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Glycation of Nail Keratin as an Index of Long Term Glycemic Control and Comparison with Other Indices

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Abstract **Original Research Article**

A study was conducted in Government Stanley Medical College and Hospital, in the Department of Biochemistry, to assess nail keratin glycation as an indicator of long term glycemic control. A total of 70 individuals, 35 known diabetic patients and 35 healthy individuals were enrolled for the study. The patients were longitudinally followed up for three months to assess their glycemic status. The aim was to establish whether the nail fructosamine could be used to determine the glycemic status of the diabetic patients over the preceding three months which is the turnover time of the nail from root to the free edge. HbA1C estimation was used to confirm the changes in the patient's glycemic status and compared with nail fructosamine. A linear relationship was established after analysis of the results (p value 0.009). There was also a significant difference of nail fructosamine between cases and controls (p value 0.09).The mean value of nail fructosamine in controls (2.18µmol/g equivalents of nail) was within the physiological range (2.0-2.5µmol/g) as reported by Goldsmith et al in 1985.The nail fructosamine levels determined in diabetic cases were above the physiological range (2.68 and 3.1µmol/g).The fasting blood glucose and serum fructosamine were also measured for assessing the blood glucose levels in both cases and controls. These parameters on comparison with nail fructosamine showed linear relationship. To conclude, the nail fructosamine assay could be standardized for determining the long term glycemic status of an individual as well as in people with Diabetes Mellitus. The reference range in south Indian population needs to be established. The interference like drugs and other dietary substances which might react with NitroBlueTetrazolium need to be studied and the procedure can be used as routine laboratory investigations to assess the blood glucose levels on long term basis. The method is cheaper, easier to perform and sample collection and preservation is easier (nail clippings form finger nails) and noninvasive. Pre analytical variation is practically nil. Precision of the assay is good.

Keywords: Nail Fructosamine, Serum Fructosamine, HbA1c, Glycemic control, Diabetes Mellitus.

INTRODUCTION

Diabetes Mellitus a global health issue now has become the ninth major cause of death .Our sedentary life style, physical inactivity, faulty food habits ultimately led us to become leader nation with high incidence of Diabetes. Type 2 DM affects one in every eleven persons worldwide [1].The cost of extending health care to diabetics is rising [2]. HbA1C testing considered as the index of glycemic control is not only costly but also becomes unreliable with many conditions like anemia, CKD, Hemoglobinopathies. A search for a cost effective method to determine glycemic status, this study aims at finding out the relationship between nail keratin glycation i.e Fructosamine in nail and HbA1C levels in Diabetic and nondiabetic individuals and its evaluation as an indicator of long term glycemic control.

Keratin belongs to the class of fibrous proteins. Nail comprises of Nail matrix, Nail plate, Cuticular system, Nail bed, Nailfolds & Lunula. The nail plate is made up of hard keratin and is of horny and translucent nature. It varies in thickness about 0.5 and 0.75 mm**.** Three layers are identified; they are thin dorsal lamina, thick intermediate layer, and Ventral layer. The ventral layer is about one-fifth of the nail thickness is derived from the nail bed and the rest from the nail matrix. The nail plate is showing closely packed flattened squamous cells in close opposition because of tortuous and interlocking plasma membranes [3].The soft tissue where the nail overlies is called the nailbed. It consists of a vascular matrix contiguous with the periosteum of the distal phalanx, two or three celled layer of epidermis (living layer of cell) above which dead cells of the ventral nail plate lies. As the cells differentiate, they are incorporated into the ventral surface of the nail plate

and move distally with this layer. Finger nails grow at a rate of 0.1mm per day and toe nails at one third of this rate [3], approximately the time taken for the ventral nail plate cells to reach the free edge will be 3 months. Recent studies showed that fructosamine in nail estimation can be used as a measure of long term control of blood sugar in diabetic patients [4]. Hence glycation in keratin present in finger nails gives an average estimate of blood glucose levels over the previous three months. Nail clippings are taken for testing. For comparison, HbA1C determination by immuno-turbidimetry is used .This study aims at testing nail fructosamine levels in nail protein of finger nails by Nitro Blue Tetrazolium method and whether it can be useful as an indicator of long term glycemic control over the previous 3months in diabetic patients and to compare with serum fructosamine levels by NBT method :(Endpoint) Baker *et al*.

