

Original Research Article

Behaviour Of Foetal Haemoglobin And Its Correlation To Haemoglobin A1c Levels In Sudanese Adult Insulin-Dependent Diabetes Mellitus Patients

ABSTRACT

Aims: To estimate the foetal 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.

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. 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, ($R = -0.964$, $R^2 = 0.929$, $p < 0.05$). There was weak negative correlation between Hb F level and Hb Alc according to age in IDDM, ($R = 0.580$, $R^2 = 0.337$, $p < 0.05$).

Conclusion: It is noted in this study, the high level of HbF accompanied by a decrease 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

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].But recent estimates place the diabetes population at around one million – around 95% of whom have type 2 diabetes. Patients with diabetes make up around 10% of all hospital admissions in Sudan.[1]The major complications of diabetes occur as result of hyperglycemia which is the major source of morbidity and mortality in both type 1 and type 2 diabetes. 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 haemoglobin in red blood cells, thus the more glucose binds to red blood cell the higher is the glycosylated haemoglobin. 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[8,9].

Regular measurements of haemoglobinA1C lead to changes in diabetes treatment and improvement of metabolic control, indicated by a lowering of haemoglobin A1c values [10].The accuracy of HbA1c methods can be affected adversely by the presence of haemoglobin variants or elevated levels of foetal haemoglobin (HbF) ^[11].

Fetal haemoglobin (HbF, $\alpha_2\gamma_2$) 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 ($\alpha_2\beta_2$).Normal adults have less than 1% of HbF [12] 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. Some recently published studies found elevated foetal haemoglobin (HbF) levels in insulin-dependent diabetes mellitus (IDDM) compared to non-diabetic subjects.^[13-15]

^{15]}Thus, physicians must be aware that if their diabetic patients have elevated HbF.

In the present study, we describe the behaviour of foetal 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. MATERIAL 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 Type1 (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: All diabetic patient male and female who are 40 years old or more were included.

2.4 Exclusion criteria: Adult diabetic patients already diagnosed with (Haemoglobinop- athy, Renal failure, Leukaemia, anaemia, Thalassaemia and Hereditary persistence of foetal haemoglobin) were excluded.

2.5 Collection of Blood Samples: Under a septic condition 2.5 milliliters venous blood was collected in EDTA containers. Then the sample were placed in ethylenediethyltetra acetic acid (EDTA). Haemolysate prepared immediately and tests done within 4 Hours.

2.6 Estimation of HbA1c: HbA1c measured with a high-performance liquid chromatography (HPLC) ion-exchange analyzer[16].Kit manufactured by BioSystems code 1104.[17]

2.6.1 The principle of method: After preparing the haemolysate, where the labile fraction is eliminated, haemoglobins were retained by a cationic exchange resin. Haemoglobin A1c was specifically eluted after washing away the haemoglobin A1a+b fraction1 (HbA1a+b), and was quantified by direct photometric reading at 415nm.

The results obtained with the present method can be converted into equivalent to a US National Glycohemoglobin Standardization Program certified method (NGSP)[17] or equivalent to the

International Federation of Clinical Chemistry standardized method (IFCC), using the following formulas: ^[17]

$$\% \text{HbA1C-NGSP} = 0.86 \times \% \text{HbA1C-BioSystems} + 0.24$$

$$\% \text{HbA1C-IFCC} = 0.94 \times \% \text{HbA1C-BioSystems} - 2.09.$$

2.6.2 Reference values: The following cut-off points have been established by the Diabetes Control and Complications Trial Research Group (DCCT) and have been adopted by many countries for a reference population (Non-diabetic) and for the evaluation of the degree blood glucose control in diabetic patients. ^[18,19]

DCCT / NGSP ^[18,19] % HbA1c	IFCC ^[18,19] % HbA1c	Bio Systems ^[18,19] % HbA1c	Degree of control ^[18,19] % HbA1c
4.0 - 6.5	2.0 - 4.2	4.4 - 6.7	Non Diabetic
6.0 - 6.5	4.2 - 4.8	6.7 - 7.3	Goal
6.5 - 8.0	4.8 - 6.4	7.3 - 9.1	Good Control
> 8.0	> 6.4	9.1	Action suggested

2.7 Modified BETKE method for the estimation of Hb F:^[20]

2.7.1 Principle: To measure the percentage of Hb F in a mixture of haemoglobins, sodium hydroxide is added to a lysate and, after a set time, denaturation is stopped by adding saturated ammonium sulphate. The ammonium sulphate lowers the pH and precipitates the denatured haemoglobin. After filtration, the absorbance of the alkali-resistant and total haemoglobin measured in spectrophotometer at 420 nm. Then quantity of undenatured (unprecipitated) haemoglobin is measured. The proportion of alkali-resistant (fetal) haemoglobin is then calculated as a percentage of the total amount of haemoglobin present.

