

Fructosamine and Glycosylated Haemoglobin Levels in Patients with Different Glucose Status

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ABSTRACT: A total of 45 (21 males and 24 females) patients studied were divided in three groups, depending upon their postprandial blood sugar status (Group I, ≤ 140 mg%; Group II, 141 to 199 mg% and Group III, ≥ 200 mg %). Both Fructosamine and Glycosylated haemoglobin differed significantly between the groups. The Fructosamine concentration increased significantly from 320.96 to 684.87 (μ mole/l), when the postprandial blood glucose levels increased from <140 to > 200 mg%. Glycosylated haemoglobin significantly differed between the groups with a range from 5.52 to 9.47% with increase in postprandial level blood glucose from <140 mg% to >200 mg%.

KEYWORDS: Fructosamine, Glycosylated haemoglobin.

I. INTRODUCTION

The number of people with diabetes in India is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken [1]. Monitoring and controlling the levels of diabetes indicators, is an integral component of diabetes care from prevention to early diagnosis, to prevention or delay of serious complications. However, universal screening is not recommended because of the no specificity of traditional biochemical tests and the high cost of case detection [2]. Amongst the various biochemical estimates, fructosamine determination is most preferred test in addition to monitoring of the blood glucose levels. Fructosamine (FA) measurement has been used as an alternative to measuring glycosylated haemoglobin (HbA1c) for assessment of glycemic control. Important advantages of the fructosamine test include low cost [3], ease of analysis with routine biochemical analysers [4] and ready incorporation into existing external quality-assurance programs [5]. A higher fructosamine value indicates poor glycemic control over the preceding 2 to 3 weeks, since half life time of albumin is about 20 days. Glycosylated haemoglobin estimation is used primarily to identify the average blood glucose concentration over the previous 2-3 months.[6],[7].

The aim of the present study was to evaluate the HbA1c, fructosamine(FA) and glycation gap(GG) levels in diabetes mellitus patients under hypoglycaemic medication and having PP2BS ranging from ≤ 120 to ≥ 200 mg%. Although HbA1c and fructosamine have been used in diabetes mellitus, less information about their significance and association is available both with the fasting blood glucose and PP2BS levels and hence this information was assessed through this study.

II. MATERIAL AND METHODS

The study was performed on forty five patients (21 male, 24 females) having age between 22 to 82 years referred to the clinical laboratory. The necessary information on name, age and sex of patients, duration, and treatment were obtained. The patients depending upon their PP2BS levels were grouped in three groups [8],[9] viz. Group I ≤ 140 mg%, Group II 141 to 199 mg% and Group III ≥ 200 mg% having equal numbers in each group. The patients were also

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classified according to HbA1c levels in group A having HbA1c <7.0% and group B having HbA1c having HbA1c >7.0 %. This classification of HbA1c was done in light of multiple clinical trials reported for the vast majority of patients with diabetes and substantial life expectancy [10].

The data obtained on blood Glycosylated haemoglobin (HbA1c) estimated using Nycocard Reader; on Fructosamine (FA) estimated using Standard nitro-blue tetrazolium chloride salt kit and blood glucose estimated by Glucose Oxidase-Peroxidase (GOD-POD) enzymatic end point method. (Kit:-Trinder GOD-POD, Mfg by Spinreact) were analysed and glycosylation gap (GG) was calculated as the difference between measured HbA1c and HbA1c predicted [11]. The following formula was used for calculation of GG [12]:

Predicted HbA1c= 0.017x Fructosamine + 1.61

Glycosyl gap(GG)=measured HbA1c–predicted HbA1c

The data of present study were arranged group wise for calculation of mean values. Statistical analysis was done by using t-test to find out significance of difference between two groups and correlation coefficient to find out statistical correlation between two variables and its significance. Interpretation was done according to p-value as follows: p< 0.05 is considered significant p ≥ 0.05 is considered not-significant.

III. OBSERVATION

The details statistics of patients for age wise distributions and the absolute numbers of patients for a given sex, groups and age are given in table I and IA. The percentage of patients between 20-30 years was 6.66%, between 31-40 years was 4.45%, between 41-50 years was 11.11%, between 51-60 years was 33.34%, between 61-70 years was 35.55% and above 70 years was 8.89%. This present study covered only 22.22% (n=11) of patients between 20-50 years and it was 77.78% (n=35) from 51 years onwards (Table-I).

Table I. Age wise distribution of patients

Age groups(yrs)	20-30	31-40	41-50	51-60	61-69	Above 70
Patients (%)	6.66	4.45	11.11	33.34	35.55	8.89

Table IA. Groups, age and sex wise number of patients.

