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Assessment of Serum Neuregulin 4 Level and Several Metabolic Parameter in Gestational Diabetes[1]

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ABSTRACT

Background: The prevalence of gestational diabetes mellitus has increased dramatically during the previous few decades. Neuregulin 4 is a new protein that protects against diet-induced insulin resistance and steatosis in the liver. Neuregulin 4 levels in subcutaneous and visceral adipose tissues were significantly lower in patients with impaired glucose tolerance or diabetes than in healthy people, according to a recent study. **Aim:** To evaluate the clinical significance of serum Neuregulin 4 and other metabolic parameters in gestational diabetes mellitus. **Methods**: A case-control study was conducted during the period from February to October 2021. The study included 90 pregnant women in the second trimester and was divided into two groups; the gestational diabetes mellitus group (included 45 women who presented with gestational diabetes during current pregnancy) and control group (included 45 healthy pregnant women matched for age, gestational age). Neuregulin 4 and other parameters were measured in the two groups.

Results: The mean fasting blood sugar, Hemoglobin A1c, oral glucose tolerance test, Homeostatic Model Assessment for Insulin Resistance, and triglyceride were significantly higher among the cases compared to the control groups. According to the cut-off point of 0.992 ng/ml of Neuregulin 4, the sensitivity was 91.11%, and the specificity of 66.67%. There was a significant negative association between the Neuregulin 4 levels and fasting blood sugar and Homeostatic Model Assessment for Insulin Resistance, while there was a positive significant association between the Neuregulin 4 levels and high-density lipoprotein. **Conclusion:** Circulating Neuregulin 4in pregnant women with gestational diabetes mellitus was significantly lower as compared with healthy pregnant women and this may be considered a risk factor for developing gestational diabetes mellitus.

Keywords: *Serum Neuregulin 4; Several Metabolic ; Gestational Diabetes*

INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as diabetes or glucose intolerance occurring or first recognized in pregnancy, a definition that includes those with previously unrecognized diabetes **(1)**

GDM is frequently described as the most common metabolic disorder of pregnancy with prevalence increasing at epidemic proportions. Accurate estimation of GDM prevalence is important for service planning, funding allocation, and research**(2)**. **g**lobally, GDM is estimated to affect 13.9% of all pregnancies, approximately one in 10 pregnant women in Eastern and Southeastern Asia had GDM**(3)**. A review of 77 studies showed that the Middle East and North

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Africa region had the highest prevalence of GDM with a median estimate of 12.9% (range 8.4–24.5%), followed by Southeast Asia, Western Pacific, South and Central America, Africa, and North America and the Caribbean (median prevalence 11.7, 11.7, 11.2, 8.9, and 7.0%, respectively), while Europe had the lowest prevalence[(4)]

In Iraq, a prospective study done in 2014 on 100 pregnant women, the GDM prevalence was 7%[(5)]. In a recent 2020 study on 120 pregnant women between the ages of 20-45 years, the prevalence of GDM was found to be 13.3%[(6)], usually GDM results from β-cell dysfunction on a background of chronic insulin resistance during pregnancy and thus both β-cell impairment and tissue insulin resistance represent critical components of the pathophysiology of GDM. In most cases, these impairments exist before pregnancy and can be progressive representing an increased risk of type two diabetes mellitus (T2DM) after pregnancy[(7)].

GDM is most commonly a forerunner of T2DM. In a meta-analysis of Bellamy *et al*, women with GDM have a sevenfold risk of T2DM for several years compared to women with normal glucose tolerance during pregnancy. Longitudinal studies longer than 10 years indicate that more than 25% of GDM will develop T2DM. In addition, GDM is found to carry more T2D risk alleles[(8)].

During the last few decades, the prevalence of GDM has increased significantly. As GDM contributes to an increased risk of acute and chronic complications in both the mother and newborn, the pathophysiological mechanisms behind this metabolic disorder during pregnancy are of great interest[(9)]. A recent study demonstrated that NRG4 mRNA levels in subcutaneous and visceral adipose tissues were significantly lower in patients with IGT or T2DM than in normal individuals [(10)].

