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Clinical Research

GLYCATED PLASMA PROTEINS AS SHORT TERM GLYCOMETABOLIC MARKERS.

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ABSTRACT Glycated plasma proteins estimation which is akin to HbA1c is a cost-effective investigation to estimate the glycometabolic condition of a diabetic patient. Given the Socioeconomic strata of a country wherein this noncommuicable disease is observed irrespective of urban or rural populace, it is important to identify, short term diagnostic and prognostic markers of diabetes and its complications .Glycated plasma proteins is a time tested marker even in developed countries such as Japan, hence it can safely be sought as an investigative tool in the management of diabetes .

KEYWORDS : Glycate Plasma Proteins , Diabetes

INTRODUCTION:

Glycated proteins are formed by a non-enzymatic reaction between reducing carbohydrates (e.g. glucose, fructose, ribose, or derivatives such as ascorbic acid) with amino groups which are located in the N-terminal position or in lysine and arginine residues.

Unlike Glycosylation which is involved in many biological processes, glycation in contrast is a completely undesired modification from a clinical point of view. Because of the crucial role of glucose as an energy source in humans, it is the main circulating sugar and thus the most relevant molecule in terms of protein glycation. The effects on biological function will depend on the extent of glycation. . These carbonyl compounds are generally more reactive than the original carbohydrate and act as propagators by reactions with free amino groups, leading to the formation of a variety of heterogeneous structures irreversibly formed and commonly known as advanced glycation end products. The impact of glycation encompasses alterations of the structure, function, and turnover of proteins the glycemic status of diabetics is influenced by two classical factors, glucose concentration and half-life of the protein [15] From a clinical point of view, the detection of this Post Translational Modification at the initial stage would be helpful for both prognostic and diagnostic purposes. Glycation has often been related to chronic complications of diabetes mellitus, renal failure, and degenerative changes occurring in the course of aging. Even in non-diabetic patients [14],[12].

Studies have demonstrated that glycated plasma proteins induce oxidative stress [18], enhance, pro-inflammatory endothelial response [20]. This study was conducted to check the hypothesis that glycated plasma proteins can be used as short term diagnostic and prognostic markers based on the age of the patient, duration of diabetes and their association with different complications associated with diabetes mellitus.

MATERIALS and METHODS: The study group included 52 randomly selected diabetics attending the government hospital at Visakhapatnam, and 30, healthy controls. The blood samples of the above subjects were analyzed for Fasting blood sugar, Glycated plasma proteins and Glycated hemoglobin's, after obtaining their consent.

In 30 of the diabetic's glucose tolerance test was performed and in 2 fasting and 2hr blood glucose value after glucose load. 9 patients were followed up with weekly sample analyses during their stay in the hospital for treatment (3-4 weeks). Glycated plasma proteins [13] and Glycated hemoglobin's [11] were estimated by spectrophotometric method using thiobarbituric acid. The method is selected for its feasibility in the routine clinical biochemistry laboratory in the hospital, and because thiobarbituric acid reacts specifically with stable ketamine linkages which will be advantageous especially when using them as short term markers of glycaemic control and eliminating unstable schiff base adducts.

RESULTS: Data related to controls and patients
Table 1

Study Subjects	Number
Total diabetics studied	52
Type 1 diabetics	10
Type 2 diabetics	42
Diabetics with complications	28
Diabetics without complications	24
Diabetics under control	19
Diabetics not under control	33

The blood sugar values are expressed in mg/dL and Glycated plasma proteins as absorbance per gram protein (A/g protein). Glycated hemoglobin values as % of total hemoglobin. The data is analyzed statistically. The mean and standard deviation values are calculated. The significance of difference in mean values of different parameters in different study groups is assessed using ANOVA (F and p) and Student t test (t and p). The correlation between different parameters is assessed by calculating correlation coefficient (r) and p values. The data is also presented graphically.

