemistry and Laboratory Medicine, using a 2-point calibration.

2.5.3   Estimation of Neuron specific enolase (NSE)

Neuron specific enolase was determined by ELISA method using a kit obtained from Calbiotecth Inc, USA. It is based on a solid phase direct sandwich ELISA method. The diluted samples and the conjugate reagents (anti-NSE, Biotin and horse-raddish peroxidase) are added to the wells which have been pre-coated with streptavidin. Neuron specific enolase in the sample is sandwiched between two specific antibodies to NSE. The unbound protein and HRP are washed off. Upon the addition of TMB substrate, the intensity of the color developed is in direct proportion to the concentration of NSE in the samples. It produces a standard curve that relates the intensity of the color to the concentration of NSE.

2.6   Study size

Sample size was determined according to the method of Sullivan [24], using the formula $\frac{(Z_\alpha + Z_\beta)^2 \bar{p}(1-\bar{p})}{(p0 - p1)2}$. The power of 0.84 was calculated at beta error of 80%. The sample size of 105 patients was arrived at, while 60 apparently healthy individuals who served as controls were selected for the study.

2.7   Quantitative variables

BMI, diastolic blood pressure, systolic blood pressure, FPG, Glycated haemoglobin, neuron specific enolase.

2.8   Statistical analyses

Results were presented as mean ± standard deviation. Data were analyzed using the statistical package for social sciences (SPSS version 23.0, IBM, USA). One way analysis of variance (ANOVA) was used to test the variations within and among group means and Fisher's least significant difference (LSD) post-hoc test was used for the comparison of multiple group means. Pearson's correlation was used to determine the associations between variables. The confidence interval was set to 95%. The significance level of the tests was set at $\alpha=0.05$.

3.   Results

3.1   Participants

A total of 165 participants comprising of 105 patients with diabetic peripheral neuropathy and 60 non-diabetic controls were recruited into this study.

3.2   Descriptive data

The profile of patients with diabetic peripheral neuropathy is as shown in table 1, in this study 41% of the patient with diabetic peripheral neuropathy (DPN) study participants were females while 59% were males. Sixty six per cent of the study participants were 60 years and below while 34% were above sixty years and less than 70 years old. From this study diabetes patients with unsteady gait, burning sensation, hypersensitivity response, numbness, spontaneous response and tingling constituted 15.24%, 20.95%, 11.43%, 24.73%, 19.05% and 8.57% respectively.

3.2.1   Outcome data

Unsteady gait (n=16), burning sensation (n=22), hypersensitivity response (n=12), numbness (n=25), spontaneous response (n=21) and tingling (n=9) constituted respectively. The controls were 60.

3.3   Main results

The comparison of age, body mass index, blood pressure, fasting plasma glucose, glycated haemoglobin and neuron specific enolase in patients with diabetic peripheral neuropathy and control were shown in table 2. Body mass index, blood pressure, fasting plasma glucose, glycated haemoglobin and neuron specific enolase concentrations were significantly different ($P=<0.05$) between the diabetic patients and the controls. The comparison of age, blood pressure, body mass index, glycated haemoglobin and neuron specific enolase in diabetic patients based on the dominant peripheral neuropathy expressed were shown in table 3.

Table 1 Profile of patients with diabetic peripheral neuropathy studied

	Population profile	Category	Frequency	Percentage
1	Sex	Males	62	59
		Females	43	41
2	Age	≤60	69	66
		>60	36	34
3	Unsteady gait		16 (F=11, M=5)	15.24
4	Burning sensation		22 (F=8, M=14)	20.95
5	Hypersensitivity response		12 (F=3, M=9)	11.43
6	Numbness		25 (F=12, M=13)	24.73
7	Spontaneous response		21 (F=6, M=15)	19.05
8	Tingling		9 (F=2, M=7)	8.57

