Association of Maternal Serum Nesfatin-1 Level with Gestational Diabetes Mellitus (GDM) in a Tertiary Care Hospital

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Abstract

Background: Gestational diabetes mellitus (GDM) is a highly prevalent disease characterized by a disorder in glucose and insulin metabolism first recognized during pregnancy. In Bangladesh, there is an increasing burden of GDM that substantially impacts maternal and fetal health through its short- and long-term consequences. Nesfatin-1 is an anorexigenic peptide secreted by peripheral tissues and also the central and peripheral nervous systems. It stimulates glucagon and insulin secretion thereby a reduction in serum Nesfatin-1 level could impact on the regulation of blood sugar levels during pregnancy.

Objective: The objective of the study was to evaluate the association between maternal serum Nesfatin-1 level and GDM.

Methods: This case-control study was carried out in the Department of Fetomaternal Medicine, in collaboration with the Department of Biochemistry Bangabandhu Sheikh Mujib Medical University, Dhaka during the period between October 2021 to September 2022. The study included 48 diagnosed (according to WHO 2013 criteria) women with GDM as cases and 48 healthy pregnant women without GDM as the controls. Then Maternal serum Nesfatin-1 level was measured. Descriptive and inferential analysis was done using the analytic software SPSS 27.0 for Windows.

Results: The mean serum Nesfatin-1 level was relatively low in the case group (1.50±1.26ng/ml) compared to that in the control group (2.36±1.95 ng/ml), and this difference between the two groups was statistically significant (P=0.013). Respondents with a reduced level of Nesfatin-1 have 6.1 times more chance of developing GDM (OR=6.067; 95% CI=2.407-15.291; P-value<0.001). A significant inverse correlation was also observed between the Nesfatin-1 level with maternal FBS (r= -0.205, p=0.045) and between Nesfatin-1 level with maternal 2HAG blood sugar (r= -0.239, p=0.019).

Conclusion: It may conclude that low maternal serum Nesfatin-1 level was found to be associated with an increased risk of developing GDM.

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Keywords: Gestational diabetes mellitus (GDM), serum nesfatin-1 level

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Introduction

Gestational diabetes mellitus is a condition of glucose intolerance diagnosed in the second or third trimester of pregnancy that was not overt diabetes before gestation.[1] Unrecognized and untreated, GDM can increase the risk of health problems for pregnant women and the fetus and the risk of death for the fetus. It is well established that a GDM diagnosis is a factor associated with a 30% to 70% increased risk to develop the disease in future pregnancies and type 2 diabetes later in life.[2] Therefore, it is crucial to understand GDM pathogenesis, identify risk factors, and provide early prevention and appropriate treatment.[3]

. The prevalence of GDM among 20-49 years of aged women in Southeast Asia (SEA) was reported higher (25%) compared to North America and the Caribbean Regions (10.4%).[4] Several studies revealed that the GDM prevalence ranged from 6 to 14% in Bangladesh.[5] Although, pregnancy is associated with an increase in the beta-cell mass and increase in insulin level but certain women are unable to up-regulate insulin production relative to the degree of insulin resistance, and consequently become hyperglycemic, developing gestational diabetes.[6] Nesfatin-1 (nucleobindin2-encoded satiety and fat-influencing protein-1) is an 82-amino acid peptide derived from its larger precursor protein, non-esterified fatty acid/nucleobindin-2 (NUCB2), which was discovered in 2006 as a potential novel anorexigenic modulator and first identified in the hypothalamus. Some studies found that circulating nesfatin-1 concentrations increased in nondiabetic humans in proportion to adiposity and increased during T2DM, indicating that nesfatin- 1 may play a role in the regulation of glucose homeostasis.[7]

Rationale for the study

The incidence of GDM in Bangladesh is increasing day by day. Nesfatin-1 plays an important role in carbohydrate metabolism by inhibiting glucagon secretion. It has glucose-dependent insulinotropic effect. If the association between Nesfatin-1 and GDM could be established, it might use for the treatment for GDM patients. Furthermore, the study findings can pave the way towards early diagnosis of the risk of developing GDM among the women of our county.

Research hypothesis

A low level of maternal nesfatin-1 level is associated with gestational diabetes mellitus.

Objectives

General objective

To determine the association between maternal serum Nesfatin-1 level and gestational diabetes mellitus.

Specific Objectives:

1. To measure serum Nesfatin-1 level of women with gestational diabetes mellitus (cases)
2. To estimate serum Nesfatin-1 level of normal healthy pregnant women (controls)
3. To compare serum Nesfatin-1 levels between the case and control groups
4. To find out the association of low serum Nesfatin-1 level with gestational diabetes mellitus.