Sampling method and inclusion criteria

A convenient sample of 90 pregnant women presented in the second trimester who attended the consultation clinic or collected from the inpatient ward, the gestational age confirmed by accurate last menstrual period and/or early ultrasonography. The sample included two groups:

Group A (case group): Includes 45 women who presented with gestational diabetes during the current pregnancy, the diagnosis was based on the IADPSG guidelines. **Group B (Control group):** Include 45 euglycemic pregnant women matched for age and gestational age.

Exclusion Criteria: Chronic diseases (pre-existing diabetes, chronic respiratory disease, chronic liver disease, or chronic kidney disease; Risk factors such as smoking; Endocrine causes (congenital adrenal hyperplasia, androgen-secreting tumors, Cushing syndrome and thyroid dysfunction); Use of immunosuppressive drugs and Hypertension includes chronic hypertension, pregnancy induced hypertension and\or preeclampsia.

The investigations include: NRG4; Fast blood sugar (FBS) after 12 hours of fasting; OGTT, all women were given 75gm glucose dissolved in 300 ml water, and then the blood sugar was measured at one hour (OGTT1) and 2 hours (OGTT2) after drinking the dissolved sugar; Fasting insulin levels; HbA1c; The lipid profile includes total cholesterol, HDL, LDL, and triglyceride; Alanine aminotransferase (ALT); Creatinine levels were measured by standard laboratory methods; The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was used to measure insulin resistance.

Statistical Analysis: The data was entered and analyzed by Microsoft Excel 2016 and software package of social science (SPSS), version 22. The descriptive analysis focused on frequencies and percentages. Continuous variables were presented as mean (± Standard deviation (SD)) and were compared between the two study groups using the independent t-test. Categorical data were presented as proportion and the chi-square test for the difference between two proportions were used to test the statistical difference. A P. Value of less than 0.05 was considered statistically significant.

Results:

A total number of 90 women were enrolled in the current study. There was no significant difference between the study groups regarding age (p-value>0.05). No significant difference was obtained between the study groups regarding gravidity, parity, abortions, and gestational age (p-values>0.05). In addition, there was no significant difference between the study groups regarding SBP and DBP (P-value>0.05) as shown in table (1).

Table (1): Demographic and clinical characteristics of the study groups

Variables		**Group A (N=45) N (%)**	**Group B (N=45) N (%)**	**P-value**
Age groups (years)	15-25	12 (48%)	13 (52%)	0.96
	26-35	22 (51.16%)	21 (48.84%)	0.99
	>35	11 (50%)	11 (50%)	1
		Group A (N=45) Mean (±SD)	**Group B (N=45) Mean (±SD)**	
Gravida		3.24± 2.22	3.67± 2.39	0.89
Para		1.53± 1.46	1.62± 1.68	0.78
Abortion		0.76± 1.09	0.73± 1.03	0.92
Gestational Age (week)		25.56± 0.72	26.78± 0.45	0.98
SBP (mmHg)		123.56 (±12.34)	128 (±11.67)	0.12
DBP (mmHg)		77.6 (±5.66)	75.89 (±6.12)	0.87

The means of FBS, OGTT1, and OGTT2 were significantly higher in group A compared to group B (P-value<0.001 for all). As shown in table.2 ,So the mean of HbA1c, HOMA-IR, and triglyceride was significantly higher among group A compared to the group B groups (P-value<0.001), while fasting insulin level was lower in group A compared to group B (P-value<0.001), as shown in table (2).

