Assessment of Neuron Specific Enolase Level and some Related Biochemical Factors in Patients with Diabetic Peripheral Nerve Disorders

Zina J. Ghaib[1], Kadhim K. Ghudhaib[2], Firas Y. Mohsen[3]

[1]Post graduate, [2]Asst. Prof. Dr. Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad – Iraq, [3]Lecturer Dr. National Diabetes Centre, Al-mustanseriah University, Baghdad – Iraq

Abstract

Background: the disorder of neuron system by the action of hyperglycemia is Diabetic neuropathy considers a common diabetic complication which is associated with a wide scope of clinical cases. Peripheral neuropathy (PN) represents a clinical case, wherein the peripheral nervous system is damaged. Accordingly, biochemical parameters have importance to apply as good predictor factors. Accordingly we attempt to assess the biochemical factors of Neuron Specific Enolase NSE, Calpotectin CALP, Xanthine Oxidase XOD in patients suffering from this type of diabetes complications as early predictors and study their correlations.

Methods: Fifty eight patients are known to have diabetic mellitus, which is classified into three groups. Group I represents DPN of 30 patients, group II referred to 28 diabetic patients without DPN, in addition to 30 healthy subjects as a control group, with range age (38-70) years old. BMI was determined for all the studied groups. Levels of FBS, lipid categories and HbA1C were estimated. NSE, CALP and XOD levels were measured by ELISA method. hs-CRP also determine for patients and control.

Results: The results revealed that levels of both FBS and HbA1C are increased significantly ($p<0.05$) in both patients groups rather than healthy subjects. Moreover, results recorded an important increase of cholesterol, TGs, LDL and VLDL levels ($p<0.05$). in contrast the HDL decrease in the two patient groups related to healthy group. Increased levels of NSE, CALP, XOD and hs-CRP in DPN group and diabetic group compared with control group. The results of this study showed a direct correlation between BMI and FBS, in addition to BMI and hsCRP which possess the same correlation. Analysis by ROC curve showed that both NSE and CALP are representing the strongest markers for diagnosis of diabetic neuropathy followed by XOD and hs-CRP respectively.

Conclusion: Levels of the studied factors consider good indicators to use them as suitable markers for early detection of diabetic sensory neuropathy. The results of ROC curve analysis revealed that NSE and CALP is the strongest biomarker for diagnosis of diabetic neuropathy.

***Key words**: Neuron Specific Enolase, Calprotectin, Xanthine Oxidase, diabetic neuropathy*

Introduction

In this field, our previous studies were included the relation between diabetes with osteoporosis [1,2], complications of diabetes with nephropathy and neuropathy [3,4,5,6,7] together with the in vivo studies of diabetic treatment by plant extracts [8,9].

Diabetic neuropathy represents a major type in parallel to diabetic nephropathy and diabetic retinopathy which is classified as microvascular diabetic complications. It is results in typical sign and symptoms [10]. Diabetic neuropathy can cause deteriorating affects on the central nerves as well, resulting in subsequent vestibular system impairments[11]. Patients with diabetic neuropathy have certain symptoms like tingling and numbness. some types of abnormalities that concerned with this disease may accompanied with autonomic and sensory neurons in the body [12].

However, all peripheral nerves can be affected by diabetic neuropathy, consequently long nerves of feet and hands may be damage due to this type of diabetic disorder as a result to damage small nerve fibers. Many studies have shown that the small fibres damage might lead to large fibre damage and then too in neuropathy. Apart from this nerve damage there are several other pathogenesis of systemic and cellular disturbances in glucose and lipid metabolism leads to other complex biochemical pathways in the end leading to neuropathy (13).

So, it is very important to know about the different biomarkers of neuropathy for the early diagnosis of condition and to manage the condition with appropriate and effective way. Thus extensive research has been done in this area although appropriate and widely utilizable biomarkers have not been identified for diabetic neuropathy. Accordingly this work aimed to study some relevant factors with diabetic neuropathy to study their relation with this type of disease as predictor factors for Iraqi patients with diabetic peripheral neuropathy.

