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Afamin as an Independent Diagnostic Marker of Gestational Diabetes Mellitus

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ABSTRACT

Objectives: To assess utility of afamin (AFM) as an independent marker of Gestational Diabetes Mellitus (GDM) in comparison with Oral Glucose Tolerances Test (OGTT), and to determine cut off value for afamin.
Study Design: Diagnostic accuracy study.
Place and Duration of Study: Chemical Pathology and Gynecology Department, Army Medical College and Pak Emirates Military Hospital, Rawalpindi Pakistan, from Jan to Dec 2021.
Methodology: Ninety-six pregnant females with history of 24 to 28 weeks of gestation were included in this study, out of which 48 were cases of GDM (Group-A) and 48 were included in age and gestation matched control group (Group-B). Fasting plasma glucose was taken for 75g OGTT and Afamin (AFM) levels. AFM concentrations were compared with OGTT results between the two groups.
Results: Median age of cases was 30.0(3.0) years and of controls was 29.5(5.0) years. A significant difference was found in the median values of serum afamin, fasting plasma glucose and 1- and 2-hours plasma glucose post-glucose load between Group-A and Group-B. The optimal cut off level of serum afamin for diagnosing GDM was determined as 71.2 mg/L, with Area Under Curve (AUC) 0.993, sensitivity 97.9% and specificity 79.0%. AUC for serum afamin levels was greater than HbA1c, fasting plasma glucose and 1-hour plasma glucose post-glucose load.
Conclusion: Median second trimester serum afamin levels were significantly higher in Group-A as compared to Group-B, making it a reliable test for GDM in second trimester.

Keywords: Afamin, Gestational Diabetes Mellitus, Oral Glucose Tolerance Test.

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INTRODUCTION

Gestational diabetes mellitus (GDM) is defined as diabetes diagnosed in the second or third trimester of pregnancy that was not clearly overt diabetes prior to gestation.[1] It is a common complication during pregnancy that may lead to various adverse outcomes. The prevalence of GDM is on the rise, paralleling the prevalence of type-2 DM and obesity. Based on recent estimates from the International Diabetes Federation (IDF), 20.4 million or 15.8% of live births to women in 2019 had some form of hyperglycemia in pregnancy, of which, 83.6% were due to GDM.[2] The true prevalence of glucose intolerance during pregnancy in Pakistan is still to be determined. However small hospital-based studies have given figures of 3.2% for GDM.[3]

Currently, oral glucose tolerance test (OGTT) is currently used as gold standard for GDM screening.[4] OGTT is a sensitive test that has the ability to detect early glucose impairment in pregnant females. However, it has certain limitations. It is time-consuming, requires fasting state, multiple pricks for serial glucose testing, can lead to nausea and vomiting, and is unsuitable in dextrose allergy.[5] For these reasons, the International Association of the Diabetes and Pregnancy Study Groups (IADPSG) recommend alternate, single-biomarker testing.[6]

Newer biomarkers that do not require fasting, multiple sampling and have better sensitivity, specificity and reproducibility profile are being introduced like Adiponectin, Visfatin, Leptin, IL-6 and Afamin.[7] Afamin (AFM) is an Emerging GDM Biomarker, which is mainly of hepatic origin.[8] Several studies have recently shown that high levels of serum afamin are associated with development of GDM and insulin resistance in pregnancy.[7-9] Evidence suggests that afamin has the potential to be a biomarker for early prediction of GDM ant type 2 diabetes mellitus.[10] Therefore, this study was planned to assess the clinical utility of AFM as a single screening/diagnostic marker for GDM in our population.

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METHODOLOGY

This diagnostic accuracy study was conducted in the Department of Chemical Pathology and Endocrinology, Army Medical College and Department of Gynecology PEMH, Rawalpindi, Pakistan from Jan 2021 to Dec 2021 after taking approval from ethical research committee (ERC/ID/134).

Inclusion Criteria: Pregnant females with history of 24 to 28 weeks of gestation were included.

Exclusion Criteria: Morbidly obese women, those with twin pregnancy, pregnancy induced hypertension, history of fetal anamolies, premature rupture of membranes, those already diagnosed with diabetes mellitus, those who had abnormal thyroid functions, those with deranged serum lipid profile, known patients of chronic liver and kidney disease and females taking oral supplements containing vitamin E were excluded.

The sample size calculated was 48 per group, using the WHO calculator keeping prevalence of GDM as 3.2%.[3] Out of these, 48 were confirmed cases of GDM on basis of 75g oral glucose tolerance test results, were placed in Group-A. The remaining 48 were taken as controls without GDM and were age and gestation matched, and were placed in Group-B (Figure-1).

