ROLE OF GLYCATED HEMOGLOBIN IN THE DIAGNOSIS OF DIABETES MELLITUS

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ABSTRACT

Objective: To evaluate glycated hemoglobin as a marker for diagnosis of diabetes mellitus.

Design: Comparative cross-sectional study

Place and Duration of Study: This study was carried out between July 2005 to April 2006 at department of chemical pathology and endocrinology, Armed Forces Institute of Pathology (AFIP) Rawalpindi.

Subjects and Methods: Subjects (n=104) demonstrating impaired glucose regulation at the endocrine clinic of AFIP were selected. Forty seven age and sex matched normoglycemic healthy looking controls were also included in the study. Samples under complete medical fasting state were collected for glucose and glycated hemoglobin analysis, and then samples were collected at 1 hour and 2 hour for plasma glucose as part of oral glucose tolerance testing (OGTT). These subjects were later stratified to have all the disease ranges in terms of severity by 75 g OGTT results into following groups: Group-1: Controls, Group-2: Subjects with impaired fasting glucose (IFG), but became normoglycaemics on performing OGTT (Plasma glucose fasting result = 5.6 -6.9 mmol/L, but 2 h OGTT result < 7.8 mmol/L), Group-3: Subjects with impaired glucose tolerance (IGT) as per ADA recommendations (2 h OGTT result between 7.8-11.1 mmol/L), and Group-4: Subjects with diabetes mellitus as per American diabetic association (ADA) recommendations (2 h OGTT result >11.1 mmol/L). Receiver operating curve (ROC) curve analysis and diagnostic performance in terms of sensitivity, specificity, predictive values were used to evaluate the performance characteristic of glycated hemoglobin, in comparison with plasma glucose fasting and 1 hour OGTT results against 2 hour OGTT results taken as gold standard.

Results: ROC curve analysis showed an area under the curve (AUC) of 0.722 for plasma glucose fasting, 0.607 for 1 hour OGTT and 0.564 for glycated hemoglobin. Moreover, the diagnostic performance as measured by sensitivity, specificity, predictive values and efficiency stood higher for plasma glucose fasting than glycated hemoglobin in the diagnosis of diabetes mellitus.

Conclusion: Glycated hemoglobin, as determined by ion exchange resin chromatography is less useful for the diagnosis of diabetes mellitus than plasma glucose fasting. So the present approach for using plasma glucose fasting for diagnosing diabetes mellitus must remain in vogue.

Keywords: Oral glucose tolerance test, glycated hemoglobin, diabetes mellitus

INTRODUCTION

Diabetes mellitus is one of the largest emerging pandemic of the modern age [1]. Developments and progression in lifestyle has on one side allowed the human skills to develop all leading to a luxurious life style, but the other view saw the increased frequency of metabolic diseases [2]. Diagnosing "diabetes mellitus" is desirable for early intervention through changes in life

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styles and medications, but also remains essential to avoid its long-term micro and macro vascular complications [3]. The disease "diabetes mellitus" has undergone the initial stages of recognition to diagnostic criteria development, and now has entered into the phase of molecular diagnosis and improvements into the previously defined aspects. The recent criteria word health organization and American diabetic association (WHO and ADA) recommends initial screening through plasma glucose fasting state followed by (if required) an oral glucose tolerance test (OGTT) [4].

Measurement of glucose in plasma over the years has become very reproducible, and has been measured through very well characterized methods [5]. However; the inherent in vivo minute to minute variability of glucose in response to multitude of exogenous and endogenous factors can result in marked variation in the results in the same individual [6,7]. Moreover the glucose in plasma is only a representation of the balance between intakes versus utilization, which may be different on very other day [8]. On any given day the reported plasma glucose results may not be indicative of the true endogenous glycemia index, and definitely can be shadowed by a multitude of variables. It was therefore always required that a test for diagnosis of diabetes mellitus be free of all these in vivo and in vitro factors and which should always give a reflection of overall glucose homeostasis over a prolong duration pf time [9-11]. In this regard glycated hemoglobin has emerged as a potential candidate marker [12].

