Serum levels of fructosamine in healthy non-diabetic Nigerians

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Abstract

Objectives: *Fructosamine (glycated serum proteins) was estimated in 255 healthy non-diabetic Nigerians. This was aimed at developing a new reference interval (RI) for this intermediate-term index of glycaemic control. The RI that is currently in- use in most laboratories across Nigeria were either wrongly derived or are values derived in other countries of the world.*

Methodology: *Fructosamine was estimated using the nitroblue tetrazolium (NBT) assay method as evaluated by Isah in 1990. Random blood glucose, albumin, total proteins and total bilirubin were also measured in all the participants by routine laboratory methods. Non-parametric method was employed for the determination of RI for fructosamine. The new RI for fructosamine was 0.7-1.8 mmol/L as against the RI (0.9-1.8 mmol/L) currently in-use.*

Results: *This study showed a difference in the 2.5th percentile value between male and female participants. Fructosamine also showed positive correlation with glucose, total proteins and albumin. No correlation was observed between fructosamine and total bilirubin.*

Conclusion: *This study described a new reference interval for fructosamine in our indigenous population.*

Keywords: Fructosamine, Glycated serum protein, Reference interval

Introduction

Fructosamine is a ketoamine, introduced into Clinical Chemistry practice by Johnson and Colleagues in 1982.[1] It is a derivative of the non-enzymatic reaction product of sugar (usually glucose) and a protein (usually albumin).[2] Fructosamine is the trivial name for 1-amino-1-deoxyfructosamine, also called isoglucosamine by Emil Fischer, who first synthesized the compound in 1886.[3]

Fructosamine (glycated serum protein) and HbA_1c (glycated haemoglobin) have been

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advocated as glycaemic indices in the long-term assessment of blood glucose control in diabetics. Fructosamine is an intermediate-term indicator of average blood glucose to which the serum proteins have been exposed, while HbA_1c is a long-term indicator of average blood glucose to which the red cells have been exposed to during their life span.[2] Fructosamine has been found to be a more responsive marker of average blood glucose values than HbA_1c. This is because the half-life of albumin and the other serum proteins are considerably shorter than that of haemoglobin. The concentration of fructosamine will change more rapidly than that of glycated haemoglobin.[2,4] Fructosamine thus serves as an index of intermediate-term diabetic control (1-3 weeks) that can alert the Physician to deteriorating glycaemic control before changes in glycated haemoglobin can be detected, which may not be apparent for at least 4 weeks after improvement of glycaemic control.[5,6] Besides being a measure of intermediate-term diabetic control, serum fructosamine may even be useful as a screening test for diabetes and for managing diabetes of pregnancy.[6,7,8]

Although, fructosamine assay is yet to be widely or routinely used in Nigeria, there is the need to provide appropriate reference values that are derived using indigenous healthy reference population.

The reference values in use in Nigeria today were either wrongly derived or are values that were derived in other countries like the United States of America and Europe. These intervals should only serve as guidelines because each country's population differ from one another due to differences in life style and diet.

This study was therefore designed to develop a new reference interval for fructosamine in healthy non- diabetic Nigerians using the most appropriate statistical method.

Materials and Methods

This study was a cross sectional quantitative study aimed at determining a new reference range for fructosamine conducted at the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria-Nigeria.

Ethical approval for the study was obtained from the Ethical Committee of the Ahmadu Bello University Teaching Hospital, Zaria. Participants recruited for the study were informed of the aim, objectives, procedure and clinical implications of the study and their consent obtained accordingly. Participation was entirely voluntary and participants were free to withdraw at any time during the course of the study with no obligations.

Two hundred and fifty-five healthy volunteers (165 males, 90 females) were recruited for the study. They were adults aged between 21 and 60 years of age. They were mainly patients' relations and those who presented at the blood transfusion unit of the Ahmadu Bello Teaching Hospital, Zaria. All the participants were free of both history and clinical evidence of diabetes mellitus, cardiovascular and kidney diseases. None of the participants was on any form of drug therapy. Excluded from the study were individuals less than 18 years of age and those suffering from acute or chronic illnesses. Cigarette smokers and alcoholis were also excluded from the study.

Venous blood specimens were collected from the participants by venepuncture with minimum stasis, into plain tubes containing no anticoagulant. The blood samples were centrifuged at 1000 x g for 10 minutes, within one hour of blood collection and the sera harvested into plain bijou bottles. Measurements of glucose, albumin, total proteins and total biliruin were made on the same day of blood collection and the remaining sera stored frozen at -20^{o}C until

required for fructosamine assay, which was done weekly.

Serum fructosamine was estimated by the method of Johnson et al, using nitroblue tetrazolium (NBT) semi-manually as evaluated in our laboratory by Isah.[1,4] Fructosamine in an alkaline medium reduces NBT to produce a reddish brown colour which can be measured colorimetrically at 540 nm. The intensity of the colour produced is directly proportional to the concentration of fructosamine in the sample.