The subjects were recruited using non-probability consecutive sampling technique, and informed consent was taken from each respondent.

Age, weight, height and gestational age of all the ladies were recorded, and fasting plasma glucose, Hba1c, lipid profile and thyroid function tests were done.

After explaining the procedure to the patient, 2ml of blood sample was taken in fluoride tube for fasting glucose and 75g oral glucose tolerance test. 2ml of blood was collected in plain/gel tube for AFM levels and allowed to clot. After centrifugation the supernatant was collected in separate tube and frozen at -20°C. AFM analysis was done by bioassay technology laboratories commercial kit based on the methodology of Enzyme-Linked Immunosorbent Assay (ELISA) for quantitative detection of human AFM in serum. Two levels of controls normal and abnormal were run before analysis of batch. Plasma glucose analysis for OGTT was measured by a commercial kit by Roche diagnostics as a 2-point end assay based on the principle of enzymatic hexokinase reference method. HbA1c was measured on commercial kit by Roche diagnostics based on the principle of immunoturbidimetric assay.

Statistical package for Social Sciences (SPSS) version 21 was used for data analysis. Distribution of data was assessed by Shapiro Wilk test which showed non parametric distribution. Median (IQR) was calculated for continuous variables and Mann Whitney U test was used to compare medians between groups. A *p*-value of <0.05 was considered as significant. For diagnostic accuracy 2x2 table was made and sensitivity, specificity, Positive Predictive Value, Negative Predictive Value and overall accuracy of serum afamin levels was calculated. Receiver Operating Characteristic curve was used to determine the cut off for serum afamin levels for GDM in second trimester.

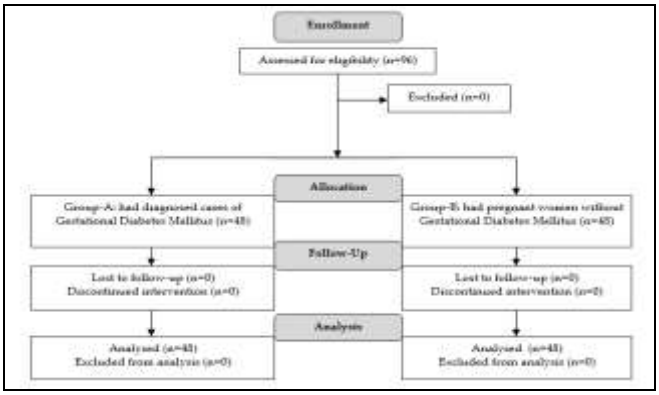

Figure-1: Patient Flow Diagram (n= 96)

RESULTS

Ninety-six pregnant females with history of 24 to 28 weeks of gestation were included in this study, out of which 48 were cases of GDM (Group-A) and 48 were included in control group (Group-B). Median age of cases was 30.0(3.0) years and of controls was 29.5(5.0) years. Median second trimester serum afamin levels were significantly higher in GDM group as compared to control group [99.1(65.1) mg/L vs 58.0(10.2) mg/L, *p*< 0.001]. Other baseline characteristics of GDM and control group are given in Table-I.

For study of diagnostic accuracy 2x2 table was made. Out of 48 cases of GDM, 37 cases had raised serum afamin levels and were labeled as true positive, whereas 11 had low serum afamin levels, therefore labeled as false negative. Similarly in the control group only one patient had falsely increased serum afamin levels and was labeled as false positive whereas 47 patients had low serum afamin levels and were

labeled as true negative which depicts high specificity of this test for GDM. Sensitivity, specificity, Positive Predictive Value, Negative Predictive Value and overall accuracy of serum afamin levels have been shown in Table-II. Serum afamin levels showed a high specificity of 97.9% for diagnosing GDM, similarly this test showed a high positive predictive value of 97.3% which makes it an excellent test for the diagnosis of GDM.