Age	Up to 50 years		Above 50 years	
	Male	Female	Male	Female
Group I	Zero	5	3	7
Group II	1	zero	7	7
Group III	2	2	7	4
Total	10		35	

Groups wise mean values of various biochemical estimates are shown in table II. The group differences for various biochemical estimates validated by t-test are presented in table – III and the correlation values between two estimates in different groups are given in table – IV.

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Table II. Groups wise mean values of various biochemical estimates.

Biochemical estimates	Groups as per PP2BS mg%		
	I (≤ 140)	II (141 to 199)	III (≥ 200)
Blood glucose----Fasting (mg %)	84.45	120.66	141.29
Blood glucose----PP2BS (mg %)	105.46	168.62	273.45
Fructosamine ((μ mole/l)	320.96	498.53	684.87
HbA1c (%)	5.52	8.06	9.47
Fructosamine/ HbA1c ratio	58.15	61.85	72.32

Table – III Group differences for various biochemical estimates validated by t-test.

Biochemical estimates	Group I and II t value	Group I and III t value	Group II and III t value
Fasting	4.73 ****	6.16 ****	1.94*
Postprandial	9.46 ****	7.86 ****	4.84 ****
HbA1c	8.19 ****	17.74 ****	4.17 ****
Fructosamine	3.36 ***	5.95 ****	3.21 ***
Glycosylation Gap	0.51 NS	2.25**	1.86 *

Test of significance :

* (P<0.1), ** (P<0.05), *** (P<0.01), **** (P<0.001)

Table – IV Correlation values between two estimates in different groups.

Biochemical estimates	Group I	Group II	Group III
Fasting blood sugar and HbA1c	0.58 *	-0.28	0.23
Postprandial blood glucose and	0.68 **	0.64 **	0.16
Fasting blood sugar and	-0.06	0.49	0.46
Postprandial blood and	-0.01	0.46	-0.20
HbA1c and Fructosamine	-0.13	0.09	0.56 *

Test of significance :

* (P<0.05), ** (P<0.01), *** (P<0.001)

Blood Glucose levels:

The postprandial blood glucose levels reported as fasting and postprandial levels. The postprandial blood glucose levels were ≤ 140 mg% in Group I, between 141 to 199 mg% and in Group II and it ≥ 200 mg% in group III. Fasting as well as postprandial blood glucose levels were lowest in Group I and highest in Group III. The mean levels of fasting and postprandial blood glucose was 84.45 mg% and 105.46 mg% in Group I, 120.66mg% and 168.62 mg% in Group II, 141.29mg% and 273.45 mg% in Group III, respectively. The observed differences for fasting and postprandial levels between the groups of were significant (Table-III).The fasting blood glucose levels were lower than those of postprandial levels in all groups. The correlation coefficient between all forty five observations of fasting and postprandial.

Glycosylated haemoglobinA1c (HbA1c)

Glycosylated HbA1c was 5.52 % in group I, 8.06 % in group II and 9.47% in group III patients. In other words, the Glycosylated haemoglobin level increased with the increase in blood glucose from group I to group III. The observed group differences in HbA1c levels between any two groups were highly significant. (Table III).There was a

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significant correlation between Glycosylated haemoglobin and fasting blood glucose only in group I. Glycosylated HbA1c also correlated significantly with postprandial blood glucose in group I and group II but not in group III (Table IV). The correlation coefficient between HbA1c and all forty five observations (irrespective of the groups) of fasting blood glucose ($r=0.63, P<0.01$) and postprandial blood glucose ($r=0.77, P<0.01$) were as positive and highly significant. Correlation coefficient values between HbA1c % and PP2BS blood glucose for Group A ($<7.0\%$ HbA1c) and Group B (HbA1c $>7.0\%$) revealed positive significant correlation coefficient between fasting and PP2BS levels. The correlation coefficient values (0.81 with fasting blood glucose and 0.85 with PP2BS) in group A having HbA1c $<7\%$ were highly significant and the corresponding values was .17 with fasting blood glucose and 0.46 with PP2BS) in group B having HbA1c $>7\%$ were significant with only in PP2BS and not in case of fasting blood glucose level.

