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Original Article

Serum Fructosamine and Glycosylated Haemoglobin in Diabetic Subjects and their Non-Diabetic First-Degree Relatives

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ABSTRACT

Aim: To Assess Serum fructosamine and glycosylated haemoglobin in diabetic subjects and their non-diabetic first-degree relatives, a comparative evaluation. **Background:** Glycosylated Hemoglobin has been used for decades for the diagnosis of diabetes. However, it is altered in situations including red blood cell wall defects, hemoglobinopathies and chronic renal failure. Therefore, the utility of short-term glycemic markers like serum fructosamine is gaining popularity. **Objectives:** The objective of this research is to assess Serum fructosamine levels in patients with Type II diabetes and their non-diabetic first-degree relatives. **Materials and Methods:** This research enrolled 300 participants. Group A comprised 100 diabetic patients, Group B contained 100 non-diabetic first-degree relatives (test group) of type II diabetic patients and 100 healthy controls with no immediate family history of Diabetes Mellitus were included in Group C. **Results:** Fasting blood sugar, postprandial sugar, glycosylated hemoglobin, serum fructosamine levels and fasting insulin levels were tested in each case. The Serum Fructosamine levels (362.95±71.06 µmol/L) and HbA_{1c} (8.59±1.99%) were raised in diabetes patients. The fasting blood sugar and postprandial blood glucose and glycated hemoglobin levels were within the target levels in the first-degree relatives. There is a noteworthy difference in serum fructosamine levels between test and controls at $p<0.05$. **Conclusion:** Serum fructosamine can be used as a more robust marker than glycated hemoglobin for the early detection of diabetes in firstdegree relatives.

Keywords: Type II diabetes mellitus, Serum fructosamine, HbA_{1c}, First-Degree relatives, HOMA-IR.

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INTRODUCTION

Diabetes mellitus has been recognized as a serious illness since ancient times. Ayurveda acknowledged it and described it in detail; references to prameha/madhumeh may additionally be found in various works of literature. The macrovascular and microvascular effects of the illness, which result in significant morbidity and mortality, are the primary source of the burden of diabetes. Examining the prevalence and causes of diabetes mellitus is crucial. Our understanding of the condition has grown dramatically over time and hyperglycemia is now recognised as the tip of the iceberg that results from a variety of metabolic abnormalities. The large multicentric landmark trials like Diabetes Control and Complications Trial (DCCT), UK Prospective diabetes study (UKPDS) and Kumamoto's trial have conclusively shown that the correction of dysglycemia led to a significant reduction or slowing down the progression of complications of diabetes.[1-3]

The advancement of medical technologies has enriched our ability to monitor and regulate blood sugar levels, aiming for physiological norms. Despite these advancements, there exists a considerable gap in achieving desired near-physiological levels due to short-term and long-term glycemic variability. Conventionally, blood sugar levels are measured retrospectively through fasting, postprandial and random tests using capillary blood, leaving a significant gap between readings. The glycosylated hemoglobin, known as HbA_{1c}, has emerged as a reliable modality for maintaining euglycemia and diagnostic purposes, considering advanced glycation end products. However, it provides an

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average of blood glucose levels over the previous two and a half to three months, overlooking short-term variations and gestational diabetes. Continuous Glucose Monitoring (CGM) offers a closer 24 hr monitoring, but its cost and application challenges hinder extensive use, especially in developing countries. Recognizing diabetes as a metabolic syndrome influenced by genetics, environment, lifestyle and dietary habits, the study emphasizes the shared risk factors among first-degree relatives. Identifying parameters to evaluate glycemic variations in these relatives, who are not yet diagnosed with diabetes or metabolic syndrome but are at a higher risk, is crucial.[4,5]

The study aims to assess the efficacy of short-term modalities like fructosamine levels, a marker of recent glycemic control lasting approximately 20 days, in both diabetic individuals and their first-degree relatives. The goal is to pinpoint a single or combination of markers that can measure glycemic variations in non-diabetic first-degree relatives, shedding light on the heightened diabetes risk within families beyond genetic factors.