MATERIALS AND METHODS

After getting approval from the Institute's Ethical Committee, 35 Type 2 Diabetic patients (ADA Criteria FBS ≥ 126 mg %) attending Diabetology OPD and 35 Healthy non diabetic controls of age between 22 years and 72 years were enrolled for the study. Those with nephropathy [5], Retinopathy, persistent hyperproteinemia due to any cause, those with diseased fingernails and artificial coloring on nails were excluded.

Nail clippings about 10 mg, were taken twice for estimation of nail fructosamine by Nitroblue tetrazolium method(NBT) using spectrophotometer. HbA1C is estimated by using Immunoturbidimetric 2nd Generation kits in fully automated chemistry analyser. Serum fructosamine assayed by NBT method kinetic assay in fully automated chemistry analyser.Two samples analysed with interval of 2-3 weeks [6].Total serum proteins and urinary spot PCR were done to decide over the exclusion criteria.Fasting Blood glucose levels were done to confirm the Diabetes mellitus for cases and also for controls.

Nail Fructosamine assay:(Endpoint) Baker et al. using NBT reagent ,pH10.3 .Incubation time allowed was one hour after addition of the reagent and readings taken at 530nm in a double beam UV 1800 spectrophotometer of Fructosamine standard (ROCHE) was used for assay and results are calculated. Serum fructosamine assay is a kinetic assay and the rate of formation of formazan compound is proportional to the amount of fructosamine present in the serum. The study is observational and the selected patients are followed up longitudinally for three months for determination of their glycemic status either weekly or biweekly follow-ups. The indices determined to assess the glycemic status in this study are 1.Fasting blood glucose, 2.Serum Fructosamine, 3.HbA1C.Two values taken as PRE/(1) and POST/(2) before and after the specified duration above were performed for all controls after obtaining informed and written consent. The average estimated glucose (derived value from HbA1C- MBG) is also taken. The three indices are correlated with nail fructosamine of the same individual to evaluate this promising cost effective glycemic index and its usefulness in longterm glycemic control.

RESULTS AND STATISTICAL ANALYSIS

The long term glycemic control of diabetic patients were analysed using a newer parameter-glycated nail protein (fructosamine) in finger nails and the results were compared with the controls.In the present study 35 cases of diabetic patients, 35 controls were studied and their Fasting plasma glucose, HbA1C levels, serum fructosamine levels and nail fructosamine levels were analysed.

Most of the patients belonged to the age group of 41-50. The mean age of the study group was 51.1years among the diabetics and in controls maximum was in 30-50 yrs. Females constituted 77% of the study population in the cases and 74% of the total control population.Family history was positive in 65.7% of the patients in 60% of controls.In the present study most of the patients were in oral hypoglycemic drugs mainly(88.57%). Others were given OHA in a combination with insulin.Duration of Diabetes among the patients belonged maximum to less than 5 years (71.5%) and > 5years (28.5%). Hypertension was found to be the most common associated comorbidity (22.8%) followed by hypothyroidism.

Table-1: Comparison of means – glycemic indices

S.no	Variable	Mean- controls	Mean- case	T value	P value	Significance
1	Fbs mean	97.2	170.24	-8.967	0.0001	Strong significance
2.	Mean blood glucose	100.77	202.17	-8.388	0.0001	Strong significance
3.	HbA1C	5.583	7.806	-8.632	0.0001	Strong significance
4.	Serum fructosamine	267.43	333	-5.562	0.0001	Strong significance
5.	Nail fructosamine	2.177	2.679	-2.706	0.009	Significant

