Netrin-1 and Insulin Resistance as markers in Type 2 Diabetes Mellitus

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Abstract

The study's goals were to evaluate blood Netrin-1 levels in people with and without type 2 diabetes, to examine the relationship between Netrin-1 levels and insulin resistance (IR), and to determine whether Netrin-1 might be a sensitive and accurate marker to identify people who will develop Type 2 Diabetes Mellitus (T2DM). Insulin Resistance is a physiological condition, while Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) is a mathematical model used to estimate insulin resistance. HOMA-IR can be used as a diagnostic tool to identify insulin resistance and help with appropriate management and treatment. Forty-four type 2 diabetics who were matched for age and gender with thirty-five non-diabetics as controls, participated in the cross-sectional pilot study. Using the Enzyme Linked Immunosorbent Assay (ELISA) approach, the levels of fasting plasma glucose, insulin, and netrin were assessed. The Statistical Package For The Social Sciences (SPSS) 16 statistical analysis programme was used. Cases had lower serum Netrin levels than controls (P = 0.76). When T2DM patients' insulin levels were compared to controls, they were considerably lower (P=0.03). Netrin and (HOMA-IR) as well as Fasting Blood Sugar (FBS) were significantly ($p<0.0001$) correlated, according to a linear regression analysis. Although not significantly, netrin levels reduced in diabetics. A strong positive connection between netrin and insulin sensitivity shows that netrin has a protective role. Although netrin alone may not be a sensitive and specific marker to predict diabetes, it may be an effective marker when combined with Insulin Resistance.

Key words: netrin-1; insulin resistance; diabetes mellitus

Introduction

Overall incidence of Type 2 Diabetes mellitus (T2DM) is escalating worldwide. Increased prevalence of T2DM has inflicted huge psychological and financial burdens on society. Global count of 463 million adults who are currently living with diabetes have proved that T2DM is no less to be called an epidemic (IDF., 2020). At the ground level it is either Insulin resistance or insufficient insulin synthesis are the key causatives of hyperglycaemia typically paired up with obesity, sedentary life, age, family history along with other environmental factors and even geography. Patients with T2DM commonly presented with constellation of comorbidities such as cardiovascular disease, hypertension, dyslipidaemia, diabetic foot ulcers and renal insufficiency which may tighten the choices of anti-hyperglycaemic therapies. Studies have also reported that detrimental effects of diabetes are associated among Cardio Vascular Disease (CVD) patients with pronounced hyperglycaemia (Capes et al., 2000; Furnary et al., 2003). Early diagnosis and proper clinical management in Diabetes is of utmost importance as to fight shy of further comorbidities as the cost of illness of diabetes with other complications has always been high and progressing every passing year which states the necessity of efficient biomarker. However, recent studies have demonstrated the importance of biomarkers in evaluation of most effective therapeutic regimes. Netrin 1 is one such promising biomarker which has been found to have a role in the pathogenesis of diabetes mellitus and its complications. In this study we have tried to plot the association of insulin resistance with Netrin 1.

Insulin Resistance and Diabetes

Insulin is a peptide hormone of 51 amino acids and synthesized by β cells of pancreas. The hormone assists in metabolic functions which includes promoting glucose utilization by peripheral tissues, transport and storage of glucose, maximizing its absorption by adipose and muscular tissues. Thus, decreases gluconeogenesis in liver via gene expression and promotes protein synthesis in muscular tissues. Along with glucose, lipid metabolisms insulin has been contributing majorly to the cardiac contractility and vascular tone by activating endothelial Nitric Oxide enzyme (Bertrand et al., 2008; Muniyappa et al., 2007). Even brain is not devoid of insulin assistance for its smooth functioning. Prof. Havrankova J for the first time gave insight via his animal experiments that insulin is present in brain also found that insulin contents in brain are not dependent on plasma insulin levels (Havrankova et al., 1979). Insulin acts this by binding to its receptor (tyrosine kinase family) located on the plasma membrane of target cells (Haeusler et al., 2018). Inability of insulin to assist in metabolic fluxes is termed as Insulin resistance (IR). Lillioja S et al studied non diabetic Pima Indians for ten years and came to a conclusion that Insulin resistance is predictor of T2DM (Lillioja et al., 1993).

