Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 03, pp.48279-48282, March, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

WHETHER STORAGE FACILITIES ALTER HbA1c VALUES?

[1,*]Malligai, E., [2]Divya Sri, L.R., [3]Murugavel, S.K. and [4]Manikandan, N.

[1]Professor and head of the Department of Biochemistry, Chettinad Academy of Research Institute, Kelambakkam Chennai
[2]Postgraduate, Department of Biochemistry, Chettinad Academy of Research Institute, Kelambakkam Chennai
[3]Assistant Professor, Department of Biochemistry, Chettinad Academy of Research Institute, Kelambakkam Chennai
[4]Lab Supervisor, Department of Biochemistry, Chettinad Academy of Research Institute, Kelambakkam Chennai

ARTICLE INFO

Article History:
Received 15th December, 2016
Received in revised form
12th January, 2017
Accepted 19th February, 2017
Published online 31st March, 2017

Key words:
HbA1c,
HPLC ion,
Chromatography.

ABSTRACT

Hemoglobin A1C (HbA1c) has been recognized as a diagnostic marker for diabetes mellitus by the ADA in 2013. Accuracy is extremely important to diagnose the disease. In case of HbA1c measurements, accuracy may be affected by various factors like transportation, storing facility and temperature alterations etc. Therefore, we designed this study to find out whether temperature and storage timings alter HbA1c results.

Aım: The aim of the study was to find the comparison of HbA1c levels at different time intervals and storage temperatures.

Materıals and Methodology: Paired Samples were collected from subjects attending master health check-up program irrespective of their diabetic status. In the first step, samples were processed immediately and the values were noted down. Then the first set was stored at 24°C and re -estimated after 8 hours and 16 hours. The second set was kept at 4°C and estimated after 8 hours and 16 hours. 18 samples alone from second set (4°C) were measured at 30 hours. All the samples were measured using BIO-RAD D10 machine based on HPLC ion exchange chromatography.

Results and Conclusıon: Statistical analyses were done using Paired student t test and Pearson correlation coefficient in SPSS software version 21. Linear regression analysis was used to visually represent the difference. Our study did not show any major difference in HbA1c values irrespective of temperature and duration of the storage.

Citation: Malligai, E., Divya Sri, L.R., Murugavel, S.K. and Manikandan, N. 2017. "Whether storage facilities alter hba1c values?", *International Journal of Current Research*, 9, (03), 48279-48282.

INTRODUCTION

Hemoglobin A1c (HbA1c) has been recognized as a tool for the diagnosis of diabetes. Hemoglobin A1c (HbA1c) has been used for many years to assist in the monitoring and treatment of diabetes (Chris Florkowski, 2013). The case for HbA1c for as a diagnostic test was put forward as early as the mid-1980s, but concerns regarding its availability and poor assay standardization prevented its uptake. In 2009, an international expert committee brought HbA1c as diagnostic criteria (≥6.5%). This recommendation was then taken over by the American Diabetes Association (ADA) later then by the WHO. Increased emphasis has been placed upon the use of this marker as a diagnostic tool. Glycated haemoglobin (HbA1c) is a biomarker that is fundamental for the diagnosis of diabetes and for monitoring glycaemic control (Cas Weykamp, 2013).

****Corresponding author: Malligai, E.***
Professor and head of the Department of Biochemistry, Chettinad Academy of Research Institute, Kelambakkam Chennai.

The following are the HbA1c values given by the ADA, 4.5-5.6 as normal, 5.7-6.4 as pre-diabetic or the high risk group and ≥ 6.5 as diabetic. Even a mild variation in the values by 0.5 is said to affect the diagnosis part. Its measurement depends on venepuncture, and on processing, transportation and storage of samples, which can be logistically challenging. Since the above said factors can affect its accuracy, we designed this study. Fasting blood glucose is the routine test to identify Diabetes. A new criteria introduced by ADA in 2010 known as HbA1c (Glycosylated Hb). The formation of HbA1c is a post translational, non-enzymatic, substrate concentration dependent, irreversible process of combination of aldehyde group of glucose with amino terminal valine of the beta chain of Hb. It can be measured by several methods like Enzymatic, Turbidimetry, Immunoassay, Capillary electrophoresis and High performance liquid chromatography (Rajni Dawar Mahajan *et al*., 2011). In 2004, IFCC standardized the test and has added value for HbA1c estimation (Miedema, 2004).

