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# Comparison of Serum Levels of Fructosamine and Erythrocyte Sodium Potassium ATPase ($Na^+/K^+$ ATPase) in Gestational Diabetes Mellitus (GDM) and non-Gestational Diabetes Mellitus (non GDM) Patients

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## Abstract

***Background:*** The study aims at the comparison and correlation of serum levels of fructosamine and erythrocyte $Na^+/K^+$ ATPase in Gestational diabetes mellitus (GDM) and Non Gestational Diabetes Mellitus (Non GDM).

***Methods:*** A total of 326 samples were divided into 4 groups. Pregnant women between the age group of 20-40 years who gave samples for Oral Glucose Tolerance Test (OGTT) were included as the subjects. Anonymized and left over fasting and 2 hours' samples were collected from biochemistry laboratory, Kasturba Hospital, Manipal.

***Results:*** In the comparison of fructosamine levels in GDM and Non GDM, fructosamine was found to be significant (p value<0.001) in both fasting and 2 hours (G2) blood glucose condition. Na+/ K+ ATPase did not show any significant variation between the groups. Correlation was not significant between the parameters.

***Conclusions:*** Fructosamine showed significant increase when compared between the groups, whereas significant correlation is not obtained between the parameters. Thus, the use fructosamine as a diagnosis tool becomes inconclusive. Further studies must be carried out to identify a marker which reduces the interferences observed in fructosamine and to find out the exact relationship between hyperglycaemia and $Na^+/K^+$ ATPase activity.

**Keywords:** Fructosamine, Gestational Diabetes Mellitus, Na+/ K+ ATPase Enzyme Activity, Screening Test.

## Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first detection during pregnancy (1). Between three to nine percent of pregnancies are affected with gestational diabetes. It is especially common during the last three months (3rd trimester) of pregnancy (2). Gestational diabetes mellitus begins during pregnancy and disappears following delivery of the infant. Adverse maternal and fatal outcome has association with the degree of glucose intolerance during pregnancy, which leads to complications like preeclampsia, urinary tract infection, hydramnios, hypertension, increased operative intervention and occurrence of diabetes mellitus in future. Macrosomia, metabolic abnormalities, increased operative anomalies, adolescent obesity are associated with fetal outcome (3).

In defined circumstances, various screening and diagnostic tests have been used to detect high levels of glucose in plasma or serum. Non-challenge blood glucose test such as fasting blood glucose, 2-hour postprandial glucose test, and random glucose test, screening glucose challenge

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test, oral glucose tolerance test (OGTT) are the most commonly used diagnostic tests (3). Other indices like fructosamine and sodium potassium ATPase ($Na^+$/ $K^+$ ATPase) has acquired research importance in screening, diagnosis and action of gestational diabetes mellitus.

Glycation reactions between sugars like fructose or glucose and a primary amine, followed by isomerization results in formation of compounds like fructosamine. Fructosamine is commonly referred as a laboratory test used in diabetes management. The fraction of total serum proteins that have undergone glycation i.e., the glycated serum proteins can be determined by testing the levels of fructosamine (2). Measurement of glycated proteins include fructosamine and glycated haemoglobin (HbA1c) and they have been employed in the assessment of short term and long term glycaemic control, respectively. Fructosamine levels increase in states of abnormally elevated glucose concentrations such as GDM and hence it can be used for assessing glucose control over a short time frame as the level of fructosamine in blood reflects glucose levels over the previous 2-3 weeks (4).

Sodium potassium ATPase ($Na^+/K^+$ ATPase) is an electrogenic transmembrane enzyme consisting of alpha, beta and gamma subunits. It is found in the plasma membrane of all the animal cells (5). Deportation of $Na^+$ from cells in exchange for $K^+$ at a ratio of 3:2 takes place (6). This is carried out to maintain the cell membrane potential where cells keep low concentration of sodium and high concentration of potassium intracellularly. The energy released after hydrolysis of ATP is utilised for transport of three sodium ions to outside of the cell and two potassium ions into the cell from outside. Various functions like effect transport, signal transducer or integrator to regulate MAPK pathway, intracellular calcium, regulating cellular volume and maintaining resting potential is carried by $Na^+/K^+$ ATPase (7). The activity of $Na^+/K^+$ ATPase acts as a driving force for secondary active transport of solutes like phosphate, vitamins and glucose. Studies suggests that many hormones like insulin regulate the activity of $Na^+/K^+$ ATPase (7).

