Original Article

Study of neuregulin-4 levels in newly diagnosed type 2 diabetes mellitus

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Abstract

Background: Neuregulin-4 is a recently recognized adipokine acting as ligands to tyrosine kinases receptor of the Erb B family. This adipose tissue augmented endocrine factor participates in the modulation of lipid and glucose metabolism and energy homeostasis. This novel adipokine is associated with insulin resistance, dyslipidemia, obesity, oxidative stress, and inflammation. **Objective:** The study aimed to compare plasma levels of neuregulin-4 in newly diagnosed type 2 diabetes mellitus as compared to matched controls and to correlate with glycemic and lipid parameters. **Materials and Methods:** 100 newly diagnosed T2DM patients and 100 age, sex, and BMI-matched controls after fulfilling all exclusion and inclusion criteria were included in the study. Fasting and postprandial blood glucose levels, glycated hemoglobin (HbA1c), and fasting plasma insulin levels were measured in both cases and controls. HOMA-IR values in both groups were calculated using fasting glucose and insulin levels. **Results:** Mean levels of plasma neuregulin-4(pg/mL) in newly diagnosed T2DM were 7949.76 ± 949.76) pg/ml, which was significantly lower as compared to 9143 ±949.76) pg/ml in the control group (P-value <.0001). In the present study, a significant negative correlation was seen between plasma neuregulin-4 (pg/mL) with fasting blood sugar, postprandial blood sugar, HbA1C, and HOMA-IR with a correlation coefficient of -0.303, -0.416, -0.433, and -0.514, respectively. Moreover, a significant positive correlation was seen between plasma neuregulin-4 (pg/mL) with HDL with a correlation coefficient of 0.216. A significant negative correlation was seen between plasma neuregulin-4 (pg/mL) and LDL, with a correlation coefficient -0.208. **Conclusion:** Neuregulin levels are significantly lower in diabetics as compared to controls. There levels correlated inversely with HbA1C and HOMA IR.

Keywords: Dyslipidemia, glycemic parameters, neuregulin-4, type 2 diabetes mellitus

Introduction

Diabetes is a group of metabolic diseases distinguished by hyperglycemia resulting from inadequate insulin production, insulin action, or both. Type 2 diabetes, previously referred to as "non-insulin-dependent diabetesabolic diseases distinguished by hyperglycem-95% of all diabetes. This form encompasses individuals with relative (rather than absolute) insulin deficiency and peripheral insulin resistance.[1]

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According to International Diabetes Federation, 537 million adults aged between 20 and 79 have diabetes worldwide. The prevalence of diabetes mellitus in India at present is 74.2 million, and by 2045, the majority is projected to be 124.9 million. India has an estimated number of 39.4 million undiagnosed diabetes patients in the age group 20-79 years, with a proportion of 53.1.%.[2]

Adipose tissue is accepted as an active metabolic organ that primarily stores energy and secretes various bioactive compounds known as adipokines or adipocytokines.[3] White adipose tissue predominantly secretes adipokines that mediate unfavorable metabolic effects contrary to various adipokines with favorable systemic metabolic effects originating from brown adipose tissue.[4] Neuregulin-4, a recently recognized brown adipose

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tissue secreted adipokine, is involved in insulin resistance, obesity, dyslipidemia, oxidative stress, and inflammation.[5,6]

Wang *et al.* showed that mice lacking in NRG4 had amplified insulin resistance and steatosis of the liver and also demonstrated that expression of lipogenic genes was attenuated in mice upregulating NRG4.[7] Cai *et al.* study revealed lower levels of NRG4 in metabolic syndrome subjects with obesity compared to controls, and NRG4 levels were inversely associated with fasting plasma glucose.[8] Helda *et al.* also demonstrated a similar finding where NRG4 levels were decreased in NAFLD subjects, and the lowest quartile group of NRG4 had higher BMI, triglyceride, waist circumference, and HOMA-IR.[9]

Contrary to the above studies, Chen *et al.* detected higher levels of NRG4 in T2DM.[10] Similarly, Kang *et al.* showed a similar correlation with higher NRG4 levels in subjects with deranged FBS and raised HOMA-IR.[11] Our present study explores the relationship between NRG4 and metabolic parameters in newly diagnosed T2DM.

Materials and Methods

The study was conducted after taking ethical clearance from the institutional ethics committee of ABVIMS and Dr. RML Hospital.

Study design: Cross-sectional observational study.