Table-I: Comparison of Baseline Characteristics across Groups (n=96)

Parameter	Group-A (n=48) Median (IQR)	Group-B (n=48) Median (IQR)	*p*-value
Age (years)	30(3)	29.5(5)	0.035
Gestational age (weeks)	26(1)	25(2)	0.045
Height (Feet)	5.5(0.1)	5.5(0)	0.977
Weight (Kg)	66(9)	67(8)	0.740
Serum Afamin (mg/L)	99.9(65.1)	58.4(10.2)	< 0.001
Fasting plasma glucose (mmol/L)	5.9(1)	5(0.7)	< 0.001
1hour post glucose load (mmol/L)	10.6(1.5)	7.6(1.8)	< 0.001
2hour post glucose load (mmol/L)	9.4(1.6)	6.4(1.4)	< 0.001
Total Cholesterol (mmol/L)	4.62(1.11)	4.55(1.16)	0.631
HDL – Cholesterol (mmol/L)	1.14(0.42)	1.21(0.41)	1.000
LDL – Cholesterol (mmol/L)	2.84(1.17)	2.68(0.86)	0.711
Triglycerides – Cholesterol (mmol/L)	2.08(1.22)	1.91(1.12)	0.066

*HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein

Table-II: Measures of Diagnostic Accuracy of Serum Afamin for GDM (n=96)

	Group-A (n=48) (OGTT Positive)	Group-B (n=48) (OGTT Negative)
Serum Afamin High	True Positive (37)	False Positive (1)
Serum Afamin Low	False Negative (11)	True Negative (47)
Sensitivity	TP/TP+FN = 37/37+11 =0.77(77%)	
Specificity	TN/TN+FP = 47/47+1 =0.979(97.9%)	
Positive Predictive value (PPV)	TP/TP+FP = 37/37+1 =0.973(97.3%)	
Negative predictive value(NPV)	TN/TN+FN = 47/47+11 =0.81(81.0%)	
Overall Accuracy	TP+TN/TP+TN+FP+FN=37+47/37+47+1+11 =0.875(87.5%)	

*TP: True Positive, FP: False Positive, TN: True Negative, FN: False Negative

Receiver Operating Characteristic (ROC) curve analysis demonstrated excellent diagnostic accuracy for second trimester afamin levels for GDM with AUC to be 0.993(95% CI (0.978-1.000; p<0.001), which makes second trimester afamin levels a reliable test for diagnosing GDM as compared to HbA1c, fasting plasma glucose, and 1-hour post glucose load plasma glucose levels as shown in Figure-2. AUC for afamin was found to be 0.993 which was greater as compared to other parameters. HbA1c had an AUC of 0.756, fasting plasma glucose had AUC of 0.83 and 1-hour post 75g glucose load plasma glucose had AUC of 0.939. Two hours post glucose load plasma glucose had the highest AUC of 1 as shown in Table-III.

The optimal cut off level of serum afamin for diagnosing GDM was determined as 71.2 mg/L with sensitivity 97.9% and specificity 79% using ROC curve.

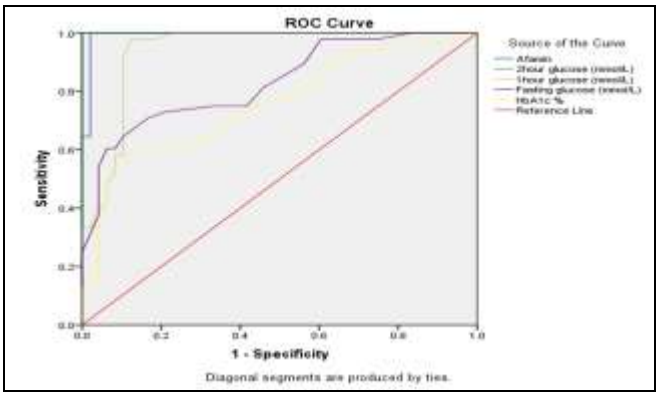

Figure-2: ROC Curve for Serum Afamin Levels, HbA1c and OGTT

Table-III: Comparison of AUC for S. Afamin with OGTT and HbA1c

Parameter	AUC (Area under the curve)
Afamin	0.993
Fasting Plasma Glucose	0.830
Plasma glucose 1-hour post 75g glucose load	0.939
Plasma glucose 2-hour post 75g glucose load	1.00
HbA1c	0.756