Neuron specific enolase vary significantly ($P<0.05$) among the diabetic patients with different forms of diabetes peripheral neuropathy. From the LSD post hoc test, the neuron specific enolase level of patients with unsteady gait was significantly higher ($P=<0.05$) than those of patients with burning sensation, numbness, spontaneous response and tingling. The neuron specific enolase level of patients with burning sensation was significantly lower ($P=<0.05$) than

those of patients with hypersensitivity response and spontaneous response and higher than that of patients with numbness, while no significant difference ($P=>0.05$) was observed between those having burning sensation and those having tingling. Neuron specific enolase was significantly higher in those having hypersensitivity response compared with those having numbness, while the levels were significantly lower ($P=<0.05$) in those having numbness compared with those spontaneous response and tingling

Table 2, Age, blood pressure, body mass index, fasting plasma glucose, glycated haemoglobin and neuron specific enolase in patients with diabetic peripheral neuropathy and control

Parameters	Diabetic patients (n=105)	Controls (n=60)	Cal. T	P-Value
Age(years)	59.14±4.39	59.42±4.96	0.367	0.714
BMI (Kg/m^2)	24.88±4.04	27.36±3.07	4.103	<0.001*
DBP(mmHg)	84.85±5.91	77.10±5.24	8.433	<0.001*
SBP (mmHg)	144.39±11.47	131.38±9.63	7.412	<0.001*
FPG (mmol/L)	10.12±4.16	4.37±0.79	10.571	<0.001*
HbA1C (%)	12.89±5.02	3.01±1.33	14.936	<0.001*
NSE(ng/mL)	64.57±40.13	8.26±2.95	10.838	<0.001*

Key: Result presented as mean±SD, *, significant at $P<0.05$; SBP= systolic blood pressure, DBP= diastolic blood pressure, BMI= body mass index, FPG=fasting plasma glucose, HbA1c= glycated haemoglobin, NSE=neuron specific enolase

Table 3 Age, blood pressure, body mass index, glycated haemoglobin and neuron specific enolase in diabetic patients based on the dominant peripheral neuropathy exhibited

Parameters	Altered gait (n=16)	Burning sensation (n=22)	Hyper-sensitivity (n=12)	Numbness (n=25)	Spontaneous response (n=21)	Tingling (n=9)	Cal. F	P-Value
Age(years)	59.56±4.63	58.81±4.71	58.75±3.84	57.73±3.87	60.45±4.50	60.89±4.65	1.266	0.285
BMI(Kg/m^2)	25.55±4.67	24.96±3.17	24.96±3.50	25.55±4.67	23.14±3.73	26.23±3.83	1.106	0.362
DBP(mmHg)	84.63±6.49	86.14±5.38	86.25±9.36	83.46±5.23	84.35±5.26	85.33±3.57	0.664	0.652
SBP(mmHg)	144.87±10.50	144.82±11.23	145.75±15.96	142.73±11.47	143.80±10.38	146.77±8.77	0.234	0.947
NSE(ng/mL)	101.54±41.76[a]	51.26±21.43[b]	88.14±47.63[c]	31.03±15.39[d]	77.01±42.42	69.13±14.18	12.547	<0.001*

Key: Result presented as mean±SD, *, significant at $P<0.05$; a=significant difference between patients with altered gait and burning sensation, numbness, spontaneous response, tingling b=significant difference between burning sensation and hypersensitivity, numbness, spontaneous response, tingling, c=significant difference between hypersensitivity and numbness, d= significant difference between numbness and spontaneous response, tingling, SBP= systolic blood pressure, DBP= diastolic blood pressure, BMI= body mass index, FPG=fasting plasma glucose, HbA1c= glycated haemoglobin, NSE=neuron specific enolase

Table 4 Correlation between the various parameters in patient with diabetic peripheral neuropathy

Correlation Parameters	r	P-value
Age vs NSE	0.456	<0.001
SBP vs FPG	0.517	<0.001
SBP vs NSE	0.233	0.017
SBP vs HbA1C	0.267	0.006
DBP vs FPG	0.640	<0.001
DBP vs NSE	0.224	0.022
DBP vs HbA1C	0.354	<0.001

FPG vs NSE	0.441	<0.001
FPG vs HbA1C	0.238	0.001
HbA1C vs BMI	0.412	<0.001
HbA1C vs NSE	0.328	0.001

Key: SBP= systolic blood pressure, DBP= diastolic blood pressure, BMI= body mass index, FPG=fasting plasma glucose, HbA1c= glycated haemoglobin, NSE=neuron specific enolase