Methods

Study design: Case control study.

Place of study: In the Department of Fetomaternal Medicine and Department of Obstetrics and Gynecology, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka.

Period of study: From October 2021 to September 2022 (one year)

Study population:

The study population included pregnant women between 24 to 28 weeks of gestation attending in the outpatient department of Fetomaternal Medicine and Obstetrics and Gynecology of BSMMU, Dhaka. The cases and controls were selected according to inclusion as well as exclusion criteria, 1:1 matched by age and gestational age for this study, as following-

Case: Pregnant women aged – 18 to 40 years with gestational age between 24 to 28 weeks with GDM

Control: Age and gestational age matched healthy pregnant women.

Sampling technique:

Purposive (non-random) sampling was done

Sample size determination

The sample size was determined by using this formula. Comparison of two means as follows-

Sample size (n) = $(u+v)2\ (\sigma12+\sigma02)(\mu1-\mu0)2$

Here,
u = Z value of standard normal distribution at 5% level of significance = 1.96
v = Z value of standard normal distribution at 80% power = 0.842
μ0 = 7.9, μ0 = Mean Nesfatin-1 level of the cases
μ1 = 11.2, μ1 = Mean Nesfatin-1 level of the controls
σ0 = 2.8, σ0 = Standard deviation of Nesfatin-1 level of the cases
σ1 = 7.7, σ1 = Standard deviation of Nesfatin-1 level of the controls
(All the values were taken from Ademoglu et al. 2017)[16]
Therefore,
𝑛=(1.96+0.85)2𝑥 {(7.7)2+(2.8)2}(11.2−7.9)2
= 48.39773
≈ 48

Therefore, total sample size, n = 96 (case = 48, control = 48).

Sample size determination

The sample size was determined by using this formula. Comparison of two means as follows-

$$\text{Sample size (n)} = \frac{(u+v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

Here,

u = Z value of standard normal distribution at 5% level of significance = 1.96
v = Z value of standard normal distribution at 80% power = 0.842
μ0 = 7.9, μ0 = Mean Nesfatin-1 level of the cases μ
σ0 = 2.8, σ0 = Standard deviation of Nesfatin-1 level of the cases σ1 = 7.7, σ1 = Standard deviation of Nesfatin-1 level of the controls
(All the values were taken from Ademoglu et al. 2017)[16]

Therefore,

$$n = \frac{(1.96+0.85)^2 \times \{(7.7)^2 + (2.8)^2\}}{(11.2-7.9)^2}$$

= 48.39773

≈ 48

Therefore, total sample size, n = 96 (case = 48, control = 48).

Results

This case control study was carried out to assess the association of serum Nesfatin-1 level with gestational diabetes mellitus. Forty-eight pregnant women diagnosed with GDM were included in the study as cases and 48 healthy pregnant women were considered as the controls and were evaluated for their serum Nesfatin-1 levels. Findings of the study are presented by graphs and tables

Table I: Distribution of the respondents according to their socio-demographic characteristics by group (N= 48 X 2 = 96)

Socio-demographic variables	Case n (%)	Control n (%)	p-value
Age (in years)			
18-25	11 (22.9)	19 (39.6)	
26-34	30 (62.5)	24 (50.0)	0.209[a]
35-40	7 (14.6)	5 (10.4)	
Mean ± SD	28.29 ± 4.63	26.83 ± 4.16	0.108[b]
Religion			
Islam	45 (93.8)	41 (85.4)	0.181[a]
Hinduism	3 (6.3)	7 (14.6)	
Education qualification			
Up to primary	13 (27.1)	8 (16.7)	
S.S.C.	9 (18.8)	8 (16.7)	0.393[a]
H.S.C. and above	26 (54.2)	32 (66.7)	
Occupation			
Housewife	37 (77.1)	39 (81.3)	
Student	3 (6.3)	6 (12.5)	0.217[c]
Service holders & others	8 (16.7)	3 (6.3)	
Monthly family income (in BDT)			
<10,000 Tk	2 (4.2)	4 (8.3)	
10,000 – 25,000 Tk	32 (66.7)	28 (58.3)	0.599[c]
>25,000 Tk	14 (29.2)	16 (33.3)	

[a]Chi square test was done to measure the level of significance. [b]Unpaired-t test was done to measure the level of significance. [c]Fisher's exact test was done to measure the level of significance. Within parenthesis are percentages over row total.

Table I shows the socio-demographic characteristics of the patients. In this study there was no statistically significant difference in between the case and control groups regarding their age, religion, educational qualifications, occupation and monthly family income ($p > 0.05$).