The fasting blood sugar and Glycated plasma protein values are significantly higher in diabetics compared to controls.[Table 2]
Table 2

Study Subjects	Number	Fasting Blood Glucose (in mg/dl), Mean+/- SD mg/dL	Glycated Plasma protein (in A/g protein) Mean+/- SD
Controls	30	74 +/- 6.96	5.45 +/- 1.12
Diabetics	52	185+/- 89.27	10.15 +/- 3.21
t		6.72	7.67
p values		<0.0001	< 0.0001

The glycated plasma proteins in diabetics correlated with fasting blood glucose, blood glucose at 1 hr. And 2 hrs. after glucose load and with glycated hemoglobins , which is statistically significant . No statistically significant correlation was observed between Glycated plasma proteins and fasting plasma glucose and glycated hemoglobin's

controls. [Table 3]

Table 3

S.No	Number	Analytes Correlated	Diabetics		Number	Controls	
			r	p		r	p
1	52	Glycated Plasma Proteins Vs Fasting Blood Sugar	0.45	<0.001	30	0.012	Not Significant
2	30	Glycated Plasma Proteins Vs Fasting Blood Sugar	0.32	< 0.01	-	-	
3	32	Glycated Plasma Proteins Vs Fasting Blood Sugar	0.4	< 0.001	-	-	
4	52	Glycated Plasma Proteins Vs Glycated Hemoglobins	0.24	<0.01	30	0.21	Not Significant

No statistically significant difference in fasting Blood glucose and Glycated Plasma proteins was observed in different age groups of diabetics. [Table 4]

Table 4

Age	Number Of Patients	Fasting Blood Glucose (mg/dl) Mean+/- SD	ANOVA	Glycated Plasma Proteins (A/gm Protein) Mean +/- SD	ANOVA
< / = 40 Years	9	177 +/- 57		10.09 +/- 2.88	
41 - 50 Years	17	171 +/- 78.34	F = 0.691	9.04 +/- 3.3	F = 1.375
51 - 60 Years	13	161 +/- 57.69		10.88 +/- 2.78	
> 60 Years	3	117 +/- 24.25	P = 0.563	7.92 +/- 0.54	P = 0.265

No statistically significant difference in glycated plasma proteins was observed in relation to duration of diabetes. However the blood glucose values were significantly different in different groups with varying duration [Table 5]

Table 5

Duration	Number Of Patients	Fasting Blood Glucose (mg/dl) Mean+/- SD	ANOVA	Glycated Plasma Proteins (A/gm Protein) Mean +/- SD	ANOVA
Freshly Detected	18	153+/- 76.37		10.08 +/- 2.88	
02 Years	10	157 +/- 66.41	F = 5.585	10.06 +/- 4.71	F = 0.027
02 – 05 Years	9	280 +/- 129		10.03 +/- 2.76	
> 05 Years	15	172 +/- 54.22	P = 0.002	10.35 +/- 3.06	P = 0.994

Glycated plasma proteins in different groups of diabetics with and without complications were compared, the glycated plasma protein values were significantly different. No significant differences were observed when blood glucose values in these groups were compared [Table 6]

Table 6

Diabetics Complications	Number Of Patients	Fasting Blood Glucose (mg/dL) Mean+/- SD	ANOVA	Glycated Plasma Proteins (A/gm Protein) Mean +/- SD	ANOVA
Acute And Chronic Complications	28	210 +/- 89.93		10.87 +/- 2.80	
Chronic Complications	20	206 +/- 143.04	F = 1.134	9.94 +/- 2.37	F = 3.305
Acute Complications	8	219+/- 48.11		13.19 +/-2.52	
Retinopathy	14	215 +/-108.3	P = 0.271	11.16 +/-2.51	P = 0.014
No Compliactions	24	155 +/- 78.40		9.30 +/- 3.53	

DISCUSSION:

This is a study based in Southern India.