Correlation between various parameters studied was depicted in table 4. A significant positive correlation was observed between age and NSE (r=0.456, P=0.000) in patients with DPN. Systolic blood pressure correlated significantly and positively with FPG (r=0.517, P=0.000), NSE (r=0.233, P=0.017) and glycated haemoglobin (r=0.267, P=0.006) correspondingly. Diastolic blood pressure correlated positively with FPG (r=0.640, P=0.000), NSE (r=0.224, P=0.022) and glycated haemoglobin (r=0.354, P=0.000) in that order. Fasting plasma glucose correlated positively with NSE (r=0.441, P=0.000) and glycated haemoglobin (r=0.328, P=0.001) respectively. Glycated haemoglobin correlated positively with BMI (r=0.412, P=0.000) and NSE (r=0.328, P=0.001) in that order.

4. Discussion

This study evaluated the level of neuron specific enolase and how it relates to glycemic indices in patients with diabetes peripheral neuropathy. Neuropathy is one of the most common complications of both type 1 and type 2 diabetes, affecting over 60% of the diabetic patients. In this study fasting plasma glucose, glycated haemoglobin and neuron specific enolase levels were significantly (P<0.05) higher in patients with diabetic neuropathy compared with the non-diabetic controls. The significantly higher level of neuron specific enolase in patient with DPN compared with the controls may be attributed to hyperglycemia related metabolic and oxidative stress-induced nerve damage and the release of the neuronal derived protein (NSE) into the circulation. This finding is similar to that of Elsharkawy et al.,[25], who reported increased level of NSE in diabetic patients. Also, Li [26], had reported increased level of NSE in diabetic patients which correlates with diabetic peripheral neuropathy and also in accordance with the finding of Pandey et al., [27], who observed that hyperglycemia predicts an increased risk of poor outcome after ischemic stroke and reflected by a significantly increased level of neuron specific enolase. Hyperglycemic and ischemic/hypoxic environments induce oxidative stress in the nervous system generally. Hyperglycemia and oxidation of free fatty acids inactivate several glycolytic enzymes, including key glycolytic enzyme such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and enolase (in neurons). To meet the fairly high-energy requirements under such conditions, the glycolytic enzymes are compensatively upregulated to increase survival of the neurons. Chronic exposure to hyperglycemia or its related ischemia/hypoxia with oxidative stress leads to an increased risk for peripheral neuropathy, typified by neurodegeneration, neuroregeneration, myelin sheath alteration and the release of neuron specific enolase into circulation. Also during this process, the rate of synthesis of the enolase in the affected neurons increases and it is likely to cause the NSE to leak into the endoneurial fluid and into circulation.

Neuron specific enolase vary among diabetic patients having different forms of neuropathic manifestations. The levels of HbA1C and NSE may not be directly related with the distinctive neuropathic disorder manifested but may be related with the nature and extent of nerve damage. The nature of nerve damage affect the nerve response and the signal the residual nerve transmits, which may be related with the neuropathic disorder manifested. Glycated haemoglobin level may fluctuate following strict glycemic control but damaged nerve repairs may not be readily achieved in the diabetic patient. Inhibition of GAPDH activity by hyperglycemia increases the level of all the glycolytic intermediates that are upstream of GAPDH. This then increases glucose flux into the 5 pathogenic pathways described earlier and inactivates anti-atherosclerosis enzymes eNOS and prostacyclin synthase via ROS in these patients. Through these pathways, increased intracellular ROS cause defective angiogenesis in response to ischemia/hypoxia, activating a number of pro-inflammatory pathways. Hyperglycemia-induced increase in polyol pathway flux can damage the tissues involved. The polyol pathway involves a family of aldo-keto reductase enzymes with the ability to utilize a diversity of carbonyl compounds as substrates, and reduce them by nicotinic acid adenine dinucleotide phosphate (NADPH) to their respective sugar alcohols (polyols). Glucose is converted to sorbitol by the enzyme aldose reductase, with sorbitol then oxidized to fructose by the enzyme sorbitol dehydrogenase (SDH), using NAD+ as a cofactor. Aldose reductase is found in tissues such as nerve, retina, lens, glomerulus and vascular cells, where glucose uptake is mediated by insulin independent GLUTs; thus, intracellular glucose concentrations may rise in parallel with hyperglycemia. Increased aldose reductase and consumption of NADPH, a cofactor required to regenerate reduced glutathione (GSH), an important scavenger of reactive oxygen species (ROS). This could lead to the accumulation of toxic species and induce intracellular oxidative stress, and microvascular atherosclerosis, nerve ischemia and nerve damage. Damaged nerve endings are thought to contribute to pain in DNP, since the upset action potentials produced by damaged nerve endings,