Glycated hemoglobin forms when glucose in the plasma combines with the amino group of proteins (hemoglobin) by non-enzymatic glycation. Initially the complex is labile, but later the amodari rearrangements lead to permanent nature of this glycated product [13]. Already this investigation has been in clinical application for monitoring treatment in subjects with diabetes mellitus. The major clinical advantages which can be anticipated from the clinical application of this marker include the fact that it provides an insight over 3-4 months of glycemic status and does not suffer from the minute to minute in vivo variations. Moreover, a medical fasting state is not required for its measurement. Very recently the debate regarding the use of glycated hemoglobin for routine diagnosis of diabetes mellitus has re-emerged with various studies appearing on the pubmed with contrasting results [10,11,14,15] Keeping in view the anticipated clinical utility of glycated hemoglobin, the results from various contrasting studies and probably the social and racial differences which could be there in our society, a study is planned to evaluate the diagnostic performance of glycated hemoglobin in the diagnosis of diabetes mellitus in our set up.

MATERIALS AND METHODS

This validation study was conducted at the department of chemical pathology and endocrinology, Armed Forces Institute of Pathology from July 2006 to April 2006. The target sample population constituted those subjects who reported at AFIP for evaluation of their plasma glucose fasting. The subjects who demonstrated plasma glucose fasting results > 5.6 mmol/L were considered further. These selected subjects were requested to report for evaluation by adding queries in their lab reports. Seven hundred and forty- eight subjects reported. Individuals already diagnosed to have diabetes mellitus, or other secondary disorders or using any kind of medication were straight away excluded from the study. Finally selected individuals were requested to report in medical fasting state on any working day. By October 2006 one hundred and four subjects reported. These individuals were formally consented, explained the procedural details, interviewed, examined, and sampled. Forty-seven age and sex matched normoglycemic controls were also included in the study. The initial sample comprised plasma glucose fasting and glycated hemoglobin. Later 2 hour

oral glucose tolerance test was started and sampling was done after one hour and two hours. Plasma glucose was analyzed by method of GOD-PAP on Selectra–2 clinical chemistry analyzer, Glycated hemoglobin were analyzed by ion-exchange chromatography (Stanbio kit) on Microlab – 300.The labile aldamine fraction was removed by pre-incubation of the sample with isotonic saline for thirty minutes. Later, HbA1c fraction was calculated using the conversion formulas available in the kit literature.

The 2 hour OGTT results were considered the gold standard in the diagnosis of diabetes mellitus. Based upon the results of 2 hour OGTT, the data were grouped into four categories, as:

- Group-1 (n=42): Age and sex-matched normoglycemic controls (All three plasma glucose results were within reference range). Five subjects, who demonstrated normoglycemia on plasma glucose fasting, were found to have impaired glucose regulation and diabetes as per 2 hour OGTT results.
- Group-2 (n=34): Subjects with impaired fasting glucose (IFG), but became normoglycemics on performing OGTT (Plasma glucose fasting result = 5.6 -6.9 mmol/L, but 2 h OGTT result < 7.8 mmol/L).
- Group-3 (n=44): Subjects with impaired glucose tolerance (IGT) as per ADA recommendations (2 h OGTT result between 7.8-11.0 mmol/L).
- Group-4 (n=31): Subjects with diabetes mellitus as per ADA recommendations (2 h OGTT result > 11.1 mmol/L).

STATISTICAL ANALYSIS

The data were entered in the SPSS-version 11 program, and descriptive statistics were calculated. The results of glycated hemoglobin were evaluated with plasma glucose fasting (the present day recommended marker of diagnosis of diabetes mellitus) and 1 hour OGTT results against 2 hour OGTT. Receiver operating curve (ROC) analysis was carried out to know the area under the curve (AUC)for glycated hemoglobin, one hour OGTT result and plasma glucose fasting, keeping 2 hour OGTT results as gold standard. Finally the diagnostic performance characteristics in terms of sensitivity, specificity, predictive values and overall efficiencies were calculated at different cut-offs for these markers.

