Evaluation of Serum Fructosamine as a Screening Test for Gestational Diabetes Mellitus in Nigerian Women

[1]Haladu H & [2]Yahaya IA
[1]Department of Chemical Pathology & Immunology,
Federal Medical Centre,
Katsina
sadiamuallim@yahoo.co.uk
[2]Department of Chemical Pathology & Immunology,
Bayero University/Aminu Kano Teaching Hospital,
Kano.
yahadagiri2@yahoo.com

Abstract
Background: *The use of serum fructosamine as a screening test for gestational diabetes mellitus (GDM) may provide a simpler alternative to oral glucose tolerance test (OGTT) which is tedious, as it requires multiple venepunctures, overnight fasting and may be associated with unpleasant side effects.*
Aim: *This study evaluated the clinical usefulness of serum fructosamine as a screening test for the detection of GDM in Nigerian women.*
Methods: *This descriptive cross sectional study recruited a total of 313 subjects, made up of 193 pregnant women with risk factors for GDM referred from antenatal clinic for oral glucose tolerance test (OGTT) and 120 apparently healthy non pregnant women as controls. A 75 g OGTT, serum glucose, fructosamine, albumin and total protein measurements were performed on the subjects.*
Results: *Forty one of the 193 pregnant women (21.2%) were diagnosed of GDM based on the World Health Organisation's (WHO) criteria for OGTT while 38.9% of the pregnant women had GDM based on cut-off point for the diagnosis of GDM using fructosamine. In comparison with 75 g OGTT, single assay of serum fructosamine gave sensitivity, specificity, positive and negative predictive values of 44%, 63%, 24% and 63% respectively for the detection of GDM.*
Conclusion: *Serum fructosamine may not be a useful screening test for the detection of GDM in the studied population, the Nigerian women.*

Keywords: *Fructosamine; Oral glucose tolerance test; Gestational diabetes mellitus.*

Introduction

Gestational diabetes mellitus (GDM) is defined as a disorder of glucose tolerance with first onset or recognition during pregnancy (Dornhorst and Rossi, 1998). GDM is a problem that affects a significant number of women during pregnancy worldwide with prevalence's that range from 1-14% depending on the study population and the diagnostic criteria employed (Ben-Haroush *et al*., 2004). In the United States of America, the prevalence of GDM ranges from 4.6 - 9.2% (Desisto *et al*., 2014). The prevalence of GDM in Africa ranged from 0% in Tanzania to 13.9% in Nigeria (Macaulay *et al*., 2014).

GDM have significant and lasting health impacts on both the mother and foetus (Kvetny *et al*., 1999). In order to circumscribe and minimise potential complications to both the mother and the child, screening, diagnosis and management of hyperglycaemia are critical. Thus, routine screening for GDM during antenatal care is of paramount importance.

Oral glucose tolerance test (OGTT) is considered worldwide as the gold standard for the diagnosis of GDM. But the procedure for OGTT is time consuming and inconvenient for most patients, as they are required to fast for a minimum of eight hours and it also involves collection of several blood samples over a period of two to three hours (Bhavadharini *et al*., 2016). In addition, it may be associated with unpleasant side effects like nausea and vomiting (Crook, 2012). The need for simpler methods for the detection of GDM therefore, becomes imperative. Some researchers have suggested that measurement of fructosamine could serve as an alternative to the glucose challenge test (GCT) and OGTT in screening for GDM, and it can also be used to monitor glucose control during pregnancy (Roberts and Baker, 1986; Agarwal *et al*., 2001; Perea-Carrasco *et al*., 2002).

Serum fructosamine assay has shown validity in monitoring recent glycaemic alterations and has been proposed to be useful in GDM patients, in whom short-term confirmation of the maternal glycaemic state is clinically warranted (Artal *et al*., 1984; Leiper *et al*., 1985; Cefalu *et al*., 1988). Furthermore, fructosamine assay does not require prior fasting and the second generation assay methods currently in use for fructosamine measurement are rapid, inexpensive and free from interferences by urates and triglycerides (Perea-Carrasco *et al*., 2002).