may be interpreted in the central nervous system (CNS) as pain and other responses related with nerve injury. Diabetic patients with diverse type of pains and varying levels of NSE, FPG and HbA1C may sustain different forms of nerve injuries. This observation is similar to that of Schreiber et al., [28], who reported that DNP may be a consequence of changes in the neurons of both peripheral and CNS. Also Duncan et al., [29], observed changes in axonal structural integrity in order to meet up with high energy requirement which may cause the release of NSE. Similarly, Haque et al.,[30], observed that NSE could be a reliable, quantitative, and specific marker of neuronal injury, with neurotrophic role as it controls neuronal survival, differentiation, and neurite regeneration via activation of mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) signaling pathways.

The levels of the indices of glycemic control correlate positively with NSE level, suggesting that rising levels of HbA1C and FPG (poor glycemic control) may be associated with progressive nerve damage in these patients, and that NSE levels, hyperglycemia or the associated metabolic aberrations are essential factors in establishing symptomatic neuropathy. This finding is in line with that of Li et al., [26], who observed that Serum NSE levels are closely related with peripheral neuropathy in diabetic patients. Similarly Binh et al., [31], who reported a correlation between neuron specific enolase and serum glucose level in diabetic patients with acute ischemic stroke. Patients with diabetes peripheral neuropathy manifest different symptoms of pains which likely transit from one form to the other and may be attributed to the form of nerve damage at the beginning of neuropathy which may progress to affect other regions on the nerve; changing the nerve response and the associated type of pain manifested. The extent of nerve damage may be related to the level of NSE released but not to the type of pain manifested. Shortage of blood supply to nerves underlies the pathogenesis of all forms of diabetic complication. Normal blood vessels have an inner lining, the endothelium that keeps blood flowing smoothly by producing local Nitrous oxide (NO). Nitric oxide aids the relaxation of smooth muscles in the walls of blood vessels and avert cells from sticking to the walls. A disturbance of this mechanism leads to the increased formation of plaques in diabetes. High blood sugar, elevated fatty acids and triglycerides in diabetics leads to stickier walls, boosting the attachment of cells that produce local tissue reaction. The local tissue reaction additionally traps floating particles and different blood cells, piling up; hardening the vessel walls. Insulin stimulates the production of NO by the cells lining the blood vessels. This stimulatory effect is lost in diabetics who are resistant to the actions of insulin, resulting in increased tendencies towards plaque formation and obstruction of normal blood flow to tissues they supply with ensuing hypoxia/ischemia and oxidative stress.

5. Conclusion

This study has shown that glycated haemoglobin and fasting plasma glucose are related with neuron specific enolase levels in patients with diabetes peripheral neuropathy. Thus, rising levels of glycated haemoglobin and fasting plasma glucose (poor glycemic control) may be associated with progressive nerve damage in patients with diabetes peripheral neuropathy, and that neuron specific enolase levels, hyperglycemia or the associated metabolic abnormalities are essential factors in establishing symptomatic neuropathy.

Limitation of the study

Patients were not screened to rule out other sources of NSE such as small cell lung carcinoma (SCLC), and non-small cell lung cancer (NSCLC).

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

This study was carried out in accordance with the ethical principles for Medical Research involving human subjects as outlined in the Helsinki Declaration in 1975 and subsequent revisions. The study protocol was approved by the Health Research Ethics Committee, Ministry of Health, Cross River State (REC.NO.CRSMOH/RP/REC/2021/156).

Statement of informed consent

A written informed consent was obtained from each of the study participants, after explaining the purpose of the study. The confidentiality of patient's information was preserved at all steps. The rights to withdraw from participation in the study at any point in time were respected.

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