MATERIALS AND METHODS

The investigation was carried out at a multi-specialty hospital in Dehradun, Uttarakhand, India for a period of 24 months. A prospective, observational, cross-sectional, investigation was carried out in the inpatient and outpatient medicine departments. The protocol was approved by the institutional ethics committee of Shri Guru Ram Rai Institute of Medical and Health Sciences Shri Mahant Indiresh Hospital (Registration No. ECR/710/Inst/UK/201 S/RR-18). The subjects agreed to sign a formal informed consent form covering the details of the research. The medical history and patient profile questionnaires were used to collect the patient's data. The study participants included those who visited the hospital for their routine check-ups and follow-ups during the study period. The study's inclusion criteria encompass individuals above the age of eighteen, irrespective of gender, who have been diagnosed with type II diabetes mellitus in accordance with the American Diabetic Association guidelines. Additionally, it includes first-degree non diabetic relatives of individuals afflicted with Type II diabetes. Conversely, the exclusion criteria comprise individuals with type I diabetes mellitus, secondary diabetes mellitus, gestational diabetes mellitus, as well as those with hereditary red cell wall defects and haemoglobinopathies. This research enrolled 300 participants. Group A comprised 100 diabetic patients, Group B contained 100 non-diabetic first-degree relatives (test group) of type II diabetic patients and 100 healthy controls with no immediate family history of Diabetes Mellitus were included in Group C. Fasting blood sugar, postprandial sugar, glycosylated hemoglobin, serum fructosamine levels and fasting insulin levels were tested in each case. After a 10 hr fast, vacutainers were filled with venous blood that had been drawn from the antecubital vein. The datasets were analyzed and evaluated using IBM SPSS Statistics 20 software. Statistical significance was considered at p-values less than 0.05. The independent sample t test was used for normally distributed data while the Mann-Whitney U test was used for non-normally distributed data.

RESULTS

This research enrolled a total of 300 participants. Group A comprised 100 diabetic patients, Group B contained 100 non-diabetic FDRs (test group) of type II diabetic patients and 100 healthy controls with no immediate family history of Diabetes Mellitus were included in Group C.

Data is represented as mean±standard deviation. Statistical significance is defined at a p-value of less than 0.05 (Table 1). All the parameters, including age, fasting blood glucose, postprandial blood glucose, HbA1c, serum fructosamine and insulin resistance/HOMAIR, show greater readings in patients, followed by the test and control group. The p-value of 0.16 indicates that the age distributions of the test group and non-diabetic healthy controls are similar. The Mann Whitney U test explains that glycated haemoglobin values for the first-degree relatives (median=5.4, n=100) and the control group (median=5.3, n=100), U=3845.5, z=-2.838, p=0.005, r=0.20, (where r is the effect size), differ significantly. Similarly, there is a significant difference between serum fructosamine values for the first-degree relatives (median=293.06, n=100) and the control group (median=231.74, n=100), U=828, z=-10.194, p=0.000, r=0.720, where r is the effect size. "Effect size is a quantitative measure of the strength of a phenomenon. It emphasizes the size of the difference or relationship between two variables being compared. According to Cohen's 1988 criteria, the R-value of 0.1 shows a small effect, 0.3 shows the medium effect and 0.5 shows a large effect size". As a result, the effect size/size of difference for serum fructosamine values between the test and control group is greater than for the glycated haemoglobin values for the two groups.[6]

The male and female diabetic patients are significantly different on certain studied variables (Table 2). The fasting blood sugar (p=0.928), postprandial blood sugar (p=0.408) and fructosamine levels (p=0.063) did not differ significantly among males and females. However, female diabetic patients had higher values of glycated haemoglobin (p=0.018*), Fasting insulin (0.001*) and Insulin resistance (HOMA-IR values) (p=0.02*) as compared to their male counterparts.

p-value<0.05 is considered statistically significant. The male and female subjects are significantly different on almost all the variables under consideration (Table 3). The females had higher values of fasting blood sugar (p=0.002*), postprandial blood sugar (p=0.026*), fructosamine levels (p=0.023*) glycated haemoglobin (p<0.05*), Fasting insulin (0.004*) and Insulin resistance (HOMA-IR values) (p=0.002*) as compared to their male counterparts (Table 3).