Studies have further confirmed that glucose is inflammatory whereas insulin is anti-inflammatory. Insulin induces vasodilatation which in turn helps in diminishing leucocyte – endothelium adhesion as well as retarding adhesion and aggregation of platelets (Grover et al., 1995; Muniyappa et al., 2007). Development of IR might affect the anti-inflammatory function of insulin.

Netrin 1 and Diabetes

Netrin is an extracellular, laminin related secretory proteins known to form neuronal circuit during embryogenesis. It is found to be acting as neuronal guidance cue in developing pancreas (Sun et al., 2011.) Along with pancreas, Netrin 1 also reported to be actively participating in development of nervous system, mammary glands, lungs also in angiogenesis and tumorigenesis (Srinivasan et al., 2003; Wilson et al., 2006; Arakawa., 2004). It exhibits itself as both chemo attractive and chemo-repellent in axonal guidance (Colamarino et al., 1995). It inhibits cellular adhesion and migration pancreatic cells by coupling with the integral proteins α6β4 and α3β1 (Yebra et al., 2003). There are references of its involvement in modulation of inflammation based conditions, suppression in production of inflammatory cytokine and chemokine (Tadagavadi et al., 2010; Mao et al., 2014). Inflammation is one of the major contributors in development of diabetes and Netrin 1 has reported to inhibit inflammation by acting on inflammatory cytokine and chemokines. This led us to hypothesized that serum Netrin 1 levels might show association with Insulin resistance and come out as a promising biomarker in prediction of T2DM.

Objectives of the study were

- To compare serum Netrin-1 levels in patients with and without Type 2 DM
- To find the correlation between Netrin-1 levels and insulin/IR in diabetics
- To assess whether netrin 1 could be a sensitive and specific marker to predict T2DM in insulin resistant subjects

Materials and Methods

Study subjects

The study was conducted in the department of Biochemistry, KS Hegde Medical Academy, Deralakatte, Mangalore after obtaining ethical approval from Institutional Ethics Committee.

It is crosses sectional study involving 79 subjects who visited hospital from January 2020 to march 2020. Of the total study subjects 44 were cases diagnosed with T2DM as per American Diabetes Association 2019 guidelines. Samples were collected on their visit to clinical laboratory for biochemical investigation. Subjects with renal diseases other than diabetic nephropathy were excluded from the study. Samples from age and gender matched 35 subjects visiting Medicine OPD for routine health check-up were taken as study controls. All of them were healthy and non- diabetic. Informed consents were obtained from all the study subjects prior to sample collection.

Methods

Baseline characters and demographic details for all the study subjects were collected from their respective medical records. After 8 hours of fasting, 2 ml of blood sample was collected in fluoride and plain vials. Fasting plasma glucose was estimated using fully automated chemistry analyser, Cobas C311. Samples in plain vials were centrifuged at 3000rpm for 10 min to separate serum. In due course serum samples were stored in aliquots without preservative at -30◌ C for an average of two months until analysis of Insulin and Netrin 1 were performed. Insulin and Netrin-1 level were determined using a commercially available human enzyme-linked immune sorbent assay (ELISA) kit (Manufacturer: Sincere Biotech Co., Ltd, Beijing, China).

Measurement of IR was carried out by Homeostatic model assessment (HOMA). HOMA-IR was calculated using the following formula, fasting insulin multiplied with fasting glucose divided by 22.5 where, insulin is expressed in ml U/L and glucose in mg/dL.

Study Definitions

Diagnosis of diabetes was done as per American Association of Diabetes guidelines. Known diabetic subjects without taking anti-diabetic medications and those devoid of pre-history hyperglycaemia but with fasting glucose level ≥126 mg/ dL were defined as cases (n=44). Controls were the subjects with fasting glucose level <126mg/ dL (n=36).

Statistical Analysis

Analysis of the collected data was performed by SPSS version 16. The continuous variables following normal distribution were expressed as Mean ±SD. The variables which are not normally distributed (insulin, fbg, netrin and homa) in both cases and controls were also expressed as Mean ±SD. Independent samples tests viz. Levene's Test and t tests were done to estimate equality of variances and equality of means respectively among non-normally distributed variables. Mann- Whitney U test has been used to examine the differences between different independent groups (Netrin 1 and Insulin resistance). Spearman's correlation analyses were used to assess the relationships. A p value <0.05 was considered statistically significant.