Table (2): Oral glucose tolerance test and Biochemical characteristic of the study groups

Variables	**Group A (N=45)**	**Group B (N=45)**	**P-value**
	Mean (±SD)	Mean (±SD)	
FBS (mg/dl)	95.15 (±13.45)	77.11 (±3.71)	<0.001
OGTT1 (mg/dl)	187.97 (27.35)	113.62 (6.70)	<0.001
OGTT2 (mg/dl)	157.91 (±5.75)	105.17 (±7.29)	<0.001
HbA1C (%)	6.80 (±0.27)	4.77 (±0.46)	<0.001
Fasting insulin level	14.95 (±2.13)	20.33 (±2.12)	<0.001
HOMA-IR	2.23 (±0.48)	1.04 (±0.14)	<0.001

Total cholesterol (mg/dl)	249.42 (±10.92)	247.42 (±9.95)	0.365
HDL-C (mg/dl)	61.75 (±3.19)	62.93 (±2.42)	0.052
LDL-C (mg/dl)	158.02 (±16.59)	152.13 (±17.41)	0.104
Triglyceride (mg/dl)	238.13 (±24.22)	223.04 (±20.27)	**0.002**
ALT (U/L)	14.84 (±3.27)	14.51 (±3.20)	0.627
Creatinine (mg/dl)	0.71 (±0.67)	0.61 (±0.17)	0.340

There was a significant difference between the study groups regarding the mean of NRG4, the mean was higher among group B compared to group A (Pvalue<0.001), as shown in table(3).

Table (3): NRG4 level in the study groups

Variables	Group A (N=45)	Group B (N=45)	P-value
	Mean (±SD)	Mean (±SD)	
NRG4 (ng/ml)	0.77 (±0.25)	1.27 (±0.44)	<0.001

According to the ROC test, the NRG4 level of ≤0.992 ng/ml was considered a cutoff point for the diagnosis of GDM, and according to this cut-off point, the sensitivity of NRG4 as a diagnostic test for GDM was 91.11%, specificity 66.67%, positive predictive value 73.21, and negative predictive value was 88.23, as shown in figure (1)and table (4).