Table 1: Baseline characteristics of the subjects.

Variables	Group A	Group B	Group C
Age (Years)	69.96±6.29	43.04±4.56	44.35±5.08
Fasting Blood Sugar (FBS) (mg/dL)	161.61±51.58	94.11±5.24	89.54±6.29
Post-Prandial Blood Sugar (PPBS) (mg/dL)	285.80±46.65	127.39±8.31	123.91±6.18
Glycated Hemoglobin (HbA_{1c}) (%)	8.59±1.99	5.48±0.36	5.33±0.25
Fructosamine (µmol/L)	362.95±71.06	289.61±35.96	232.99±17.19
Fasting Insulin (µIU/mL)	14.31±3.88	12.42±4.41	9.13±2.67
Homeostatic Model Assessment for Insulin Resistance (HOMAIR)	5.63±2.09	2.92±1.14	2.05±0.72

Table 2: Comparison of baseline characteristics of males and females in Group A-Type II Diabetic Patients.

Variables: Number of males Number of females		Mean±SD	*p*-Value
Fasting blood glucose (mg/dL)	Male	161.30±50.49	0.928
	Female	161.87±52.97	
Post prandial blood sugar (mg/dL)	Male	280.15±42.54	0.408
	Female	290.61±49.77	
HbA_{1c} (%)	Male	8.05±1.72	0.018*
	Female	9.05±2.10	
Fructosamine (µmol/L)	Male	347.79±67.48	0.063
	Female	375.87±72.08	
Fasting Insulin (µIU/mL)	Male	12.95±4.19	0.001*
	Female	15.46±3.31	
HOMAIR	Male	5.14±2.18	0.020*
	Female	6.05±1.94	

Table 3: Comparison of baseline characteristics of males and females in Group B-Nondiabetic First-degree relatives of Type II Diabetic Patients.

Variables: Number of males Number of females		Mean±SD	*p*-Value
Fasting blood glucose (mg/dL)	Male	92.42±5.26	0.002*
	Female	96.02±4.55	
Post prandial blood sugar (mg/dL)	Male	125.62±8.37	0.026*
	Female	129.38±7.85	
HbA_{1c} (%)	Male	5.32±0.32	<0.05*
	Female	5.66±0.32	
Fructosamine (µmol/L)	Male	281.91±34.68	0.023*
	Female	298.31±34.75	
Fasting Insulin (µIU/mL)	Male	11.16±4.33	0.004*
	Female	13.84±4.09	
HOMAIR	Male	2.58±1.10	0.002*
	Female	3.30±1.07	

The healthy male and female controls without any immediate family history of diabetes do not differ significantly from one other as far as glycemic markers are concerned (Table 4). The mean values for fasting blood sugar (p=0.458), postprandial blood sugar (p=0.129), glycated hemoglobin (p=0.797) and serum fructosamine (p=0.113), are higher in males than females. However, the values are statistically significant for fasting insulin (p=0.006*) and HOMA-IR (p=0.015*). The males in the control group are showing comparatively higher fasting insulin levels and insulin resistance as compared to females (Table 4).

