

Credibility of measurement of fructosamine and hemoglobin A_{1c} in estimating blood glucose level of diabetic patients with thalassemia major

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ABSTRACT

Background and Aim: Patients with thalassemia major are classified in high risk group for diabetes mellitus, and therefore monitoring blood glucose level has a vital importance in these people. As high fetal hemoglobin level in thalassemia patients interferes with measurement of glycosylated hemoglobin (hemoglobin A_{1c}), fructosamine evaluation as an alternative approach is suggested.

Materials and Methods: This descriptive study was carried out on 33 diabetes mellitus patients with beta-thalassemia (21 female and 12 male cases). The following biochemical measurements were done: blood glucose level through biochemical glucose oxidation method, fructosamine by colorimetry, hemoglobin A_{1c} by immunoturbidimetry, serum ferritin by chemiluminescence and fetal hemoglobin by HPLC methods. Using SPSS software v18.0, statistical analysis was done and correlation between fructosamine and hemoglobin A_{1c} (Pearson's correlation) and linear regression were investigated. $p < 0.05$ was considered as statistically significant.

Results: In female and male patients, blood glucose levels were 204 ± 103 mg/dL and 224 ± 101 mg/dL ($p = 0.63$), fetal hemoglobin were $9\% \pm 7\%$ and $13\% \pm 9\%$ ($p = 0.22$); serum ferritin levels were 1744 ± 1534 ng/mL and 3253 ± 1773 ng/mL ($p = 0.96$), respectively. Mean serum fructosamine level was 442 ± 124 mmol/L and glycosylated hemoglobin amount was $8.9\% \pm 1.8\%$. These two parameters showed significant correlation ($r = 0.69$, $p < 0.01$). Blood glucose level with hemoglobin A_{1c} ($r = 0.75$, $p < 0.01$) and fructosamine ($r = 0.54$, $p < 0.01$) showed a significant correlation.

Conclusion: In diabetic patients with thalassemia major who have frequent blood transfusion, evaluation of serum fructosamine and glycosylated hemoglobin levels are both reliable approaches for estimating blood glucose levels and the two methods can be used alternatively.

INTRODUCTION

Beta-thalassemias are hereditary and molecularly heterogeneous blood disorders. Insertions in the β -globin gene sequence, deletions or point mutations are molecular defects responsible for most β -thalassemia. Most patients come from Mediterranean countries and Middle East, South-East Europe, and Asia. Hematological changes can be detectable from between the ages of three months and six months onwards. Three main types of beta thalassemia are identified: thalassemia major, thalassemia intermedia and thalassemia minor. Individuals with thalassemia major usually present within the first two years of life with severe anemia, requiring regular blood transfusions [1, 2, 3].

Elevated serum iron level and iron toxicity are among common complications in patients with thalassemia major who have frequent blood transfusion. If iron chelating approaches are neglected, the patient may encounter a variety of health problems, including diabetes mellitus [4, 5]. For thalassemia patients who have already suffered from morbidity, this problem is an extra burden and would further affect their life quality [6, 7, 8, 9]. Like other diabetic patients, diabetic patients with thalassemia must be continuously monitored for the progress of treatment and managing complication [10].

Measurement of glycosylated hemoglobin (Hb) - also known as Hb A_{1c} - is a widespread method for estimation of blood glucose level in diabetic patients. Hb A_{1c} which is an extensively used marker in monitoring diabetes and management of the disease, is a glycosylated protein synthesized by binding of free amine group of glucose molecule to beta chain of globin protein through a two step reaction. In the first reversible step, a bond between free aldehyde group of glucose and amine group of the protein is formed. Subsequently, an intra-molecular rearrangement - known as Amadori reaction - ensues which results in formation of a stable ketoamine molecule. The reaction at this stage is irreversible. Bound glucose molecules would be removed from metabolism cycle and accumulated in circulating blood and increased by any prolongation in erythrocytes life span. Transitory

changes in blood glucose level and sugar consumption prior to Hb A_{1c} test does not affect the result of examination. Average life span of erythrocytes is about 120 days and hence the amount of accumulated glycosylated Hb in blood may represent the available glucose concentration within the past 3 to 4 months. Existence of such relationship is confirmed by laboratory examinations and has become a basis for estimating mean plasma glucose concentration [11]. 1% change in Hb A_{1c} content is proportionate to 28 to 34 mg/dl change in blood glucose [12]. It is also demonstrated that health problems such as cerebral and cardio-renal dysfunctions affiliated with diabetes, are associated with high amount of this molecule [10]. Increased amount of Hb A_{1c} to 6% and higher, is a valuable marker in identifying diabetes mellitus patients. This method also helps identify asymptomatic patients, who previously had to be tested twice for fasting blood sugar or undergo glucose tolerance test [13]; however there are some studies raising questions about credibility of measurement of Hb A_{1c} in estimating blood glucose storage in hemolytic patients and hemoglobinopathy carriers. In such cases measurement of fructosamine - another serum protein - as an alternative approach is suggested [14, 15, 16].