Study group: 100 newly diagnosed T2DM patients and 100 controls matched for age, sex, and ethnicity from medicine OPD, wards, and emergencies after fulfilling all inclusion and exclusion criteria were taken for the study.

Study period: January 1, 2021, to May 31, 2022.

Calculation of sample size

The study by Lei Zhang *et al.* observed that the median neuregulin-4 level in the control group was 2.32 (1.11-3.67), and the median neuregulin-4 level in the diabetes mellitus group was 1.37 (0.86-2.43).[12] Taking this value as a reference and at 95% confidence interval and 90% power, the sample size was calculated as 78 per group using the formula-

$$n = (\sigma_1 + \sigma_2)^2 \frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{(m_1 - m_2)^2}$$

- where m_1 = mean neuregulin-4 levels in the control group = 2.35
- m_2 = mean neuregulin-4 levels in diabetes mellitus group = 1.50
- σ_1 = SD of the outcome variable in the control group
- σ_2 = SD of the outcome variable in the diabetes mellitus group

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The formula for mean and SD by using median and range: -

Mean = (a + (2 × m) + b)/4

SD = square root of $[((1/12) \times ((a-(2 \times m) + b)^2/4) + (b - a)^2))]$

where m = median; a and b are interquartile range

Calculation of sample size:

$$N = \frac{(1.12 + 1.18)^2 \times (1.96 + 1.28)^2}{(2.35 - 1.50)^2}$$

n = 78 per group

Inclusion criteria

100 cases of newly diagnosed drug-naive type 2 diabetes mellitus patients as defined by ADA 21 guidelines as follows were taken

- A fasting plasma glucose level of 126 mg/dL or higher, *or*
- A 2-hour plasma glucose level of 200 mg/dL or higher during a 75-g oral glucose tolerance test, *or*
- A random plasma glucose level of 200 mg/dL or higher in a patient with signs and symptoms of DM or
- A HbA1c level of 6.5% or higher

100 control subjects, matched for age, gender, ethnicity, body mass index, and with fasting blood glucose of less than 100 mg/dl AND 2-hour postprandial glucose of less than 140 mg/dl and HbA1c less than 5.7 with no known co-morbidities as per exclusion criteria.

Exclusion criteria

- Known cases of cerebrovascular accidents
- Known chronic alcoholics
- Smokers
- Thyroid diseases
- Known cases of SLE, leprosy, vasculitis, malignancy
- Known HIV+ ^{ve} patients
- Patients on chronic glucocorticoid therapy

The study participantls baseline data about age, sex, race, ethnicity, and family history of diabetes or hypertension were recorded in a questionnaire. Thereafter, their height (using a stadiometer), weight (using a weight measurement scale), BMI, and waist circumference (using a standard measuring tape) were measured.

Resting systolic and diastolic blood pressures in right arm in sitting position were recorded two times after a gap of 5 minutes using an automated sphygmomanometer. The mean of the two values (both systolic and diastolic) of blood pressure was considered.

Laboratory investigations

Around 10 ml of fasting blood sample was collected after venipuncture. Blood samples were collected in EDTA vials for HbA1c and red (plain) vials for the rest of the parameters.

The following investigations were performed:

- Fasting plasma insulin levels
- Fasting plasma glucose
- HbA1c
- Serum lipid profile
- Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as:
  HOMA-IR = (FPI × FPG)/22.5,
  where FPI is fasting plasma insulin (mIU/L), and FPG is fasting plasma glucose (mmol/L)
- Plasma Neuregulin-4

Kits for plasma neuregulin-4 were imported from Shanghai Coon Koon Biotech (CK-bio-12611) Test was performed by ELISA. Prepared samples, standards, and horse radish peroxidase were mixed for 60 minutes at 37°C incubation. After washing the plate five times, chromogen A and B were added and incubated for 15 minutes at 37°C for developing color. After adding the stop solution, optical density value was read within 15 minutes. According to the standard concentrations and the corresponding OD values, the linear regression equation of the standard curve was calculated. Then, according to the OD value of the samples, the concentration of the corresponding sample was calculated. Values were obtained in pg/ml and multiplied by the dilution factor 5 to get each sample's final plasma neuregulin-4 concentrations.