Glycated haemoglobin levels positively correlate with other glycemic markers that have been evaluated in the three groups of Type II diabetic patients, their first-degree relatives and healthy controls. Glycated haemoglobin and serum fructosamine show a robust correlation (r_s=0.807) in Group A diabetic patients and moderate correlation ($r_{s=}$0.653 and $r_{s=}$0.610) in Group B first degree relatives and Group C control groups, respectively. The glycated haemoglobin and HOMA-IR show moderate to very strong Correlation (r_s=0.717) in Group B while Group A has r_s=0.621(moderate) and Group C has r_s=0.458 (fair) correlations. In Group A(r_s=0.677) and B(r_s=0.696), the fasting blood glucose moderately correlated with glycated haemoglobin. The control group C shows a fair to moderate correlation (r_s=0.503) of fasting glucose and glycated haemoglobin. For postprandial blood sugar, Group A gives r_s=0.732 (moderate to very strong), Group B gives r_s=0.677 (moderate) and Group C gives r_s=0.651 (moderate) correlations. The Correlation was significant at the 0.01 level (2 tailed) (Table 5 and Figure 1).

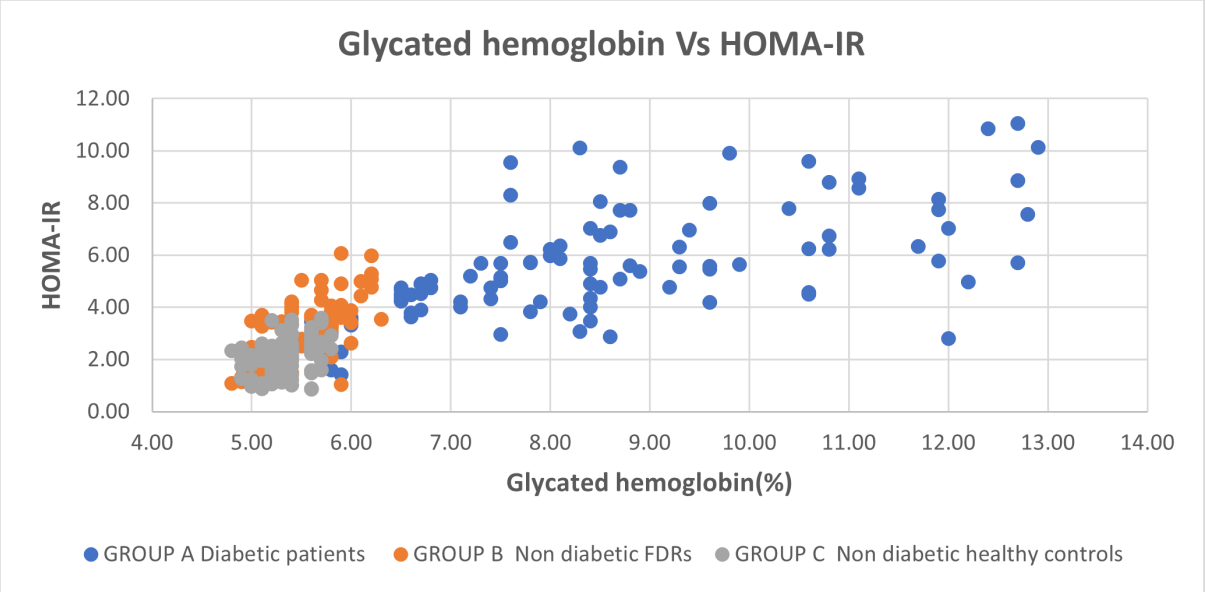

Figure 1: Graphical representation of Glycated hemoglobin Vs HOMA-IR in three groups.

Table 4: Comparison of baseline characteristics of males and females in Group C-Non-diabetic healthy controls.

Variables: Number of males Number of females		Mean±SD	*p*-Value
Fasting blood glucose (mg/dL)	Male	90.02±6.29	0.458
	Female	88.88±6.31	
Post prandial blood sugar (mg/dL)	Male	124.74±5.69	0.129
	Female	122.76±6.68	
HbA_{1c} (%)	Male	5.34±0.22	0.797
	Female	5.32±0.28	
Fructosamine (µmol/L)	Male	235.20±16.47	0.113
	Female	229.94±17.89	
Fasting Insulin (µIU/mL)	Male	9.72±2.87	0.006*
	Female	8.32±2.15	
HOMAIR	Male	2.20±0.78	0.015*
	Female	1.85±0.58	

