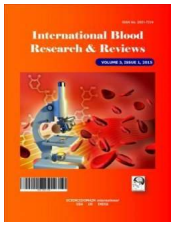

International Blood Research & Reviews
5(4): 1-7, 2016, Article no.IBRR.26078
ISSN: 2321–7219

SCIENCEDOMAIN *international*
www.sciencedomain.org

Behaviour of Fetal Haemoglobin and Its Correlation to Haemoglobin A1c Levels in Sudanese Adult Insulin-Dependent Diabetes Mellitus Patients

Sahar Elbager[1*], Tahani Abbas[2], Mahir Mohamed[1], Rania Ahmed[1], Sana Abdalla[3] and Adil Babiker[1]

[1]Department of Haematology, Faculty of Medical Laboratory Sciences, University of Medical Sciences and Technology, Sudan.
[2]Department of Haematology, Faculty of Medical Laboratory Sciences, University of Karary, Sudan.
[3]Department of Pathology, Faculty of Medicin, Al Neelain University, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. Author GSE designed and performed the research, collected and analyzed data, wrote the protocol and wrote the manuscript. Author ATA provided the technical support. Authors EMM and HRA performed the statistical analysis. Authors ESA and AAB supervised the research. Author AAB reviewed the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2016/26078
Editor(s):
(1) Dharmesh Chandra Sharma, Incharge Blood Component & Aphaeresis Unit, G. R. Medical College, Gwalior, India.
Reviewers:
(1) Mamta Soni, Apollo Hospitals, India.
(2) Moacir Marocolo Júnior, Federal University of Juiz de Fora, Brazil.
(3) Maria Demetriou, University of Thrace, Greece.
Complete Peer review History: http://sciencedomain.org/review-history/14849

Original Research Article

Received 31st March 2016
Accepted 25th May 2016
Published 31st May 2016

ABSTRACT

Aims: To estimate the fetal haemoglobin (HbF) in diabetes mellitus patients and its correlation to haemoglobin A1c levels as it is one of the effective diagnostic tools used in monitoring of diabetic patients.
Study Design: Analytical, laboratory, hospital-based, cross-sectional study.
Place and Duration of Study: The study was carried out during the period March-June 2013. In Dreby Diabetic Centre, Khartoum, Khartoum State.
Methodology: A total of 150 samples were obtained; 50 insulin dependent diabetic patients, 50 non-insulin-treated diabetic patients and 50 control subjects with the same age and sex distribution.

**Corresponding author: E-mail: Saharelbagir@ymail.com;*

Hb A1c was estimated by ion exchange method and HbF was estimated by alkaline denaturation methods.

Results: Results showed significant increase in HbF and HbA1c in insulin-dependent diabetes (IDDM) in comparison to normal control ($p<0.05$). Strong positive correlation between the Hb F level and duration of treatment per year, (R 0.961) and weak negative correlation between Hb F level and Hb Alc in IDDM (R -0.436). Strong negative correlation between Hb F level and Hb Alc according to duration of insulin treatment in IDDM, (R-0.964, R2 0.929, $p<0.05$). There was weak negative correlation between Hb F level and Hb Alc according to age in IDDM, (R 0.580, R2 0.337, $p<0.05$).

Conclusion: It is noted in this study, the high level of HbF accompanied by a decreases in Hb A1c level, thus HbF must be considered when A1C measured by ion exchange method and when comparing it with other measures of glycaemic control.

Keywords: Fetal hemoglobin; HbA1c; diabetes mellitus; type 1 diabetes; type 2 diabetes; (HPLC).