RESULTS

The mean age and sex distribution between patients and controls (table-1). The comparison between plasma glucose fasting, glycated hemoglobin and one hour OGTT results in the diagnosis of diabetes mellitus (table-2 and figure). The highest area under the curve was for plasma glucose fasting. The plasma glucose fasting showed overall higher diagnostic performance than glycated hemoglobin and 1 hour OGTT result. The various detailed cut-offs along with their sensitivity, specificity, predictive values and their efficiencies (table-3).

Table-1: Descriptive statistics of data collected (n=151).

Group	Gender	Age (years)
Normoglycaemia (n=42)	Male(n=24)	43.2
	Female(n=18)	45.1
Only IFG (n=34)	Male(n=19)	41.7
	Female(n=15)	42.3
IGT (n=44)	Male(n=23)	44.1
	Female(n=21)	43.2
Diabetes mellitus (n=31)	Male(n=16)	45.6
	Female(n=15)	46.8

DISCUSSION

Glycated hemoglobin remained a less useful alternative as compared to plasma glucose fasting in the diagnosis of diabetes mellitus. This finding is in concordance with the studies of Peter's et al and Greci et al [16,17] But on the other hand there are studies which advocate the more useful nature of glycated hemoglobin in the diagnosis of diabetes mellitus. McCane et al have proved of glycated hemoglobin as a better screening

Table-2: Area under the curve for plasma glucose fasting, one hour OGTT result, and glycated hemoglobin.

Test Result Variable(s)	Area under the curve	Std. Error	Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Plasma glucose fasting	0.722	0.04	< 0.001	0.639	0.805
One hour OGTT result	0.607	0.05	< 0.05	0.507	0.707
Glycated hemoglobin	0.564	0.06	< 0.05	0.445	0.684

Table-3: Diagnostic performance at important cut-offs for glycated hemoglobin, one hour OGTT and plasma glucose fasting.

Parameters	Percentile selected	Value	Sensitivity%	Specificity%	PPV%	NPV%	Efficiency%
Plasma glucose fasting (mmol/L)	60	4.9	90.33	61.90	63.64	89.66	73.97
	65	5.5	80.65	73.81	69.44	83.78	76.71
	70	6.2	77.42	76.19	70.59	82.05	76.71
	75*	6.7	77.42	83.33	77.42	83.33	80.82
	80	6.8	67.74	85.71	77.78	78.26	78.08
	85	7.3	58.06	90.48	81.82	74.51	76.71
Glycated hemoglobin (%)	60*	5.3	70.97	45.24	48.89	67.86	56.16
	65	5.8	61.29	50.00	47.50	63.64	54.79
	70	6.0	48.39	52.38	42.86	57.89	50.68
	75	6.1	41.94	57.14	41.94	57.14	50.68
	80	6.4	29.03	61.90	36.00	54.17	47.95
	85	6.7	22.58	73.81	38.89	56.36	52.05
1 hour OGTT (mmol/L)	60	9.2	74.19	45.24	50.00	70.37	57.53
	65	10.2	58.06	47.62	45.00	60.61	52.05
	70	11.9	54.84	57.14	48.57	63.16	56.16
	75	12.5	48.39	64.29	50.00	62.79	57.53
	80	13.4	38.71	69.05	48.00	60.42	56.16
	85*	14.1	32.26	80.95	55.56	61.82	60.27

**Recommended cut-offs*

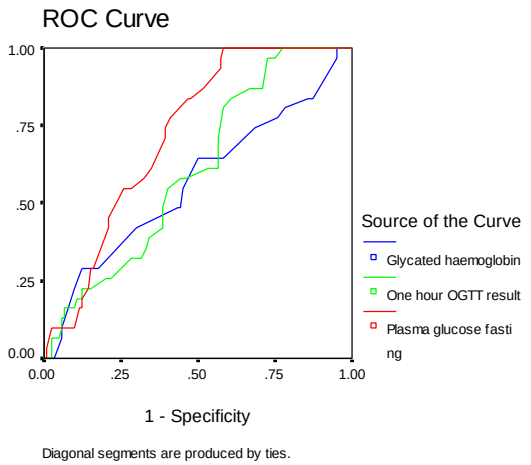

Figure: Receiver operating curve (ROC) curve analysis showing the comparison between plasma glucose fasting, one hour OGTT result, and glycated hemoglobin for the diagnosis of diabetes mellitus against 2 h OGTT results.