This study was therefore conducted to assess the usefulness of serum fructosamine as an alternative method to OGTT in the detection of GDM among pregnant women in Kano.

Materials and Methods

This was a descriptive cross sectional study conducted among two groups (I and II) of women in a tertiary hospital in northern Nigeria. Group I were 193 pregnant women at 24 to 28 weeks of gestation (with risk factors for GDM) who were referred from the antenatal clinic of the hospital for OGTT. The risk factors for GDM considered were: glycosuria, intrauterine foetal deaths, family history of diabetes mellitus, booking weight > 90 kg, macrosomia, previous history of neonatal deaths, and history of recurrent miscarriages. The controls (Group II) were 120 non-pregnant, apparently healthy women that attended immunization clinic of the hospital.

Pregnant women with established GDM, known diabetes mellitus patients, patients with disorders that can affect serum albumin level like nephrotic syndrome, liver cirrhosis and monoclonal gammopathy were all excluded from the study.

Ethical approval for the conduct of the study was obtained from the Research Ethical Committee of the hospital. Informed consent was also obtained from all the subjects and all data collected from the subjects were kept confidential.

Pre- tested structured questionnaire was used to record the bio-data of the subjects.

Subsequently, the Blood Pressure, weight and height of each subject were measured using Accoson mercury sphygmomanometer, RGZ-120 Health weighing scale and stadiometre RGZ- 120 Health scale respectively. The body mass index (BMI) of each of the subjects was calculated as weight in kilogram divided by height in squared metres (kg/m^2).

Serum samples were collected from the subjects for the measurement of serum fructosamine, albumin, total proteins and a Standard 2-hour 75 g oral glucose tolerance test (OGTT) was performed on each of the pregnant women (patients) after an overnight fast.

Quantitative measurement of serum fructosamine was done using nitroblue tetrazolium method (Siedel *et al*., 1988) on Cobas Integra 400 plus (Roche) automated chemistry analyser (Kruse-Jarres *et al*., 1989). The fructosamine results thus obtained were corrected (for total proteins) in the pregnant women using the formula below:

$Fructosamine_{corr}$ = measured fructosamine X 72/measured total protein (g/L) (Kruse- Jarres *et al*., 1989).

Plasma total proteins and glucose were estimated manually using Biuret method and glucose oxidase method respectively (Doumas *et al*., 1971; Howanitz and Howanitz, 1984).
Statistical analysis of the Data obtained were analysed using statistical package for social sciences (SPSS) version 22.0. The serum fructosamine, plasma glucose (fasting plasma glucose and serial plasma glucose during OGTT), total proteins and albumin levels were presented as mean and standard deviation in tabular form. Analysis of variance (ANOVA) was used to compare the means between the three groups (GDM patients, non-GDM patients and controls), while t-test was used to compare means between the GDM and non-GDM patients. Qualitative variables were compared using Chi-squared test. The alpha level of statistical significance was set at < 5% (0.05).

Outcome measures
1. The results of OGTT were interpreted using WHO criteria (Schmidt *et al*., 2001).
2. The result of serum fructosamine was considered to be in the diabetic range when the value is >332 µmol/L (upper reference limit established from the distribution of serum fructosamine in the controls used for this research.

Results:
The reference range for serum fructosamine concentration was established using values of the control group, as 158.2 to 332 µmol/L representing the 2.5^{th} and 97.5^{th} percentiles (Table 1). The mean and median values of fructosamine were 221 µmol/L and 212 µmol/L respectively.
The baseline characteristics of the studied subjects showed no significant difference between the patients and the controls except the body mass index (Table 2).
The serum fructosamine levels and OGTT results of the patients are presented in table 3, showing statistically significant differences ($p < 0.001$) in all the plasma glucose values between patients with GDM and those without GDM. The GDM patients had higher values of plasma glucose all through. However, there was no significant difference in the serum fructosamine levels between the GDM and non-GDM patients.
Table 4 shows significant difference in the serum fructosamine, albumin and total proteins levels between the patients and controls. The control group had lower levels of serum fructosamine but higher levels of serum albumin and total proteins.
The prevalence of GDM was 21.2% and 38.9% based on OGTT and single measurement of serum fructosamine respectively (Table 5).
Table 6 shows the performance characteristics of fructosamine as a screening test for GDM using OGTT as gold standard. The sensitivity, specificity, positive predictive value and negative predictive value were 44%, 63%, 24% and 81% respectively.