ABBREVIATIONS

DCCT : Diabetes Control and Complications Trial
Hb F : Fetal haemoglobin
HbA1c : Hemoglobin A1c
HPLC : High-Performance Liquid Chromatography
IDDM : Insulin-Dependent Diabetes Mellitus
NIDDM : Non-Insulin-Dependent Diabetes Mellitus
UKPDS : United Kingdom Prospective Diabetes Study

1. INTRODUCTION

Diabetes, the most common non-communicable disease in Sudan, is having an increasing impact on rates of morbidity and mortality in Sudan [1-4]. The spread of sedentary lifestyles and adoption of western dietary habits – high in refined carbohydrates and fat – are driving an increase in the number of people with obesity-related type 2 diabetes. Knowledge of the diabetes epidemic in Sudan is limited. The most recent data come from a small-scale study that was carried out in 1996, the results of the study indicated a prevalence of 3.4% [4]. The major complications of diabetes occur as result of hyperglycemia which is the major source of morbidity and mortality in both type 1 (IDDM) and type 2 diabetes (NIDDM). The harmful of hyperglycemia divided into macrovascular complications (coronary artery disease, peripheral arterial disease and stroke) and microvascular complications (diabetic nephropathy, neuropathy and retinopathy) [5]. Diabetes Control and Complications Trial (DCCT) [6] and the United Kingdom Prospective Diabetes Study (UKPDS) [7] demonstrated conclusively that risks for complications are related directly to glycemic control, as measured by HbA1c.

In longer hyperglycaemia, more glucose molecules attach to the N-terminus of valine residue of the β-globin chain haemoglobin in red blood cells, thus the more glucose binds to red blood cell the higher is the glycosylated haemoglobin. Glycation can also occurs on the lysine residue either on the α or β chains [8,9]. Once a haemoglobin molecule is glycated, it remains that way and glycosylated haemoglobin will accumulate within the red cell, therefore, HbA1c level reflects the average level of glucose concentration in the preceding 2 to 3 months, the average erythrocyte lifespan [10,11]. Regular measurements of haemoglobinA1c lead to changes in diabetes treatment and improvement of metabolic control, indicated by a lowering of haemoglobin A1c values [12].

The accuracy of HbA1c methods can be affected adversely by the presence of haemoglobin variants [13-16]. In addition,elevated levels of foetal haemoglobin (HbF) may interfere with HbA_{1c} measuremen [17-21]. Fetal haemoglobin (HbF, α2γ2) is the main type of haemoglobin of the fetus and newborn. Normally in the second trimester, γ chain production (and Hb F levels) decrease and β chain production increases, resulting in increasing levels of hemoglobin A (Hb A), the major normal adult hemoglobin (α2β2). Normal adults have less than 1% of HbF [22]. Elevated HbF levels can occur in patients as a result of pathologic conditions e.g. (Leukemia, anaemia, and Thalassaemia) or a hereditary persistence of fetal haemoglobin [22]. HbF it glycated at lysine residues of γ-chain

because it has no **β** chain. Glycation of HbF is approximately one-third that of HbA [23,24].

Some published studies reported elevated foetal haemoglobin (HbF) levels in insulin-dependent diabetes mellitus (IDDM) [25-28]. Thus, physicians must be aware that of existence of these conditions and it is potential interference when interpreting HbA1c.

In the present study, we describe the behaviour of fetal haemoglobin in diabetes mellitus patients and its Correlation to haemoglobin A1c levels as it one of the effective diagnostic tools used in monitoring of diabetic patients.

2. MATERIALS AND METHODS

2.1 Data Collection

Data was collected using designed interview based questionnaire to obtain general information about patient (Age and gender), clinical information.

2.2 Study Population

The study was carried out among patients diagnosed with Type 1 (Insulin-dependent diabetes mellitus) diabetic patients who have been under insulin treatment for more than 15 years and Type 2 (Non-insulin-dependent diabetes mellitus).

2.3 Inclusion Criteria

Diabetic patient male and female (40 years old or more, whose HbA1c values differed significantly from the expected results) were included. 3 had a value higher than expected and 47 had HbA1c values lower than expected

2.4 Exclusion Criteria

Adult diabetic patients already diagnosed with (Haemoglobinopathy, Renal failure, Leukaemia, anaemia, Thalassaemia and Hereditary persistence of foetal haemoglobin, etc) were excluded.

2.5 Blood Sample Collection and Analysis

Under a septic condition 2.5 milliliters venous blood was collected in Ethylenediaminetetra acetic acid (EDTA). Haemolysate prepared immediately and tests done within 4 Hours. HbA1c measured with a high-performance liquid chromatography (HPLC) ion-exchange analyzer [29]. Kit manufactured by BioSystems code 1104. [30] Hb F was estimated by: Modified BETKE method [31].

