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Correlation of Afamin with oxidative stress in GDM

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Abstract

It is becoming a public health issue to predict which expectant women will develop gestational diabetes mellitus (GDM). The goal of this case control research is to investigate the role of maternal oxidative stress levels in the first, second, and third trimesters, as well as other factors, in the development of gestational diabetes mellitus (GDM).

Methods

Between October and December 2021, 142 women participated in this research. The 101 GDM patients were split into three groups based on their gestation (T1, T2, and T3), and 41 healthy pregnant women were chosen as the comparison group. TAS and TOS levels of oxidative stress and XO were calculated using a Spectrophotometer for colorimetric techniques; fasting and random sugar levels, as well as HbA1c, were evaluated.

Results

Afamin levels were significantly greater in women with GDM in all trimester (T1=51.63 ± 3.86, T2=57.39 ± 3.17, T3=64.22 ± 3.67 ng/mL) in comparison with control (45.93 ± 2.26) with statistical significance ($P \leqslant 0.01$). Afamin levels were higher significantly in T3>T2>T1.The levels of oxidative stress were higher significantly in women with GDM in all trimester (T1=73.79 ± 18.43, T2=81.28 ± 18.06, T3=96.70 ± 20.69) in comparison with control (37.28 ± 18.95) with statistical significance ($P = 0.000$). Oxidative stress levels were higher significantly in T3>T2>T1. The measured TOS and XO levels were higher in all GDM groups compared with control (p=0.000) and high significant between patient groups (T3>T2>T1), while high significant of TAS in GDM than control but (T3<T2<T1). The correlation of Afamin and XO in T2 was positive with significance, oxidative stress with TOS in T1, T2, and T3 groups was positive with significance, while negatively correlated with TAS in T1, T2, T3 groups. **Conclusions:** From the findings of this study, a significant of increasing of Afamin levels in GDM demonstrated by overall patients tends to heighten oxidative stress.

Key word:

Gestational diabetes mellitus, Trimester, Afamin, oxidative stress, total oxidant and total anti-oxidant status, xanthine oxidase.

GDM is described as glucose resistance that begins or is discovered during pregnancy. Globally, the prevalence of GDM fluctuates but is now believed to be between 7 and 10% [1]. GDM is linked to poor

maternal, fetal, and newborn results [2]. Having previously been identified with GDM is a recognized risk factor for developing T2DM in later life [3]. The primary risk factors for developing GDM, which usually results in a spontaneous hyperglycemic condition, include obesity [4, 5], increasing overweight, a high-fat and low-carb diet, and a sedentary lifestyle [6].

Afamin mostly created in the liver and delivered into the bloodstream before being transported to the other extravascular fluids. It contains 15% carbohydrates and shares 55% of the amino acid sequence with albumin [7]

The amino acid sequence Afamin contains 33% of its sequence with AFP, 29% with Albumin, and 19% with vitamin D binding protein with another member of the albumin family [8]. This glycoprotein is found in biological fluids such as plasma, cerebrospinal fluid, ovarian follicular fluid, and seminal fluid [9].

It is a protein from the liver that participates in cellular defenses against apoptosis brought on by reactive stress, Performs a role in the apoptotic cellular processes linked with OS [10]. Afamin is a measure for oxidative stress, and elevated amounts are linked to metabolic syndrome and insulin resistance (IR). It may be used as a marker to detect aberrant metabolism of glucose during pregnancy, Afamin has been linked to pregnancy complications such as gestational hypertension GH, preeclampsia PE, intrauterine growth restriction IUGR, preterm birth PB, and gestational diabetes mellitus GDM [11, 12].

The purpose of this research was to investigate the relationship between Afamin concentrations during the first, second, and third trimesters, as well as oxidative stress, and the onset of GDM in pregnant women.

Methods:

142 subjects who attended Al-Alawiyeh Maternity Teaching Hospital in Baghdad between October and December 2021 were enrolled in a case control study. 101 GDM patients were split into three groups (T1=34, T2=34, and T3=33) based on the stage of pregnancy, with a comparison group of 41 healthy pregnant women. After a doctor performed a fasting/random blood sugar study in accordance with American Diabetes Association (ADA) guidelines, GDM was identified. Pregnancy in all three trimesters was the time frame for data gathering. In addition to receiving the permission of the scientific and ethical committee, every participant in this research was told about it and given the go-ahead [13].

Participants with persistent inflammatory disease, metabolic disorders, hypertension, coronary heart disease, a family history of T2DM, or prior GDM were removed from the study. The selection conditions were pregnancy with one fetus and HbA1C% 6.5.

A straight HbA1c measurement was performed after drawing venous blood samples, and the leftover blood was centrifuged after clotting to gather serum, which was kept in Eppendroff containers at -20 °C. According to the instructions from the manufacturer, serum Afamin concentrations were determined using a sandwich enzyme-linked immuno-sorbent assay (ELISA) reagent (Catalogue number: RDEEH2493, Bioassay Technology Company, Germany). The variance values (CV) for the intra- and inter-assays were 8% and 10%, respectively.