test for diabetes mellitus [18]. In this prospective analysis, the main outcome measure remained the progression of disease to retinopathy and neuropathy. These findings may be very important with regards to atherosclerosis, which includes in it manifold causative factors, and not just the glycemia. The most important limitation which this study may have is the fact that the study was focused on Pima Indians, who have definitive genetically determined risks for diabetes mellitus, resulting in full blown diabetes appearing very early in life [19]. Another study by Rohlfing et al, again has shown glycated hemoglobin being a superior marker for diagnosis of diabetes mellitus, but the focus target sample population ware Mexican Americans [20]. The authors have very strongly identified significant physiological variations among various communities [21].

Some of the studies which have produced results like ours have yielded higher specificities and negative predictive values for

glycated hemoglobin [22,23]. This finding may be important for glycated hemoglobin as a marker replacing OGTT in future. However our study was not primarily meant to define the various cut-offs for use of glycated hemoglobin in clinical screening program.

The few limitations to the study are as follow; firstly our sample size, however in configuration with the statistical requirements remained smaller as compared to some of the other studies. Secondly, the differences in methodologies are quite obvious between various studies [24]. In this regard we have resorted to the methodology i.e., the ion exchange resin chromatography, which is most routinely used in our country. Finally the fact that our focus was mainly an urbanized sample and urbanization has been considered important in the development of various metabolic diseases.

Clinical implication of this study remains pertinent to the fact that the medical practitioners tempting to use glycated hemoglobin for the diagnosis of diabetes mellitus should be discouraged. Moreover, keeping n view the enhanced specificity, as demonstrated by some of the studies, it is recommended that a separate study be planned to evaluate glycated hemoglobin as "rule out marker" for diabetes mellitus. This would probably help in situations like stress hyperglycemia.

CONCLUSION

Glycated hemoglobin, as measured by ion exchange resin chromatography remains a less useful investigation than plasma glucose fasting in the diagnosis of diabetes mellitus. Moreover use of glycated hemoglobin as a confirmatory like OGTT, merits further evaluation.

REFERENCES

1. Narayan KM, Zhang P, Williams D, Engelgau M, Imperatore G, Kanaya A, et al. How should developing countries manage diabetes? *CMAJ* 2006; 175(7):733.
2. Bhalla V, Fong CW, Chew SK, Satku K. Changes in the levels of major cardiovascular risk factors in the multi-ethnic population in Singapore after 12 years of a national non-communicable disease intervention programme. *Singapore Med J* 2006; 47(10): 841-50.
3. The Diabetes Prevention Program Research Group. The Diabetes Prevention Program: design and methods for a clinical trial in the prevention of type 2 diabetes. *Diabetes Care* 1999; 22: 623-34.
4. World Health Organization. *Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus*. Geneva: World Health Organization; 1999. Available at: http://Whqlibdoc. Who int/hq/1999/WHO-NCD-NCS-99.2.pdf. Accessed December 12, 2003.
5. Stuart DA, Yuen JM, Shah N, Lyandres O, Yonzon CR, Glucksberg MR, et al. In vivo glucose measurement by surface-enhanced Raman spectroscopy. *Anal Chem* 2006; 78(20): 7211-5.
6. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. Beta-cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. *J Clin Endocrinol Metab* 2005; 90(1): 493-500.
7. Stahl M, Jorgensen LGM, Peterson PH, Brandslund I, Olivarius DF, Borch-Johnsen K. Optimization of preanalytical conditions and analysis of plasma glucose. 1. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. *Scand J Clin Lab Invest* 2001; 61(3): 169-79
8. Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. *JAMA* 2006; 295(14): 1707-8.
9. Petersen PH, Brandslund I, Jorgensen L, Stahl M, Olivarius DF, Borch-Johnsen K. Evaluation of systematic and random factors in measurements of fasting plasma glucose as the basis for analytical quality

specifications in the diagnosis of diabetes. 3. Impact of the new WHO and ADA recommendations on diagnosis of diabetes mellitus. *Scand J Clin Lab Invest* 2001; 61(3): 191-204.

