A CROSS-SECTIONAL STUDY ON THE ACCURACY OF GLYCATED ALBUMIN IN DIAGNOSING PREGNANCIES COMPLICATED WITH GESTATIONAL DIABETES MELLITUS

Abstract

Background: The diagnosis of gestational diabetes mellitus is made using the oral glucose tolerance test. Conducting this test requires patient preparation. Meanwhile, glycated albumin does not require the patient to be prepared. Glycated albumin is affected by ethnicity and black Americans have higher glycated albumin levels than Caucasians. This study determined the use of glycated albumin in diagnosing gestational diabetes mellitus among pregnant women.

Materials and method: The study was a prospective cross-sectional study of 200 pregnant women between 24 to 28 weeks of gestation at the University of Port Harcourt Teaching Hospital. The diagnosis of GDM was based on the World Health Organization 2013 diagnostic criteria. The diagnostic cut-off of glycated albumin was determined using the receiver operator characteristic (ROC) curve. The comparison of means was done using the Student's T-test.

Results: The area under the receiver operator characteristic curve for glycated albumin was 0.8 and the optimal cut-off value of glycated albumin in the diagnosis of gestational diabetes mellitus was 19%. Glycated albumin was significantly elevated in women with gestational diabetes mellitus compared to women without gestational diabetes mellitus at $P<0.001$.

Conclusion: Glycated albumin has an area under the ROC curve of 0.85 with an optimal cut-off value of 19.0%. Glycated albumin is significantly elevated in women with GDM than in women without GDM with a sensitivity of 83.3% and a specificity of 86.8%. Therefore, glycated albumin can be used to diagnose GDM.

Keywords

Glycated albumin, Oral glucose tolerance test, Gestational diabetes mellitus, pregnancy complications

1. Introduction

Gestational diabetes mellitus (GDM) is a common pregnancy complication seen in about 5% of all pregnancies [1]. Gestational diabetes mellitus is varying degrees of glucose intolerance first diagnosed in pregnancy [2,3]. There is a variation in the prevalence of GDM in different regions of the world. The prevalence of GDM in Europe is 5.4% [4] while the prevalence in the Middle East and Asian region is 11.5% [5]. The prevalence in the Sub-Saharan Africa region is 14.3% [6]. The prevalence of GDM in Port Harcourt, southern Nigeria, is 10.5% [7].

Gestational diabetes puts the baby at risk of prematurity, growth restriction, fetal macrosomia, intra-uterine fetal death, shoulder dystocia, instrumental vaginal delivery, birth injuries, and stillbirth [8,9]. Neonatal complications may include electrolyte imbalance, hyperglycemia, hypoglycemia, hyperbilirubinemia, jaundice, respiratory distress syndrome, and early neonatal death [8,10]. The child is at risk of obesity, type II diabetes mellitus, and cardiovascular disease later in life [11,12]. The mother may develop hypertension, pre-eclampsia, preterm rupture of membranes, perineal injuries at delivery, and an increased risk of cesarean section [6,10,]. Obesity and type II diabetes mellitus may also occur after puerperium [12,13].

Gestational diabetes mellitus occurs when the body fails to regulate the hyperglycemic effects of hormones produced during pregnancy [13]. Most women with GDM may not have any symptoms or risk factors, [14] therefore, the World Health Organization recommends universal screening for GDM using OGTT [15]. However, screening women based on risk factors is the practice in some countries. For example, the National Institute for Health and Care Excellence recommends a selective screening for GDM for women with the following risk factors: a history of a first-degree relation with diabetes mellitus, Body mass index ≥30kg/m^2, a history of delivery of a baby weighing ≥4.5 kg, belongs to an ethnicity with a high rate of diabetes mellitus [16]. Maternal age, previous unexplained stillbirth, and previous GDM are other risk factors for GDM [2,3].