Table 1. Values of HbA1c and CV at spot, room temperature 8 and 16hrs, cold temperature 8 and 16 hrs and at 30 hrs

	SPOT(DONE IMMEDIATELY)	AT 24.C	AT 4.C
Spot 1	8.462±2.080		
Spot 2	8.455±2.081		
Room temperature 8H	8.462±2.080	8.581±2.079	
Room temperature 16H	8.462±2.080	8.574±2.094	
Cold temperature 8H	8.455±2.081		8.700±2.079
Cold temperature 16H	8.455±2.08		8.693±2.075
At 30 hours	8.455±2.081		8.661±2.071

Table 2. Data of spot samples and within each group compared using paired t test

		Mean HbA1c values	Std. Deviation	Paired Samples t-test			
				Mean Difference	SD	t	Sig.
Pair 1	Spot 1	8.462	2.080	.007	.026	1.776	.083
	Spot 2	8.455	2.081				
S1	Room (8 Hrs)	8.581	2.079	.007	.071	.650	.519
	Room (16 Hrs)	8.574	2.094				
S2	Cold (8 Hrs)	8.700	2.079	.007	.026	1.776	.083
	Cold (16 Hrs)	8.693	2.075				

Table 3. Comparison of Group 1 and Group 2 using Independent t test.

Group 1 Spot 1	Group 2 Spot 2	T value	P value
Room temperature 8H	Cold temperature 8H	-0.27801	.391344
Room temperature 16H	Cold temperature 16H	-0.26168	.397111

Table 4. Comparing the data using Pearson Correlation coefficient

Correlations

	Spot HbA1c	Room (8 Hrs)	Room (16 Hrs)	Cold (8 Hrs)	Cold (16 Hrs)
Spot	1	.999**	.999**	.999**	.999**
Room (8 Hrs)	.999**	1	.999**	.999**	.999**
Room (16 Hrs)	.999**	.999**	1	.999**	.999**
Cold (8 Hrs)	.999**	.999**	.999**	1	1.000**
Cold (16 Hrs)	.999**	.999**	.999**	1.000**	1

Correlations

	Spot	48 Hours
Spot	1	.999**
48 Hours	.999**	1

HbA1c has greater stability than plasma glucose concentration. Food, time of collection of sample and physical activities won't change its values. There are only handfuls of studies describing how the technical handlings of samples affect HbA1c value. Hence this study is designed to find out the effects of storage hours and temperature on HbA1c results using paired samples.

MATERIALS AND METHODOLOGY

This particular study was done from September 2016 to October 2016. People who attended Master health check-up programme aged between 20 to 60 years were selected. Samples were collected from 40 subjects who were selected irrespective of their diabetic status. From each subject 2 samples were obtained. Hence totally 80 samples were collected, divided into two groups according to the type of storage. Initially both the samples (S1+S2) were measured immediately and S1 group stored at room temperature (24˚C). And S2 stored at 4˚C. After 8 hours and 16 hours, samples from both the groups were analyzed. 18 samples stored at 4˚C were measured after 30 hours.

RESULTS AND ANALYSIS

Data obtained from both the group of samples were analyzed by paired t test and Pearson correlation coefficient. Linear regression curve was kept for visual comparison. All the statistics were done with computerized version of SPSS software version 21.

DISCUSSION

For decades clinicians and laboratorians are using Fasting plasma glucose (FPG) as the ideal test to diagnose Diabetes Mellitus. Occasionally Oral glucose tolerance test is preferred when the values are on the borderline with strong family history. Otherwise OGTT is done to rule out impaired glucose tolerance. Factors like Food, activity and drugs play a role in the estimation of Glucose. Inconvenient timing of sampling, multiple samplings are great hindrance in FPG and OGTT measurement. Hence Expert Committee introduced HbA1c (Glycated Hemoglobin) as a diagnostic marker in 2010.