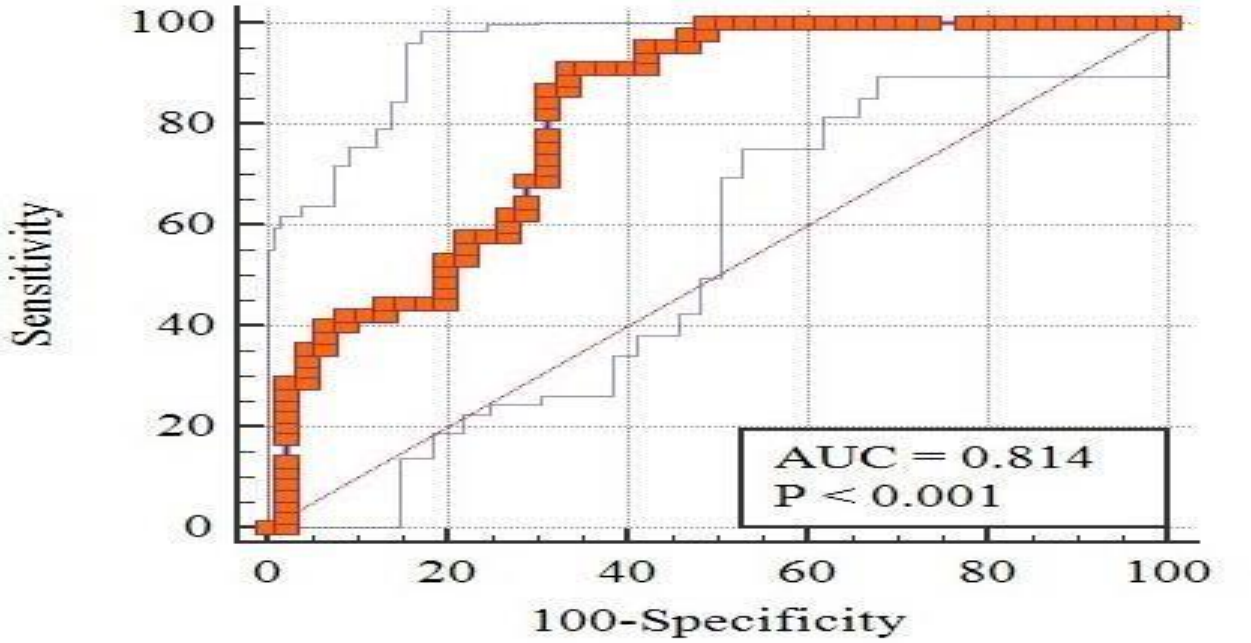

Figure (1): Cut-off point of NRG 4 according to ROC curve

Table (4): Association between NRG4 level and gestational diabetes mellitus

NRG 4	Count	Group		Total	Pvalue
		Group A	Group B		
Low (below the cut-off point)	N	41	15	56	**<0.001**
	%	91.1	33.3	62.2	
High (above the cut-off point)	N	4	30	34	
	%	8.9	66.7	37.8	
Total	N	45	45	90	
	%	100.0	100.0	100.0	

In group A, there was a significant negative correlation between the NRG4 levels and FBS and HOMA-IR, while there was a positive significant correlation between the NRG4 levels and HDL, as shown in table 5.

Table (5): Correlation between NRG4 level and fasting blood sugar , oral glucose tolerance test and biochemical parameters

Variables	Pearson Correlation *(r)*	P-value
FBS	-0.449	**0.002**
OGTT1	-0.084	0.585
OGTT2	-0.200	0.187
SI	0.257	0.088
HbA1C	-0.216	0.154
HOMA-IR	-0.295	**0.049**
Total cholesterol	0.090	0.558
HDL	0.377	**0.011**
LDL	0.183	0.229
Triglyceride	-0.246	0.103
ALT	-0.265	0.079
Creatinine	-0.050	0.743

DISCUSSION

The worldwide prevalence of GDM is increasing and is associated with adverse maternal and fetal outcomes[(11)]. According to the latest report, the increasing prevalence of GDM has caused serious harm to both mothers and fetuses[(12)]. Effective early identification of the development of GDM might reduce disease onset and associated maternal and perinatal complications [(13)]. Many studies found that Nrg4 was negatively correlated with inflammation markers in patients withT2DM, but the role of NRG4 in GDM, however, is less studied and controversial. Moreover, there is no information regarding the relationship between NRG4 and inflammatory factors in GDM [(12)]. Best to our knowledge, this is the first study in Iraq to assess serum NRG4 levels along with several metabolic parameters in patients diagnosed with GDM.

In the current study, there was a significant difference between the study groups regarding FBS, OGTT1, OGTT2, and HbA1c (P-value was <0.001). These results agreed with the results that were obtained by Pezeshki *et al*, they concluded

that HbA1C can significantly help in the primary prediction of GDM[14]. Another study concluded that the FBS and OGTT can be used in the screening and diagnosis of GDM[15].

Khalafallah *et al* revealed that the HbA1c can be used in the diagnosis of GDM at a cut-off of 5.1% with a sensitivity of 61% and specificity of 68% concluding that pregnant women with HbA1c of ≥5.4% must proceed with an OGTT, this may cause a significant decrease in the burden of testing on both patients and health system resources[16].

An important finding of the current study was a significant difference between the study groups regarding insulin resistance as detected by HOMA-IR (P-value<0.001), the HOMA-IR was significantly higher among those with GDM compared to those in the control group. These findings were in the same line with another study that was done by Winden e al and concluded that HOMA-IR is a simple measure of insulin resistance that can help doctors predict which pregnant women are at risk for GDM and which GDM patients will need medical treatment. HOMAIR could be a useful tool in trials of GDM treatments that require an insulin resistance index [17].
The logistic regression analyses of another study revealed that HOMA-IR remained independently associated with GDM[18]. Song et al revealed that GDM was linked to an increase in HOMA-IR during early pregnancy [19]. Insulin sensitivity improves immediately after delivery in women with GDM but seems to deteriorate within the first 6 months postpartum as revealed by Skajaa *et al*[20] **,** Another finding of the current study was that the triglyceride was significantly higher among pregnant women in group A compared to group B (P-value=0.002) in contrast to other lipids (including total cholesterol, HDL, and LDL) where these lipids were insignificantly different between the study groups.