DISCUSSION

Ninety-six pregnant females with history of 24 to 28 weeks of gestation were included in this study, out of which 48 were cases of GDM and 48 were included in control group. Median age, gestational age, and anthropometric parameters such as height and weight did not differ significantly across our cases and controls. We inducted the women with 24-28 weeks of gestation in both the groups. Median second trimester serum afamin levels were significantly higher in GDM group as compared to control group (*p*=0.000) in our study. Kunick *et al.*, also included the women with same gestation age, mean gestation age of women in their study was 26.05±0.35 weeks, which is similar to our study and also reported higher serum afamin levels in women with GDM.[11] Wang *et al.*, also reported a significant difference between serum concentrations of mid-trimester afamin in pregnant women with and without GDM (83.67±22.65 and 70.01±19.2 mg/L, *p*<0.001).[12] Atakul *et al.*, studied the predictive power of afamin in diagnosing GDM in both the first-trimester and the second-trimester of pregnancy.[13] Their findings were that afamin levels were higher in both the first and second trimester in women who had GDM as compared to women who had normal glucose tolerance. However, a systematic review and meta-analysis of seven studies (1,195 GDM

and 1,407 healthy women) have reported elevated first-trimester afamin levels in women with GDM but found no significant differences during the second and third trimesters.[14]

Cai *et al.,* worked on the correlation between liver factor levels and the risk of GDM. The results showed that the plasma afamin levels in women with GDM were significantly higher than those of healthy pregnant women standard mean difference (SMD) of 0.58, 95% confidence interval (CI) 0.24-0.93.[15]

Recent evidence revealed that afamin concentrations in serum are strongly related with parameters of metabolic syndrome like increased BMI, high blood pressure, high fasting plasma glucose, glucose intolerance and deranged lipid profile. Therefore, researchers are investigating the association between serum afamin levels during pregnancy and different pregnancy related diseases and complications. Our study reported significant positive correlation of afamin with other markers of GDM currently in use, which is in concordance with previous studies.[16,17] Atakul *et al.,* stated that the serum afamin concentrations of pregnant females with GDM were higher than those without GDM, in line with other studies reported in the literature.[13] They also reported that serum afamin concentrations in pregnancies complicated by GDM. with large for gestational age fetuses, were significantly higher than those in the GDM group with appropriate for gestational age fetuses. As a result, they stated that afamin is associated with fetal growth independent of glycemic control and can be used to predict large for gestational age fetuses in pregnant women with GDM. In another study, third trimester serum afamin concentration was found to be higher in pregnant women whose pregnancy was complicated with late fetal growth restriction compared to control group.[18] Similarly in a recent study, serum afamin levels in umbilical cord blood after delivery in mothers with and without GDM were compared, and it was found that median afamin levels were higher in GDM group (p<0.001).[18]

There was slight disagreement in cut off values of AFM among different studies. Our study reported a cut off of 71.2 mg/L with sensitivity 97.9% and specificity 79%. Wang *et al.,* reported similar cut off, i.e. 72mg/L but with much less sensitivity and specificity 35.6 and 77.3%.[12] Similarly, Li *et al.,* reported a slightly higher cut off i.e. 108mg/L with sensitivity of 44.83% and specificity of 85%.[19]

Our results show that afamin is an excellent marker for diagnosing GDM with AUC of 0.993 (95% CI (0.978-1.000; p<0.001). Dogan *et al.,*[18] also reported that afamin could predict GDM diagnosis with an AUC of 0.67 (95% CI 0.53–0.81). Li *et al.,* performed ROC to see if afamin can be used to predict GDM and found an AUC of 0.629 (95% CI: 0.527~0.731).[19]

These findings suggest that AFM may serve as a promising non-invasive biomarker for the early identification of GDM.

LIMITATION OF STUDY

Our main limitations included a small sample size, and potential confounders.

CONCLUSION

Median second trimester serum afamin levels were significantly higher in GDM group as compared to control group. Serum afamin levels outshines other parameters with AUC of 0.993 (95% CI (0.978-1.000; p<0.001) making it a reliable test for GDM in second trimester.

Conflict of Interest: None.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

JA & UN: Data acquisition, data analysis, critical review, approval of the final version to be published.

AB & QK: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

ZS & RI: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Staimez LR, Kipling LM, Ham JN, Legvold BT, Jackson SL, Wilson PW, et al. Potential misclassification of diabetes and prediabetes in the US: mismatched HbA1c and glucose in NHANES 2005–2016. Diab Res Clin Pract 2022; 189: 109935. https://doi.org/10.1016/j.diabres.2022.109935
2. Ruszała M, Pilszyk A, Niebrzydowska M. Novel biomolecules in the pathogenesis of gestational diabetes mellitus. Int J Mol Sci 2022; 23(8): 4364. https://doi.org/10.3390/ijms23084364
3. Adnan M, Aasim M. Prevalence of gestational diabetes mellitus in Pakistan: a systematic review and meta-analysis. BMC Pregnancy Childbirth 2024; 24(1): 108. https://doi.org/10.1186/s12884-024-06290-9
4. Casas LA, Alarcón J, Urbano A, Peña-Zárate EE, Sangiovanni S, Libreros-Peña L et al. Telemedicine for the management of diabetic patients in a high-complexity Latin American hospital. BMC Health Serv Res 2023; 23(1): 314. https://doi.org/10.1186/s12913-023-09267-0