Statistical analysis

The categorical variables were demonstrated as percentages (%) and numbers. On the other hand, the quantitative data with normal distribution were presented as the means n SD and the non-normal distribution as the median with 25th and 75th percentiles (interquartile range). The data normality was checked by using Kolmogorov–Smirnov test. In cases where the data were abnormal, we used nonparametric tests. The following statistical tests were applied to the results:

1. The comparison of the variables, which were quantitative in nature, was analyzed using an independent *t*-test (for two groups) and ANOVA (for more than two groups). A post hoc comparison was made using the Bonferroni correction.
2. The qualitative variable comparison was analyzed using the Chi-square test. Fisher's exact test was used if any cell had an expected value of less than 5.
3. Pearson correlation coefficient was used to correlate plasma neuregulin-4 (pg/mL) with various parameters.

Microsoft EXCEL spreadsheet was used for data entry, and the final analysis was performed using Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 25.0.

For statistical significance, a *P* value of less than 0.05 was considered statistically significant.

Results

The study aimed to assess the plasma levels of neuregulin-4 in newly diagnosed T2DM, compare the same in healthy controls, and correlate its levels with glycemic and lipid parameters. It was an observational case–control study, and 100 patients and 100 controls were enrolled. Matching concerning age, sex, blood pressure, and BMI was ensured.

The mean tudy, and 100 patients and 100 controls were enrolled. Matching concerning age, sex, blood pressure, and BMI was ensured. ed by using Kolmogo *P* value < .0001). The mean t SD of HOMA-IR in the case was 4 and 100 controls were enrolled. Matching concerning age, sex, blood pressure, an *P* value < .0001). A significant difference was seen in total cholesterol (mg/dL), LDL (mg/dL), VLDL (mg/dL), and triglycerides (mg/dL) between cases and controls (*P* value < .05). Mean r SD of total cholesterol (mg/dL), LDL (mg/dL), VLDL (mg/dL), and triglycerides (mg/dL) in the cases were 197.37 LDL (mg/dL), L), LDL (mg/dL), VLDL (mg/dand 158.44 e 61.8, respectively, which was significantly higher as compared to controls (135.72 LDL (mg/*P* value < .0001), 70.26 ificant (*P* value < .0001), 25.62 ifican (*P* value < .0001), and 127.85, 25 (*P* value < .0001)), respectively.

Mean levels of plasma neuregulin-4 (pg/mL) in newly diagnosed T2DM was 7949.76 ± pg/mL) in newly diagnosed T2DM was 7949.76 to controls (135.72 L ± pg/mL) pg/ml in the control group (*P* value < .0001). In the present study, a significant negative correlation was seen between plasma neuregulin-4 (pg/mL) with fasting blood sugar, postprandial blood sugar, HbA1C, and HOMA-IR with a correlation coefficient of -0.303, -0.416, -0.433, and -0.514, respectively [Figures 1 and 2].

Moreover, a significant positive correlation was seen between plasma neuregulin-4 (pg/mL) with HDL with a correlation

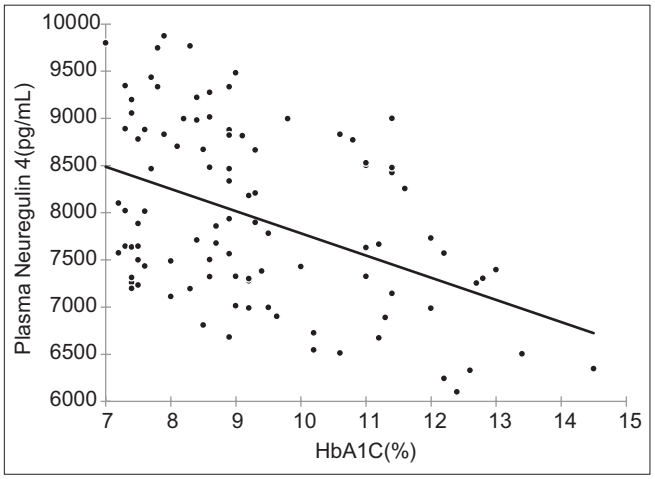

Figure 1: Correlation of HbA1C (%) with plasma neuregulin-4 (pg/mL) in cases

coefficient of 0.216. A significant negative correlation was seen between plasma neuregulin-4 (pg/mL) and LDL, with a correlation coefficient -0.208 [Figures 3 and 4]. A mild negative nonsignificant correlation was obtained with total cholesterols.