Materials and Methods

Blood samples were taken from thirty patients (15 male and 15 female) with diabetic sensory neuropathy, twenty eight patients (14 male and 14 female) diabetic without neuropathy whom attended to National Diabetes Center, University of Al-Mustansiritya and 30 persons (15 male and 15 female) as control group at the period from December 2019 until Fabruary 2020, all subjects with age range 38-70 years. Venous blood (8 ml) was withdrawn for each sample. Two ml were collected in EDTA tube for determine HbA1C by using Ichroma, and 6 ml collected in an anticoagulant serum separator tube, then the blood left at room temperature for a period of 30 minutes to clot and separated at 3000 rpm for 10 minute using a suitable centrifuge, then, the obtained serum was removed and divided into numerous parts that stored in suitable tubes. One part was used to evaluate the biochemical parameters, which are including FBS, Lipid profiles directly, using UV-Visible spectrophotometer and hs-CRP determination by using Ichroma. The remaining three parts were kept in a very cold place at -20°C to estimate other parameters include NSE, XOD and CALP that were evaluated by ELISA, with kits supplied by Shanghai Biological/ China and Cusabio / China. BMI was enumerated for all the studied groups.

Statistical Methods

Our statistics in this work were analyzed by the Analysis System - version 9.1. The assessment of significant differences ($P < 0.05$) is achieved by ANOVA test and LSD together with SPSS-19 program which is used to calculate correlation coefficient (r). ROC curve analysis was used also in this study to estimate the strength for each marker to be useful in diagnosis of the disease.

Results

The results of age and BMI in table -1 revealed that there are no significant differences ($p>0.05$) between patients and control groups and the patients groups themselves. The results in table (1) revealed significant increase in FBG and HbA1C levels ($p<0.05$) in DPN group related to diabetic and healthy groups, where the mean values for DPN and DM groups are higher than their levels in control group.

Table (1): mean values of Age , BMI, FBG and HbA1C of the studied groups.

Categories	AGE (Year) mean ± SD	BMI (Kg/m2) mean ± SD	FBS (mg/dl) Mean ± SE	HbA1C % Mean ± SE
DPN Group	55.96±9.04a	31.62±4.51a	200.05±11.73a	9.18 ± 0.25 a
Diabetic Group	53.46±10.81a	30.50±5.22a	170.28±9.67b	8.13±0.22b
Control Group	51.13±11.22a	29.03±5.68a	92.44±0.93c	4.34±0.07c
LSD	5.2868	2.69	27.14	0.6076
Mean with same letters of AGE and BMI are not significantly (P>0.05). Considerable significantly (P<0.05) are represented by changed letters in each column.				

The result of cholesterol was showed a significant increase (p<0.05) between DNP and healthy subject groups as recorded in table 2 , but there are no significant differences between patient groups for this parameter. The results of triglyceride and VLDL levels in patients with diabetic neuropathy (DPN) increased considerably (p<0.05) related to healthy subjects group. There are no considerable differences (p>0.05) in the case of HDL and LDL between both patient groups together with healthy subjects group.

Table (2) mean values of T.Ch., TGs, HDL, LDL and VLDL of all the studied groups

Categories	Chol. mg/dl mean ± SE	TGs mg/dl mean ± SE	HDL mg/dl mean ± SE	LDL mg/dl mean ± SE	VLDL mg/dl mean ± SE
DPN Group	186.30±7.55a	167.38±13.08a	42.53±2.41a	110.01±6.75a	33.91±2.64a
D. Group	177.64±8.03ab	161.00±13.28a	42.81±3.06a	107.96±9.30a	32.80±2.82a
C. Group	161.11±5.09b	73.92±7.22b	43.40±2.00a	103.97±3.34a	14.90±1.43b
LSD	20.445	34.066	7.2047	19.845	7.0039

Considerable differences (P<0.05) are represented by changed letters in each column

Table (3) showed the results of CALP, NSE, XOD and hs-CRP. The mean values of CALP and hs-CRP increased significantly (p<0.05) in DPN group related to healthy subjects group, but there are no considerable differences (p>0.05) between DPN and diabetic groups. Also, the results recorded considerable differences increased (p<0.05) for the parameters of NSE and XOD between DPN group and control group, but there are no important differences (p>0.05) noticed between diabetic and healthy subject groups.