Results

Baseline characteristics of the population studied is as follows. Mean age of study subjects (n=79) are 48.71± 14.19 years. Among the total population, subjects with impaired fasting hyperglycaemia and T2DM were cases (n=44) and with normal fasting glucose were study controls (n=35). Demographic profile is as shown in table 1.

Correlation analysis showed an insignificant positive correlation between HOMA-IR and netrin (r=0.2655 p=0.1638). There was a significant negative correlation between Quantitative Insulin Sensitivity Check Index (QUICKI) and netrin (r= -0.4279, p = 0.0059). Receiver Operating Characteristic (ROC) curve for netrin 1 levels and type 2 diabetes mellitus cases showed an area under the curve (AUC) of 0.699, with a sensitivity of 0.778 and specificity of 0.581, cut off value of netrin being 382pg/mL (fig 1).

Levene's test for Insulin, FBG and Insulin resistance are significant and equal variances are not assumed whereas for Netrin 1 the F test is non-significant with (F=0.589, at 0.445 significance) hence equal variances are assumed. Biochemical parameters are as depicted in table 2.

Table 1: Demographic profile of the subjects

	Diabetics	Non-diabetics
Age in years	49.38±2.29	47.78±2.69
Gender		
Male	26	24
Female	18	11

Table 2: Biochemical parameters in cases and controls

	Diabetics	Non-diabetics	P value
FBS (mg/dl)	166.6 ±7.96	96.09 ±1.77	<0.0001
Insulin (µIU/L)	5.98 ± 1.61	8.47±1.99	0.03
HOMA-IR	2.89±1.05	1.23±0.18	0.21
HOMA B cell	18.78±3.54	46.35±6.65	<0.0001
HOMA1-%B Cell	20.75±3.83	61.43±9.19	<0.0001
QUICKI	0.37±0.009	0.65±0.10	0.0063
Netrin (pg/ml)	532.9±78.38	578.21±87.17	0.76

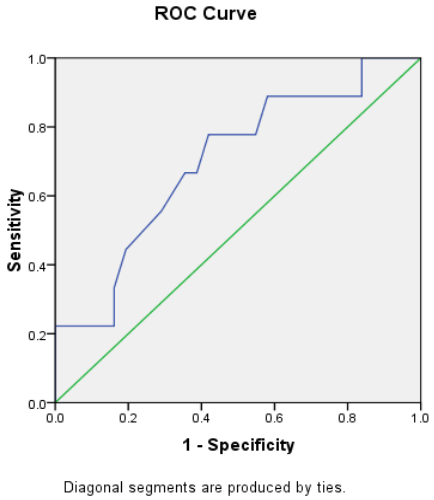

Fig 1: ROC for netrin as a marker of T2DM

Discussion

Netrin is a secretary protein found to have an important role in complications associated with diabetes. High expression of Netrin has been noted in acute kidney damage. Few animal studies done in this regard showed that higher levels of Netrin in most of the kidney damages are due to the Netrin secreted from epithelial cells of Proximal tubule (Ramesh et al., 2010; Brian Reeves et al., 2008). Jayakumar et al., (2014) reported that in the course of diabetes, early and higher expression of Netrin-1 is detected in urine among patients with diabetic nephropathy which may assist as a biomarker for predicting development of chronic kidney disease (CKD) among diabetics. Contrasting to the previously mentioned result, Liu C et al arrived at the result that plasma Netrin-1 levels were decreased in newly diagnosed type 2 diabetics. Here a negative association was observed between Netrin 1 and IR (Liu et al., 2016).

Our present study showed that there is no significant difference in the serum Netrin 1 level in T2DM and healthy controls. An insignificant positive correlation was seen between Netrin 1 and insulin resistance in T2DM patients. A study by Marakoglu et al. (2016) reported elevated Netrin-1 levels in diabetic-microalbuminuria patients. The same study shares similarity with our results by stating that there was no noticeable significant difference in Netrin-1 levels among controls and diabetics without albuminuria. The final conclusion drawn was, increased plasma Netrin1 level could be due to glomerular damage that occurs in diabetic nephropathy.