2.7.2 calculation : The percentage alkali-resistant haemoglobin calculate as follows:

$$\% \text{ Alkali resistant haemoglobin} = \frac{A^{420}_{\text{alkali - resistant Hb}}}{A^{420}_{\text{total Hb}} \times 20} \times 100$$

2.7.3 Reference values: 0.2 – 1.0 %

3. RESULTS

Table 1 shows mean $\pm$ St. Dev. of HbA1C% ,in IDDM group was found to be $7.2 \pm 1.2\%$ St. Dev. and in the control group $5.5 \pm 0.7\%$; this difference was found to be statistically significant ($P = 0.02$). In NIDDM ,The mean $\pm$ St. Dev. of HbA1C% was found to be $8.1 \pm 0.4\%$ St. Dev. and in control group 5.5 ± 0.7 , this difference is statistically significant ($P = 0.02$).

Table 1: Mean and standard deviation of haemoglobin A1c levels among the study groups

T-test	Type	Mean	P-value
HbA1c	IDDM	7.2 ± 1.2	0.025
	Control	5.5 ± 0.7	
	NIDDM	8.1 ± 0.4	0.022
	Control	5.5 ± 0.7	

Figure 1 shows mean HbF levels , in IDDM group was found to be $2.38\% \pm 2.1$ St. Dev., in control subjects it was found to be $0.6\% \pm 0.3$; these differences were found to be statistically significant ($P = 0.000$). In the NIDDM group the mean of HbF was found to be $0.7 \pm 0.4\%$ and in the normal control subjects it was $0.6 \pm 0.3\%$ and these differences were found to be statistically insignificant at ($P = 0.33$).

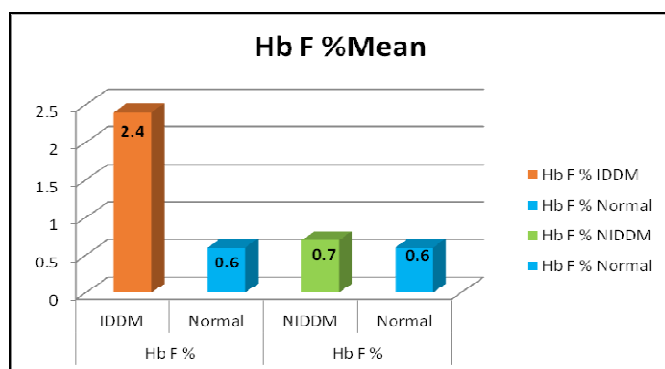


Figure1 Mean and standard deviation of haemoglobin F levels among the study groups

Table 2.a shows mean of HbA1c% in IDDM patients, of age group 40-44 years was found to be 7.9%, in the group of 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, the of mean of HbF% in IDDM patients within age group 40-44 years was found to be 0.8%, in the group of 45-49 years it was found to be 2% and in age group 50-54 years it was found to be 4.2%. The differences between groups were found to be statistically significant ($P= 0.002$)

Table 2.b shows mean of HbA1c% in NIDDM patients of age group 40-44 years was found to be 8%, in the group of 45-49 years it was found to be 8.2% and in age group 50-54 years it was found to be 8.1%; this difference was found to be statistically insignificant ($P=0.83$) For HbF, the of mean of HbF% in IDDM patients within age group 40-44 years was found to be 0.5%, in the group of 45-49 years it was found to be 0.4% and in age group 50-54 years it was found to be 0.6%. The differences between groups were found to be statistically insignificant ($P=0.91$).

Table 2.a: Mean of HbA1c% and HbF% in the specific age groups in IDDM

T-test	Age group (in years)	N	Mean± SD %	P-value
HbA1c%	40-44	3	7.9±0.6	0.025
	45-49	40	7.3±1	
	50-54	7	5.8±1.5	
HbF%	40-44	3	0.8±0.1	0.002
	45-49	40	2±1.9	
	50-54	7	4.2±2	

Table 2.b: Mean of HbA1c% and HbF% in the specific age groups in NIDDM

T-test	Age group (in years)	N	Mean± SD %	P-value
HbA1c%	40-44	3	8±0.5	0.831
	45-49	40	8.2±0.3	
	50-54	7	8.1±0.4	
HbF%	40-44	3	0.5±0.3	0.917
	45-49	40	0.4±0.2	
	50-54	7	0.6±0.3	

Table 3 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 ($P=0.70$). No significant differences were seen when result values were correlated to gender.