Fructosamine (1-amino-1-dioxy-fructose) is a ketoamine and the product of non-enzymatic reaction of a carbohydrate (usually glucose) and a protein (generally albumin). Post synthesis, fructosamine undergoes non-enzymatic changes; this is a characteristic difference between fructosamine and glycoproteins [13, 14]. As glycation occurs during the whole course of serum protein life span, the amount of glycosylated proteins reflects the available quantity of blood sugar during this period. Measurement of these biochemical molecules is a reliable approach to estimate blood sugar level for a certain time window in diabetic patients. The quantity of available glycosylated albumin and fructosamine correlates to mean plasma glucose for the past one or two weeks, as half lives of plasma proteins are relatively short [17].

The current study investigates the relationship between fructosamine and Hb A_{1c} and their correlation with plasma glucose content in thalassemia patients referred to thalassemia

research center in Mazandaran University of Medical Sciences (Sari, Iran).

MATERIALS AND METHODS

This descriptive study was carried out in the summer of 2010. First, diabetes mellitus patients suffering from thalassemia major were selected and after receiving their consent, entered the study group. Medical history of patients was extracted from their records and peripheral blood samples were collected for laboratory examination.

Blood glucose concentration was measured via glucose oxidation based method by biochemistry auto-analyzer (Hitachi, Japan). Other measured biochemical molecules were: serum ferritin via chemiluminescence method (Liaison auto-analyzer, DiaSorin S.p.A., Italy), Hb F via high performance liquid chromatography (Drew Scientific Ltd, United Kingdom), Hb A_{1c} via immunoturbidimetry method (Cobas Integra 400, Roche Diagnostic GmbH, Mannheim, Germany).

Glycosylated Hb concentration below 7% was considered as optimal, 7% to 8.5% as intermediate and above 8.5% as poor control of blood sugar level. Serum fructosamine was detected by colorimetric method based on its reaction with nitrobluetetrazolium (Cobas Integra 400, Roche Diagnostic GmbH, Mannheim, Germany). 350-400 µmol/L fructosamine was considered as excellent, 400-450 µmol/L as good, 450-500 µmol/L as intermediate, and above 500 µmol/L as poor control of blood glucose level.

Statistical analysis was carried out by SPSS software version 18.0 (SPSS Inc., Chicago, U.S.A.) and in order to investigate meaningful correlation between serum fructosamine and Hb A_{1c} levels, Pearson’s correlation test was done and linear regression examined. In all calculations p<0.05 was considered as statistically significant.

RESULTS

This study was carried out on 33 beta patients with thalassemia major, consisting of 21 female (63.6%) and 12 male (36.4%) cases, all suffering from diabetes. Patients were diagnosed with thalassemia at the average age of 1.5 years and with diabetes at the average age of 19.5 years. Mean age of diagnosis of diabetes in male cases was 21.2 years and in female patients 18.3 years. On average, it has been 7.8 years since patients were diagnosed with diabetes. Mean blood glucose levels in male and female patients were 224±101 mg/dL and 204±103 mg/dL respectively. The two values were not significantly different (p=0.63). Fetal Hb (Hb F) content were 13%±9% in male and 9%±7% in female patients (p=0.22); and serum ferritin levels were 3253±1773 ng/mL in males and 1744±1534 ng/mL in females (p=0.96). Mean serum fructosamine level in the whole group was 442±124 mmol/L and this value for Hb A_{1c} was 8.9%±1.8%. This amount for fructosamine in males was 450±116 mmol/L and 436±131 mmol/L in females, and for Hb A_{1c} it was 8.8%±1.8% in males and 9.0%±1.8% in females ([table 1](#)).