Utilizing the Spectrophotometer PD-303 for colorimetric techniques, serum TAS and TOS were measured in order to compute the level of oxidative stress, xanthine oxidase was measured by using UV-Visible spectro-photometer (Japan) [14].

(Japan), Also Fasting and random glucose concentrations, were measured by using kits from HUMAN (Germany) for colorimetric methods, HbA1C by using DCA® analyzer Siemens (Ireland).

Statistical analysis: All statistical evaluations used the SPSS version 26 software. Mean and standard variation was used to show descriptive data. (SD). One-way ANOVA, post leg, was used to compare the groups. The link between oxidative stress levels and other factors was determined using Pearson correlation analysis. The threshold of importance when all statistical analyses were conducted with a P value (<0.05).

Results:

The results of measuring the biochemical parameters are shown in Table 1, where fasting/random blood glucose and HbA1c levels in the

first, second, and third trimesters showed noticeably higher levels in GDM than those of controls (P=0.043, P=0.001, and P=0.000, respectively). With statistical significance (P = 0.032), the HbA1C values were higher in the GDM T1 phase than in the GDM T3.

At the end of the third trimester, GDM patients had statistically significantly greater mean Afamin levels than the comparison group (P = 0.000). However, GDM group members who were in T3 had higher Afamin levels than those in T2 and T1, with statistical significance for three semesters (P = <0.000).

Additionally, with statistical significance, TOS, oxidative stress and XO were higher in GDM patients at three trimesters compared to controls (P = 0.000); additionally, higher significance was seen in the GDM group at T3 compared to T2 and T1 with statistical significance for three semesters (P = < 0.000). While in contrast to TAS which higher in control than GDM Patients (T3<T2 and T1) with statistical significance (P=0.000).

Table1 the characteristics of the biochemical parameters levels between GDM patients (three semesters) and control groups.

Parameter	Control n=41	T1 n=34	T2 n=34	T3 n=33	P value
Age range (year)	16-43	19-45	18-43	20-45	---
FBG mg\dL	79.45 ± 5.22	113.68 ± 32.38[a]	116.22 ± 34.27[a]	113.61 ± 25.01[a]	0.043
RBG mg\dL	99.25 ± 17.20	148.80 ± 61.87[a]	126.73 ± 42.80[a]	133.53 ± 42.93[a]	0.001
HbA1C %	3.56 ± 0.38	6.13 ± 0.91[a]	6.00 ± 0.91[a]	5.74 ± 0.63[ab]	0.000
TAS (mmol glutathioneEq\L)	1.41 ± 0.51	0.92 ± 0.26[a]	0.94 ± 0.22[a]	0.88 ± 0.19[a]	0.000
TOS (mmol H_2O_2 Eq\L)	46.18 ± 15.95	66.49 ± 13.43[a]	74.72 ± 15.42[ab]	82.60 ± 15.08[abc]	0.000
OSI	37.28 ± 18.95	73.79 ± 18.43[a]	81.28 ± 18.06[ab]	96.70 ± 20.69[abc]	0.000
XO U\L	30.79 ± 5.65	55.32 ± 9.16[a]	70.13 ± 6.52[ab]	78.31 ± 6.53[abc]	0.000[**]
AFM ng\ml	45.935 ± 2.265	51.634 ± 3.865a	57.395 ± 3.176ab	64.223 ± 3.670abc	0.000

Statistical analysis using ANOVA, significant difference when (P< 0.05). a: significant when compared with control group, b: significant when compare T1 with T2 and T3 group, c: significant when compare T2 with T3 group.

Afamin correlated positively with XO in T2 (r= 0.538, P=0.014), also with RBG and HbA1c levels in T3 group (r =0.737; P =0.023, and r =0.612; P =0.002,) respectively, and positive correlation between Afamin and No other correlations were found between Afamin levels with other parameters, as shown in Table 2 and figure 1.

Table2: Pearson correlation coefficients of Afamin with other biochemical parameters in the semester groups.