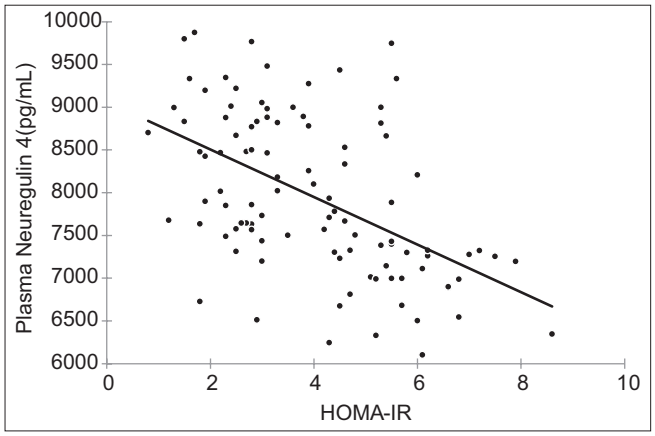

Figure 2: Correlation of HOMA-IR with plasma neuregulin-4 (pg/mL) in cases

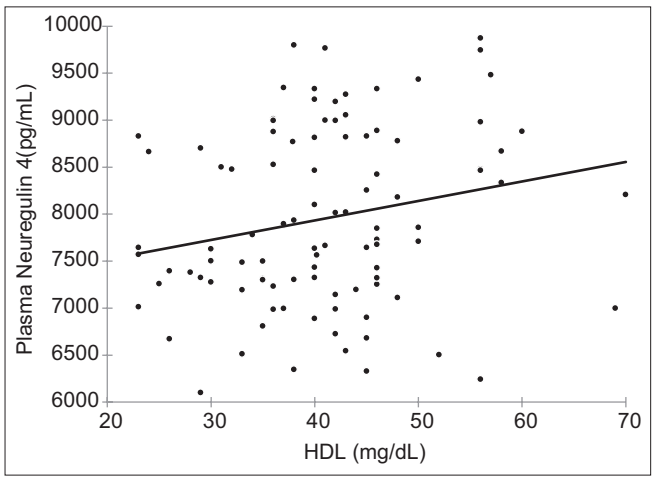

Figure 3: Correlation of HDL (mg/dL) with plasma neuregulin-4 (pg/mL) in cases

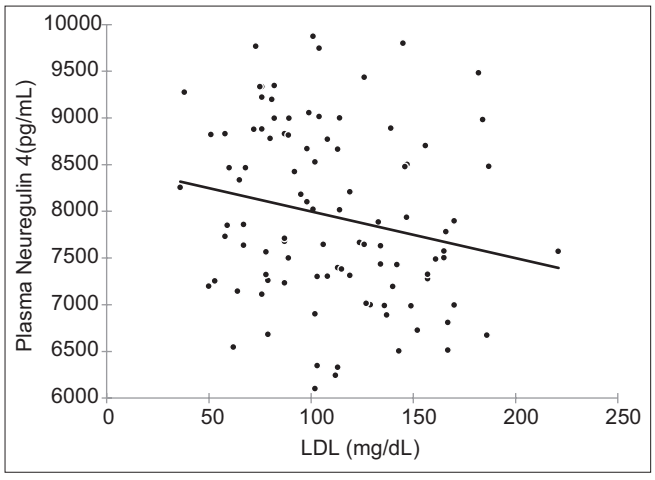

Figure 4: Correlation of LDL (mg/dL) with plasma neuregulin-4 (pg/mL) in cases

No correlation was seen between NRG4 with BMI, VLDL, or triglycerides.

Discussion

Diabetes mellitus and its various complications, apart from being a big threat to the wellbeing of the individuals, also post a major burden on the global healthcare resources. Though there are various clinical and laboratory parameters to aid in the diagnosis of diabetes, a predictor molecule, a laboratory value that can be useful in predicting the occurrence of diabetes mellitus has been a challenge. Neuregulin-4 (NRG 4) is one of the recent advances in that path, a molecule whose level is decreased in the serum of newly diagnosed diabetics, compared to general population, according to various studies. As our study has shown, the levels of NRG 4 have been found in many studies, to be negatively correlating with various parameters associated with metabolic syndrome. Apart from its values in a diagnostic perspective, whether NRG 4 can be a therapeutic target, in diabetes mellitus and metabolic syndrome, is an interesting aspect to look forward to.