Table 1: Biochemical evaluation and hemoglobin electrophoresis of the patients

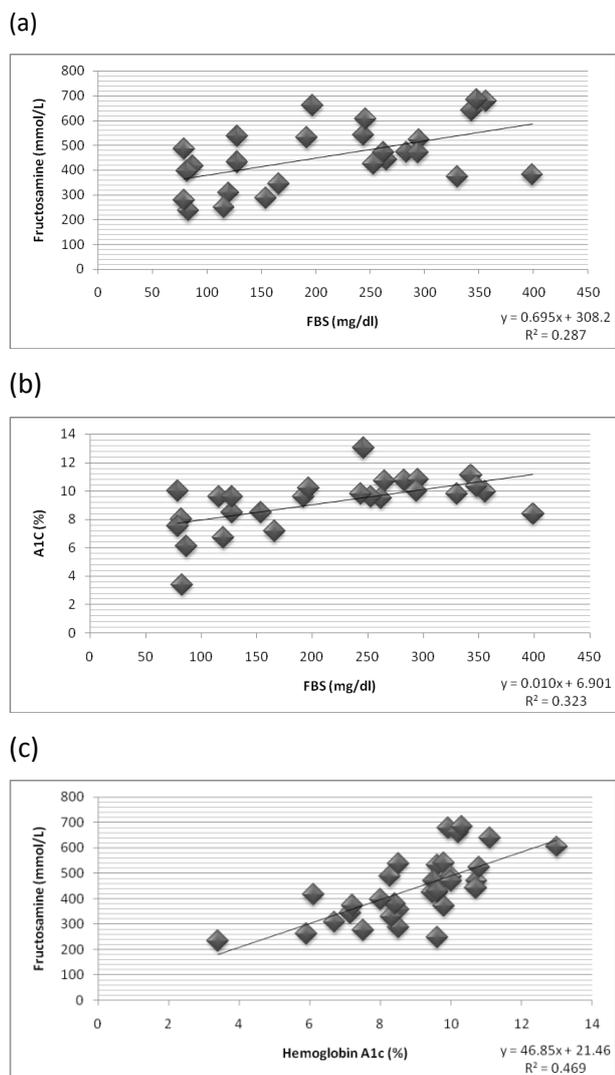
Group	FBS (mg/dL)	A1C (%)	Fructosamine (mmol/L)	Ferritin (ng/mL)	Hb F (%)
Female (63.6%)	204±103	9.0%±1.8%	436±131	1744±1534	9%±7%
Male (36.4%)	224±101	8.8%±1.8%	450±116	3253±1773	13%±9%
Whole group	213±101	8.9%±1.8%	442±124	2310±1741	10.6±8.5

Fructosamine and Hb A_{1c} values between male and female cases were not significantly different (p=0.61 and p=0.89 respectively). The result of Pearson’s correlation test for these two

parameters was meaningful (r=0.69, p<0.01) and linear regression was defined with the formula “Fructosamine=21.46+46.85Hb-A_{1c}” (R²=0.47, p<0.001) ([Fig 1](#)). There were significant

correlations among patients' blood glucose and Hb A_{1C} marker ($r=0.75$, $p<0.01$) and blood glucose and fructosamine ($r=0.54$, $p<0.01$).

Figure 1: linear regression between (a) fructosamine and FBS, (b) Hb A_{1C} and FBS, and (c) Hb A_{1C} and fructosamine in diabetes mellitus patients with thalassemia major.



DISCUSSION

The current study addresses an alternative methodology to evaluate blood glucose levels in patients afflicted by beta thalassemia major. The outcome showed that the two approaches – Hb A_{1C} and fructosamine – are both reliable in patients who have regular blood transfusion.

Using Hb A_{1C} to determine blood glucose level has its own limitations. Conditions that affect the rate of erythrocyte synthesis (hemolysis, bleeding) and presence of Hb variants may change

the results. Such conditions must be particularly considered when Hb A_{1C} content is not compatible with patient's clinical symptoms [18].

There have been several studies to evaluate the validity of the results of Hb A_{1C} in diabetic patients. Cohan and colleagues compared half life of erythrocytes and Hb A_{1C} content in diabetic patients and healthy people and showed erythrocyte life span in diabetic patients is reduced. This decrease, results in a meaningful reduction of Hb A_{1C} - compared to healthy population - affecting its linear correlation with blood glucose [19]; however in another study on 371 diabetic patients, Guillausseau and colleagues showed a direct correlation between fasting blood sugar and the two markers: fructosamine and Hb A_{1C}. This study was followed up for 6 months and the results were reproducible [20]. In this study, the only clinical difference in interpreting the results of fructosamine and Hb A_{1C} was due to longer half life of Hb in comparison with other serum proteins and therefore in monitoring glucose level in diabetic patients it is better to use fructosamine test as a complimentary to Hb A_{1C}.

In a Study on 139 patients with diabetes mellitus, a linear correlation among blood glucose and the two markers Hb A_{1C} and fructosamine were found; however in that study, the results of Hb A_{1C} and fructosamine in 19 cases who were afflicted by thalassemia was not correlating with fasting blood sugar levels [21].