Table II: Distribution of the respondents according to obstetrical characteristics by group (N=96)

Obstetrical characteristics	Case n (%)	Control n (%)	p-value
Gravida			
Primigravida	17 (35.4)	26 (54.2)	0.065[a]
Multigravida	31 (64.6)	22 (45.8)	
Gestational age (in weeks)			
Mean ± SD	25.60 ± 1.41	26.17 ± 1.42	0.054[b]

[a]Chi square test was done to measure the level of significance. [b]Unpaired t test was done to measure the level of significance. Within parenthesis are percentages over row total.

On evaluation of the obstetrics characteristics, the distribution of the respondents was found statistically not significant ($p>0.05$).

Table III: Distribution of the respondents according to BMI by group (N=96)

BMI (kg/m^2)	Case *n* (%)	Control *n* (%)	*p*-value
Normal (18.5 - 24.9)	10 (20.8)	18 (38.3)	
Overweight (25.0-29.9)	37 (77.1)	28 (59.6)	0.108[c]
Obese (≥30.0)	1 (2.1)	1 (2.1)	
Mean ± SD	26.25 ± 1.99	25.34 ± 2.76	0.063[b]

The distribution of the respondents according to their BMI before delivery was found statistically not significant (p>0.05).

Table IV: Distribution of the respondents according to family history of diabetes mellitus by group (N=96)

Parameter	Case *n* (%)	Control *n* (%)	*p*-value
Family history of diabetes in parent or sibling			
Present	9 (18.8)	4 (8.3)	0.136[a]
Absent	39 (81.3)	44 (91.7)	

[a]Chi-square test was done to measure the level of significance.

Table IV illustrates that there was no statistically significant difference in distribution of the respondents according to their family history of diabetes mellitus (p>0.05).

Table V: Comparison of the respondents according to blood glucose level and insulin resistance by group (N= 96 = 48 + 48)

Blood sugar parameters	Case (Mean ± SD)	Control (Mean ± SD)	*p*-value
Fasting blood sugar (mmol/L)	5.76 ± 0.36	4.31 ± 0.61	<0.001[b]
2hr after 75g glucose (mmol/L)	9.47 ± 0.65	5.83 ± 0.52	<0.001[b]

[b]Unpaired t test was done to measure the level of significance.

Table V shows, A statistically significant difference was observed between the case and control group of patients in table 4.5 based on their blood glucose and HOMA-IR levels (p < 0.001).

Table VI: Comparison of mean (±SD) maternal Nasfetin-1 level in study subjects (N= 96 = 48 + 48)

Parameter	Case (Mean ± SD)	Control (Mean ±S D)	*p*-value
Serum Nesfatin-1 level (ng/mL)	1.50 ± 1.26	2.36 ± 1.95	0.013[b]

[b]Unpaired t test was done to measure the level of significance

Table VI demonstrates the distribution of study subjects according to their mean (±SD) serum Nesfatin-1 level. Here, the mean Nesfatin-1 levels for case and control group of respondents were 1.50±1.26 ng/mL and 2.36±1.95 ng/mL, respectively. The mean difference of serum Nesfatin-1 between the two groups was statistically significant (p=0.013).

Table VII: Odds ratios (OR) and 95% confidence intervals (CI) for GDM according to serum Nesfatin-1 level in pregnancy (Case = 48, Control = 48)

Nesfatin-1 (ng/ml)	Groups		p-value	Odds ratio (95% CI)
	Case N (%)	Control N (%)		
<1.68	39(81.3)	20(41.7)	<0.001[a]	6.607
>1.68	9(18.7)	28(58.3)		(2.407-15.291)

[a]Chi-square test was done to measure the level of significance.

There was significant difference in regards of serum Nesfatin-1 level in between case and control groups (p= <0.001) and the respondents with serum Nesfatin-1 <1.68 ng/mL had 6.1 times more chance to develop GDM compared to that of the healthy controls (OR=6.067; 95% CI=2.407-15.291).

Discussion

Ninety-six singleton pregnant women between 18-40 years of age in their 24-28 weeks gestation attending the outpatient department of the Fetomaternal Medicine and Department of Obstetrics and Gynecology, Bangabandhu Sheikh Mujib Medical University (BSMMU) were included in this study. Among them, 48 women diagnosed with GDM were considered as the cases, and the rest of the 48 healthy pregnant women without GDM were considered as the controls.