The diagnosis of GDM is usually made following a one-hour 75g oral glucose tolerance test (OGTT): fasting plasma glucose (FPG) value of ≥5.1mmol/L but ≤ 6.9 mmol/L, one-hour OGTT value of ≥10.0mmol/L, or two-hour OGTT value of ≥8.5mmol/L but ≤11.1mmol/L [15,17]. The drawbacks of OGTT are patient preparation before the test, drinking of a glucose solution, and multiple sample collections. The patient preparation requires the woman to have her normal diet for three days and to have an overnight fast of 8 to 15 hours [18,19]. Some health workers don't know how to prepare women for the procedure and some women are not motivated to fast [18,20]. Oral Glucose Tolerance Test may also be affected by exercise, physical stress, acute illness, and medication. [18,19]. Nausea and vomiting, a side effect of drinking the glucose solution during OGTT, have been indicated as a reason some women withdraw from the test [21]. The cumbersome nature of the OGTT procedure has led to the search for other methods of screening and diagnosis of GDM. Glycemic markers that have been evaluated for diagnosis of GDM are glycated hemoglobin, glycated albumin, B-cell activating factor, tumor necrosis factor, platelet-activating factor, and methylglyoxal [22,23].

Glycated albumin is produced by a non-enzymatic reaction between albumin and reducing sugars [24]. Albumin has a high content of glycine and lysine which makes it susceptible to a non-enzymatic reaction with reducing sugars in plasma [24,25]. Since albumin is an abundant extracellular plasma protein, it is glycated 9 to 10 times more than other proteins [22,24]. The amount of glucose attached to the albumin during this reaction is dependent on the degree and duration of hyperglycemia, therefore, serum glycated albumin is a reflection of the degree and duration of hyperglycemia for a period of 14 to 20 days [22,26]. Fasting is not required before the measurement of glycated albumin, it is not affected by abnormal hemoglobin metabolism, insulin use, and renal failure [25,27]. Disease conditions that affect albumin metabolism, age, ethnicity, and body mass index may influence the level of glycated albumin [24,28]. Most studies on glycated albumin use in the diagnosis of hyperglycemic states were done among Asians and Caucasians [29], however, studies have shown that blacks have higher glycated albumin levels than Caucasians. In a cross-sectional community-based study in the United States of America, a total of 1295 Caucasians and 424 African Americans were studied. Caucasians without diabetes mellitus had a mean glycated albumin of 13.4% while African Americans without diabetes mellitus had a mean glycated albumin of 14.5%. The study group diagnosed with diabetes mellitus had means glycated albumin values of 16.5% and 20.1% for Caucasian and African

Americans respectively. The group concluded that GA is higher in the black population [28]. A review of publications on glycated albumin and its role in the diagnosis of GDM suggested that Asians may have lower glycated albumin. [30] This study was conducted among women of African origin.

2. Objectives

The objective of this study was to determine the diagnostic cut-off of glycated albumin in the diagnosis of GDM and to determine whether glycated albumin can differentiate women with GDM from women without GDM.

3. Methodology

The study was conducted at the antenatal clinic of the University of Port Harcourt Teaching Hospital from February 2021 to March 2022. The hospital is a referral center for Rivers State Nigeria and neighboring states. The study was a prospective cross-sectional study of pregnant women between 24 to 28 weeks of gestation. The women were given information on the purpose of the study, and the study procedure including potential harms and benefits of the study. Those who expressly declared their intentions to be part of the study were recruited. Inclusion criteria: Women who gave consent, and women between 24 to 28 weeks of gestation. Exclusion criteria: Women with diabetes mellitus, chronic liver disease, or chronic kidney disease, women with unsure last menstrual period, and no early ultrasound scan determination of their gestational age. The sampling technique was a simple random sampling technique. On each clinic day, all the pregnant women who met the inclusion criteria were given a serial number, and their serial numbers were entered into a computer. Using a table of random numbers, the computer randomly selected four (4) women for the study each day. The process continued until the sample size of 200 women was achieved. The study instrument was a case record form which contained the following section demographic characteristics, fasting plasma glucose, one hour and two hours oral glucose tolerance test and the glycated albumin values.

The participants were told to have their usual diet for at least three days, and they fasted for 8 to 16 hours from the previous day before blood sample collection the next morning. The time of arrival was 7:00 AM each day and sample collection commenced by 8:00 AM (after 30 minutes of rest). Fasting blood samples were collected and the women drank 75g of glucose (in 300ml of sterile water) in less than five minutes. Two more blood samples were then collected at an interval of one hour. The samples were collected in a Fluoride Oxalate sample container and sent to the chemical pathology laboratory. Within 4 to 6 hours of sample collection, the oxidase method was used for the analysis of the plasma glucose. The diagnosis of GDM was based on the WHO 2013 diagnostic criteria.

The blood samples for glycated albumin were collected the same day blood glucose samples were collected. The glycated albumin samples were collected into an Ethylenediamine tetra-acetic acid bottle and transported to the Laboratory. The glycated albumin was analyzed with the Enzyme-Linked Immunosorbent Assay technique.

