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Comparison of Mean Level of Hemoglobin A1C Between β-thalassemia Traits and Normal Non-Diabetic Individuals

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Abstract

Objective: To compare the mean level of hemoglobin A1C between β-thalassemia traits and normal non-diabetic individuals.

Study design: Case-control study.

Place and duration of the study: Department of pathology, PAEC general hospital H/11-4 Islamabad for a duration of 6 months from January 2021 to June 2021.

Methodology: Complete blood count by light scatter principle and hemoglobin A1C by High-Performance Liquid Chromatography (HPLC) of fifty normal (controls) and fifty known β-thalassemia traits (cases) were determined. Data were analyzed using SPSS version 16.

Results: A total of 50 known β-thalassemia traits and normal non-diabetic individuals were included in the study as cases and controls respectively. Cases and controls were age-gender matched. The mean hemoglobin level of cases i.e known β-thalassemia traits was 11.37+1.56 g/dl ranging from 8.3-14.5 g/dl. Mean hemoglobin level of normal non-diabetic controls was 12.5+1.86 g/dl with range of 10.1-17.1 g/dl (p-value>0.005). The mean corpuscular volume of red blood cells of β-thalassemia traits was 61.74+7.25fl. The range of mean corpuscular volume among β-thalassemia traits was 52.2-93.9fl.Mean corpuscular volume of controls was 74.4+7.6 fl ranging from 61.1 to 92.6 fl (p-value >0.005). Red cell distribution width among cases and controls was 40.54 +4.27 fl and 42.38+4.24 fl. The range of red cell distribution width among cases and controls was 32.7-57.9 and 30.5-53.7 fl (p-value >0.005). The mean red cell count of β-thalassemia traits was 6.23+ 0.80*10^6/million with a range of 4.5-8*106/million.Mean red cell count of cases and was 5.14+0.80*10^6/million ranging from 3-6.5*10^6/million (p-value>0.005). The mean value of glycosylated hemoglobin A1C in non-diabetics i.e controls and β-thalassemia traits i.e cases was 5% and 5.04% (p-value>0.005) i.e mean level of glycosylated hemoglobin A1C were comparable in both cases and controls.

Conclusion: Hemoglobin A1C can be used to monitor glycemic control in β-thalassemia traits.

Keywords: β-thalassemia traits; Glycosylated hemoglobin A1C; High-performance liquid chromatography

Introduction

Hemoglobin molecule (tetramer constituted in adults by 2 α-globin and 2-β globin chains) is responsible for providing and transporting oxygen to all tissues. There are different molecular forms of human hemoglobins including normal i.e Hb A (α 2β 2), Hb A 2(α 2 δ2) Hb F (α 2 γ2) and numerous hemoglobin variants e.g HbS, HbE, etc resulting from various genetic mutations [1]. In 1955, researchers described the heterogeneous nature of hemoglobin. After more than a decade, In 1968, Rahbar and associates reported an elevated level of glycated hemoglobin in diabetic patients [2]. Glycohemoglobin refers to hemoglobin glycosylated at any of its amino groups [3]. Hemoglobin A1C is defined by the International Federation of Clinical Chemistry working group (IFCC) as hemoglobin that is irreversibly glycated at one or both N-terminal valines of β chains. It is formed from the irreversible, slow, non-enzymatic addition of sugar residues to the hemoglobin with a rate of production being directly proportional to ambient glucose concentration. The long life of red blood cells (mean 120 days) enables Hemoglobin A1C to be used as an index of glycemic control over the preceding two to three months and as the adequacy of treatment in diabetic patients [4].

Thalassemias refer to the group of disorders characterized by alterations in the synthesis of either of two globin chains i.e α or β thalassemia. α-thalassemia is characterized by defective α-globin chain synthesis. β-thalassemics have defective β-globin chain synthesis and the amount of β-globin chain is diminished. Every individual has two β gene loci. The complete absence of β-globin synthesis from the single gene is denoted as $β^o$ and reduced synthesis levels as β+thalassemia. More than 180 different mutations are affecting β gene loci resulting in β thalassemia disorders. Every mutation has a different consequence on the rate of production of the β globin chain from the affected β gene locus. The heterozygous state of β thalassemia is associated with very extremely mild anemia and morphological changes in erythrocytes, causing ineffective erythropoiesis. As the amount of β globin is diminished in β thalassemia, there is a relative excess of α-globin chains. This alteration promotes mechanical damage of erythrocytes precursors as well as oxidative membrane destruction [5]. Various factors may affect the accuracy of Hemoglobin A1C measurements according to assay and method used. Hemoglobin variants (structural and production disorders) are one of them [6]. For Hemoglobin A1C determination, two confounding effects of thalassemias should be considered: the alteration of hemoglobin molecule as an analytical target and the potential effect of anemia [7].