HbA1c values were grouped in to two groups as Group A having HbA1c $<7\%$ and as Group B having HbA1c $>7\%$. The HbA1c values were correlated with fasting and postprandial blood glucose levels (Table V). The analysis revealed that in Group A, the correlation coefficient values were highly significant in fasting as well as postprandial blood glucose levels. In case of Group B, the correlation coefficient values behaved differently and found to be non significant in case of fasting blood glucose level but was found to be highly significant in case of postprandial blood glucose level. An overall correlation coefficient value was 0.81 with fasting and was 0.85 with postprandial blood glucose levels as compared to their value in group B. The results were indicative that the correlation coefficient was more in group A having lower HbA1c ($<7\%$) the group B having more HbA1c ($>7\%$).

Table – V HbA1c groups related blood glucose and correlation coefficient

Characters	Group A (HbA1c $<7\%$) N=19,MEAN HbA1c5.72%		Group B (HbA1c $>7\%$) N=28 ,MEAN HbA1c 8.98	
	Fasting Blood glucose	Postprandial Blood glucose	Fasting Blood glucose	Postprandial Blood glucose
Observations	19	19	28	28
Blood glucose(mean)	91.20	111.54	131.62	229.59
HbA1c and Blood glucose Correlation coefficient (r value)	0.81****	0.85****	0.17 NS	0.46***

Fructosamine :

The fructosamine levels expressed in $\mu\text{mole/l}$ were 320.96 in Group I, 498.53 in Group II and 684.87 in Group III. In other words as the mean blood glucose increased, the level of fructosamine also increased from group I to group III. The observed group differences in levels between any two groups were highly significant (Table III). The correlations between fructosamine and blood glucose levels were not significant in any of the three groups. However the levels of fructosamine and HbA1c correlated significantly only in group III having highest blood sugar level (Table IV). Fructosamine/HbA1c ratio was 58.15, 61.85 and 72.32 in group I, group II and group III, respectively. The correlation coefficient between all forty five observations of fasting blood glucose and fructosamine was positive ($r=0.66$) and highly significant ($P<0.01$) like that with PP2BS and fructosamine was positive ($r=0.54$) and highly significant ($P<0.01$).

Glycosylation gap(GG):

The GG values were negative in all the three groups. The mean GG values were -1.55,-2.03 and -3.79%, respectively for group I, group II and group III, respectively (Table II). The recorded GG values when compared for group

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differences, it was significant between groups I and III as well as between groups II and III but it was non-significant between groups I and II. The correlation coefficient between all forty five observations of fasting blood glucose and GG was negative ($r = -0.66$) and not significant so also was recorded with PP2BS and GG was negative and not significant ($r = -0.21$). The correlation coefficient was also recorded between GG, fructosamine and HbA1c. The correlation values between GG and fructosamine, between GG and HbA1c was negative and not significant with corresponding values of $r = -0.87$ and $r = -0.24$ respectively.

IV. DISCUSSION

In the present study, total 45 patients with different postprandial blood glucose levels ranging from ≤ 140 to ≥ 200 mg% were studied which included 21 males and 24 females. The percentage wise, 22.22% ($n=11$) of patients were between 20-50 years and 77.78% ($n=35$) from 51 years onwards (Table-I). Three study groups having equal numbers ($n=15$ in each group) were made depending upon their postprandial blood sugar levels (Group I, ≤ 140 mg%; Group II, 141 to 199 mg% and Group III ≥ 200 mg %).

Blood Glucose Levels

The observed differences between any two groups were significant both for the fasting and the postprandial blood glucose levels (Table-III). The fasting blood glucose levels were lower than those of postprandial levels in all groups. The mean fasting and postprandial bloods glucose increased from group I to group III. Given the emphasis on fasting blood glucose, it was reported by other workers that the fasting capillary glucose (FCG) was the most effective predictor of 2-h post-load glucose and the best criterion for discriminating diabetes and other glucose tolerance abnormalities from normal glucose tolerance. Further, a combined use of fructosamine and fasting capillary glucose (FCG) predicted 2-h post-load glucose better than combined use of fasting capillary glucose and HbA1c. It was also reported that HbA1c correlated stronger with fasting capillary glucose than with 2-h post-load glucose [12]. Blood glucose level with haemoglobin A1C ($r=0.75$, $p<0.01$) and fructosamine ($r=0.54$, $p<0/01$) showed a significant correlation [13]. Generally, FBS was a more accurate predictor for HbA1c compared with HbA1c as a predictor of FBS. FBS sounds more reliable to separate diabetic from non-diabetic subjects than HbA1c [14]. In the present study the correlation coefficient between all forty five observations of fasting and postprandial blood glucose (irrespective of the groups) was positive and highly significant ($r=0.60$, $P<0.01$). Comparative results could not be found in literature.