Table (3): mean values of CALP, NSE, HDL, XOD and hs-CRP of all the studied groups

Categories	CALP (ng/mL) Mean ± SE	NSE (ng/mL) Mean ± SE	XOD (ng/mL) Mean ± SE	hs-CRPmg/dl Mean ± SE
DPN Group	89.69±20.071a	10.98±2.07a	1.07±0.22a	6.82±0.28a
D. Group	78.04±8.84a	4.70±1.44b	0.91±0.20b	6.08±0.15a
C. Group	16.25±2.26b	2.06±0.16b	0.44±0.06b	3.90±0.43b
LSD	54.12	4.01	0.541	0.866

Considerable differences (P<0.05) are assigned by changed letters in each column

The results of correlation showed positive correlation between FBG and BMI. Furthermore, a direct correlation was found between hs-CRP and BMI in diabetic group; however, no correlations among other studied parameters were recorded as shown in table (4).

Table (4): correlation coefficient of BMI Levels in DM group

Parameters		AGE	HbA1C	FBS	NSE	CALP	XOD
BMI in diabetic peripheral neuropathy Group	r	-0.221	-0.293	0.389*	0.047	0.120	0.112
	p	0.277	0.130	0.041*	0.811	0.544	0.570
		T.Chol	TGs	HDL	LDL	VLDL	hsCRP
	r	0.191	0.243	0.329	0.007	0.139	0.526*
	p	0.329	0.212	0.087	0.971	0.481	0.004*

* Correlation is significant at P value < 0.05 level.

Analysis by ROC curve was conducted as shown in figure of (1). ROC curve is a diagram, which can be drawn between sensitivity and specificity. The area under the curve was adopted in the interpretation of the results. It was found to be 0.989, 0.96, 0.897 and 0.819 for NSE, CALP, XOD, hs-CRP respectively. In the case of the NSE test, the cut-off point derived from the ROC curve showed a sensitivity of (95.2%) and specificity of (87.5%) in the value of criterion >2.794 ng/ml. accordingly, the test value above 2.794 ng/ml considers abnormal case (disease condition) and the value below 2.974 ng/ml represents the healthy condition, as shown in fig. (1).

Analysis of CALP, XOD and hs-CRP have revealed the best cut off points at values of criterion (>22.279 ng/ml, 1.019 ng/ml and >5mg/dl), respectively, while the analogous values of sensitivity and specificity for the mentioned cut-off points were found to be (100.0%, 90.9%), (77.8%, 88.9%) and (91.7%, 81.5%) respectively.

Discussion

In this work, our findings revealed that no-considerable differences (p>0.05) in the parameters of age and BMI between patients and healthy subject groups, as shown in table(1). This result is agreed with other studies [14,15]. The rising of serum cholesterol, triglycerides, LDL and VLDL levels and decrease in HDL level in patient groups than control group may be attributed to the associating with the case of disease and its complications. However, our results agree with earlier studies [16].

Elevation of triglycerides with a decrease of HDL levels in diabetic patients is also documented by several authors [17,18]. The results of HbA1c showed significant differences among the studied groups, where the mean values for DPN and diabetic group higher than the control group. This result agrees with [19]. That means the HbA1c test provides important information that can be used for the management of diabetes.

In the same time, there is a considerable increase (p<0.05) in level of FBG in DPN and diabetic groups (200.05±11.73), (170.28±9.67) respectively, in comparison with control group (92.44±0.93). This result is in consistence with other study [20]. Consequently, the present study confirmed a strong association between the disease (Neuropathy) and hyperglycaemia that produce advanced glycation end products (AGEs) as a high risk for disease (diabetic complications) formation [21].

The values of the hs-CRP showed significant differences between patients and the control groups, this result agrees with [22]. This result has shown that serum hs-CRP level is considered a useful marker for long term risk assessment, besides that, it provides a sensitive marker for the increasing inflammation activity.