Serum fructosamine levels positively correlate with other glycemic markers that have been evaluated in the three groups of Type II diabetic patients, their first-degree relatives and healthy controls. Serum fructosamine show moderate to very strong Correlation with fasting blood sugar in Group A diabetic patients' moderate Correlation in first degree relatives (r_s=0.669), fair to moderate correlation in Group C (r_s=0.592). Serum fructosamine and PPBS show a very strong Correlation (r_s=0.839) in Group B non-diabetic first-degree relatives, moderate to very strong Correlation ($r_{s=}$0.764) in Group A diabetic patients and moderate Correlation ($r_{s=}$0.645) in Group C control group, respectively. HOMA-IR values have a very strong Correlation ($r_{s=}$0.832) with serum fructosamine in Group B first degree relatives and moderate correlations in Group C ($r_{s=}$0.608) and Group A ($r_{s=}$0.657). The Correlations were significant at 0.01 level (2 tailed) (Table 6 and Figure 2).

Table 5: Correlations of Glycated hemoglobin with FPG, PPBG, SF and HOMA-IR.

Investigations		HbA_{1c}		
		Group A	Group B	Group C
FPG	r value	0.677	0.696	0.503
	p value	<0.01	<0.01	<0.01
PPBG	r value	0.732	0.677	0.651
	p value	<0.01	<0.01	<0.01
SF	r value	0.807	0.653	0.610
	p value	<0.01	<0.01	<0.01
HOMA-IR	r value	0.621	0.717	0.458
	p value	<0.01	<0.01	<0.01

Table 6: Correlations of Serum Fructosamine with FBG, PPBS and HOMA-IR.

Investigations		SF		
		Group A	Group B	Group C
FPG	r value	0.713	0.669	0.592
	p value	<0.01	<0.01	<0.01
PPBG	r value	0.764	0.839	0.645
	p value	<0.01	<0.01	<0.01
HOMA-IR	r value	0.657	0.832	0.608
	p value	<0.01	<0.01	<0.01

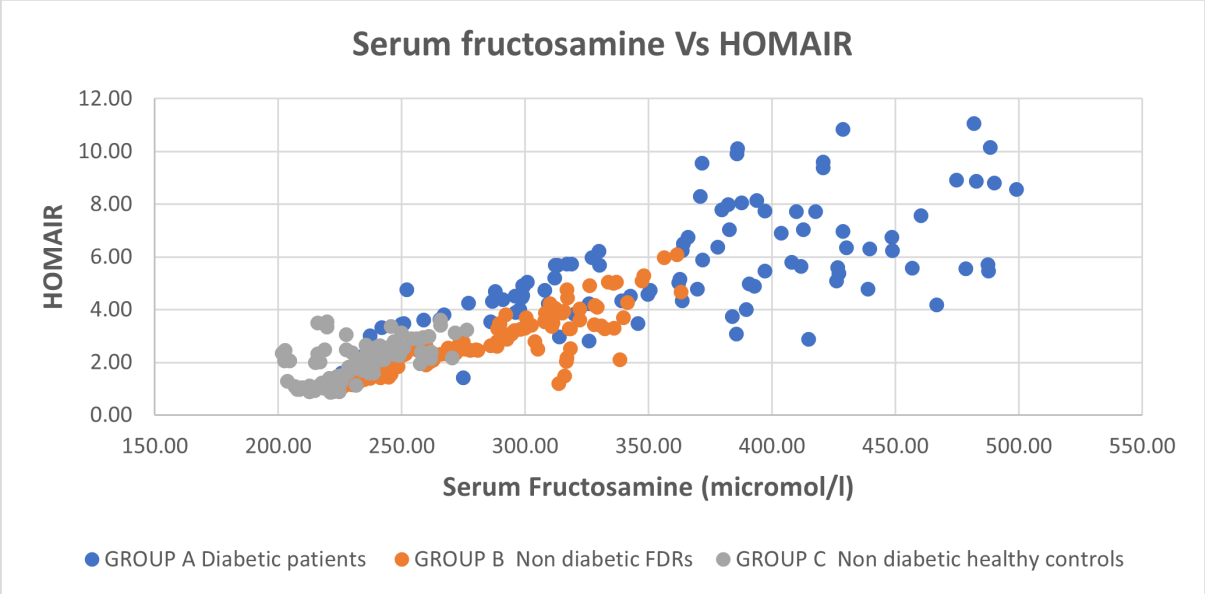

Figure 2: Graphical representation of Serum fructosamine Vs HOMA-IR in three groups.