The analysis of the data was done using the Statistical Product and Services Solutions version 25.0. The diagnostic cut-off of glycated albumin was determined using the receiver operator characteristic (ROC) curve. The comparison of means was done using the Student's T-test. The confidence interval was at 95% and the significance was at a p-value of <0.05.

4. Results

4.1. Demographic characteristics

Table 1 shows the demographic characteristics of the women. The mean age of the women was 31.08 (±5.12) years. Almost half (42.5%) of the women were nulliparous and most of the women (52.0%) were overweight.

Table I: Demographic characteristics of the study population

	Frequency (n=200)	Percentage (%)
Age (years)		
≤19	3	1.5
20 - 34	148	74.0
≥35	49	24.5
Parity		
P0	85	42.5
P1	53	26.5
P2	37	18.5
P3	10	5.0
P4	12	6.0
≥P5	3	1.5
Body Mass Index		

18.5 - 24.9	18	9.0
25 – 29.9	104	52.0
≥ 30	78	39.0

4.2. Optimal cut-off of glycated albumin in the diagnosis of GDM

Figure 1 shows the ROC curve with the area under the curve (AUC) above the diagonal line. **Table 2** shows the AUC value of the ROC curve and glycated albumin cut-off value. **Table 3** shows the cross-tabulation for the determination of the sensitivity and specificity of glycated albumin using an optimal cut-off of 19.0%.

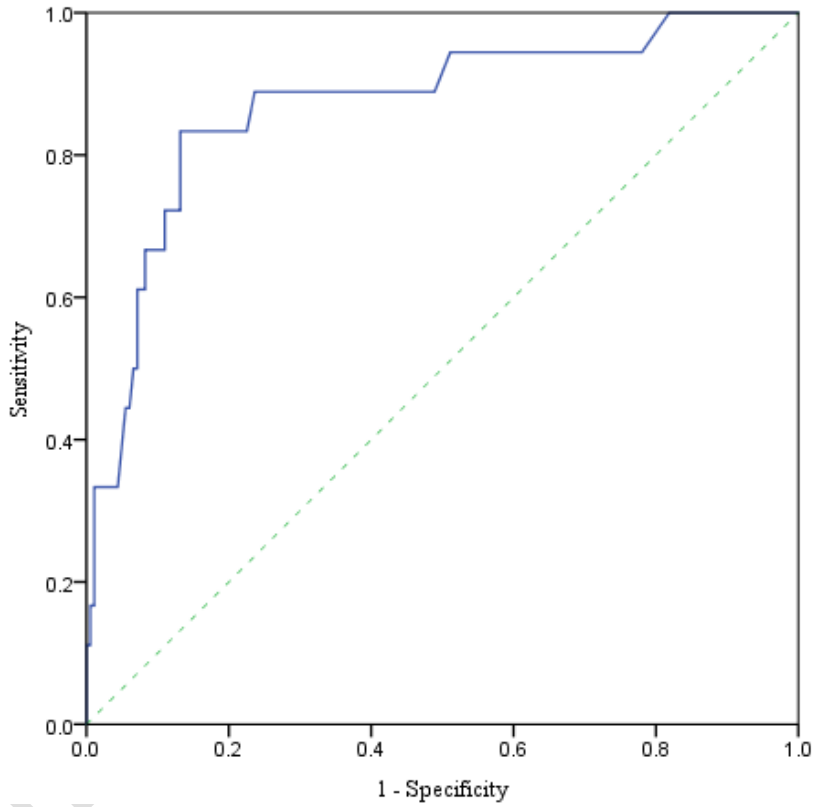

Figure 1: ROC curve showing the different cut-offs of glycated albumin in the diagnosis of GDM.

Table 2: Summary of ROC findings on Glycated albumin in the diagnosis of GDM

ROC findings	**Values**
AUC (95% CI)	0.8 (0.7 – 0.9)
p-value	<0.0001*
Optimal cut-off value of GA	19.0%

AUC – Area under the Curve; CI – Confidence intervals; *Statistically significant.