5. Rathnayake H, Han L, daSilva F. Advancement in predictive biomarkers for gestational diabetes mellitus diagnosis and related outcomes: a scoping review. BMJ Open 2024; 14: e089937. https://doi.org/10.1136/bmjopen-2024-089937
6. Hu SL, He BT, Zhang RJ. Association between maternal alcohol use during pregnancy and gestational diabetes mellitus: a meta-analysis. Int J Diabetes Dev Ctries 2021; 41(2): 189-195. https://doi.org/10.1007/s13410-020-00877-6
7. Sweeting A, Hannah W, Backman H, Catalano P, Feghali M, Herman WH, et al. Epidemiology and management of gestational diabetes. Lancet 2024; 404(10448): 175-192. https://doi.org/10.1159/000480161
8. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33(3): 676-682. https://doi.org/10.2337/dc09-1848
9. Adam S, Pheiffer C, Dias S, Rheeder P. No association between gestational diabetes and biomarkers: a role in diagnosis. J. Biomark 2018; 23(4): 386-391. https://doi.org/10.2147/DMSO.S294328
10. Wu S, Li L, Hu KL. A Prediction Model of Gestational Diabetes Mellitus Based on OGTT in Early Pregnancy: A Prospective Cohort Study. J Clin Endocrinol Metab 2023; 108: 1998–2006. https://doi.org/10.1210/clinem/dgad052
11. Kunick Mi, Rzewuska N, Sopońska P, Pawłosek A, Sowińska I, Kloska A. Novel serum biomarkers for early diagnosis of gestational diabetes mellitus—a review. J Gynaecol Endocrinol 2025; 41(1): 2455472. https://doi.org/10.1080/09513590.2025.2455472
12. Wang X, Zheng X, Yan J. The Clinical Values of Afamin, Triglyceride and PLR in Predicting Risk of Gestational Diabetes During Early Pregnancy. Front Endocrinol 2021; 12: 723650. https://doi.org/10.3389/fendo.2021.723650
13. Atakul N, Atamer Y, Selek S, Kılıç B, Unal F: Novel Metabolic Marker Afamin: A Predictive Factor for Large-for-Gestational-Age (LGA) Fetus Estimation in Pregnancies with Gestational Diabetes Mellitus? J Gynecol Obstet Hum Reprod 2021; 102201(2): e102201. https://doi.org/10.1016/j.jogoh.2021.102201
14. Yuan Y, He W, Fan X. Serum afamin levels in predicting gestational diabetes mellitus and preeclampsia: a systematic review and meta-analysis. Front Endocrinol 2023; 14(2): 1157114. https://doi/org/10.3389/fendo.2023.1157114
15. Cai Z, Yang Y, Zhang J. Hepatokine levels during the first or early second trimester of pregnancy and the subsequent risk of gestational diabetes mellitus: A systematic review and meta-analysis. J Biomark. 2021; 26(6): 517-531. https://doi.org/10.1080/1354750X.2021.1928754
16. American Diabetes Association Professional Practice Committee. Standards of care in Diabetes- 2023. Diabetes Care 2023; 48(1): 542-543. https://www.bluecirclehealth.org/wp content/uploads/2023/06/ADA_SoC_ch5.pdf
17. Chen LW, Soh SE, Tint MT, Loy SL, Yap F, Tan KH, et al. Combined analysis of gestational diabetes and maternal weight status from pre-pregnancy through post-delivery in future development of type 2 diabetes. Sci Rep 2021; 11(1): 5021. https://doi.org/10.1038/s41598-021-82789-x
18. Dogan Y, Arslan O, Oztas B, Kurtali A, Daryal AS, Eser MD. Comparison of Afamin Values in Umbilical Cord Blood After Delivery in Pregnancies With and Without Gestational Diabetes Mellitus. Fetal Pediatr Pathol 2024; 43(2): 83-93. https://doi.org/10.1080/15513815.2023.2300981
19. Li Q, Li C, Jin J, Shen Y, Wang M. Clinical significance of neuregulin 4, afamin, and SERPINB1 in gestational diabetes mellitus and their relationship with insulin resistance. J Evid-Based Complement Altern Med 2022; 2022(1): 2829662. https://doi.org/10.1155/2022/2829662