The efficient diagnosis and accurate monitoring of gestational diabetes are the cornerstones for reducing the risk of gestational diabetes complications. The introduction of other indices of glucose homeostasis such as fructosamine and sodium potassium ATPase ($Na^+/K^+$ ATPase) may be regraded. Few studies have researched on the correlation between fructosamine and $Na^+/K^+$ ATPase in diabetes mellitus patients, in the current study we will be focusing on the combined comparison and correlation of fructosamine and erythrocyte $Na^+/K^+$ ATPase in gestational diabetes patients.

## Materials and methods

After receiving approval from the Institutional Ethics Committee, (**IEC 801/2017)** the study was conducted at Department of Biochemistry, Kasturba Medical College, Manipal.

Study Subjects Pregnant women between the age group of 20-40 years who gave samples for OGTT were included as the subjects for the current study, the anonymized and left over fasting samples and 2 hours samples were collected from the Clinical Biochemistry Laboratory, Kasturba Hospital, Manipal. Pregnant women above the age for 40 years and below 20 years, hyperthyroidism and hypothyroidism subjects were excluded from the study.

This cross-sectional study includes 326 samples divided into 4 groups, which includes:

Group 1: Fasting blood glucose of GDM subjects (n=60),

Group 2: 2 hours (G2) blood glucose of GDM subjects (n=60),

Group 3: Fasting blood glucose of Non GDM subjects (n=103),

Group 4: 2 hours (G2) blood glucose of Non GDM subjects (n=103)

Here, group 1 and group 2 were together considered as the cases as they included pregnant women with GDM whereas group 3 and group 4 were together considered as the controls as they included subjects who are Non GDM.

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***Laboratory Investigations (8- 10)***

In the current study fasting blood glucose and 2 hours (G2) blood glucose of both cases and controls were collected from the clinical biochemistry laboratory. Whole blood for determination of erythrocyte $Na^+/K^+$ ATPase was freshly processed, and the serum samples were stored at -80 °C for the estimation of fructosamine.

Fructosamine was estimated spectrophotometrically at 550 nm by Nitro Blue Tetrazolium method (9) using a standard graph. Measurement of erythrocyte $Na^+/K^+$ ATPase activity includes few steps. Firstly, erythrocyte membrane pellet was extracted. Then, estimation of phosphate released by $Na^+/K^+$ ATPase activity by Fiske Subbarow method (8) is performed. Lastly, proteins are estimated using Lowry's method (10), standard graphs are plotted for both phosphate estimation and protein estimation.

***Statistical Analysis***

As per the statistical analysis, the current study consisted of non-parametric data and they were not normally distributed, therefore Mann Whitney U test was conducted to compare between the groups. Mean±SD, Mean Ranks are listed and p value<0.001 were significant SSPS software version 16 is used for statistical analysis.

## Results

In the current study we have compared the serum levels of fructosamine and erythrocyte $Na^+/K^+$ ATPase among groups.

It was observed that fructosamine significantly varies between group 1 and group 3 with p value<0.001 (Table 1 and Fig. 1).

$Na^+/K^+$ ATPase was not significant between the groups as its p value did not satisfy the condition (Table 1)

Serum fructosamine significantly varies between group 2 and group 4 with p value<0.001 (Table 2 and Fig. 2). $Na^+/K^+$ ATPase was not significant between the groups as its p value did not satisfy the condition (Table 2). Correlation between the groups with fructosamine and

Na+/K+ ATPase was found to be not significant (Table 3)

**Table 1.** Comparison of Fructosamine and $Na^+/K^+$ ATPase in GDM and Non GDM patients under Fasting Condition. (FBG: Fasting Blood Glucose)

| FBG | Groups | Median | Mann Whitney U Value | P Value |
|---|---|---|---|---|
| Fructosamine | 1<br>3 | 97 (93, 109.7)<br>80 (77, 86) | 431 | 0.00* |
| $Na^+/K^+$ ATPase | 1<br>3 | 37 (31.2, 40.98)<br>21 (18.3, 26.4) | 2974 | 0.690 |