Serum glucose, albumin, total proteins and total bilirubin were estimated by glucose oxidase method, modified method of Bartholomew and Delaney as modified by Doumas et al, biuret method and the method of Jendrassik and Grof respectively.[9,10,11,12]

Statistical analysis

An Excel spreadsheet (Excel 2007, Microsoft Corporation) was used to capture the data. Statistical analysis of the data was performed using the statistical package for the social science (SPSS), version 16.0. The central tendencies of descriptive data are presented as median, means and the variability from central tendency is presented as standard error of the mean (SEM), range, percentiles and 95% confidence interval. For data where the normality assumptions were suspect, the Mann Whitney U test was used. Linear logistic regression analysis was used to study association between fructosamine and the other variables. A p-value less than 0.05 was considered significant.

Results

The biochemical characteristics of the participants are summarized in table 1. The results are not significantly different from the established reference values on our Laboratory's repertoire except that the lower limit of the new RI for fructosamine (0.7 mmol/L) was lower than the lower limit of the RI (0.9 mm/L, p = 0.02) currently in-use.

The 2.5th percentile value for fructosamine observed among the female participants was lower (0.5 mmol/L, p= 0.01) than the value observed among the male participants (Table 2).

Table 1: Biochemical characteristics of the participants

Participants (n= 255)	Median	Mean ± SEM	Range	95% Confidence Interval
Fructosamine (mmol/L)	1.3	1.3 ± 0.02	0.2-2.0	0.7-1.8
Glucose (mmol/L)	4.4	4.5 ± 0.07	1.8-5.7	2.5-5.4
Albumin (g/L)	38.5	39.1 ± 0.3	27-60	29.4-49.6
Total Protein (g/L)	71	71.4 ± 0.6	56-97	55.8-90.6
Total Bilirubin (µmol/L)	7.0	7.4 ± 0.4	4.0-8.0	6.0-12.0

Table 2: Biochemical characteristics of the participants by sex

	Male (n=165)				Female (n=90)			
	Median	Mean ± SEM	Range	Confidence Interval	Median	Mean ± SEM	Range	95% Confidence Interval
Fructosamine (mmol/L)	1.4	1.3 ± 0.03	0.2-2.0	0.7-1.8	1.3	1.3 ± 0.03	0.4-1.8	0.5-1.8
Glucose (mmol/L)	4.5	4.6 ± 0.09	1.8-8.1	2.5-7.4	4.1	4.3 ± 0.09	2.0-7.2	2.6-6.6
Albumin (g/L)	38.0	38.5 ± 0.4	28-53	30-49	41	40.2 ± 0.6	27-60	29-53.2
Total Protein (g/L)	69.0	69.8 ± 0.6	53-97	55.3-89.4	75	74.3 ± 1.1	56-94	54.4-93.7
Total Bilirubin (µmol/L)	6.6	7.0 ± 0.6	4-18	6-11.4	7.1	7.2 ± 0.3	6-17	6.2-11.7

Fructosamine showed a positive correlation with glucose (r= 0.52, p < 0.00001), total protein (r=0.37, p Â 0.00001) and albumin (r= 0.42, p Â 0.00001). However, no correlation was demonstrated between fructosamine and total bilirubin (r= -0.11, p= 0.23)

Linear logistic regression analysis was used to study association of fructosamine (as dependent variable) with random blood glucose level, total protein, albumin and total bilirubin. The effect of all the independent variables on fructosamine levels was 26.4%, R^2 = 0.264, but the significant contributors were glucose, total protein and albumin.

Discussion

Reference intervals (RI) play important roles in clinical practice as they are required for assessing the health status of patients. Furthermore, they are basic tools of clinical Laboratory practice, both in quality control and in providing reference values according to the protocols used in each case. Ultimately, reference intervals are essential for clinical laboratory test interpretation required for optimal patient care.[13] RI is typically defined as the range between the 2.5th and 97.5th percentiles of data distribution from a given reference population. Accordingly, this interval estimates the expected values that would contain central 95% of the subjects in the considered population.[14] By implication therefore, 5% of all results from healthy people will fall out of the reported RI and, as such, will be flagged as being abnormal.

It is hard to underestimate the importance of clinical laboratory test results. Nearly 80% of physicians' medical decisions are based on information provided by laboratory reports. A test result by itself is of little value unless it is reported with the appropriate information for its interpretation. Typically, this information is provided in the form of a reference interval (RI) or medical decision limit.[15]

RI vary considerably from one laboratory to another and are dependent on the methodology and instrumentation utilized. As a consequence, published reference values may not be valid for results generated in other laboratories. Hence RI should be established

by each Laboratory. It has been recommended that RI be established by selecting a statistically sufficient group (a minimum of 120) of healthy reference subjects.[16]

Generally, there are two statistical methods (i.e parametric and non-parametric) for determining reference intervals based on the distribution of the data obtained from healthy reference individuals in a population. The parametric methods are performed on data with Gaussian distribution while the non-parametric methods are performed on data with the non-Gaussian distribution.