Table 3: Cross-tabulation for determination of sensitivity and specificity of glycated albumin

	OGTT (Gold standard)	

		Yes	No	Total
Glycated Albumin (screening test)	Elevated GA (≥19.0%)	15 True positive	24 False positive	39
	Normal GA (<19.0%)	3 False-negative	158 True negative	161
	Total	18	182	200

4.3. Glycated albumin level among women with GDM and women without GDM

Figure 2 is a bar chart showing the percentage of the study population who has elevated glycated albumin. Women who had GDM and an elevated glycated albumin value above the optimal cut-off value of ≥19% were 39 (19.5%). **Table 4** shows that women with GDM have a significantly elevated glycated albumin level compared to women without GDM.

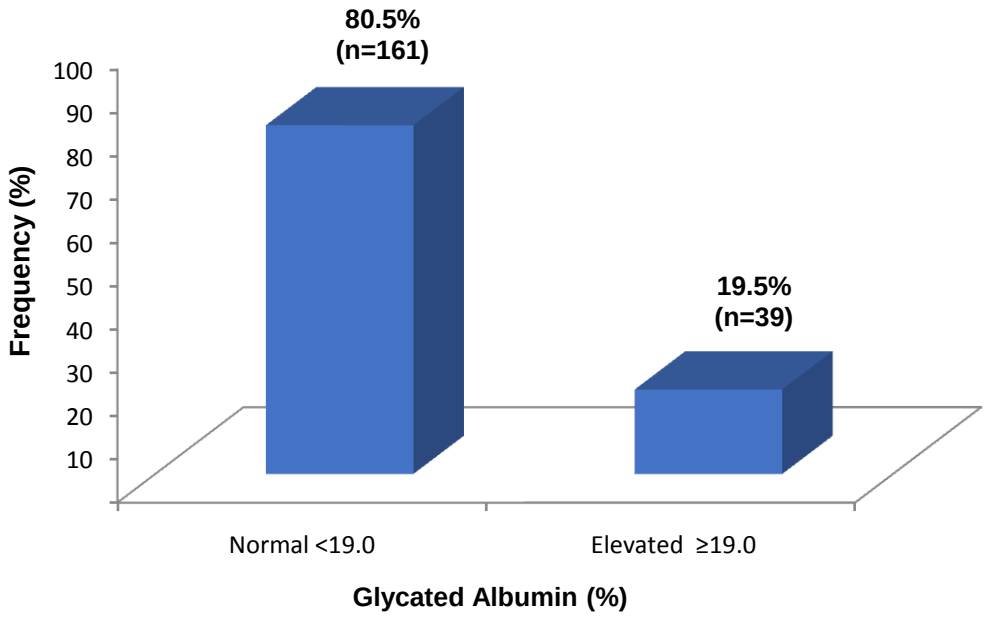

Figure 2: Glycated albumin level among women with GDM and women without GDM

Table 4: Comparison of glycated albumin levels among women with GDM and women without GDM

	With GDM Mean ±SD	**Without GDM Mean ±SD**	**p-value**	**95% CI**
Glycated albumin	**21.4 ±3.2**	**16.5 ±2.3**	**<0.0001***	**3.7 - 6.5**

SD-Standard deviation; *=Statistically significant at p<0.05

5. Discussion

This study shows that glycated albumin is significantly elevated in women who have GDM compared to women without GDM. The mean glycated for women with GDM in this study was higher than the values reported in studies done among Caucasians and Asians [31,32]. A Report from China showed that the mean glycated albumin level of women with GDM was 11.7% (±1.5) [31] which is lower compared to the mean value of 21.4% (±1.5) obtained in this study. This may be due to racial differences associated with glycated albumin. A study that compared the mean glycated albumin among Black Americans and Caucasians reported that Black Americans had significantly higher glycated albumin levels in participants without diabetes mellitus and participants with diabetes mellitus [28].

The optimal cut-off can be determined from the area under the ROC curve: it is the point along the curve where the diagnostic test has the best sensitivity and specificity [33,34]. The Optimal cut-off is chosen based on the need for a diagnostic test to have a higher sensitivity and lower specificity or vice versa [34,35]. In our study, the diagnostic cut-off of glycated albumin was chosen to be at ≥19.0%. This cut-off value was chosen because it is the point where the sensitivity and specificity of glycated albumin have a fair balance in diagnosing GDM. A value above this cut-off will reduce sensitivity and increase specificity, while a point below this cut-off will increase sensitivity and reduce specificity. The calculated sensitivity and specificity based on the glycated albumin diagnostic cut-off value of ≥19.0% were 83.3% and 86.8% respectively. The diagnostic cut-off in this study was higher than the values reported in some studies. In Shanghai a diagnostic cut-off value of ≥11.6% was gotten and for a sensitivity of 75.9% and specificity of 86.4% [30].