*Mann Whitney U test*, *P value<0.001: Significant

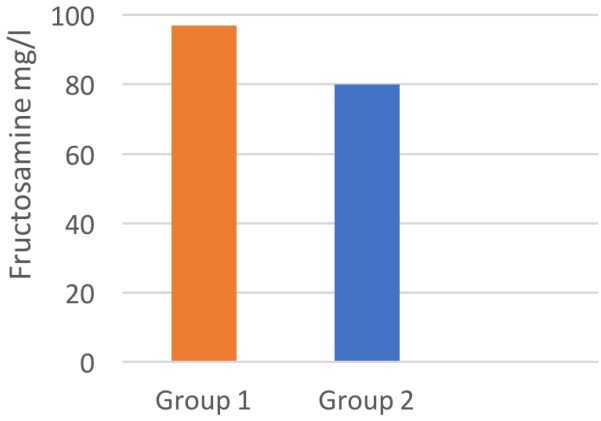


**Fig. 1.** Levels of Serum Fructosamine in GDM (group 1) and Non GDM (group 3) under fasting condition.P value<0.001: Significant.

**Table 2.** Comparison of Fructosamine and $Na^+/K^+$ ATPase in GDM and Non GDM patients under 2 hours (G2) blood glucose condition (OGTT: Oral Glucose Tolerance Test).

| 2 Hours (OGTT) | Groups | Median | Mann Whitney U Value | P Value |
|---|---|---|---|---|
| Fructosamine | 2 | 39.3 (32.6,42) | 235 | 0.00* |
| | 4 | 21.6 (18.3,26.4) | | |
| $Na^+/K^+$ ATPase | 2 | 6.1 (4.6,7.4) | 2808 | 0.331 |
| | 4 | 6.4 (5.7,6.8) | | |

*Mann Whitney U test*, *P value<0.001: Significant

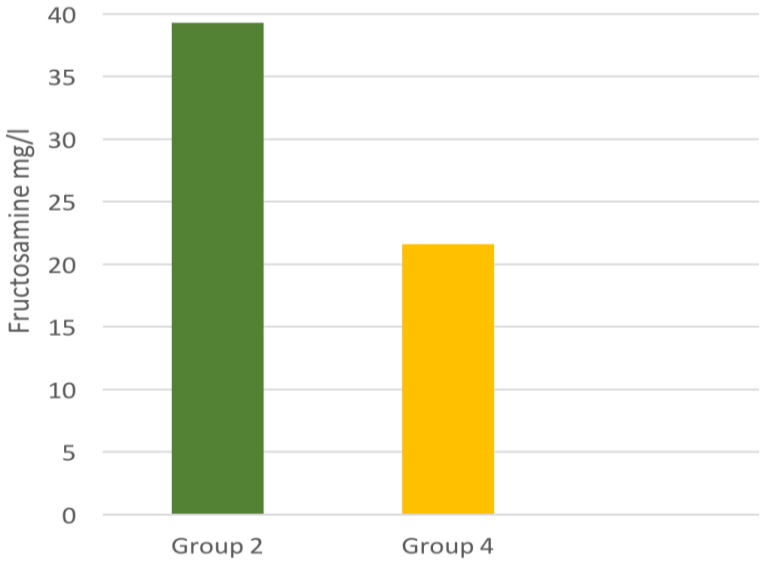


**Fig. 2.** Comparison of Serum Fructosamine levels between GDM and Non GDM under 2 Hours blood glucose condition. *P value<0.001: Significant.

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**Table 3.** Correlation of Fructosamine and $Na^+/K^+$ ATPase with groups.

| Parameters | Groups | Z Value | P Value |
|---|---|---|---|
| Fructoasmine | 1 | -0.584 | 0.28 NS |
| | 2 | 0.224 | 0.411 NS |
| Na+/K+ ATPase | 1 | 0.157 | 0.438 NS |
| | 2 | -0.097 | 0.462 NS |

NS: Not Significant

## Discussion

The present study is mainly focused on the comparison of serum fructosamine and erythrocyte $Na^+/K^+$ ATPase between the groups and to correlate the parameters between the groups. Basically, the subjects were divided into four groups as explained in the study subjects of materials and methods.