3. RESULTS

Table 1 shows mean ± St. Dev. of HbA1C%, in IDDM group was found to be 7.2±1.2% St. Dev. and in the control group 5.5±0.7%; this difference was found to be statistically significant (*P*= 0.02). In NIDDM, The mean ± St. Dev. of HbA1C% was found to be 8.1±0.4% St. Dev. and in control group 5.5±0.7, this difference is statistically significant (*P*= 0.02).The mean HbF levels, in IDDM group was found to be 2.38% ±2.1 St. Dev., in control subjects it was found to be 0.6% ±0.3; these differences were found to be statistically significant (*P* = 0.000). In the NIDDM group the mean of HbF was found to be 0.7±0.4% and in the normal control subjects it was 0.6±0.3% and these differences were found to be statistically insignificant at (*P* = 0.33).

Table 2 shows the mean of HbA1c in IDDM male subjects was found to be 7.1%, while it was found to be 7.2% in female subjects and this difference was found to be statistically insignificant(*P*=0.85). In the male subjects with NIDDM, the mean of HbA1c level was found to be 8.1±0.4 St. Dev. and in female subjects it was found to be 8.1±0.4 a difference found to be statistically insignificant (*P*=0.91). For HbF%, In IDDM male subjects, the mean of HbF was found to be 2.8%±2.3 St. Dev. and in female subjects it was 2.7±2.4. The difference between the two groups was found to be statistically insignificant (*P*=0.83). In the NIDDM male subjects, the mean of HbF was found to be 0.7±0.3 St. Dev., in female subjects it was 0.8±0.2. The difference between the two groups was found to be statistically insignificant (*P*=0.70).

Table 3 shows mean of HbA1c% in IDDM patients, In group had HbA1c % higher than expected (age 40-44 years) mean was found to be 7.9%, in the group had HbA1c % lower than expected (45-49 years) it was found to be 7.3% and in age group 50-54 years it was found to be 5.8%; this difference was found to be statistically significant (*P*= 0.02).For HbF%, In group had HbA1c % higher than expected(age 40-44 years) mean was found to be 0.8%, in the group had HbA1c % lower than expected (45-49 years) it was found to be 2% and in age group 50-54 years it was found to be 4.2%; this difference was found to be statistically significant (*P*= 0.002).

Regarding the duration of insulin treatment in years, the mean of HbA1c% in case group (IDDM) in the group subjected to insulin treatment of 15-23 years was found to be 7.9%, in the age group of 24-32 years it was 6.1% and in the age group 33-42 years was 4.7%; these differences were found to be statistically significant (*P*=0.04). For HbF%, in the group subjected to insulin treatment for a duration of 15-23 years was found to be 0.8%, in the group subjected to treatment for a duration of 24-32 years it was found to be 3.8% and in group subjected to insulin treatment for a duration of 33-42 years it was found to be 7.1%. The differences between these groups were found to be statistically significant (*P*=0.02). In NIDDM the mean of HbA1c% in the age group subjected to treatment of 1-4 years was found to be 8.2%, in the group of 5-9 years it was 8.1% and in the group 10-14 years was 8.0%; these differences were found to be statistically insignificant (*P*=0.92). For HbF%, in the age group subjected to treatment of 1-4 years was found to be 0.5%, in the group of 5-9 years it was 0.6% and in the group 10-14 years was 0.4%; these differences were found to be statistically insignificant (*P*=0.73) Table 4.

Analysis of correlations indicates that there is a statistically significant negative correlation between HbF level and HbA1c in IDDM, where R = – 0.964, R2 = 0.929 (p-value = 0.000). On the other hand there was a positive correlation between the HbF level and duration of treatment per year, where R = 0.961, R2 = 0.923 (*P* = 0.000) Table 5.

4. DISCUSSION

Hemoglobin A1c (HbA1c) is used routinely in the management of diabetes, as it is related directly to risks for diabetic complications. The accuracy of some HbA1c methods can be affected adversely by the presence of hemoglobin variants or elevated levels of (HbF). It is important to consider hemoglobinopathy in patients when the HbA1c value does not correlate with clinical expectations.