The Gaussian type of distribution is described when the data is normally distributed, i.e. the distribution of the data is symmetrical around the mean. The reference interval is calculated as the mean ± 2 standard deviations (SD), which encompasses central 95% of the observations in healthy individuals. The top 2.5% and bottom 2.5% of the results from healthy individuals will thus, fall outside an established reference interval.[17]

For the data that is non-Gaussian, the distribution is not normally distributed around the mean. It is skewed. This data can be mathematically transformed, e.g. to logarithms, to yield a normal or Gaussian distribution. The geometric mean ± 2 SD is used for reference interval determination. However, percentiles are used to create reference intervals from non-Gaussian data, in most instances. The top 97.5 and bottom 2.5 percentiles are used as the limits of the reference range. As most biochemical data are not normally distributed, this is the optimal technique for reference interval determination.[17]

In the present study, the non-parametric method was used to determine the reference interval for fructosamine in 255 healthy participants because the distribution of the data obtained was slightly skewed to the right. This exercise became necessary because the previous reference interval in use was not properly derived.[17] It was derived based on the formular, mean ± standard deviation, which is meant for use on data that is normally distributed i.e Gaussian.

The reference interval (RI) obtained from this study was 0.7-1.8 mmol/L as against the reference interval that was previously derived for our local population (0.9- 1.8 mmol/L). The 2.5th percentile of the new RI is lower than the lower limit of the previously reported RI. The difference may be explained by the different methods employed in their derivation. This study also showed a difference in the 2.5th percentile value between the male and female participants. This shows that the interpretation of fructosamine results among the females in this population should be approached with caution especially when it is being used in assessing diabetic individuals (on treatment), at risk of developing hypoglycaemia.

All the participants in this study were normoglycaemic with mean random serum glucose value of 4.5 mmol/L. This constitute a major criteria in the selection of participants into this study as fructosamine is a by-product of the irreversible non-enzymatic reaction between glucose and serum proteins, mainly albumin. The serum levels of albumin found in the participants of this study were within the reference interval. The rate of fructosamine synthesis is directly dependent on the rate of protein synthesis and composition as well as the concentration of glucose during the life time of the circulatory proteins. A positive correlation between fructosamine and total serum proteins and particularly albumin concentration was also observed in this study. This observation is in agreement with previous reports by Lloyd et al and Hindle et al.[18,19] It was also reported that serum fructosamine level may not be valid in hypoalbuminaemia, but in individuals with albumin level greater

than 30 g/L, serum fructosamine concentrations are valid and independent of albumin concentration.[6,18,20] Albumin is expected to play a significant role in fructosamine formation, being the most abundant protein in circulation and in view of its relatively long half-life (17-20 days) and possession of several lysine residues.[21] About 80% of the fructosamine in serum is said to be accounted for by albumin.[1]

The concentration of total bilirubin in each of the participants in this study was found to fall within the reference range with mean value of 7.4 ìmol/L. This was done to ensure that none of the participants had raised level of total bilirubin which has been found to interfere with the measurement of fructosamine in vitro.[4,22] However, Dominiczak and Colleagues carried out an in- vivo study from which they did not observe significant difference between the concentrations of fructosamine in the patients with mild to moderate hyperbilirubinaemia and normal controls.[23] They however reported a significant positive correlation between fructosamine and bilirubin.[23]

The measurement of fructosamine allows more frequent assessment of glycaemic control than glycated haemoglobin hence its use as intermediate glycation index for monitoring glycaemic control in diabetics.[24] The value of an intermediate glycation index is that it allows retrospective evaluation of changes in diet and exercise habits as well as faster evaluation of changes in medication dosages and other control measures.[25]

The NBT assay method used in this study for fructosamine estimation is highly sensitive, simple, fast, precise, fairly free of interferences and easily automated for use with microsample volumes.[26,27,28] The method has been adapted to a wide variety of automated chemistry analysers, such as Cobas Bio, Cobas PARA, Cobas Mira, Hitachi 405, Hitachi 405, Abbott ABA-100,Abbott ABA-VP, Technicon RA-1000, IL Monarch, IL Multistat-3, Centrichem 300, Centrichem 400 to mention but a few.[29,30,31]

In anaemic conditions, haemolytic or otherwise, marked changes in the life span of haemoglobin molecules may make glycated haemoglobin values unreliable, whereas the glycated serum protein (fructosamine) is stable in such instances.[32,33] Haemoglobinopathies and recent blood transfusion can also adversely affect the usefulness of glycated haemoglobin values.[34] Fructosamine is especially more useful than HbA1c in our environment with high prevalence of haemoglobinopathies, such as sickle cell disease.[35]

Conclusion and Recommendations

This study has described a new reference interval for fructosamine for our population using appropriate statistical method. A single reference range (0.7-1.8 mmol/L) for both adult males and females is recommended. However, interpretation of fructosamine results in female individuals should be handled with caution due to the lower 2.5th percentile value observed in them. This is especially important when fructosamine is being used as screening test for gestational diabetes mellitus and in monitoring response to drug therapy in female diabetic individuals.[8]

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