The fasting blood sugar and Glycated plasma protein values were significantly higher in diabetics compared to controls however the blood glucose values were significantly different in different groups with varying duration.

Glycated plasma proteins in different groups of diabetics with and without complications were compared, and the glycated plasma protein values were statistically significantly.

The Amadori-albumin, a major glycated plasma proteins, in experimental hyperglycemia, induced microvascular complications, and is associated with advanced nephropathy in Type I diabetic patients. Amadori-albumin was also associated with early nephropa thy and with retinopathy [10]. Thereby making it biomarker of diagnostic and prognostic value.

Its common knowledge that in diabetes management, Hb A1c is specifically requested by clinicians, for assessment of the efficacy of their treatment protocols and patient compliance. One of the advantages of using HbA1c is, the sample can be drawn independent of patients fasting /post prandial states

Selvin.E et al and other Borg ([21,3]) have suggested ,there is racial differences in non-fasting glycaemia wherein in case of glycated plasma proteins it was observed that there were no differences between the sexes similar to our study ; however, race differences were observed (higher levels in blacks relative to whites)

Given the incidence of diabetes [[2]] and CAD [[10]] among Asian Indians, more so in certain South Indian populace as reported by Anjana et al & Krishnan et al respectively , it's prudent to use blood glucose and glycated plasma protein values, in managing diabetic patients especially in southern India. Where the staple main carbohydrate is rice. It's ideal to use glycated plasma proteins as a short term prognostic marker of the patient's drug compliance. Given the exorbitant cost in using an HbA1c for monitoring diabetes. Lifestyle changes has effected tribes ,who prefer treatments in Government general hospitals and medical college attached hospitals for the low cost of treatment , it's been observed that they too suffer from non-communicable diseases like diabetes[[7,1]] . Since their physical activity has seasonal variations compared to their urban cousins as well as amount of carbohydrate intake ,it becomes imperative that a 3 months turnover RBC suggested HbA1c would not be as effective as glycated plasma proteins with shorter half-life of 14–20 days [e.g. albumin] .Daily monitoring is more ideal but not feasible in all set ups.

According to some studies the HbA1c test / glycated plasma proteins has not yet been shown to be related to the risk of the development or progression of chronic complications of diabetes [[4]], but in our study there was significant changes in the glycated plasma proteins values in the diabetic patients based on the duration of disease and between the patients with complications. The results of our study agree with Jamil-ur Rahman et al that glycated plasma proteins, were significantly raised in diabetic patients. They further identified that glycated plasma proteins, levels in patients on oral hypoglycemic drugs their glucose level was comparatively more stable and was least correlated with insulin treated patients where glucose levels were more labile [[16]]. This finding further emphasizes that glycated plasma proteins, are good short term prognostic markers of patients on oral hypoglycemic agents.

Atherosclerosis Risk in Communities (ARIC) Study found that HbA1c does not change rapidly in response to changes in treatment, and a number of conditions affect the validity of the test result (e.g., anemia, altered red cell lifespan, transfusion, kidney disease, liver disease, and abnormal forms of hemoglobin). They also found that elevated baseline concentrations of glycated plasma proteins, were associated with vascular outcomes and mortality in the community, even after adjustment for traditional cardiovascular risk factors, with especially strong associations in persons with diabetes mellitus [[18]]

Further studies are required in these communities on their glycometabolic control based on their glycation gap [[6,9]] which is an empirical measure of the extent of the difference between HbA1c and glycated plasma proteins levels., but possible artifacts can be caused by dependence of the glycated plasma proteins , level on the extent of serum protein metabolism or half-life as already suggested in this article , hence it requires careful consideration by an alert clinicians to manage patients under unstable glycemic control .

CONCLUSION: Glycated plasma proteins are biomarkers of short-term glycemic control [[20]]. And can provide prognostic information similar to HbA1c for risk stratification and prediction of diabetes and its microvascular complications as already in practice in developed countries like Japan. [[19]]

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