On evaluating the respondents' socio-demographic characteristics in this present study, the majority of the respondents were found within 26-34 years age group (case: 62.5% vs. control: 50.0%) followed by 18-25 years (case: 22.9% vs. control: 39.6%). As maternal age was taken matched for this study, their mean age distribution was almost similar (case: 28.29±4.63 years, and control: 26.83±4.16 years). Most of the participants were housewives (case: 77.1% and control: 81.3%), followed by service holder and others (case: 16.7% and control: 6.3%). Most of the study participants monthly family income was within 10,000-25,000Tk. (case: 66.7%, vs. control: 58.3%), followed by more than 25,000 Tk. Monthly family income (case: 29.2% vs. control: 33.3%). But none of these differences of socio- demographic characteristics was found statistically significant (p>0.05). Siddiqui et al. carried out a study in northern India that average age of GDM patients in Bhilai and Muzaffarpur were 28.531±4.51 and 26.015±5.75 years respectively.[8] According to, Khan et al. the monthly income, female occupation, and education level of GDM women were not significantly different from healthy pregnant women.[9] Yang et al. also did not find an association between GDM and education in Chinese pregnant women.[10] Low maternal education was linked to greater rates of overweight and obesity, which in turn raised the risk of GDM.[11] In accordance, Gandhewar et al. exhibited that the incidence of GDM was higher among multigravida (80.7%) compared to primigravida (19.4%).[12] A total of 77.1 percent of cases and 59.6 percent of controls were overweight. It was determined that none of these distinctions were statistically significant (p>0.05). Obesity and overweight have been cited as risk factors for GDM and associated consequences, but this study couldn't confirm it, perhaps because pre-pregnancy weight couldn't be ascertained. Conversely, Zhang et al. elucidated that body mass index (BMI) before delivery (GDM group 27.50±3.91 kg/m^2 vs. normal glucose tolerance group 26.98±3.34 kg/m^2, p<0.001) were independently associated with serum nesfatin-1.[13] Furthermore, in the present study, 18.8% of the women with GDM had a positive family history of diabetes mellitus compared to 8.3% of the healthy pregnant women. But this was not statistically significant (p=0.136). A similar study Zhu et al. revealed a family history of diabetes was present in 25.0% of GDM cases vs. 8.3% of the healthy controls (p=0.058) Another study also reported that 58 in 86 GDM patients had a family history of diabetes mellitus.[14-15] Both the fasting and 2hr after 75g of glucose blood sugar levels were significantly higher among the patients of case group were compared to healthy controls (FBG: 5.76±0.36 mmol/L vs. 4.31±0.61 mmol/L, and 2HABG: 9.47±0.65 mmol/L vs. 5.83±0.52 mmol/L, respectively), with the p-values <0.001. At the same time, mean(±SD) maternal serum nesfatin-1 level was observed significantly low in women of case group (1.50±1.26 ng/mL) in comparison to the control group of healthy pregnant participants (2.36±1.95 ng/mL), *P*=0.013. In accordance, Ademoglu et al. found that pregnant women who were diagnosed with GDM between 24 and 28 weeks had a substantially lower serum Nesfatin-1 level than healthy controls (7.92.8 ng/mL vs.11.27.7 ng/mL, P=0.020).[16] Odds ratio calculation in the present study revealed, in pregnant mothers at their late second trimester (24-28 weeks), the serum Nesfatin-1 level < 1.68 ng/mL (cut-off value was selected through receiver operating characteristic curve) had 6.1 times more chance to develop GDM compared to that of the pregnant women with Nesfatin-1 level ≥1.68 ng/mL (OR= 6.067; 95% CI=2.407 - 15.291). Therefore, a negative correlation between the GDM women's FBS with maternal Nesfatin-1 level (r=-0.205, p=0.045) and 2hrs after 75 g glucose blood sugar level with Nesfatin-1 (r=-0.239, p=0.019) were observed, denoting that a decline of serum Nesfatin-1 level in pregnant women might result in subsequent rise of blood glucose level through dysregulation of insulin release in GDM patients. These findings provide credence to the hypothesis that low levels of Nesfatin- 1 in maternal blood are associated with an increased risk of gestational diabetes mellitus. Several previous study results supported these findings –Aslan et al. in their study found maternal serum nesfatin-1 were lower (5.5±8.1 vs. 8.1±23.9 ng/ml, *P* = 0.001) in pregnant women with GDM compared with gestational age matched healthy pregnant subjects.[17]

Conclusion

Based on the results presented in this study, findings suggest that pregnant women with GDM have relatively low serum Nesfatin-1 level than healthy pregnant women. Serum Nesfatin-1 level is also found negatively correlated with both maternal fasting and 2hABG. Therefore, low level of maternal serum nesfatin-1 can be considered an important biomarker responsible for the development of GDM.

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