In 2017, a cross-sectional study was conducted by Zhang L *et al.* on circulating levels of NRG 4 among newly diagnosed diabetics. The study showed a significant difference ($P < 0.001$) in levels of NRG4 between newly diagnosed T2DM (1.37 ng/ml) and healthy controls (2.32 ng/ml).[12] Similar results were acquired in the cross-sectional study conducted by Yan *et al.* among newly diagnosed T2DM patients; the levels of NRG 4 in cases were 3.28 ± 1.50 ng/ml, which was significantly lower compared to 4.17 ± 1.97 ng/ml in controls (P value < 0.05. The study also showed a negative correlation between the levels of NRG 4 and levels of high-sensitivity C-reactive protein in newly diagnosed diabetics, thus indicating a protective role of NRG 4 against systemic inflammation, which is a feature of diabetes mellitus, and by extension, metabolic syndrome, and also showed that the highest quartile of the NRG4 group had significantly lower BMI and triglyceride levels but more increased HDL.[13]

The landmark study by Zhang L *et al.* apart from showing the decreased NRG 4 levels among newly diagnosed diabetics also showed that the NRG 4 levels were negatively associated with fasting glucose ($P = 0.046$), HOMA-IR ($P < 0.001$), and fasting plasma insulin levels ($P < 0.001$). The study also showed a positive correlation of NRG 4 with the levels of HDL ($r = -0.197$, $P = 0.046$).[12] Similar findings were shown by the Kralisch *et al.* study, a case control study among patients of GDM, on correlation between NRG 4 levels and the status of gestational diabetes in the sample population, conducted in 2017. The study showed a negative correlation of NRG4 with 1-h and 2-h glucose during OGTT and HbA1c by univariate correlation. The glucose area under the curve during the 75 gm OGTT test had an independent and negative association with NRG4 levels after age, BMI, and Blood pressure adjustment.[14]

The 2021 case–control study by Tutunchi H *et al.* on the levels of NRG 4 in NAFLD patients showed a negative correlation with triglycerides and a positive correlation with HDL-C with NRG 4 levels, identical to our research.[9] Similar results were obtained by the Yi-Ning *et al.* study on NRG4 levels in NAFLD.[15]

The protective role of NRG4 in cardiovascular diseases was demonstrated in Rahimzadeh *et al.* study, a case–control study conducted in 2020. The study showed a higher prevalence of triple vessel disease in the lowest quartile group of NRG4 and, thus, a negative association with an acute coronary syndrome,[16] and likewise, lower levels of NRG4 in subjects having carotid plaques and augmented carotid intima-media thickness (CIMT) were shown by Jang *et al.*[17]

The studies mentioned above concord with Wang *et al.*, who reported that brown adipose tissue secreted novel adipokine NRG 4 attenuates hepatic lipogenic signaling and safeguards lipid and glucose homeostasis in obesity. It is interesting to note that in the study, the prospect of white adipose tissue being a potential source of NRG 4 is being put forward, thus showing the possibility of biologics targeting NRG 4 can be potential treatment options for type 2 diabetes mellitus and NAFLD.[7]

The results shown by our study are in concordance with various studies conducted on the association between NRG 4 levels and parameters associated with metabolic syndrome. Our study showed a decreased serum level of NRG 4 in newly diagnosed type 2 diabetics, compared to the healthy controls. The levels also correlated negatively with the FBS, PPBS, HbA1C, and HOMA-IR values, along with the levels of LDL, and showed a positive correlation with the HDL levels.

Our study showed that the NRG4 had an inverse association with long-term glycemic parameters and an unfavorable lipid profile. This is a new opening in the avenue of diabetes mellitus, as the levels can be used for the early prediction of occurrence of diabetes mellitus, thus enabling the primary care physicians to take steps to delay, or even altogether prevent the occurrence of diabetes in the patients. It is also interesting to note that the NRG 4 associated biologics can be studied, for the treatment of diabetes mellitus and metabolic syndrome. These features warrant an extensive and thorough research on the molecule NRG 4.

Limitations

A sample size of 100 is too small to establish a definite correlation among the parameters used in the study. There has yet to be an agreement regarding the exact plasma range of neuregulin-4 that can be used in clinical practice. This study was conducted in a subset of the Indian population in a particular geographic area. More studies in a broader and mixed population are needed to take ethnic and geographic distribution into account and evaluate their effect on various parameters.

Conclusions

Plasma neuregulin-4 may serve as a novel marker for T2DM and associated glycemic and lipid parameters. Earlier detection of decreased neuregulin-4 levels can predict patients at risk for insulin resistance and dyslipidemia.

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Conflicts of interest

There are no conflicts of interest.

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