Dong et al report that women with GDM had a higher GA level than women without GDM, but the difference was not significant [36]. In this study, the glycated albumin level was significantly higher in women with GDM than in women without GDM. The method used in the analysis of glycated albumin levels may explain the difference in the findings between these studies. While glycated albumin was analyzed by an enzymatic method in this study, Dong et al used the peroxidase analysis method. The finding in our study is in keeping with other studies where analysis of glycated albumin was done using an enzymatic method. The glycated albumin of women with GDM and women without GDM were analyzed by Li et al using an enzymatic method,

they found that women with GDM had significantly higher GA levels [31]. Findings by Dong et al may also be different from our findings because of the difference in the method of patient selection. Dong et al recruited pregnant women in all trimesters of pregnancy and they excluded women who had a BMI of ≥25kg/M^2. A high body mass index is a known risk factor for GDM [22,27]. Women with a BMI of ≥25kg/M were not excluded in our study and participants were between 24 to 28 weeks of gestation.

The sampling method was a simple random sampling which gave an equal chance of selection of the participants. This method eliminated bias and allowed a balanced selection of participants that can give the best representation of the general population. The pregnant women selected for the study did not do a liver or kidney function test. An underlying metabolic abnormality may affect the levels of plasma proteins including glycated albumin. It is recommended that screening for GDM should be done at first contact with a pregnant woman,[3] but this study was restricted to pregnant women between 24 to 28 weeks of gestation.

Conclusion

Gestational diabetes mellitus causes maternal and fetal morbidities and mortalities. Oral glucose tolerance test which is used for diagnosis of GDM requires patient preparation, drinking glucose solution, and multiple sample collection, therefore is cumbersome. Glycated albumin has an area under the ROC curve of 0.85 with an optimal cut-off value of 19.0%. Glycated albumin is significantly elevated in women with GDM than in women without GDM with a sensitivity of 83.3% and a specificity of 86.8%. Therefore, glycated albumin can be used to diagnose GDM.

References

1. Chong, H. P., Alazzani, H., and Boelaert, K. (2019). Endocrine disorders in pregnancy. *Obstet Gynecol Reprod Med*, **29**, 301-305. doi:10.1016/j.ogrm.2019.08.005. https://doi.org/10.1016/j.ogrm.2019.08.005

2. Ogu, R.N., John C.O., Maduka, O., Chinenye, S. (2017) Screening for Gestational Diabetes Mellitus: Findings from a Resource Limited Setting of Nigeria. *Br J Med Med Res*, **20**, 1-8. https://doi.org/10.9734/BJMMR/2017/31966
3. John, D.H., Awoyesuku, P.A., MacPepple, D.A. and Kwosah, N.J. (2019) Prevalence of Gestational Diabetes Mellitus and Maternal and Fetal Outcomes at the Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Nigeria. *JAMMR*, **31**, 1-16. https://doi.org/10.9734/jammr/2019/v31i930319
4. Eades, C.E., Cameron, D.M. and Evans, J.M.M. (2017) Prevalence of Gestational Diabetes Mellitus in Europe: A Meta-analysis. *Diabetes Res Clin Pract,* **129**, 173-181. https://doi.org/10.1016/j.diabres.2017.03.030
5. Lee, K.W., Ching, S.M., Ramachandran, V., Yee, A., Hoo, F.K., Chia, Y.C et al. (2018) Prevalence and risk factors of gestational diabetes mellitus in Asia: a systematic review and meta-analysis. *BMC Pregnancy Child B*,**18**, 1-20. https://doi.org/10.1186/s12884-018-2131-4
6. Muche, A.A., Olayemi, O.O. and Gete, Y.K. (2019) Prevalence and Determinants of Gestational Diabetes Mellitus in Africa Based on the Updated International Diagnostic Criteria: A Systematic Review and Meta-analysis. *Arch Public Health,* **77**, 1-20. https://doi.org/10.1186/s13690-019-0362-0
7. John, D.H., Awoyesuku, P.A., MacPepple, D.A. and Kwosah, N.J. (2019) Prevalence of Gestational Diabetes Mellitus and Maternal and Fetal Outcomes at the Rivers State University Teaching Hospital (RSUTH), Port Harcourt, Nigeria. *JAMMR*, **31**, 1-16. https://doi.org/10.9734/jammr/2019/v31i930319
8. Billionnet, C., Mitanchez, D., Weill, A., Nizard, J., Alla, F., Hartemann, A. et al. (2017) Gestational Diabetes and Adverse Perinatal Outcomes from 716,152 Births in France in 2012. *Diabetologia*, **60**, 636–644. https://doi.org/10.1007/s00125-017-4206-6
9. Battarbee, A.N., Venkatesh, K.K., Aliaga, S. and Boggess, K.A. (2020) The Association of Pregestational and Gestational Diabetes with Severe Neonatal Morbidity and Mortality. *J Perinatol,* **40**, 232-239. https://doi.org/10.1038/s41372-019-0516-5