Table 3: Mean and standard deviation of HbA1c% and HbF % in IDDM and NIDDM subjects further classified by gender

T-test	Type	Gender	Mean±SD %	P-value
Hb A1c %	IDDM	Male	7.1±1.2	0.859
		Female	7.3±1.1	
	NIDDM	Male	8.1±0.4	0.914
		Female	8.1±0.4	
HbF %	IDDM	Male	2.8±2.3	0.832
		Female	2.7±2.4	
	NIDDM	Male	0.7±0.3	0.707
		Female	0.8±0.2	

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 ($P=0.02$). **Table 4.a**

Table 4.b shows the mean of HbA1c% in case group (NIDDM) 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.a: Mean of HbA1c% and HbF % according to the duration of testament in IDDM group

T-test	Duration of insulin treatment (in years)	Mean± SD %	P-value
HbA1c%	15-21	7.9±0.5	0.040
	22-32	6.1±1	
	33-42	4.7±0.4	
HbF%	15-21	0.8±0.2	0.025
	22-32	3.8±0.7	
	33-42	7.1±0.4	

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

T-test	Duration of treatment (in years)	Mean± SD %	P-value
HbA1c%	1-4	8.2±0.3	0.921
	5-9	8.1±0.4	
	10-14	8±0.5	
HbF%	1-4	0.5±0.3	0.732
	5-9	0.6±0.3	
	10-14	0.4±0.2	

Figure 2 Reflects that there was strong positive correlation between the HbF level and duration of treatment per year, where $R = 0.961$, $R^2 = 0.923$ ($P = 0.000$) .

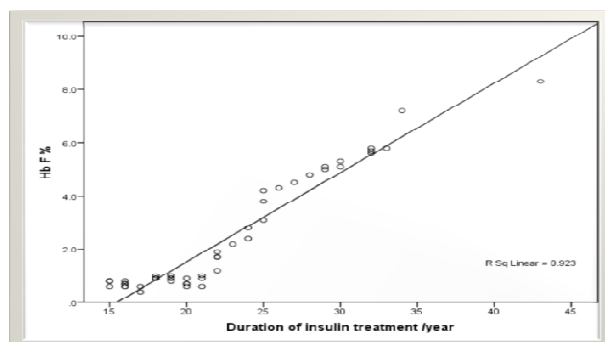


Figure 2 Correlation between HbF level and the duration of insulin treatment in years in IDDM group

Correlations (R)	0.961**
Regression (R2)	0.923
P-value	0.000

** Strong positive correlation, Correlation is significant at the 0.01 level (2-tailed)

Table5 Shows there was a strong negative correlation between HbF level and HbA1c according to the duration of insulin treatment in IDDM, where $R = -0.964$, $R^2 = 0.929$ (p-value = 0.000).

Table 5 :Correlation between HbF level and Hb A1c according to duration of insulin treatment in IDDM group

Correlations (R)	-0.964**
Regression (R2)	0.929
P-value	0.000

**Strong negative correlation, Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

To the best of our knowledge, Behaviour of Foetal Haemoglobin in Sudanese Adult Insulin-dependent Diabetes Mellitus Patients has never been addressed. An increased of HbF levels been reported in type 1 diabetes subjects in many studies.^[14,15,21] Our data are in agreement with these reports. We have observed greater values of HbF in subjects with type 1 than do non-insulin-treated and control subjects. Increased proportions of HbF have been described in autoimmune diseases such as pernicious anemia and thyrotoxicosis. Because Type I (insulin-dependent) diabetes mellitus has an autoimmune component, increases in HbF concentrations may it is possibly be present^[15,21,22]. Furthermore, we have observed IDDM a direct correlation between HbF and age duration of insulin treatment in IDDM, particularly in age more than 42 years and duration more than 24 years. The mechanisms leading to high HbF levels in diabetes are unknown. It has been suggested that high HbF levels could be related to a direct effect of insulin therapy (14,15,18). They suggested that a reactivation of the γ -globin gene can be induced by insulin, thus increasing HbF levels in insulin-treated patients seems more probable. In NIDDM, HbF concentrations do not appear dependent on age,sex, or degree of glycemic control

4. 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. It is noted in this study that the high level of HbF accompanied by a decrease in Hb A1c level, that reaches the level of goal or good Control of glycaemic control and in some cases to the normal level of non diabetic people.This leads to the wrong treatment and given the patient an incorrect dose of insulin and therefore the occurrence of complications in the long term. 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.

CONSENT (WHERE EVER APPLICABLE)

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

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

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

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APPENDIX