DISCUSSION

The study has been designed as an experimental single-blind study involving the diabetics and their first-degree relatives from Uttarakhand's hills and plain regions. The cohort has been planned to evaluate and compare the glycaemic parameters in type 2 diabetics and the first-degree relatives.

The subjects enrolled in the study were categorized into three groups, namely Group A included subjects with T2DM based on the American diabetic association criteria. The group B had first-degree relatives who are non-diabetics and not on any medications which are likely to affect the test results, including serum fructosamine, glycated haemoglobin and insulin resistance. Finally, group C included controls who were non-diabetic with no immediate family history of diabetes mellitus and have presented for the routine medical checkup in the hospital. The mean age of diabetics in the study group is 69.96±6.29 yrs.

In comparison of Group B with Group A (Table 1), there was a significant difference in the FBS, PPBS, glycated haemoglobin, serum fructosamine and HOMA-IR levels. This statistical difference is understandable because it is a non-diabetic subset.

Sathipriya *et al.*, 2009 included thirty healthy volunteers with a positive family history of type 2 diabetes and thirty-two controls with no family history of type 2 diabetes. The average fasting glucose, fasting insulin and HOMA-IR were considerably higher in type 2 diabetes' first-degree relatives. Both glycated haemoglobin and fructosamine levels were significantly higher in the test group than in the controls.[7]

The two parameters that emerge are serum fructosamine levels and HOMA-IR. That is to say, the serum fructosamine level in the first-degree relatives is intermediate between frank diabetes and completely healthy individuals. The HOMA-IR values in Group B are also intermediate between frank diabetes and healthy controls.

Manjrekar *et al.*, 2012 compared 23 non-diabetic first-degree relatives of type 2 diabetes to 20 healthy controls and 23 type two diabetics and found that first degree relatives had higher serum fructosamine (533.62 mg/dL), an increased serum fatty acid/total protein ratio and a greater waist circumference as compared to the healthy control.[8] However, the serum fructosamine levels were highest in the first-degree relatives in this study, unlike ours, where the fructosamine levels are highest in the diabetic patients (362.95±71.06 µmol/L) followed by first-degree relatives (289.61±35.96 µmol/L) and the non-diabetic controls (232.99±17.19 µmol/L).

There is a significant difference in blood fructosamine levels between first degree relatives and controls at $p<0.05$ and r=0.720, where r is the effect size. Thus, the effect size/size of the difference in serum fructosamine values between first-degree relatives and the control group is more powerful than the effect size/size of the difference in glycated haemoglobin values between the two groups. Therefore, serum fructosamine can be used as a more robust marker than glycated haemoglobin for the early detection of diabetes in first-degree relatives.

The "Atherosclerosis Risk in Communities-Studies in the US" examined the role of serum fructosamine in predicting the risk of diabetes. They suggested that the alternative markers, including serum fructosamine, are suitable for risk prediction in the populations at risk.[9,10] The serum fructosamine levels in the first-degree relatives were also found to be elevated in the studies by Sathipriya *et al.*, Ahmed *et al.* and Manjrekar *et al.*[11,12]

Glycated haemoglobin and serum fructosamine show a very strong correlation in Group A diabetic patients and moderate correlation in Group B first degree relatives and Group C control groups, respectively.