Table 1. Mean and standard deviation of haemoglobin A1c % and HbF % among the study groups

Parameter (unit)	Study group			*P*-value
	IDDM n=50(A)	NIDDM n=50(B)	Control group n=50(C)	
HbA1c %	7.2±1.2	8.1±0.4	5.5±0.7	A vs. C ;*P*=0.025; B vs. C ;*P*=0.022
HbF %	2.38±2.1	0.7±0.4	0.6% ± 0.3	A vs. C ;*P*=0.000; B vs. C ;*P*=0.330

Table 2. Mean and standard deviation of HbA1c% and HbF % in IDDM and NIDDM subjects further classified by gender

Parameter (unit)	Study groups				P-value	
	IDDM n=50		NIDDM n=50			
	Male	Female	Male	Female	IDDM	NIDDM
HbA1c %	7.1±1.2	7.3±1.1	8.1±0.4	8.1±0.4	0.859	0.914
HbF %	2.8±2.3	2.7±2.4	0.7±0.3	0.8±0.2	0.832	0.707

Table 3. Mean of HbA1c% and HbF% in IDDM groups further classified according to unexpected HbA1c results

Parameter (unit)	IDDM group			P-value
	Patient had HbA1c % higher than expected	Patient had HbA1c % lower than expected		
	Age (40-44) n=3	Age (45-49) n=40	Age (50-54) n=40	
HbA1C%	7.9±0.6	7.3±1	5.8±1.5	0.025
Hb F%	0.8±0.1	2±0.9	4.2±2	0.002

Table 4. Mean of HbA1c% and HbF % according to the duration of testament in IDDM and NIDDM group

Parameter (unit)	Duration of treatment (in years)						P-value	
	Duration of insulin treatment (in years) IDDM			Duration of treatment (in years) NIDDM				
	(15-21) y	(22-32)y	(33-42)y	(1-4)y	(5-9)y	(10-14)y	IDDM	NIDDM
HbA1c %	7.9±0.5	6.1±1	4.7±0.4	8.2±0.3	8.1±0.4	8±0.5	0.040	0.921
HbF %	0.8±0.2	3.8±0.7	7.1±0.4	0.5±0.3	0.6±0.3	0.4±0 .2	0.025	0.732

Table 5. Correlation of HbF% level with HbA1c % and the duration of insulin treatment in years and in IDDM group

Parameter	Study group (n=50)		P-value
	HbF%		
	Correlations (R)	Regression (R2)	
HbA1c %	– 0.964	0.929	0.000
Duration of insulin treatment	0.961	0.923	0.000

In this study, we observed a direct correlation between HbF, age and duration of insulin treatment in IDDM. No significant differences were seen when result of Hb A1C and Hb F values were correlated to gender in IDDM patients. In NIDDM, HbF concentrations do not appear dependent on sex, or degree of glycemic control.

We observed significantly increased of HbF in subjects with IDDM than do NIDDM and control subjects. An increased of HbF levels been reported in IDDM subjects in many studies [25-28]. Increased proportions of HbF have been described in autoimmune diseases such as pernicious anemia and thyrotoxicosis. Because IDDM has an autoimmune component [32,33], increases in HbF concentrations may it is possibly be present.

There was a statistically significant negative correlation between HbF level and HbA1c in IDDM. We have observed lower levels of HbA1c in diabetic patients presented with high levels of HbF. The 47 patients with lower HbA1c results than expected had high levels of HbF. The 3 patients with higher HbA1c results than expected had normal levels of HbF. Elevated levels of HbF are reported to interfere with the HbA1c measurements by falsely decreasing the HbA1c results [34,35]. The result may be falsely low because HbA1c (%) is expressed as HbA1c/total Hb or due to a lower glycated rate for HbF than HbA. Therefore, it is essential to eliminate HbF to reduce the possibility of any potential interference of HbA1c measurement. One way to eliminate the effect of HbF is to determine the HbA1c level corrected by HbF (HbF corrected HbA1c) by the formula: HbA1c/(total Hb-HbF), resulting in the correction of an apparently low HbA level.

