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Association of Neuron specific enolase And Insulin Resistance in Type 2 Diabetes Mellitus: A Pilot Study.

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ABSTRACT

Type 2 Diabetes mellitus (T2DM) is a metabolic disease characterized by insulin resistance resulting in hyperglycaemia. Chronic exposure to hyperglycemia with oxidative stress may affect peripheral nerves resulting in leakage of neuron specific enolase (NSE) in to the blood. Aim of the study is to compare serum NSE levels in diabetics and non-diabetics and find the association between NSE and insulin resistance. A prospective cross-sectional study was conducted in Dept of Biochemistry. Forty-five T2DM patients with/without complications visiting clinical laboratory for biochemical investigations, diagnosed as per ADA 2017 guidelines as cases and 45 age and gender matched healthy volunteers as controls were included in the study. Fasting blood sugar (FBS), insulin and NSE levels were assayed. Insulin resistance was calculated by HOMA-IR. Statistical analysis was carried out using the software SPSS 16. NSE levels were significantly high ($P<0.0001$) with NSE levels being 14.48±1.24µ gm/L in diabetics compared to that in controls, 8.22±0.76 µ gm/L.NSE had a significant positive correlation($p = 0.045$) with HOMA-IR, Pearson's correlation coefficient, $r =0.3622$. Elevated NSE levels and and its positive correlation with insulin resistance (HOMA-IR) in diabetics suggest that NSE may be an important biomarker in early detection of diabetes and may be its complications.

Keywords: NSE, Insulin resistance, T2DM

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INTRODUCTION

Neuron specific enolase or enolase 2 or NSE , is a highly soluble intracellular enzyme located in the cytoplasm of neuroendocrine cells (1). This enzyme is mainly located in neuronal tissues. Following neuronal injury, NSE is readily released into the cerebrospinal fluid and blood (2). Hyperglycemic and ischemic or hypoxic environments induce oxidative stress in the nervous system (1-3). Previous experimental and clinical studies report that oxidative stress has a key role in the pathogenesis of DM and development of complications of DM. Hyperglycemia induced free radicals may contribute to the neuronal damage in diabetes mellitus.

Glycolytic enzymes like enolase are inhibited by oxidation in neurons (4). A compensatory upregulation of this enzyme was noted so as to increase survival of neurons (5). Chronic exposure to hyperglycemia or its related ischemia/hypoxia with oxidative stress may lead to an increased risk for peripheral neuropathy in diabetes mellitus (6). As a result, the rate of enolase synthesis may change in the affected neurons and may cause the NSE to leak into the endoneurial fluid and serum. These logical events suggested necessity for a study in which serum levels of NSE are compared in diabetic and non-diabetics as well as to find out whether enolase bears any association with insulin resistance.

Experimental reports on neuronal injury and DM

NSE protein at higher concentrations is a marker of neuronal damage (7). It is well documented that long-standing diabetes mellitus is associated with neuronal damage and its clinical consequences. Studies by Skogseid et al found that higher levels of NSE were associated with exacerbation of oxidative stress and neuronal apoptosis (7). Animal experiments suggested neuronal apoptosis and suppression of neuronal cell proliferation in the hippocampus of diabetic rodents under uncontrolled hyperglycemic conditions (8-11). In experimental rat models, Manning et al reported that onset of T2DM is associated with a neurite degeneration and neuronal loss (12).

Neuronal injury and DM – Clinical Studies

Clinical studies have reported cognitive deficits, structural brain atrophy as seen in MRI brain (13,14) in patients with T2DM (14,15) . These changes were reported to be reversed with insulin replacement therapy (16). Insulin competitively inhibits insulin degrading enzyme (17) and hence persistent elevations in insulin may interfere with peripheral Aβ clearance and its higher concentrations in the brain (18). It has also been suggested that chronic elevation of insulin concentrations in the periphery may paradoxically cause a relative hypoinsulinized state in the brain (19) and thus resultant hyperinsulinemia could actually impair brain functions (20).

In an Egyptian study, Hamed et al reported that cognitive test scores of diabetics were significantly correlated with NSE concentrations regardless the level of glycemic control. This study suggests that poor glycemic control and chronic hyperglycemia can directly damage brain. It also showed a positive associations between total scores of cognition, levels of NSE and HbIAc and HOMA-IR derived from linear regression analyses. They observed an association between total scores of cognitive testing and higher NSE concentrations in association with poor glycemic control and presence of insulin resistance (IR). This relationship disappeared when they controlled for IR (21) .

In a study, Sandhu et al studied the association of NSE and diabetic peripheral neuropathy (22). But this study had certain limitations as there were no meticulous analyses of this relationship. A Chinese data is available, however results are inconclusive (23). In another study,Li J et al reported an elevated NSE levels in diabetic retinopathy patients. The study also concludes that NSE can also be a biomarker for diabetic retinopathy (24).

To the best of our knowledge, there are not many references for NSE levels in diabetics in Indian scenario.

Insulin resistance and DM

Insulin resistance (IR) is a condition where cells are non-responsive to insulin. IR is associated with type 2 diabetes mellitus (25). IR measured by HOMA-IR, is found to have certain limitations in patients with low BMI, decreased β cell function and high fasting blood glucose (26).

Research hypothesis

We hypothesize that

- Serum level of neuron specific enolase is associated with insulin resistance
- Diabetics have a altered NSE levels compared to non-diabetics.

Objectives

In this study, we aim to

- Find the association between neuron specific enolase (NSE) and insulin resistance
- Compare serum neuron specific enolase levels in diabetics and non-diabetics

METHODOLOGY

Study design

Type of study: Prospective cross-sectional.