10. Sathiamma, P.K. and Lalithambica, K. A. (2017) Prospective Study on Maternal and Perinatal Outcome of Gestational Diabetes Mellitus. *Int J Reprod Contracept Obstet Gynecol*, **6**, 2933-2938. https://doi.org/10.18203/2320-1770.ijrcog20172910
11. Farahvar, S., Walfisch, A. and Sheiner E. (2019) Gestational Diabetes Risk Factors and Long-term Consequences for Both Mother and Offspring: A Literature Review. *Expert Rev Endocrinol Metab*, **14**, 63-74. https://doi.org/10.1080/17446651.2018.1476135
12. Bianco, M.E. and Josefson, J.L. (2019) Hyperglycemia During Pregnancy and Long-Term Offspring Outcomes. *Curr Diab Rep*, **19**, 143. https://doi.org/10.1007/s11892-019-1267-6
13. McIntyre, H.D., Catalano, P., Zhang, C., Desoye, G., Mathiesen, E.R. and Damm, P. (2019) Gestational Diabetes Mellitus. *Nat Rev Dis Primers*, **5**, 47. https://doi.org/10.1038/s41572-019-0098-8
14. Hymavathi, K., Tadisetti, S., Polisetty, K., Gottipatti, M.D. (2016) Gestational Diabetes Mellitus-Universal Versus Selective Screening. *Int J Reprod Contracept Obstet Gynecol,* **5**, 2155-2160. https://doi.org/10.18203/2320-1770.ijrcog20162083
15. World Health Organization. (2013) Diagnostic Criteria and Classification of Hyperglycemia First Detected in Pregnancy. http://apps.who.int/iris/bitstream/10665/85975/1/WHO_NMH_MND_13.2_eng.pdf.
16. National Institute for Health and Care Excellence. (2020) Diabetes in pregnancy: management from preconception to the postnatal period. nice.org.uk/guidance/ng3.
17. Hod, M., Kapur, A., Sacks, D.A., Hadar, E., Agarwal, M., Di Renzo, G.C. et al. (2015) The International Federation of Gynecology and Obstetrics (FIGO) Initiative on gestational diabetes mellitus: A Pragmatic Guide for Diagnosis, Management, and Care. *Int J Gynecol Obstet,* **131**, S173–S211. https://doi.org/10.1016/S0020-7292(15)30007-2
18. Jagannathan, R., Neves, J.S., Dorcely, B., Chung, S.T., Tamura. K., and Rhee, M. (2020) The Oral Glucose Tolerance Test: 100 Years Later. *Diabetes Metab Syndr Obes,* **13**, 3787-3805. https://doi.org/10.2147/DMSO.S246062
19. Bogdanet, D., O'Shea, P., Lyons, C., Shafat, A., and Dunne, F. (2020) The Oral Glucose Tolerance Test—Is It Time for a Change?—A Literature Review with an Emphasis on Pregnancy. *J Clin Med*, **9**, 3451. https://doi.org/10.3390/jcm9113451