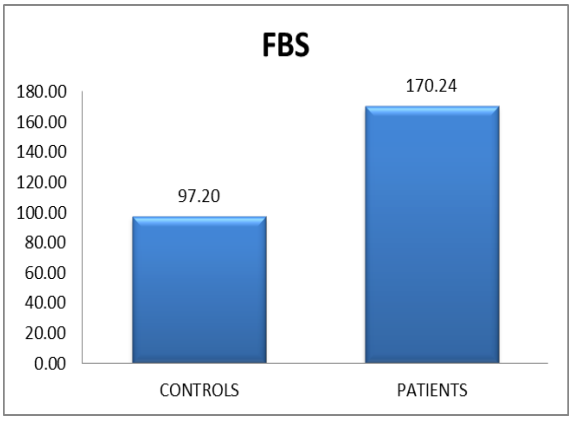

Fig-1: Comparison of FBS Mean

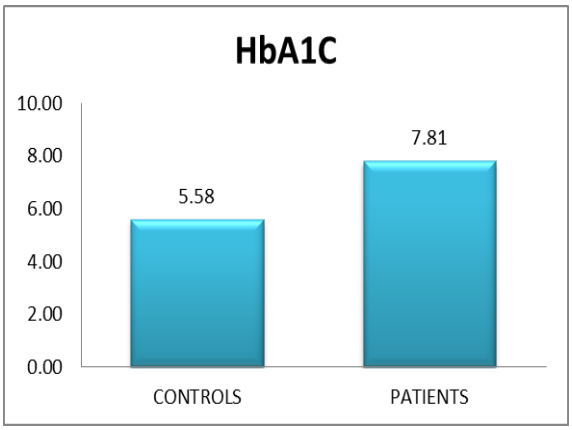

Fig-2: Comparison of HbA1C

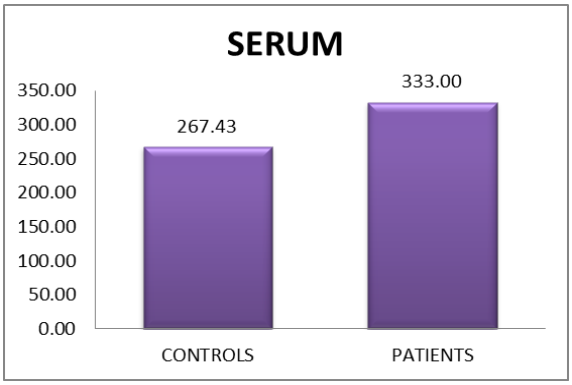

Fig-3: Serum Fructosamine

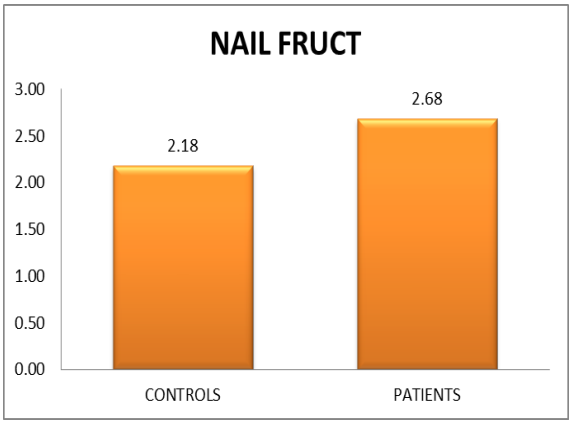

Fig-4: Nail Fructosamine

Table-2: Comparison of Means initial and final values

	N	Minimum	Maximum	Mean	Std. Deviation
HbA1C1	35	6.1	15.1	8.654	2.1203
HbA1C2	35	6	11	7.81	1.480
MBG1	35	117	417	202.17	70.742
MBG2	35	107	274	173.80	49.282
Nailfruct1	35	1.40	7.50	3.1017	1.39401
Nailfruct2	35	1.30	4.80	2.6786	.90304

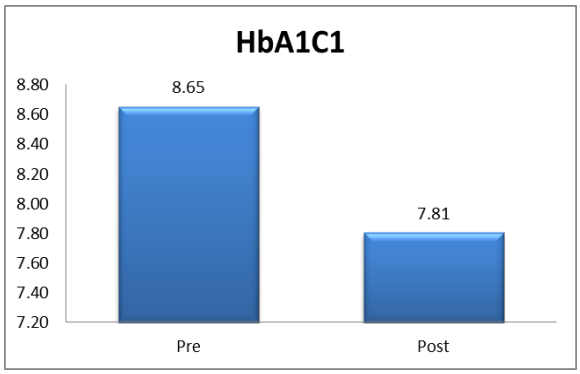

Fig-5: HbA1C (1) & (2) values

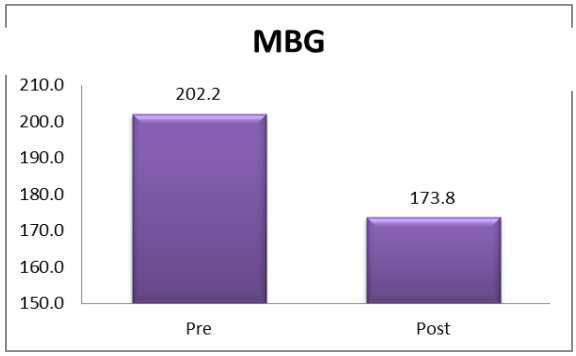

Fig-6: Mean Blood Glucose (1) & (2)