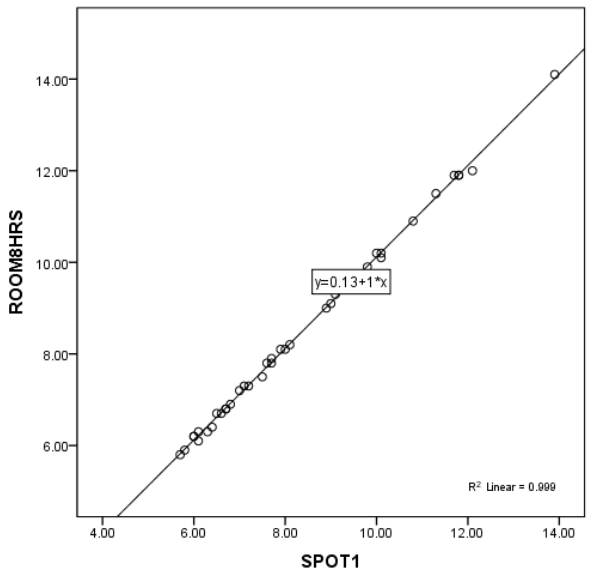

Figure 1. Effect on HbA1c values measured immediately and after 8 hours of storing at 24˚C

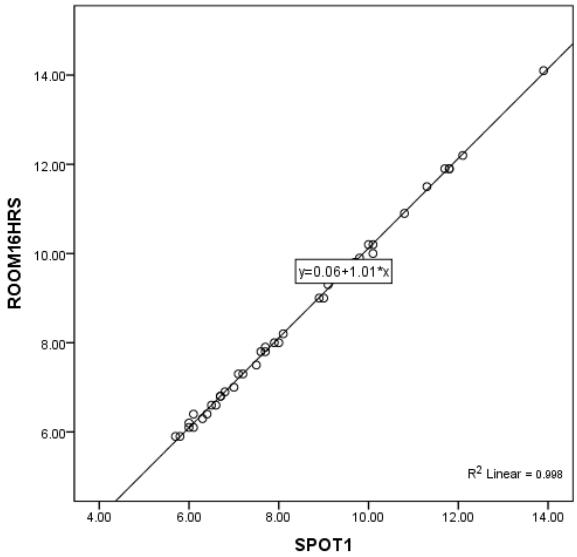

Figure 2. Effect on HbA1c values measured on spot and after 16 hours of storage at 24˚C

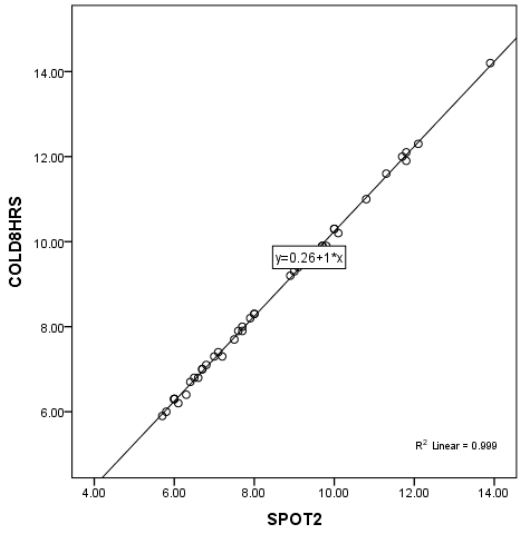

Figure 3: Effect on HbA1c values at spot and 8 hours of storage at 4˚C.

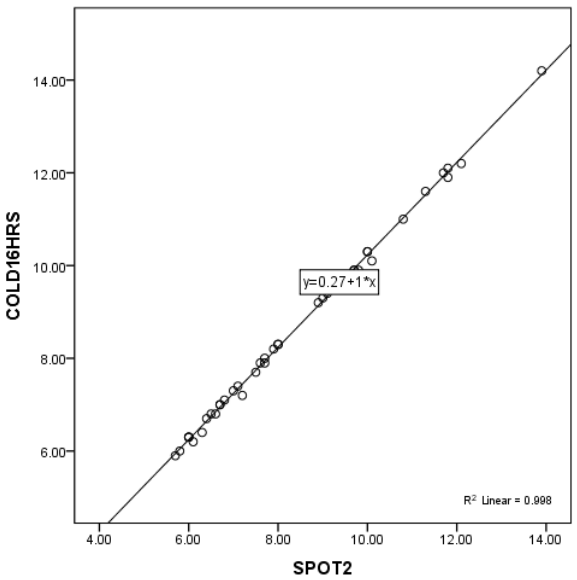

Figure 4. Effect on HbA1c values estimated at spot and after 16 hours of storage at 4˚C.