20. Nanda, R., Patel, S., Sahoo, S., and Mohapatra, E. (2018) Review of Pre-Analytical Errors in Oral Glucose Tolerance Testing in a Tertiary Care Hospital. *J Nepal Health Res Counc*, **16**, 6-10. https://doi.org/10.3126/jnhrc.v16i1.19353
21. Agarwal, M.M., Punnose, J., and Dhatt, G.S. (2004) Gestational Diabetes: Problems Associated with the Oral Glucose Tolerance Test. *Diabetes Res Clin Pract*, **63**, 73-4. https://doi.org/10.1016/j.diabres.2003.08.005
22. Freitas, P.A.C., Ehlert, L.R., and Camargo, J.L. (2017) Glycated albumin: a potential biomarker in diabetes. *Arch Endocrinol Metab*, **61**, 296–304. https://doi.org/10.1590/2359-3997000000272
23. Piuri, G., Basello, K., Rossi, G., Soldavini, C.M, Duiella, S., Privitera, G. et al. (2020) Methylglyoxal, Glycated Albumin, PAF, and TNF- Possible Inflammatory and Metabolic Biomarkers for Management of Gestational Diabetes. *Nutrients*, **12**, 479. https://doi.org/10.3390/nu12020479
24. Zendjabil, M. (2019) Glycated albumin. *Clinica Chimica Acta*. **502**, 240-244. https://doi.org/10.1016/j.cca.2019.11.007
25. Neelofar, N., Ahmad, J. (2017) An overview of in vitro and in vivo glycation of albumin: a potential disease marker in diabetes mellitus. *Glycoconj J*, **34**, 575-584. https://doi.org/10.1007/s10719-017-9789-0
26. Dozio, E., Di Gaetano, N., Findeisen, P., and Corsi, R.M.M. (2017) Glycated Albumin: From Biochemistry and Laboratory Medicine to Clinical Practice. *Endocrine*, **55**, 682-690. https://doi.org/10.1007/s12020-016-1091-6
27. George, J.A., and Erasmus, R.T. (2018) Haemoglobin A1c or Glycated Albumin for Diagnosis and Monitoring Diabetes: An African Perspective. *Ind J Clin Biochem*, **33**, 255–261. https://doi.org/10.1007/s12291-018-0755-9
28. Selvin, E., Steffes, M.W., Ballantyne, C.M., Hoogeveen, R.C., Coresh, J., and Brancati, F.L. (2011) Racial Differences in Glycemic Markers: A Cross-sectional Analysis of Community-Based Data. *Ann Intern Med*, **154**, 303–309. https://doi.org/10.7326/0003-4819-154-5-201103010-00004

29. Xiong, J.Y., Wang, J.M., Zhao, X.L., Yang, C., Jiang, X.S., Chen, Y.M., et al (2021) Glycated albumin as a biomarker for diagnosis of diabetes mellitus: A systematic review and meta-analysis. *World J clin Cases*, **9,** 9520-9534. https://dx.doi.org/10.12998/wjcc.v9.i31.9520

30. Liu, X., Wu, Na., Al-mureish, A. (2021) A Review on Research Progress in the Application of Glycosylated Hemoglobin and Glycated Albumin in the Screening and Monitoring of Gestational Diabetes. *Int J Gen Med*, **14,** 1155-1165. https://doi.org/10.2147/IJGM.S296316

31. Li, H., Wang, F., Tao, M., Huang, Y. and Jia, W. (2016) Association between glycemic control and birthweight with glycated albumin in Chinese women with gestational diabetes mellitus. *J Diabetes Investig*, **7**, 48–55. https://doi.org/10.1111/jdi.12383

32. Zhu, J., Chen, Y., Li, C., Tao, M. and Teng, Y. (2018) The diagnostic value of glycated albumin in gestational diabetes mellitus. *J Endocrinol Invest*, **41**, 121–128. https://doi.org/10.1007/s40618-016-0605-7

33. Leeflang MMG, Allerberger F. How to: evaluate a diagnostic test. Clin Microbiol Infect. 2019; 25(1): 54-59. https://doi.org/10.1016/j.cmi.2018.06.011

34. Chikere, C.M.U., Wilson, K., Graziadio, S., Vale, L., Allen, A.J. (2019) Diagnostic test evaluation methodology: A systematic review of methods employed to evaluate diagnostic tests in the absence of gold standard – An update. *PLoS One*, **14**, e0223832. https://doi.org/10.1371/journal.pone.0223832

35. Obuchowski NA, Bullen JA. Receiver operating characteristic (ROC) curves: review of methods with applications in diagnostic medicine. Phys Med Biol. 2018; 63(7): 07TR01. https://doi.org/10.1088/1361-6560/aab4b1

36. Dong Y, Zhai Y, Wang J, Chen Y, Xie X, Zhang C et al. Glycated albumin in pregnancy: reference intervals establishment and its predictive value in adverse pregnancy outcomes. BMC Preg Childbirth. 2020; 20(12): 1-9. https://doi.org/10.1186/s12884-019-2704-x