Existence of hemoglobinopathies and Hb variants may affect total amount of Hb A_{1C}. In mature person and under physiologic conditions, 98% of Hb molecules are Hb A with two alpha and two beta globin chains and the other 2% is Hb A₂ with two alpha and two delta globin chains. So far more than 700 Hb variants are identified and some of them are capable of affecting measurement of Hb A_{1C}. Hb S, C, D, E, Graz, Sherwood Forest, O Padova, Okayama, and beta thalassemia are among hemoglobinopathies and Hb variants that greatly influence Hb A_{1C} content. For instance the presence of Hb Graz led to lower than expected values of Hb A_{1C}, or Hb J-Meerut in a diabetic case resulted in Hb A_{1C} of 3.7% which was not in proportion with blood glucose level [21]. Hemoglobinopathies may also change half life of red blood cells. Short half life of erythrocytes in a patient with thalassemia minor

led to Hb A_{1c} of 1.6% despite high blood glucose level [23].

Hb F encompasses half of Hb molecules in infants. It has two alpha and two gamma globin chains. Remnants of Hb F which is traceable in 1% of adult Caucasians may also change the results of Hb A_{1c} measurement. In the presence of Hb F, electrophoresis-based methods of detection of glycosylated Hb may not be reliable; as Hb A_{1c} and Hb F have similar electrical charges, and it is difficult to separate them through these techniques. Affinity chromatography is an alternative approach, giving a more accurate result. In a report of two diabetic patients with high levels of Hb F, the results of Hb A_{1c} by agarose gel electrophoresis were higher than expected ones, but through affinity chromatography method the results were well-matched with patients' blood glucose levels [11].

Hb A_{1c} measurement by HPLC method may also be doubtful in the presence of some types of hemoglobinopathies. In 43 diabetic patients carrying hemoglobinopathy of one type or another, Nasir and colleagues compared the results of measurement of Hb A_{1c} by two different methods - HPLC and immunoassay [24]. Even in patients with Hb F content above 10%, the results for Hb A_{1c} by the two methods were similar; however in Hb E patients, Hb A_{1c} measurement by HPLC had lower results than immunoassay method [12]. The study of Higgins and colleagues, however, reached a different conclusion. They demonstrated in cases with Hb F below 8%, immunologic and HPLC methods were both reliable and the differences negligible, but with Hb F above 10% and 20%, there were meaningful differences of 1% and 2% respectively, for the measurement of Hb A_{1c} by two methods [25].

In patients with thalassemia intermedia, who do not receive regular blood transfusion and in particular are recipients of hydroxyl urea treatment, Hb F may reach to 98% of the total amount of Hb. In patients afflicted by "hereditary persistence of Hb F", Hb F content may have accumulation of 10% to 100% of the total Hb. In such cases, plasma glucose estimation based on measurement of Hb A_{1c} may not be accurate and alternative approaches such as evaluation of serum fructosamine are applicable [26].

In brief, measurement of Hb A_{1c} in patients

with thalassemia may lead to an unreliable result, as in one hand Hb F acts as an interfering factor and on the other hand patients with beta thalassemia have reduced or no synthesis of beta globin chain - the binding site of glucose molecule - to form Hb A_{1c}. Reduced production of beta globin chain leads to remarkable decrease in Hb A_{1c} synthesis.

In Thailand, Sridama *et al* examined 19 thalassemia patients suffering from diabetes. 18 cases had Hb A_{1c} results non-correlative to their fasting blood glucose levels. Fructosamine test also showed to be unreliable, as 6 cases had higher and one person lower results than expected ones - based on glucose content. They concluded in patients with abnormal hemoglobins or hemolytic disorders, none of these tests are reliable and have to be interpreted along with other related markers [21]. This finding is different from our results. It seems that frequent blood transfusion and in turn increased amount of mature Hb A and low levels of Hb F in our patients would be the main explanation for such discrepancy. In our study, Hb A_{1c} levels in diabetic patients with thalassemia showed a correlation with blood glucose content. We found a similar correlation with fructosamine, too. Examined patients were all patients with beta thalassemia major- subjects to frequent blood transfusion. These patients do not have bone marrow hematopoiesis activity and Hb F is in a negligible range [12]. This eliminates an important interfering factor in accurate measurement of Hb A_{1c}. Fresh blood is a natural source of Hb A, and thus through regular blood transfusion, adequate amounts of beta globin chains will be available for binding to glucose molecules. These facts may explain the results achieved in the current study.

CONCLUSION

The results of current study demonstrated that in patients with thalassemia major who are subject to frequent blood transfusion, laboratory examination of Hb A_{1c} level in blood serum is an acceptable approach to approximately calculate the amount of blood glucose storage within the past few months. Measurement of serum fructosamine is also applicable in these patients and may estimate blood glucose content for the shorter period of time. Both methods can be used

alternatively or in parallel to monitor blood glucose and eventually manage diabetes in patients with thalassemia.

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CONFLICT OF INTEREST

No authors have any conflict of interest about this study.

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