T2	XO	0.538*	0.014
T3	RBG mg\dL	0.737*	0.023

	HbA1C %	0.612**	0.002

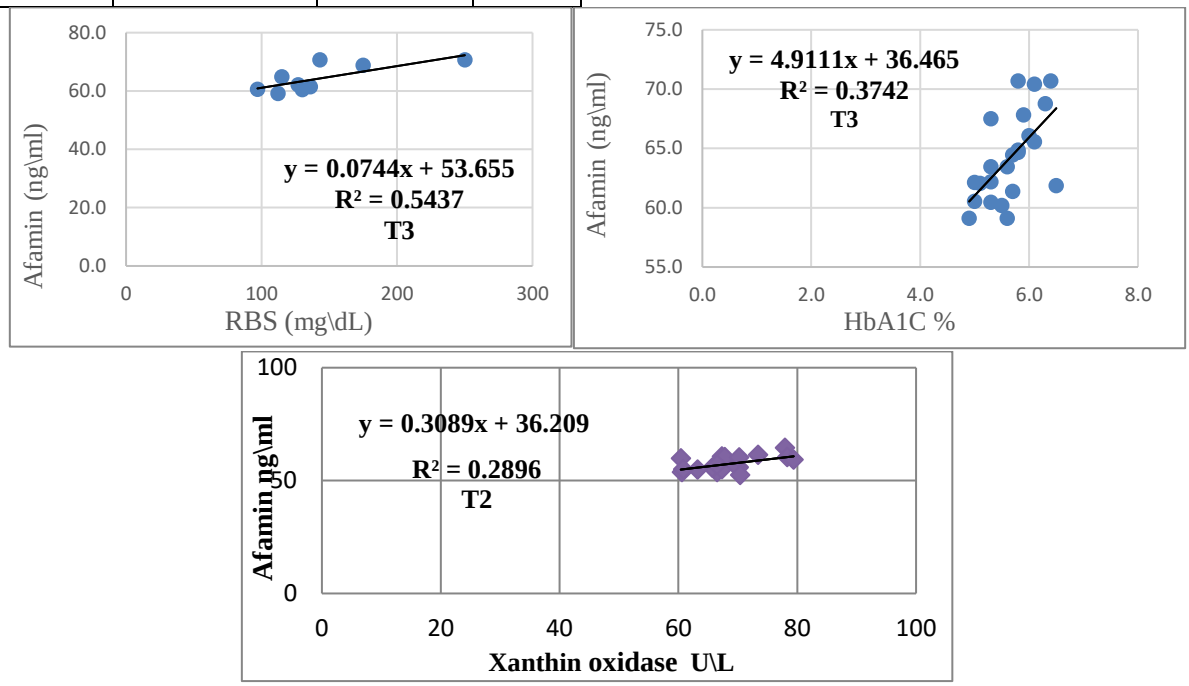

Figure 1: Pearson correlation analysis of Afamin with other biochemical parameters

Discussion

In this study, the first, second, and third trimester serum Afamin levels of pregnant women who acquired diabetes were evaluated and compared to those of pregnant women who had healthy pregnancies. According to a study looking into serum Afamin levels and pregnancy issues in the first semester only, which revealed that diabetic women have higher Afamin levels than controls, GDM groups had Afamin levels that were considerably higher than healthy pregnant women [15]

Maternal blood Afamin amounts increased linearly and roughly doubled during pregnancy in women with typical pregnancies. Furthermore, neither mRNA nor protein evidence of placental Afamin expression was observed in uncomplicated pregnancy, and no cause for this increase during pregnancy was identified. Afamin is primarily received from the mother during the course of an uncomplicated pregnancy, as evidenced by the absence of Afamin expression in embryonic rat liver and human fetuses throughout 4 to 9 weeks of gestation [16].

Therefore, it is thought that Changes in hormonal state and consequent hormonal control of Afamin gene expression in the human liver cause an increase in Afamin amounts during pregnancy. It has been determined that hepatic synthesis of lipids and lipoproteins, which causes physiological hyperlipidemia during pregnancy, is a similar process for this hormonal regulation [17].

Afamin was one of three proteins that demonstrated significant levels in GDM patients at T1 when compared with controls in a prior research that performed a proteomic analysis [18].

Afamin has been linked to the frequency of insulin resistance, pre-diabetes, and diabetes, according to a meta-analysis of eight studies on plasma Afamin amounts in people with these conditions [19, 20]

Blood Afamin concentration was found to be considerably greater in T2DM group patient blood than in that of healthy people in a study comparing T1DM, T2DM, and controls. Based on these findings, the researchers proposed using Afamin as a biomarker for T2DM [21].

Hyperglycemia caused by diabetes is associated with increased glycation, oxidative harm, and nitrosative stress. During pregnancy, GDM, a kind of glucose intolerance that can vary in severity, might appear [22].

The quantity of oxidative stress-related enzymes is especially high in the liver. The ROS, or byproducts of cellular metabolism, are well known for having both detrimental and beneficial properties. Despite the fact that ROS such hydroxyl radicals $OH^{\cdot}$, superoxide anion O_2^{-}, and hydrogen peroxide H_2O_2 are byproducts of normal oxygen metabolism, their interactions with other biomolecules lead to oxidative damage [23, 24, 25].

Suppressing XO activity is the best strategy to stop the oxidative damage caused by the buildup of free radicals. Oxidative stress and other metabolic illnesses have been linked to an increase in XO activity [26, 27].

Superoxide dismutase (SOD), an enzyme, generates these oxidants. Afamin is a vitamin E-binding glycoprotein, and hormonal changes associated with pregnancy increase its levels [28].

This protein functions as an important antioxidant in several pathways. According to studies, higher oxidative stress and illnesses associated with it (such as the metabolic syndrome, diabetes mellitus, etc.) are connected to raise levels of Afamin. Afamin, a marker for oxidative stress, participates in OS-related ant apoptotic cellular processes. Raised of Afamin levels are present in both IR and the metabolic syndrome. It might serve as a biomarker to identify irregular metabolism of glucose in pregnancy. The release of placental mediator rises in proportion to the intensity of the circumstance [29, 30].

Conclusions:

Increasing of Xanthine oxidase was association with highly levels of Afamin in GDM.

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Conflict of interest:

The authors have no conflict of interest.

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