Serum CALP level was found to increase significantly ($p<0.05$) in patients compared with healthy subjects. This result is corresponding with several previous studies [23,24]. Calprotectin, belonging to S100 protein family, is essentially implicated in proinflamatory signalling. It is suggested that calprotectin complex is a marker for inflammation, and is useful in monitoring disease activity.

Significant increase in levels of NSE in patients with neuropathy group than both diabetic and control Groups, (10.98±2.07, 4.70±1.44, 2.06±0.16) respectively, are in correspond with [25], [26].

Due to the hyperglycemia, hypoxia and hypoperfusion that lead to damage of nerve cells, the level of NSE can be increased because the damaged cells released it to blood circulation at a high level. Thus, NSE can be useful to be a sign for damage of neuron cells. This disorder of neuropathy considers the major pathological mechanism for diabetic neuropathy injuries [27].

Increase significantly in levels of XOD in neuropathies group than diabetic group and health group (1.07±0.22, 0.91±0.20, 0.44±0.06) respectively, are in corresponding with[28,29] . Formation of ROS in the case of chronic hyperglacemia may lead to reduce of nerve fibers function such as abnormal of conduction velocity and appears of disease symptoms [30].

Table (4) show a correlation coefficient of BMI Level in Diabetic Patients Groups. The results reveal that BMI has positive correlation with FBG and with hs-CRP. The increasing of BMI is associated with central obesity and coronary risk[31].

Presence of positive correlation between BMI and hs-CRP, may be attributed to the association of inflammation with its linking to obesity(32), that characterized by increased levels of hsC-RP [33].

In this work, ROC curve analysis was applied. The results revealed that NSE and CALP levels are the strongest markers for diabetic neuropathy diagnosis, because the area under the curve is 0.989 and 0.961 which represents excellent tests as shown in the figure:

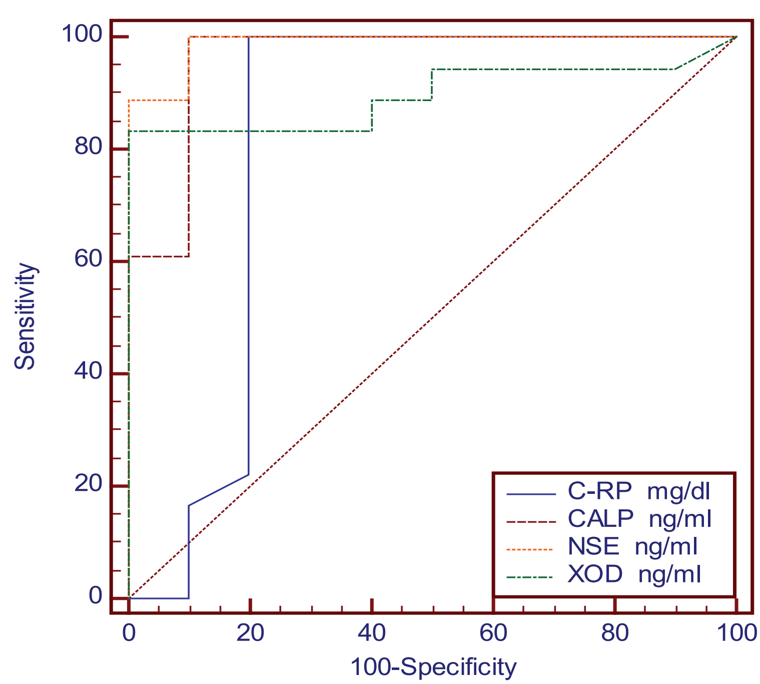

Figure (1): shows ROC curve for NSE, CALP, XOD and Hs-CRP.

Accordingly, the order of other biochemical parameters of XOD and hs-CRP found to be of 0.897 and 0.819 respectively, on the basis of their corresponding to area under the curve. Consequently, XOD and hs-CRP consider as good tests for estimating the prediction ability for developing of disease, or to compare the analytical facility between two or more tests for the same disease. (34)

Conclusions

Application of Receiver Operating Characteristic (ROC) curve analysis supports the Results of ROC curve analysis showed that NSE and CALP is consider strong marker (excellent test) to diagnose diabetic neuropathy, then (XOD and C-RP), which are found to be good tests for this purpose.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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