HbA1c is unaffected by the above mentioned factors. This test can be done at any hour of the day and this test equals the assessment of hundreds of fasting glucose levels and also captures postprandial glucose peaks. It is an ideal test to monitor glucose status. This study is designed to find out whether the technical handling of the samples (storage timings and temperature) can affect the accuracy of HbA1c results in diagnosis of Diabetes Mellitus. The data collected and analyzed by paired student t test. The paired samples measured immediately showed no difference (p value 0.083). The samples at room temperature tested at 8 hours and 16 hours gave similar values (p value 0.58). The third set of samples stored at 4˚C estimated at 8 and 16 hours exhibited p value 0.08. The above analysis showed no difference between the three pairs Spot 1 and2, Room storage at 8 and 16 hours, Cold storage at 8 and 16 hours. Spot 1 is considered as first group and spot 2 as a second group and the values of first group at 8 hours at 24˚C is compared with values of second group at 8 hours at 4˚C by independent t test. It showed a p value of 0.39 which authenticates the similarity of results.

In the same way the first group (24˚C) HbA1c values measured at 16 hours is compared with second group (4˚C) HbA1c values measured at 16 hours using independent t test. The insignificant p values (0.39) rules out the difference between them. Spot values are correlated with other values obtained at 8 and 16 hours both at 24˚C and 4˚C independently by Pearson correlation which confirms the highest strength of association (r values=.999). 18 samples stored at 4˚C were measured at 30 hours and the results are analyzed by paired t test. P values of 0.293 indicate the similarity of results. The study shows that HbA1c levels are not altered by the storage timings and temperature and it coincides with the results published by Yoshinari Nomura *et al* and Szymezak *et al* where they have concluded that no effect of temperature and timings on the measurement of HbA1c (Yoshinari Nomura *et al*., 1983; Szymezak *et al*., 2009). The above said study was carried out at different temperatures as 4˚C,-40˚C, and 80˚C at 24 and 48 hours. But Room temperature is not included. Room temperature is included in our study since in a country like India where the laboratory receives its sample through its collection centers and sometimes before transporting there are chances that it has been exposed to higher temperature.

The relationship between spot samples, room temperature at 8 and 16 hours and cold temperature at 8 and 16 hours are visually represented by linear regression graph. This study concludes that storage temperature and timings does not alter the HbA1c values grossly, but we like to point out that in spite of statistical analysis which shows the similarity of results, gross observation shows the values differ by 0.12 to 0.24 and the study should be done with huge sample size to find out whether this difference can affect the accuracy of HbA1c in diagnosing Diabetes Mellitus.

REFERENCES

Cas Weykamp. 2013. HbA1c: A Review of Analytical and Clinical Aspects. *Ann Lab Med*., 33:393-400.

Chris Florkowski. 2013. HbA1c as a diagnostic test for Diabetes Mellitus-Reviewing the evidence. *Clin Biochem Rev.,* Vol 34.

Miedema. K. 2004. Towards worldwide standardisation of HbA1c determination. *Diabetologia*, 47:1143-1148.

Rajni Dawar Mahajan, Bhawesh Mishra. 2001. Using Glycated Haemoglobin HbA1c for diagnosing of Diabetes Mellitus: An Indian Perspective. *Int J Biol Med Res*., 2(2):508-512.

Szymezak, J., Lavalard, E., Leroy, N., Gillery, P. 2009. Influence of storage temperature on the HbA1c assay by high performance liquid chromatography method. *John Libbey Eurotext*, 67:6.

Yoshinari Nomura, Kishio Nanjo, Akira Kawa, Motoshige Miyano, Kazuhiko Okai *et al*. 1983. Reliability of HbA1c Assay in the mass survey for Diabetes Mellitus, with Special Reference to the Preservation and Transportation of Blood samples. *Tohuko J.exp.Med*., 141, Suppl.,77-84.

****