Divergent results to our study have also been reported. A study by Ramkhelawon et al., (2014) reported elevated expression of Netrin-1in obese subjects. In the same study, higher Netrin levels were not derived by lean

subjects. They conducted similar study on animals by feeding mice with saturated fatty acid palmitate to induce obesity and observed higher expression of Netrin-1. From this animal study they concluded that elevated Netrin developed macrophage retention signal in adipose tissues of obese animals which ultimately caused chronic inflammation and insulin resistance. Evidences showed that Netrin is expressed in vascular epithelium (Ly et al., 2005) but, hyperglycaemic condition markedly decreases vascular netrin level in in diabetes which resulted in reduced endothelial function whereas higher expression of vascular netrin in hyperglycaemic animal prevented diabetes-induced endothelial impairment (Toque et al., 2017). Excellent work by Tadagavadi et al., (2010) and Mao et al., (2014) showed the protective action of Netrin-1 on kidney and heart against ischemia–reperfusion injury by involving in modulation of inflammation, signalling the decreased synthesis of inflammatory cytokine and chemokine.

There are three types of HOMA. HOMA-IR, measures insulin resistance levels in the body, which is the reduced sensitivity of cells to respond to insulin. Higher HOMA-IR scores indicate higher insulin resistance levels. Homeostatic Model Assessment of Beta-cell Function (HOMA-B), measures the ability of the pancreas to produce insulin. Beta cells in the pancreas secrete insulin, which helps regulate glucose levels in the blood. Lower HOMA-B scores indicate weaker beta-cell function, increasing the risk of developing diabetes. Homeostatic Model Assessment of Insulin Sensitivity (HOMA%S), measures the sensitivity of peripheral tissues (muscle and fat) to respond to insulin. Higher scores indicate better response to insulin, which reduces the risk of developing diabetes. These three types of HOMA are significant in identifying insulin resistance, beta-cell dysfunction, and the overall risk of developing diabetes. Early detection of insulin resistance can help prevent the onset of diabetes and metabolic disorders. Additionally, HOMA can help clinicians monitor the effectiveness of diabetes treatment and adjust the treatment plan accordingly. Therefore, HOMA plays an essential role in assessing insulin resistance levels and beta-cell function, which can help prevent diabetes and improve diabetes management (Radziuk, 2014).

Present study outcomes are in accordance with the opinion of Liu et al., (2016) which suggested that netrin-1 level was significantly low in T2DM patients compared to controls. The study findings demonstrate that there is a negative correlation between netrin1 and HOMA-IR by logistic regression analysis. This is in accordance with our positive correlation between QUICKI and netrin. It implies a direct relationship between insulin sensitivity and netrin. However linear regression analysis of FBS and netrin as independent and dependent variables as well as HOMA-IR and netrin as independent and dependent variables showed a significant positive relationship. It could be a compensatory mechanism, to combat the inflammatory response to IR, netrin level might get heightened.

Netrin-1 is a neuroimmune guidance cue, plays an important function in pancreatic development (Jayakumar et al., 2011). Association with several integral proteins, netrin inhibits pancreatic epithelial cell adhesion and migration (Yebra et al., 2003). Netrin also inhibits neuronal cell migration, inflammatory cytokine and chemokine production (Tadagavadi et al., 2010). IR induces inflammation by reducing the anti-inflammatory effect of insulin. At a time, cytokines enhance IR in adipose and other tissues, increasing the risk of T2DM (King et al., 2008). Lowered netrin levels as well as heightened IR may predispose to additional risk of diabetes mellitus.

Study by Natura et al., (2013) showed that netrin-1 involved inflammation, leads adverse effect on insulin secretion and contribute to β-cell dysfunction. This supports the reduced HOMA B cell and QUICKI observed in cases, in our study.

A short period of three months has put several limitations to our study. A modest sample size being the first limitation, we could not make a note of anthropometric measurements and postprandial glucose levels in our study subjects due to inadequate time. Making note of level of albumin in urine and other complications associated with T2DM would have supported our results better.

Conclusion

In conclusion, we report that in this pilot study there was no significant association noted between serum netrin 1 and insulin resistance in our study population with T2DM and Fasting plasma glucose. Further prospective study with larger samples including urine netrin levels is required to support our findings.

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