10. Nakagami T, Tominaga M, Nishimura R, Yoshiike N, Daimon M, Oizumi T, et al. Is the measurement of glycated hemoglobin A1c alone an efficient screening test for undiagnosed diabetes? Japan National Diabetes Survey. *Diabetes Res Clin Pract* 2006; [Epub ahead of print]
11. Kallner A, Waldenstrom J. Does the uncertainty of commonly performed glucose measurements allow identification of individuals at high risk for diabetes? *Clin Chem Lab Med* 1999; 37(9): 907-12.
12. Norberg M, Eriksson JW, Lindahl B, Andersson C, Rolandsson O, Stenlund H, et al. A combination of HbA1c, fasting glucose and BMI is effective in screening for individuals at risk of future type 2 diabetes: OGTT is not needed. *J Intern Med* 2006; 260(3): 263-71.
13. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM. What is hemoglobin A1c? An analysis of glycated hemoglobins by electrospray ionization mass spectrometry. *Clin Chem* 1998; 44(9): 1951-8.
14. Kuzuya T, Nakagawa S, Satoh J, Kanazawa Y, Iwamoto Y, Kobayashi M, et al. Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. *Diabetes Res Clin Pract.* 2002; 55(1): 65-85.
15. Lee TJ, Safranek S. FPIN's clinical inquiries. A1C testing in the diagnosis of diabetes mellitus. *Am Fam Physician* 2006; 74(1): 143-4.
16. Peters AL, Davidson MB, Schriger DL, Hasselblad V. A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated hemoglobin levels. Meta-analysis Research Group on the Diagnosis of Diabetes Using Glycated Hemoglobin Levels. *JAMA* 1996 Oct 16; 276(15): 1246-52. Erratum in: *JAMA* 1997 Apr 9; 277(14): 1125.
17. Greci LS, Kailasam M, Malkani S, Katz DL, Hulinsky I, Ahmadi R, et al. Utility of HbA(1c) levels for diabetes case finding in hospitalized patients with hyperglycemia. *Diabetes Care* 2003; 26(4): 1064-8.
18. McCance DR, Hanson RL, Charles MA, Jacobsson LT, Pettitt DJ, Bennett PH, et al. Comparison of tests for glycated haemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ* 1994; 308:1323-8.
19. Traurig MT, Permana PA, Nair S, Kobes S, Bogardus C, Baier LJ. Differential expression of matrix metalloproteinase 3 (mmp3) in preadipocytes/stromal vascular cells from nonobese nondiabetic versus obese nondiabetic pima indians. *Diabetes* 2006; 55(11): 3160-5.
20. Wendel CS, Shah JH, Duckworth WC, Hoffman RM, Mohler MJ, Murata GH. Racial and ethnic disparities in the control of cardiovascular disease risk factors in Southwest American veterans with type 2 diabetes: the Diabetes Outcomes in Veterans Study. *BMC Health Serv Res* 2006; 6: 58.
21. Rohlfing CL, Little RR, Wiedmeyer HM, England JD, Madsen R, Harris MI, et al. Use of GHb (HbA1c) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 2000; 23(2): 187-91.
22. Wiener K, Roberts NB. The relative merits of haemoglobin A1c and fasting plasma glucose as first-line diagnostic tests for diabetes mellitus in non-pregnant subjects. *Diabet Med* 1998; 15(7): 558-63.
23. Tavintharan S, Chew LS, Heng DM. A rational alternative for the diagnosis of diabetes mellitus in high risk individuals. *Ann Acad Med Singapore* 2000; 29(2): 213-8.
24. Matteucci E, Cinapri V, Rossi L, Lucchetti A, Giampietro O. Glycated hemoglobin measurement: intermethod comparison. *Diabetes Nutr Metab* 2001; 14(4): 217-9.