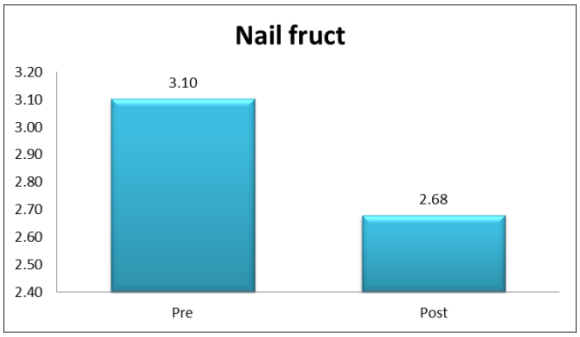

Fig-7: Nail fructosamine (1) & (2)

Table-3: Paired samples test

		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	HbA1C1 - HbA1C2	.8486	1.3565	.2293	.3826	1.3146	3.701	34	.001
Pair 2	MBG1 - MBG2	28.371	45.103	7.624	12.878	43.865	3.721	34	.001
Pair 3	Nailfruct1 - NailFruct2	.42314	1.47724	.24970	-.08431	.93059	1.695	34	.099

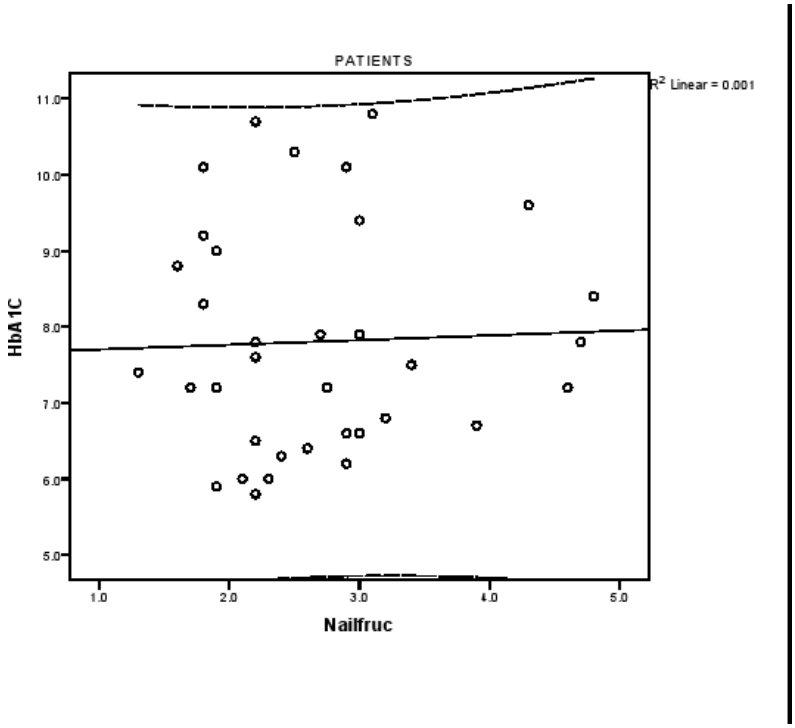

Fig-8: Least Squares Regression Analysis HbA1C and Nail fructosamine in Diabetics

DISCUSSION

Analysis of the results are summarised as follows:

- The glycation [7] in nail protein which forms 80% of the nail tissue depends on the blood glucose levels of the individual and reflects the average blood glucose over the preceeding three months ie the duration of the nail growth across the nail plate. Hence it is compared with HbA1C which also reflects the average glucose over the same duration.
- Out of the 70 individuals analysed, 35 are known diabetics and 35 of the same age group whose Fasting Plasma Glucose is <126 mg % and no known symptoms served as controls.
- The observations made are:
- Glycemic status analysis.
- Comparison with other glycemic indices.