Our study also intended to find a possible relationship between HbF level and the duration of insulin treatment (in years) in IDDM patients, we observed significant increase of HbF levels in the patient groups that had been under insulin-treatment for 24-32 and 33-42 years in compare to the group under treatment for 15-23 years. We found a positive correlation between the HbF level and duration of treatment per year. The mechanisms leading to high HbF levels in diabetes under insulin-treatment are unknown. It has been suggested that a reactivation of the γ -globin gene can be induced by insulin, thus increasing HbF levels in insulin-treated patients seems more possible [25,27,28].

5. CONCLUSION

This study demonstrates the presence of elevated HbF in IDDM patients compared to non-diabetic individuals. HbF was increased in IDDM accordance to the length of insulin treatment and age. Artificial decreasing in HbA1c placed patients at higher risk for complications of diabetes. Considering the tendency toward lower result values in Hb A1c level with elevation of the HbF, Thus HbF should be considered in the interpretation of Hb A1c results measured by iron exchange methods.

6. RECOMMENDATIONS

1. Laboratories should be aware of the limitations of their HbA1c method with respect to interference with elevated HbF.

2. IDDM patients who are older than 42 years and on insulin therapy for more than 24 years whose HbA1c values differed significantly from the expected results are recommended to be subjected for HbF screening before measuring HbA1c level.

CONSENT

Informed consent was obtained from all participants prior to sample collection.

ETHICAL APPROVAL

Before commencement of the study, the protocol was approved by SUMASRI International Review Board (SIRB) at University of Medical Sciences and Technology (UMST).

ACKNOWLEDGEMENTS

We thank the patients and staff of the Dreby Diabetic Centre who have contributed samples to this stud.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ahmed AM, Ahmed NH. Hospitalization patterns of diabetic patients in Sudan. Diabetes Int. 2000;10:18-9.
2. Ahmed AM, Ahmed NH, Abdalla ME. Pattern of hospital mortality among diabetic patients in Sudan. Pract Diabetes Int. 2000;17:41-3.
3. Ahmed AM. Diabetes mellitus in Sudan: size of the problem and possibilities of efficient care. Pract Diabetes Int. 2001;18: 324-7.
4. Elbagir M, Eltom MA. A population-based study on prevalence of diabetes in northern Sudan. Diabetes Care. 1996;24: 1126-8.
5. Michael J, Fowler MD. Microvascular and macrovascular complications of diabetes. Clinical Diabetes. 2011;29(3):116-122.
6. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993;329:977-986.
7. UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet. 1998; 352(9131):837-53
8. Lapolla A, Molin L, Traldi P. Protein glycation in diabetes as determined by mass spectrometry. Int J Endocrinol. 2013;2013:11.
9. Goodall I. HbA1c standardisation destination—global IFCC Standardisation. How, why, where and when—a tortuous pathway from kit manufacturers, via interlaboratory lyophilized and whole blood comparisons to designated national comparison schemes. Clin Biochem Rev/Aust Assoc Clin Biochem. 2005;26:5–19.
10. Goldstein DE, Little RR, Wiedmeyer H, England JD, Mackenzie EM. Glycated hemoglobin: Methodologies and clinical applications. Clinical Chemistry. 1986; 32(Suppl 1):B64-B70.
11. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycohemoglobin measured by automated affinity HPLC correlates with both short-term and longterm antecedent glycemia. Clinical Chemistry. 1994;40: 1317-1321.
12. King H, Rewers M. World Health Organization Ad Hoc Diabetes Reporting Group. Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. Diabetes Care. 1993;16:157-177.
13. Mongia SK, Little RR, Rohlfing CL, et al. Effects of hemoglobin C and S traits on the results of 14 commercial glycated hemoglobin assays. Am J Clin Pathol. 2008;130:136–140.
14. Rohlfing C, Hanson S, Weykamp C, Siebelder C, Higgins T, Molinaro R, Yip PM, Little R. Effects of hemoglobin C, D, E and S traits on measurements of hemoglobin A1c by twelve methods. Clin Chim Acta. 2016;455:80-83.
15. Lin CN, Emery T, Little RR, Hanson SE, Rohlfing CL, Jaissonc S, Gillery P, Roberts WL. Effects of hemoglobin C, D, E, S traits on measurements of HbA1c by six methods. Clin Chim Acta. 2012;413:819-21.
16. Little RR, Rohlfing CR, Hanson S, Connolly S, Higgins T, Weykamp C, D'Costa M, Luzzi V, Owen WE, Roberts