In another study, the GDM group had higher triglyceride concentrations and a higher triglyceride /HDL ratio throughout pregnancy, while having lower HDL-C concentrations. However, there were no significant differences in triglyceride and LDL concentrations between the cases group and the control group in the first, second, or third trimesters[21].

In the univariate analyses of another study that was done in the United States, pregnant women with GDM had significantly increased serum triglyceride, total cholesterol, LDL concentrations, LDL/HDL ratio, and decreased LDL concentrations, compared to control groups, while no significant correlation was observed between other lipid profiles and the risk of developing GDM were observed[22]. The discrepancy between the results of these studies might be related to the other risk factors like genetics, environmental, food habits, and social habits.

The main finding of the current study was a significant difference between the study groups regarding NRG4 level which was lower among group A 0.77 (±0.25) ng/ml) compared to group B (1.27 (±0.44) ng/ml) (P-value<0.001). In addition, in group A, there was a significant negative correlation between the NRG4 level and FBS and HOMA-IR, while there was a significant positive association between the NRG4 level and HDL. This agreed with another study that was conducted by Zhang *et al* and revealed that the NRG 4 level was significantly lower in cases than the control group. Additionally, the NRG 4 levels were negatively correlated with fasting glucose, HOMA-IR, while it was positively correlated with HDL[12] .

In the same line of the current study, Attique *et al* revealed that the median NRG4 level in the control group (0.98 (±0.1) ng/ml) was significantly high compared to cases (0.94 (±0.08) ng/ml). The mean of HOMA-IR values had a weak direct association with NRG4. Cholesterol, LDL had an inverse relationship with NRG4[23] (68). In addition, Kralisch revealed that pregnant women with GDM have significantly lower circulating NRG4 levels compared with healthy pregnant controls[24].

In contrast to the current study, Eken *et al* revealed that the serum NRG4 levels were significantly elevated in the cases group in comparison to the control group. Multivariate linear regression revealed that the BMI, OGTT, and HOMA-IR were independently and positively correlated with the NRG4 levels [11].

CONCLUSIONS

The circulating Nrg4 in pregnant women with GDM was significantly lower as compared with healthy pregnant women and this may be considered a risk factor for developing GDM , and a significant negative correlation between the NRG levels and FBS and HOMA-IR and a significant positive correlation between the Nrg4 levels and HDL.