Discussion
The similarity in the age distribution of the study subjects (patients and controls) renders the results obtained from the study comparable between the two groups and is therefore desirable.
The patients diagnosed of GDM in this study were more obese than the non-GDM patients. This observation could be explained by the fact that obesity has been strongly linked with the development of GDM (Ben-Haroush *et al*., 2004). In a population-based cohort study of about 97,000 singleton births; it was observed that obese women had a 3-fold higher risk of developing GDM than non-obese women (Bianco *et al*., 1998).
The lower mean serum level of serum fructosamine found among the controls compared to the levels found among the pregnant patients was expected as the controls were apparently healthy, non-pregnant, non diabetic women (with mean fasting serum glucose of 4.3 mmol/L) compared to the patients who were pregnant and some of them had GDM. However, the

mean serum fructosamine was not statistically different between the GDM and non-GDM patients. This finding is consistent with reports from previous studies by Cefalu *et al*. (1988) and Comtois *et al*. (1989) who found no significant difference in the levels of serum fructosamine between GDM and non-GDM patients, even though none of the researchers made mention of the *p*- values obtained in their study.

The validity of a screening test could be determined by the sensitivity, specificity, positive predictive value and negative predictive value (Gambino, 2012). Sensitivity of a screening test refers to the ability of test to designate an individual with disease as positive. A highly sensitive test would give few false negative results, and thus fewer cases of disease are missed. It is desirable for a good screening test to have high sensitivity index. In this study, the sensitivity of serum fructosamine as a screening test for GDM was 44% which means that serum fructosamine was only able to detect 44% of GDM cases as diagnosed by the gold standard test (OGTT). GDM (a disease associated with serious complications especially to the foetus), will be missed in about 6 out 10 positive cases. Thus, the sensitivity of fructosamine is quite low for the detection of GDM.

The specificity of a test is its ability to designate an individual who does not have a disease as negative. A highly specific test would give few false positive results. It may not be feasible to use a test with low specificity for screening, since many people without the disease will be screened as positive, as those individuals would potentially undergo unnecessary diagnostic procedures. The specificity of serum fructosamine in this study was 63% implying that serum fructosamine was able to identify less than two-third of those that do not have GDM as negative. It is desirable to have a test that is both highly sensitive and highly specific.

Positive predictive value (PPV) is the probability that a patient with a positive (abnormal) test result actually has the disease. The positive predictive value of fructosamine was 24% meaning that if a pregnant woman screens positive for GDM using serum fructosamine, there is only a 24% probability that she has GDM. Though, the PPV is dependent on the disease prevalence (that is, higher in diseases with high prevalence irrespective of the sensitivity and specificity), it is quite low as the observed prevalence of GDM in this study is relatively high.

Negative predictive value (NPV) is the probability that a person with a negative (normal) test result is truly free of disease. This study reported a negative predictive value of 81% which means that if a pregnant woman screens negative for GDM using serum fructosamine, there is 81% probability that she does not have GDM.