In practise, blood glucose estimation or HbA1c is preferred over fructosamine in screening and diagnosis of diabetes mellitus (11).

Fructosamine can be a better tool in monitoring glycaemic control than measuring blood glucose levels as fructosamine is not influenced by the food intake as it is a measure of glycated protein and

reflects average blood glucose over past 2-3 weeks. In pregnancy, biphasic changes are exhibited by HbA1c which decreases during first and second trimester but increases in the third trimester (12). Usually pregnancy is complicated with iron deficiency and the pattern of variation exhibited by HbA1c attributes to the decreased blood glucose in first trimester. Therefore, to reduce complications, fructosamine can be measured over HbA1c as iron deficiency does not interfere with fructosamine (13,14). Its measurement is not affected by anaemia or variant haemoglobins and its concentration does not depend on half-life of erythrocytes (15). Estimation of fructosamine is found to be technically simple and reproducible in evaluation of glycaemic control in diabetes (16). In the present study, fructosamine was found to be significantly varying when compared between group 1 and group 3 (fasting blood glucose of cases with fasting blood glucose of controls) with p value<0.001 and between group 2 and group 4 (G2 of cases and G2 of controls) with p value <0.001 (Tables 1 and 2). but there was no significant correlation of fructosamine with blood glucose in group 1 and 2 (Table 3). A recent study states that fructosamine concentration does not solely depend on the concentration of glucose but it also depends on the concentration of albumin. Due to plasma volume expansion during pregnancy there is a decrease in albumin concentration by 10 g/l.

Therefore, alterations of protein and mainly albumin concentration can interfere or affect serum fructosamine levels (17,18,19). Fructosamine is also influenced by many physiological and pathological conditions, its concentration is also affected by the levels of Immunoglobulins (IgA) (20).

Therefore, from the current study it can be stated that fructosamine can identify patients at higher risk of abnormal glucose tolerance, but it cannot be considered as a diagnosing tool in GDM, as it has poor correlation with blood glucose values. The present study gives a novel idea of conducting further studies to identify a biomarker which reduces the interferences observed in fructosamine.

$Na^{+/}K^+$ ATPase activity is defined as the amount of phosphate released per 100 µg/dl of protein per time. $Na^+/K^+$ ATPase was found to be non-significantly decreased when compared between the groups. Correlation of $Na^+/K^+$ ATPase between group 1 and group 2 was also found to be not significant or there was no correlation (Table 3). Combined correlation of the two parameters (fructosamine and $Na^+/K^+$ ATPase) between group 1 and group 2 was found to be not significant, as in, there was no correlation.

Recent study supports the current study where it states that there is no correlation between the activity of enzyme and plasma glucose and the impairment in the erythrocyte enzyme activity is not related to lesser units in the erythrocyte membrane (21,22). They suggested that variations in the erythrocyte Na+/K+ ATPase activity is not caused due to decrease in glucose tolerance. Other study stated that there was no change in enzyme activity in type 2 diabetes subjects, whereas there was a decrease in the enzyme activity in type 1 diabetes subjects (23). A recent study suggests that the impairment could be due to defect in myo-inositol metabolism which causes alteration in lipid metabolism in the membrane and hence the enzyme activity decreases (24). There is a need to carry out further studies in regard to find out the exact cause for the variations of the enzyme activity. An early detection and diagnosis of gestational diabetes mellitus and glycaemic control are the corner stones in avoiding the major serious and life threatening complications of GDM. The standard methods like OGTT are used in diagnosing GDM but measurement of other indices like fructosamine and $Na^+/K^+$ ATPase activity was carried out to check its efficiency to be used as an alternative but through the results it was found that fructosamine and $Na^+/K^+$ ATPase did not show any significant correlation. Whereas, fructosamine showed significant increase when compared between the groups, thus the use fructosamine as a diagnosis tool becomes inconclusive. Further studies must be carried out to identify a marker which reduces the interferences observed in fructosamine and to find out the exact relationship between hyperglycaemia and $Na^+/K^+$ ATPase activity.

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