REFERENCES

1. Edmonds K, Lees C, Bourne T. Dewhurst's Textbook of Obstetrics & Gynaecology: Wiley; 2018.
2. Koo BK, Lee JH, Kim J, Jang EJ, Lee C-H. Prevalence of gestational diabetes mellitus in Korea: a national health insurance database study. PLoS One. 2016;11(4):e0153107.
3. Nguyen CL, Pham NM, Binns CW, Duong DV, Lee AH. Prevalence of gestational diabetes mellitus in eastern and southeastern Asia: a systematic review and meta-analysis. Journal of diabetes research. 2018;2018.
4. Agarwal MM. Gestational diabetes in the Arab Gulf countries: Sitting on a land-mine. International journal of environmental research and public health. 2020;17(24):9270.
5. Aziz RA, Fattah CN. Maternal and fetal outcome in gestational diabetic women. Zanco Journal of Medical Sciences (Zanco J Med Sci). 2018;22(3):401-10.
6. Mohammed MK. Estimating the difference of glucose levels during 1st and 3rd trimester of pregnancy in Tikrit city in Iraq. Ann Trop Med Public Health. 2020;23:106-11.
7. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The pathophysiology of gestational diabetes mellitus. International journal of molecular sciences. 2018;19(11):3342.
8. Baz B, Riveline J-P, Gautier J-F. Gestational diabetes mellitus: definition, aetiological and clinical aspects. Eur J Endocrinol. 2016;174(2):R43-51.
9. Buchanan TA, Xiang AH, Page KA. Gestational diabetes mellitus: risks and management during and after pregnancy. Nature Reviews Endocrinology. 2012;8(11):639.
10. Wang G-X, Zhao X-Y, Meng Z-X, Kern M, Dietrich A, Chen Z, et al. The brown fat–enriched secreted factor Nrg4 preserves metabolic homeostasis through attenuation of hepatic lipogenesis. Nature medicine. 2014;20(12):1436-43.
11. Kurek Eken M, Yayla Abide C, Sahin Ersoy G, Altun Ensari T, Pekin O, Cevik O. Clinical significance of neuregulin 4 (NRG4) in gestational diabetes mellitus. Gynecol Endocrinol. 2018;34(7):605-8.
12. Zhang L, Lu B, Wang W, Miao S, Zhou S, Cheng X, et al. Alteration of serum neuregulin 4 and neuregulin 1 in gestational diabetes mellitus. Ther Adv Endocrinol Metab. 2021;12:20420188211049614-.
13. Yoffe L, Polsky A, Gilam A, Raff C, Mecacci F, Ognibene A, et al. Early diagnosis of gestational diabetes mellitus using circulating microRNAs. European journal of endocrinology. 2019;181(5):565-77.
14. Pezeshki B, Chiti H, Arasteh P, Mazloomzadeh S. Early screening of gestational diabetes mellitus using hemoglobin A1C: Revising current screening guidelines. Caspian J Intern Med. 2019;10(1):16-24.
15. Rani PR, Begum J. Screening and Diagnosis of Gestational Diabetes Mellitus, Where Do We Stand. J Clin Diagn Res. 2016;10(4):QE01-QE4.
16. Khalafallah A, Phuah E, Al-Barazan AM, Nikakis I, Radford A, Clarkson W, et al. Glycosylated haemoglobin for screening and diagnosis of gestational diabetes mellitus. BMJ Open. 2016;6(4):e011059.
17. VanWinden K, Montoro M, Korst LM, Ouzounian JG. A Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) Relates to Gestational Diabetes, Glycemic Control [1K]. Obstetrics & Gynecology. 2017;129(5).
18. Alptekin H, Çizmecioğlu A, Işık H, Cengiz T, Yildiz M, Iyisoy MS. Predicting gestational diabetes mellitus during the first trimester using anthropometric measurements and HOMA-IR. J Endocrinol Invest. 2016;39(5):577-83.
19. Song S, Zhang Y, Qiao X, Duo Y, Xu J, Peng Z, et al. HOMA-IR as a risk factor of gestational diabetes mellitus and a novel simple surrogate index in early pregnancy. International Journal of Gynecology & Obstetrics. 2021.

20. Skajaa GO, Fuglsang J, Knorr S, Møller N, Ovesen P, Kampmann U. Changes in insulin sensitivity and insulin secretion during pregnancy and post-partum in women with gestational diabetes. BMJ Open Diabetes Research & Care. 2020;8(2):e001728.
21. Wang J, Li Z, Lin L. Maternal lipid profiles in women with and without gestational diabetes mellitus. Medicine (Baltimore). 2019;98(16):e15320-e.
22. Li G, Kong L, Zhang L, Fan L, Su Y, Rose JC, et al. Early Pregnancy Maternal Lipid Profiles and the Risk of Gestational Diabetes Mellitus Stratified for Body Mass Index. Reprod Sci. 2015;22(6):712-7.
23. Attique H, Baig S, Ishtiaque S, Rehman R, Ahmed S, Shahid M. Neuregulin 4 (NRG4) – the batokine with clinical relevance in Gestational Diabetes Mellitus2020.
24. Kralisch S, Hoffmann A, Kratzsch J, Blüher M, Stumvoll M, Fasshauer M, et al. The brown-fat-secreted adipokine neuregulin 4 is decreased in gestational diabetes mellitus. Diabetes & Metabolism. 2018;44(2):150-4.