Findings relatively similar to those obtained in this study were reported by Cefalu *et al*. (1990) who obtained sensitivity and specificity of 15.4% and 86.9% respectively. Similarly, a research conducted by Bor *et al*. (1999) observed a sensitivity, specificity, positive predictive value and negative predictive value of 41.7%, 85.7%, 29.4% and 91% respectively and therefore concluded that serum fructosamine cannot be used as a screening test for GDM. Comtois *et al*. (1989) also reported that fructosamine is an insensitive screening test for the detection of GDM (in patients with risk factors) because they found that the serum fructosamine levels were not significantly different between the patients with GDM and those without GDM. Similarly, Salemans *et al.* (1987) and Corcoy *et al.* (1991) demonstrated in their studies that serum fructosamine was an insensitive parameter for GDM screening with sensitivities of 2.26% and 17% respectively. Serum fructosamine was also found to be unsuitable as a screening test for diabetes mellitus in a study conducted on a non-pregnant population by Swai *et al*. (1988).

The finding of this study was further confirmed by Agrawal and co-researchers who recruited 849 pregnant women for the evaluation of serum fructosamine as a screening test for GDM. In their study, fructosamine had a sensivity of 85.8%, a poor specificity of 23.4% and a

positive predictive value of 14.7%. They therefore concluded that serum fructosamine was a poor screening test for GDM (Agarwal *et al.*, 2011).

However, the results of this study were in contrast with those of Roberts and colleagues who reported sensitivity, specificity, positive predictive value and negative predictive value of 86%, 95%, 66% and 98% respectively (Roberts *et al*., 1986). The high sensitivity of serum fructosamine reported by Roberts and colleagues might be explained by the fact that in four out of the nine GDM patients detected by OGTT, the abnormally high serum fructosamine values were obtained much later (after 8 weeks) following the diagnosis of GDM. Therefore, the long duration of hyperglycaemia was probably responsible for the abnormal serum fructosamine levels. Weerasekera et al. (2000) also reported high sensitivity and specificity (87.5% and 94.5% respectively) for serum fructosamine but the cut-off point used by these researchers for serum fructosamine was much lower (265 µmol/L) than the cut-off point used in the current study (332 µmol/L) and serum fructosamine concentration was not corrected for serum total proteins levels in their study.

Conclusion

Serum fructosamine had low sensitivity, low specificity and low positive predictive value thereby rendering it a poor screening test for the detection of GDM.

References

Agarwal, M,M,, Dhattm G,S,, Othman, Y., & Ljubisavljevic, M.R. (2011). Evaluation of Serum Fructosamine as Screening Test in a High Risk Population. Gynecol Obstet Invest, 71, 207-212.

Agarwal, M.M., Hughes, P.F., Punnose, J., Ezimokhai, M., & Thomas, L. (2001). Gestational diabetes screening of a multiethnic, high-risk population using glycated proteins. *Diabetes Res Clin Pract*, 51, 67-73.

Artal, R., Mosley, G.M., & Dorey, F.J. (1984). Glycohaemoglobin as a screening test for gestational diabetes. Am J Obstet Gynaecol, 148, 412-414.

Ben-Haroush, A., Yogev, Y., & Hod, M. (2004). Epidemiology of gestational diabetes mellitus and its association with type 2 diabetes. *Diabet Med*, 21, 103-113.

Bhavadharini, B., Mahalakshmi, M.M., Maheswari, K., Kalaiyarasi, G., Anjana, R.M., Deepa, M. et al. (2016). Use of capillary blood glucose for screening for gestational diabetes mellitus in resource-constrained settings. *Acta Diabetol*, 53, 91–97.

Bianco, A.T., Smilen, S.W., Davis, Y., Lopez, S., Lapinski, R., & Lockwood, C.J. (1998). Pregnancy outcome and weight gain recommendations for the morbidly obese woman. Obstet Gynecol, 91, 97-102.

Bor, M., Bor, P., Cevik, C. (1999). Serum fructosamine and fructosamine-albumin ratio as screening tests for gestational diabetes mellitus. Arch Gynecol Obstet, 262, 105-111.

Cefalu., W.T., Prather, K.L., Chester, D.L., Wheeler, C.J., Biswas, M., & Pernoll, M.L. (1990). Total Serum Glycosylated Proteins in Detection and Monitoring of Gestational Diabetes. Diabetes Care, 13, 872-875.