Glycated haemoglobin (HbA1c)

Glycated haemoglobin (HbA1c) is the marker for long term glycaemic control. Intra-individual variation in HbA1c values is minimal in nondiabetic persons, but considerable inter-individual variation has been reported [15],[16],[17]. Elevated HbA1c indicates poor glycaemic control and predicts the risk for the development of cardiac complications in diabetes. In the present study Glycosylated haemoglobin significantly differed between the groups. The mean level of Glycosylated haemoglobin increased from 5.52 to 9.47 % significantly with the increase in blood sugar from ≤ 140 mg% to ≥ 200 mg%. It is reported that lower values indicate better glycaemic control. (19,20) Accordingly Lower levels of both HbA1c and blood glucose levels in group I is indicative of relatively better glycaemic control in group I (blood glucose ≤ 140 mg%, HbA1c -5.52%) than group II (blood glucose 140 to 199 mg%, HbA1c 8.06 %) and group III (blood glucose ≥ 200 mg%, HbA1c 9.47 %). In the present study the average HbA1c tends to be higher as age increased (below 50 yrs--7.25%; above 50 yrs--7.81%. The present finding of no difference between age groups was in accordance to a non-significant correlation between HbA1c and age reported [18]. It was reported that among the general healthy population, HbA1c tends to be higher as age increases [19]. Significant age difference in HbA1c was reported in subjects above 50 years old, the oldest having the highest values [20]. In contradiction to this, others found no significant relationships between age and fasting plasma glucose (FPG), glycated haemoglobin, glycated protein or glycated albumin [21]. However, these findings were not in accordance to the reported significant positive correlations between HbA1c and age as well as duration of diabetes in diabetic patients [22]. In the present study there was significant correlation between Glycosylated haemoglobin and fasting blood glucose only in group I. Glycosylated HbA1c also correlated significantly with postprandial blood glucose in group II and group III but not in

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group I. In other words, HbA1c levels correlated significantly in group I but not significant for fasting as well as postprandial blood glucose levels. A significant correlation between HbA1c and blood glucose ($r=0.75$) have been reported [13]. However, a reported positive correlation between HbA1c and fasting blood sugar was $r=0.50$ and $r=0.48$ in the Diabetic patient on haemodialysis and the Diabetic patient but no haemodialysis, respectively [23]. In the present study, a positive and highly significant correlation existed between different HbA1c groups i.e. groups A ($<7.0\%$) and HbA1c, B ($>7.0\%$) with all forty five observations (irrespective of the groups) of fasting blood glucose ($r=0.63$, $P<0.01$) and postprandial blood glucose ($r=0.77$, $P<0.01$). correlation coefficient Group A with, Group B with HbA1c between and Group C with HbA1c revealed positive significant correlation coefficient to be 0.79, 0.59, 0.35 respectively with the fasting blood glucose levels and the corresponding values were 0.87, 0.63 and 0.01 for postprandial blood glucose levels wherein the correlation coefficient values were significant for group A and B but not significant in case of Group C.

Fructosamine (FA)

The common perception is that FA results are different from HbA1c exclusively because of the difference in turnover times of the underlying glycation targets, haemoglobin versus serum proteins [24]. Fructosamine is a potentially useful post-load glycaemia index as it correlated stronger with 2-h post-load glucose concentrations than with fasting capillary glucose [12]. It was not so in the present study as correlation was stronger with fasting blood glucose levels compared to PP2BS level, when all observations were included irrespective of the groups I, II and III.

In the present study, the fructosamine concentration increased (from 320.96 to 684.87 $\mu\text{mole/L}$) with the increase in mean blood glucose levels (from ≤ 140 to ≥ 200 mg%), The observed group differences in the fructosamine levels between any two groups were highly significant. (Table III).

Ignoring the groups (I, II, III) the correlation coefficient between all observations of fructosamine and blood glucose levels (fasting and PP2Bs) in the present study findings were that a positive and stronger correlation was with fasting ($r=0.66$) than PP2Bs ($r=0.54$) levels. These findings were in accordance to the finding of a positive correlation between fructosamine and blood glucose level ($r=0.54$) [25].

GroupWise correlation coefficient between the fructosamine and blood glucose levels (fasting and postprandial) revealed that the values were more in group II having more blood glucose (140mg% to 199 mg%) compared to group I having less blood glucose (≤ 140 mg%), subsequently the correlation value decreased in group III having highest blood glucose (≥ 200 mg%). All the correlation values were not significant in all the groups and therefore, the present finding was not in agreement to the reported positive correlation between FBS and fructosamine in the Diabetic patient on haemodialysis, ($r=0.37$) and Diabetic patient but no haemodialysis, ($r=0.35$) as the groups under study were different.