WL. Effects of hemoglobin E and D traits on glycated hemoglobin (HbA1c) Measurements by twenty-three methods. Clin Chem. 2008;54:1277-82.
17. Rohfing CL, Connolly SM, England JD, Hanson SE, Moellering CM, Bachelder JR, Little RR. The effect of elevated Fetal hemoglobin on hemoglobin A1c results. American Journal of Clinical Pathology. 2008;129:811-814.
18. Shu I, Devraj S, Hanson SE, Wang P. Comparison of hemoglobin A1c measurements of samples with elevated fetal hemoglobin by three commercial assays. Clinica Chimica Acta. 2012;413: 1712-1713.
19. Little RR, Rohfing CL, Hanson SE, Schmidt RL, Lin Chia-Ni, Madsen RW, Roberts WL. The effect of increased Fetal hemoglobin on 7 common HbA1c assay methods. Clinical Chemistry. 2012;58(5): 945-947.
20. Nitta T, Yamashiro Y, Hattori Y, Ezumi T, Nishioka M, Nakamura J. The interference by HbF on HbA1c (BM test HbA1c) measurements in enzymatic method. Annals of Clinical Biochemistry. 2015;pii: 0004563214568872.
21. Felner EI, McGrath M. Inaccurate hemoglobin A1C levels in patients with type 1 diabetes and hereditary persistence of hemoglobin F. J Pediatr. 2008;153: 137–139.
22. Hoffbrand AV. Erythropoiesis and general aspects of anaemia in essential haematology. 2006;16.
23. Cas Weykamp. HbA1c: A review of analytical and clinical aspects. Ann Lab Med. 2013;33(6):393–400.
24. Little RR, Roberts WL. A review of variant hemoglobins interfering with hemoglobin A1c measurement. J Diabetes Sci Technol. 2009;3:446–451.
25. Koskinen L, Koivula T, Lahtela J. Fetal hemoglobin in diabetic patients. Diabetes Care. 1994;17:828-831.
26. Kilpatrick Michael Small, Alan Rumley, Marek H. Dominiczak, increased fetal haemoglobin in insulin-treated diabetes mellitus contribute to the imprecision glycohaemoglobin measurements. CLIN. CHEM. 1993;39(5):833-835.
27. Diem P, et al .Fetal haemoglobin levels in adult type 1 (insulin-dependent) diabetic patients. Diabetologia. 1993;36(2):129-32.
28. Pardini, et al. Fetal hemoglobin levels are related to metabolic control in diabetic subjects. Brazil J Med Biol Res. 1999; 32(6):695-701.
29. Davis JE, McDonald JM, Jarett L. A high-performance liquid chromatography method for hemoglobin A1c. Diabetes. 1978;27(2):102-7.
30. BIOCHEMISTRY | Biosystems. HbA1c measured with a high-performance liquid chromatography (HPLC). Available:http://www.biolinker.com.ar/productos/PDF_BIO/11044-11045%20Hemoglobina %20A1c.pdf
31. Barbara J Bain, Imelda Bates, Michael A Laffan, Mitchell Lewis S. Investigation of abnormal haemoglobins and thalassaemi in Dacie and Lewis. Practical Haematology. 2011;326.
32. Potter KN, Wilkin TJ. The molecular specificity of insulin autoantibodies. Diabetes Metab Res Rev. 2000;16(5):338-53.
33. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature. 2010;464(7293):1293–1300.
34. Rohlfing CL, Connolly SM, England JD, et al. The effect of elevated fetal hemoglobin on hemoglobin A1c results: five common hemoglobin A1c methods compared with the IFCC reference method. Am J Clin Pathol. 2008;129(5):811-814.
35. Chu CH, Lam HC, Lee JK, Wang MC, Lu CC, Sun CC, Chuang MJ. Common hemoglobin variants in southern Taiwan and their effect on the determination of HbA1c by ion-exchange high-performance liquid chromatography. J Chin Med Assoc. 2009;72(7):362-7.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14849