Glycemic status of the individuals is studied by comparison of Fasting blood Glucose , HbA1C, Serum Fructosamine with nail fructosamine for all patients and compared with control population.The relationship of the nail fructosamine with FBS is linear.The relationship of nail fructosamine and HbA1C is linear.

The relationship of nail fructosamine and serum fructosamine is linear.As observed by A.S.Kishabongo *et al*. the ROC analysis for nail fructosamine yielded a cut off value of 3.14 µmol/g of nail, but in this study, it was found to be 2.35µmol/g of nail the reason could be the smaller sample size. Referrence interval [6] Male: 0.58 – 3.8µmol/gm equivalent of nail Female: 0.55–3.32µmol/gm equivalent of nail. The mean for nail fructosamine initially in diabetics and control population studied were 3.1 and 2.18µmol/g respectively. As the glycemic control achieved was the aim of the study, the repeat values for the same individuals were done after 10 - 12 wks and the mean for the final values was 2.68µmol/g. This reflects the glycemic control achieved during the period of study. The same finding is also confirmed by the mean values obtained in Hb A1C initially and finally. Both initial and final values in the nail fructosamine were found to be above the physiological range 2.0 - 2.5µ mol fructosamine/gm of protein, as observed by Goldsmith in 1985, whereas the control group mean lies within the physiological range (2.18µ mol). The mean values of controls as compared to the values given by Kishabongo *et al.* (1.75µmol) done in the sub-Saharan African population is less than that done in our south Indian population because the staple food varies between these two countries. The reasons being the staple cereal is maize in sub-Saharan region (glycemic index 55) but for the population in this study is rice (glycemic index varies from 86 -109 depending upon the variety of rice).

Data from the report of CCAFS (Climate Change Agriculture and Food Security)the annual consumption of various foods in South Asian and Sub-Saharan population is shown in the following table

Table-4: Dietary variations in south Asian and sub– saharan population

S.No	Type of food	SouthAsianpopulation (Kg/person/year)	Sub-Saharanpopulation (Kg/person/year)
1.	Cereal food	150	125
2.	Roots and Tubers	40	200
3.	Sugar & Sugar crops	20	10
4.	Vegetable oil, oilseeds & products	10	10
5.	Meat	5	10
6.	Milk & Dairy excuding butter	80	30

The cereal consumption is more in proportion to the total food intake in South Asian population, rice being the staple food. The cereal consumption is less in proportion to the total food intake in the sub-Saharan population. The roots and tubers are rich in fibre. These food habits are possible reasons for the lower mean value for nail fructosamine in control population. Also the population included for study as per the sub - Saharan study were aged 1 - 91 years and mean for controls is 36 yrs and for diabetics 62 yrs .Considering the median for diabetics without complications in the literature referred, it was 3.77µ mols and the values are less in our study group because of the difference in age and duration of the diabetes. The individuals with nephropathy and retinopathy were included in the Sub-Saharan study population and their mean nail fructosamine values were in the higher range 5.64 and 5.65µmol/g of nail.In our study mean age for controls is 41.54 and cases is 51 yrs. Both nephropathy and retinopathy were excluded from our study.

The individual values [8] of the two glycemic indices were comparable and showed linear relationship except for four patients. Two were hypothyroid, on eltroxin, one patient on treatment for schizophrenia and the other was taking aspirin for treatment of cerebro - vascular complication.

Thus an evaluation for confounding factors [9] in the assay due to alcohol consumption, commonly used drugs and other substances will enlighten us on further implementation of this assay.

CONCLUSION

The nail fructosamine assay is easier,simpler and cost effective. The sample collection can be done by the patients themselves or by their relatives. Pre-analytical variation is practically nil. Transport and preservation of sample is easier.The procedure is simple to perform and can be performed in any laboratory attached to Primary Health care facility. The precision of the assay is good. On comparing with other glycemic indices like fasting plasma glucose, serum fructosamine and HbA1C, it shows linear relationship. Interference in the assay due to commonly used drugs has to be evaluated and the procedure of nail fructosamine estimation can be standardized for routine use in the laboratory to assess glycemic status of the individuals both diabetic and healthy population.

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