A GroupWise correlation coefficient between HbA1c and fructosamine was found to increase with the increase in postprandial blood glucose level in such a way that it was negative and minimum ($r= -0.13$, HbA1c 5.52%, FA 320.96 $\mu\text{mol/l}$) in group I (≤ 140 mg%), significantly maximum ($r=0.59$, HbA1c 9.47%, FA 684.87 $\mu\text{mol/l}$) in group III (≥ 200 mg%) as compared to the reported values ($r=0.09$, HbA1c 8.06%, FA 498.5396 $\mu\text{mol/l}$). in group II (140 to 199mg%) Another study on diabetic patients, reported positive correlation between duration of disease and serum fructosamine levels [24]. A correlation was reported between HbA1c and FA [11],[25]. In another study it was Cancer risks (prostate, colorectal, and lung cancer) were highest among those with increasing glucose levels and decreasing fructosamine levels [26].

The results of present study indicate that the different trends reported in the present study could be attributed to the more than one factor like different blood glucose levels, different duration of diabetes, hypoglycaemic treatments and age groups. Further work is obviously needed to appreciate if there are inter-individual differences in the activity of fructosamine 3-kinase, and if this significantly impacts on glycated haemoglobin values. Fructosamine/HbA1c ratio was 58.15, 61.85 and 72.32 in group I, group II and group III, respectively.

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The fructosamine/Hb A1c ratio was studied in diabetic mellitus patients undergoing haemodialysis (DM-HD) and was significantly higher than that in diabetic mellitus patients undergoing no haemodialysis (DM-non-HD) patients (P=0.002) [27].

Glycation gap (GG):

Despite newer, more reliable technology, however, significant differences between measures of plasma glucose and glycaemia estimated from HbA1c are still encountered [28].

Because haemoglobin is an intracellular protein and fructosamine reflects extracellular proteins, the glycation gap could result from differences between the ambient glucose concentrations or rates of glycation in the intracellular and extracellular compartments or interindividual differences in the turnover/metabolism of underlying proteins [24],[29]. Glycosylated gap mean values were 0.51, 2.25 and 1.86 in group I, group II and group III, respectively. The GG value was least in group I having least mean glucose level ≤ 140 mg%, rose to highest level in group II having glucose level between 141 to 199 mg% and then decreased in group III having glucose level \geq between 141 to 1999 but remain higher than group I mean value. A broad and higher distribution range of GG was recorded in present study indicating differences in the measured versus predicted HbA1c compared to those reported range from -3.2% to 5.5%. The glycosylation gap could be affected by the production and disappearance rate of glycated haemoglobin, glycated serum proteins, or both. we observed consistent discordances between HbA1c and an extra-erythrocyte measure of glycemic control, FA [28].

The discordances are not explained by differences in turnover of underlying proteins. GG correlated better with nephropathy than did either HbA1c or FA alone in this population [30].

The glycation gap could be affected by the production and disappearance rate of glycated haemoglobin, glycated serum proteins, or both(9). Most studies reveal that both fasting and postprandial blood glucose show an increase in the level as age advances. Fasting blood glucose increases by 1-2 mg/dl per decade while postprandial blood glucose increases up to 15mg/dl per decade increase in age [14],[30],[31],[32].

V. CONCLUSION

The present study was conducted in total 45 patients with postprandial blood glucose levels ranging from ≤ 140 to ≥ 200 mg%. Their fasting and postprandial blood glucose levels between the groups were significant. The fasting blood glucose levels were lower than those of postprandial levels in all groups. The Fructosamine concentration increased from 320.96 to 684.87 ($\mu\text{mole/l}$) with the increment in the mean blood glucose level, from ≤ 140 to ≥ 200 mg%. The differences in the fructosamine levels between any groups, were significant.

Patients with type 2 diabetes mellitus having one or more micro vascular complications had poor glycemic control compared to normal healthy persons and patient with type 2 DM without any complications. These finding indicate role of poor glycemic control in development of micro vascular complication in type 2 DM and control of hyperglycemia is very important in management of diabetes and prevention of diabetic complications. Further studies at the molecular level are required to know the how hyperglycemias modulate various pathway in producing diabetic complications.

We suggest that if fructosamine is to be used as an index of glycaemia control in diabetes, it is supplemented by a measurement of HbA1C when fructosamine measurements are stable, in order to determine whether the given value of fructosamine is consistent with the glycaemia control targets for that individual. The results were indicative that the correlation coefficient was more in group A having lower HbA1c ($< 7\%$) the group B having more HbA1c ($> 7\%$).

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