Hindle *et al.*, 1986 tested Serum fructosamine and glycated haemoglobin (HbA_{1c}) in capillary samples from diabetic children and compared with those from non-diabetic children and found a strong correlation (r=0.86) between the two.[13] Loste and Marca also found a correlation value of r=0.65 at $p<0.0001$.[14]

The glycated haemoglobin and HOMA-IR show moderate to very strong Correlation in Group B while Group A has moderate and Group C has fair correlations. Our study indicates that serum fructosamine is better correlated to HOMA-IR rather than glycated haemoglobin, which is evident from the comparative values (r for "SF with HOMA-IR" Vs r for "HbA_{1c} with HOMA-IR") "0.657" vs "0.621", "0.832" vs "0.717" and "0.608" vs "0.458" for Group A, B and C respectively.

Hom *et al.*, 1998 compared glycated haemoglobin and serum fructosamine with fasting blood glucose. Fructosamine (r=0.74) was correlated with fasting blood glucose better than HbA_{1c} (r=0.68) in 222 diabetic participants ($p<0.05$).[15] Similar findings were revealed by Lim *et al.*, 1989 Shoji *et al.*, 1989 Goyal *et al.* 2019 and Johnson *et al.*, 1983.[16-19]

The inadequacies of the HbA_{1c} test have prompted a surge in interest in unconventional glycemic indicators. It's critical to understand the circumstances in which HbA_{1c} values are challenging to interpret. As a supplement to traditional measurements, using such markers may be more beneficial in the management of diabetes.

CONCLUSION

Diabetes mellitus is a major public health issue that causes significant morbidity and mortality across the world. Our capacity to handle this condition has considerably improved because of decades of intensive study. However, there are still a lot of obstacles to overcome.[20,21] The present investigation determined the serum fructosamine and glycosylated haemoglobin levels of diabetes patients and their FDRs not having diabetes. The Serum Fructosamine levels were significantly high in type II diabetic

patients. HbA_{1c} values were observed to be raised in diabetes patients. The insulin resistance in diabetics and their first-degree relatives was significantly higher. Our study indicates that serum fructosamine is better correlated to HOMA-IR than glycated haemoglobin in all the groups. Even at normal blood sugar levels, the FDRs serum fructosamine levels were shown to be higher. This confirms serum fructosamine's superiority as a marker for detecting blood sugar variations over short time periods.

LIMITATIONS OF THE STUDY

The non-glycaemic parameters like lipid profile could not be assessed due to financial constraints. Further clinical studies should be carried out in larger and more diverse groups of first-degree relatives.

RECOMMENDATIONS

The inadequacies of the HbA_{1c} test have prompted a surge in interest in unconventional glycemic indicators. It's critical to understand the circumstances in which HbA_{1c} values are challenging to interpret. As a supplement to traditional measurements, using such markers may be more beneficial in the management of diabetes. Fructosamine can find changes in average blood glucose faster than glycated hemoglobin in conditions such as pregnancy and monitoring of diabetic patients regarding amendments in the therapy.[22,23] "The NICE (National Institute for Health and Care Excellence) guidelines recommend that fructosamine can be used as an estimator of blood glucose control where HbA_{1c} is contraindicated, e.g. disturbed erythrocyte turnover or abnormal hemoglobin type".

The use of alternative markers like serum fructosamine can either alone or in combination with the traditional markers can serve to provide a finer blood sugar tuning in diabetic patients and early detection of diabetes in the population at risk even before the development of frank diabetes. We can add two markers like, glycated haemoglobin and serum fructosamine, to develop a scoring method to monitor the blood sugar levels more closely. Across the board, this piece of research reaffirms the need to closely monitor and launch screening programmes for families of NIDDM patients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

FDR: First-degree relatives; **DCCT:** Diabetes control and complications trial; **UKPDS:** UK Prospective diabetes study; **CGM:** Continuous Glucose Monitoring; **FBS:** Fasting blood sugar; **PPBS:** Post-prandial blood sugar; **HOMAIR:** Homeostatic Model Assessment for Insulin Resistance; **SF:** Serum fructosamine; **T2DM:** Type 2 Diabeties Mellitus.

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