Cefalu, W.T., Parker, T.B., & Johnson, C.R. (1988). Validity of serum fructosamine as index of short-term glycemic control in diabetic out-patients. Diabetes Care, 11, 662-664.

Comtois, R., Desjarlais, F., Nguyen, M., & Beauregard, H. (1989). Clinical usefulness of estimation of serum fructosamine concentration as screening test for gestational diabetes. Am J Obstet Gynaecol, 160, 651-654.

Corcoy, R., Cerqueira, M.J., Pedreno, J., Matas, J., Codina, M., Pou, J.M., et al. (1991). Serum fructosamine is not a useful screening test for gestational diabetes. *Eur J Obstet* Gynecol Reprod Biol, 38, 217-220.

Crook, M.A. (2012). Carbohydrate metabolism. Clinical biochemistry and metabolic

medicine. 8th edition, London. Hodder Arnold, 176-199.

Desisto, C.L., Kim, S.Y., & Sharma, A.J. (2014). Prevalence Estimates of Gestational Diabetes Mellitus in the United States, Pregnancy Risk Assessment Monitoring System. *Prev Chronic Dis*, 11, 1304-1315.

Dornhorst, A., & Rossi, M. (1998). Risk and Prevention of Type 2 Diabetes in women with Gestational Diabetes. *Diabetes Care*, 21, 43-49.

Doumas, B.T., Watson, W.A., & Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chem Acta, 1971, 31, 87.

Gambino, B. (2012). The validity of screening tests: meet the new screen same as the old screen? J Gambl stud, 28, 573-605.

Howanitz, P.J., & Howanitz, J.H. (1984). Carbohydrates. In: Henry JB. Clinical diagnosis and management by laboratory methods. 17th edition, Philadelphia. WB Saunders, 165-179.

Kruse-Jarres, J.D., Jarausch, J., Lehmann, P., Vogt, B.W., & Rietz, P. (1989). A new colometric method for the determination of fructosamine. Lab Med, 13, 245-253.

Kvetny, J., Poulsen, H.F., & Damgaard, D.W. (1999). Results from screening for gestational diabetes mellitus in a Danish country. *Dan Med Bull*, 46, 57-59.

Leiper, J,M,, Talwar, D., Robb, D.A., Lunan, C.B., & MacCuish, A.C. (1985). Glycosylated albumin and glycosylated proteins: rapidly changing indices of glycaemia in diabetic pregnancy. Q J Med, 218, 225-231

Macaulay, S., Dunger, D.B., & Norris, S.A. (2014). Gestational Diabetes Mellitus in Africa: A Systematic Review. *PLoS One*, 9, 4 -11.

Perea-Carrasco, R., Perez-Coronel, R., Albusac-Aguilar, R., Lombardo-Grifol, M., Bassas Baena, E., & Romero-Diaz, C. A (2002). Simple index for detection of gestational diabetes mellitus. *J R Soc Med*, 95, 435-439.

Roberts, A.B., & Baker, J.R. (1986). Serum fructosamine: a screening test for diabetes in pregnancy. Am J Obstet Gynecol, 154, 1027-1030.

Salemans, T.H., Van, Dieijen-Visser, M.P., & Brombacher, P.J. (1987). The value of HbA1c and fructosamine in predicting impaired glucose tolerance-an alternative to OGTT to detect diabetes mellitus or gestational diabetes. Ann Clin Biochem, 24, 447-452.

Schmidt, M.I., Duncan, B.D., Reichelt, A.J., Branchtein, L., Matos, M.C., Forti, A., et al. (2001). The Brazilian Gestational Diabetes Study Group: Gestational diabetes mellitus diagnosed with a 2-hour 75 g oral glucose tolerance test and adverse pregnancy outcomes. Diabetes Care, 24, 1151-1155.

Siedel, J., Vogt, B., Kerscher, L., & Ziegenhorn, J. (1988). Serum fructosamine assay: Two different color reagents compared with reference to a HPLC-procedure. Clin Chem, 34, 1316.