Department: Clinical Biochemistry, KS Hegde Medical Academy, Nitte university .

Institutional ethics committee approval was sought before starting the study. Informed consent was obtained from subjects.

Inclusion criteria

Cases: 45 type 2 DM patients (18-65 years), with/without complications visiting clinical laboratory for biochemical investigations, diagnosed as per ADA 2017 guidelines.

Controls: 46 age and gender matched non-diabetics, healthy volunteers

Sample size calculation was done using the statistical formula,

$$n=4pq/d^2$$

Where p: prevalence of DM, q:100-p

Exclusion criteria

Type I diabetes, gestational diabetes, neurological disorders

Sample collection and analysis

Specimen used: Serum

Parameters analyzed: insulin, NSE

Instrument used: ELISA for insulin and NSE

HOMA –IR = fasting glucose X fasting insulin /22.5; insulin expressed in μ U/L ,glucose in mmol/l.

Statistical analysis

Software: SPSS version 16

Tests used:

Student's unpaired t test: comparison of NSE levels of diabetics and non-diabetics

Pearson's correlation: correlation between NSE &IR ,correlation between NSE & insulin

RESULTS

Table1: Comparison of Biochemical parameters in T2DM Vs Non-diabetics

	T2DM	Non-diabetics	P value
NSE(micro gm/L)	14.48±1.24	8.22±0.76	0.0001★
Insulin(micro U/L)	18.52±3.82	16.24±4.16	0.687
HOMA-IR	8.15±2.05	4.38±1.6	0.15
FBS	164.8±9.25	106.21±4.83	0.0001★

★p<0.0001 very highly significant

NSE had a significant positive correlation with HOMA-IR, Pearson's correlation coefficient, r =0.3622, p = 0.045.

There was an insignificant positive correlation between NSE and insulin, r = 0.0106, p =. 0.955.

There was no significant correlation between NSE and FBS r=0.2698 P=0.135.

DISCUSSION

A significant (p<) elevation in NSE was observed in diabetics as compared to non-diabetics. The elevation in NSE levels could be attributed to the hyperglycemia in diabetics. Though insignificant, there was a positive correlation between fasting blood sugar and NSE. The finding is in agreement with the reports by Pandey et al which states that hyperglycemia predicts an increased risk of poor outcome after ischemic stroke and it is reflected by a significantly elevated NSE(27).

The basis of elevation of NSE in diabetics could be as follows; hyperglycemia-induced lactic acidosis damages glial and endothelial cells, but may also exacerbate the biochemical events that lead to neuronal cell death and release of biochemical markers, shown by the positive correlation between NSE and the blood sugar level.

Study by Li et al suggested that serum neuron specific enolase is elevated in and indicative of diabetic retinopathy. The study also suggests that neuron specific enolase may be a potential biomarker of diabetic retinopathy(28).

Study by Jiambo et al observed that NSE was significantly elevated in those with neuropathy. The elevated NSE levels were closely related to diabetic neuropathy, and this relationship was independent of covariables. In addition, enolase levels increased with stages of neuropathy, and they were significantly correlated (29).

In T2DM,the presence of metabolic abnormalities, disturbance of vascular reactivity, hypoxia, disturbance of blood brain barrier (BBB) permeability and excitotoxic process make the quantification of neuronal derived

proteins (or NSE), a sensitive and direct biomarker of brain damage as well as its related neurological and neuropsychological outcome.

Hyperglycemic and ischemic or hypoxic environments induce oxidative stress in the nervous system (30-32). Oxidation inactivates several glycolytic enzymes, including enolase, in neurons (33). To meet the fairly high-energy requirements under such conditions, the glycolytic enzymes are compensatively upregulated to increase survival of the neurons (34). Chronic exposure to hyperglycemia or its related ischemia/hypoxia with oxidative stress leads to an increased risk for peripheral neuropathy (35), which is characterized by neurodegeneration that is often concomitant with neuroregeneration (36). During this process, the rate of synthesis of the enolase in the affected neurons may change, and it is likely to cause the NSE to leak into the endoneurial fluid and serum. This explains the elevated levels neuron specific enolase in type 2 diabetes Mellitus patients.

The study results suggest a significant positive correlation between insulin resistance (HOMA-IR) and NSE levels, which imply that more the insulin resistance, higher is the NSE levels. This is a clear implication of insulin having a major role in neuronal functions.

Brain has been considered an insulin-insensitive organ, recent reports on the location of insulin and its receptors in the brain have introduced new ways of considering this hormone responsible for several functions. The origin of insulin in the brain has been explained from peripheral or central sources, or both. Regardless of whether insulin is of peripheral origin or produced in the brain, this hormone may act through its own receptors present in the brain. The molecular events through which insulin functions in the brain are the same as those operating in the periphery. However, certain insulin actions are different in the central nervous system, such as hormone-induced glucose uptake due to a low insulin-sensitive GLUT-4 activity, and because of the predominant presence of GLUT-1 and GLUT-3. In addition, insulin in the brain contributes to the control of nutrient homeostasis, reproduction, cognition, and memory, as well as to neurotrophic, neuromodulatory, and neuroprotective effects. Alterations of these functional activities may contribute to the manifestation of several clinical entities, such as central insulin resistance, type 2 diabetes mellitus (T2DM), and Alzheimer's disease (AD).This may explain the positive correlation between HOMA-IR and neuron specific enolase.

CONCLUSION

Elevated NSE levels and and its positive correlation with insulin resistance (HOMA-IR) in diabetics suggest that NSE may be an important biomarker in early detection of diabetic complications.

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