Swai, A.B., Harrison, K., Chuwa, L.M., Makene, W., McLarty, D., & Alberti, K.G. (1988). Screening for diabetes: does measurement of serum fructosamine help? Diabetic Med, 5, 648-652.

Weerasekera, D.S., & Peiris, H. (2000). The value of serum fructosamine in comparison with oral glucose tolerance test as a screening test for detection of gestational diabetes mellitus. J Obstet Gynaecol, 2, 20, 136-138.

Table 1: Distribution of serum Fructosamine among the Controls in percentiles

Percentiles	2.5^{th}	25^{th}	50^{th}	75^{th}	90^{th}	95^{th}	97.5^{th}
Fructosamine (µmol/L)	158.2	192.2	212.0	245.0	277.6	320.9	332.0

Table 2: Baseline characteristics (mean ± SD) of the study subjects

Parameter	Patients (n= 193)	Controls (n=120)	p-value
Age (years)	38.22 ± 5.6	36.54 ± 5.3	0.052
BMI (kg/m^2)	30.01 ± 9.1	25.07 ± 5.5	< 0.001
SBP (mmHg)	125.6 ± 14.4	132 ± 14.0	0.065
DBP (mmHg)	79.01 ± 10.5	73.22 ± 10.6	0.059

SD – Standard Deviation
BMI – Body Mass Index
SBP – Systolic Blood Pressure
DBP – Diastolic Blood Pressure

Table 3: Fructosamine and OGTT Results (mean ±SD) of studied patients

Parameter	**GDM** (n=41)	**Non- GDM** (n= 152)	**P-value**
Fructosamine (µmol/l)	337.39 ± 146	300.39 ± 123	0.102
FPG (mmol/L)	5.87 ± 2.1	3.99 ± 0.7	<0.001
30 min PG (mmol/L)	9.61±2.9	6.11±1.1	<0.001
60 min PG (mmol/L)	10.83 ± 2.6	6.24 ± 1.2	<0.001
90 min PG (mmol/L)	10.75 ± 2.7	5.83 ± 1.1	<0.001
120 min PG (mmol/L)	9.82 ± 2.3	5.23 ± 1.0	<0.001

FPG – Fasting Plasma Glucose
PG – Plasma Glucose

Table 4: Serum Fructosamine, Albumin and Total Protein (mean ± SD) of the Study subjects

Variable	Patients		Controls	p-value
	GDM	Non-GDM		
Albumin (g/L)	30.88 ± 5.6	33.43 ± 7.1	42.25 ± 3.8	< 0.001
Total Proteins (g/L)	62.15 ± 6.3	61.75 ± 7.5	68.52 ± 4.3	< 0.001
Fructosamine (µmol/L)	337.39 ± 146	300.39 ± 123	221.08 ± 41.8	< 0.001

Table 5: Prevalence of GDM using OGTT and Serum Fructosamine

Parameter	Frequency (n=193)	Percentage (%)
OGTT		
Normal	152	78.8
GDM	**41**	**21.2**
Total	193	100.0
Serum Fructosamine		
Normal	118	61.1
GDM	**75**	**38.9**
Total	193	100.0

Table 6: Performance characteristics of Fructosamine as a screening test for GDM

		OGTT		
		Positive	Negative	Total
	Positive	18 (TP)	57 (FP)	75 (TP + FP)
Fructosamine	Negative	23 (FN)	95 (TN)	118 (FN + TN)
	Total	41 (TP + FN)	152 (FP + TN)	193 (TP + FN + FP + TN)

TP – True Positive, FP – False Positive, TN – True Negative, FN – False Negative
Sensitivity = TP/ (TP+FN) = 18/41 = 0.439 = 44%
Specificity = TN/ (TN+FP) = 95/152 = 0.625 = 63%
Positive Predictive Value = TP/ (TP+FP) = 18/75 = 0.240 = 24%
Negative Predictive Value = TN/ (TN+FN) = 95/118 = 0.805 = 81%