The life span of red blood cells of β thalassemia traits is said to be normal outside bone marrow circulation. Therefore, glycosylated

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hemoglobin levels should be reliable in patients with β thalassemia traits when measured by high-performance liquid chromatography. However, this hypothesis has not been tested and studies regarding the comparison of glycosylated hemoglobin levels in β thalassemia traits and normal individuals are limited in our country. This study was thus designed and carried out.

Methodology

This prospective case-control study was carried out in the pathology department of Pakistan atomic energy commission general hospital Islamabad. Study was carried out after the approval of an ethical committee of the hospital.

50 non-diabetics and non-thalassemics by normal Hb-Electrophoresis carried out at alkaline Ph of 8.6 on cellulose acetate membrane were enrolled as controls. 50 non-diabetics and β thalassemic traits by raised Hb A2 on Hb-Electrophoresis carried out at alkaline Ph of 8.6 on cellulose acetate membrane were enrolled as cases. Diabetics were excluded from both cases and control so were children <1 year of age. Cases and controls were age-gender matched.

For estimation of glycosylated hemoglobin levels, 3 ml. of venous blood samples were drawn in vacutainers containing EDTA (Ethylene Diamine Triacetic Acid) anticoagulant. Samples were directly transported to the laboratory for complete blood count followed by estimation of glycosylated hemoglobin by High-performance liquid chromatography. A graphical record of the level of hemoglobin A1C was obtained in form of % of the total.

Normal cut off values for hemoglobin values include for childrenaged 1 year to 6 years hemoglobin level <11 g/dL, children aged 6-14 years hemoglobin<12 g/dL, adults maleshemoglobin level <13 g/dL, non-pregnant females Hb <12 g/dL. Normal cut off for mean corpuscular volume, red cell count and red cell distribution width are 80-100 Fl, 3.5-5.5*10^6/million and 37-54 fL respectively.

Variables of this study include age and gender of patients, level of hemoglobin, mean corpuscular volume, red cell distribution width, red cell count and mean level of glycosylated hemoglobin A1C. Variables were expressed as mean and range. Statistical analysis was carried out using SPSS version 16. p-value<0.005 was considered significant.

Results

A total of 50 known non-diabetic β-thalassemia traits and non-diabetic and non-β-thalassemia traits were enrolled as cases and controls respectively. Cases and controls were age-gender matched.

Each group had equal numbers of males and females i.e 25 each; therefore, the male: female ratio was 1:1 (Figure 1).

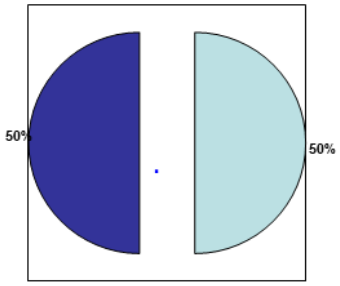

Figure 1: Gender distribution of cases and controls.
Note: ( ) Males, ( ) Females.

The mean age in each group was 32.12+12.02 years range being 13-58 years. Age distribution of cases and controls is as shown in Figure 2. The maximum number of cases and controls were in the age range 31-40 years.

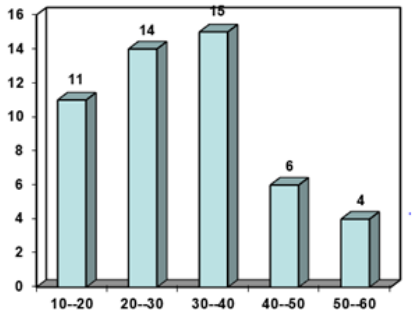

Figure 2: Age distribution of cases and controls.
Note: ( ) Age in years.

The mean hemoglobin level of cases i.e known β-thalassemia traits was 11.37+1.56 g/dL ranging from 8.3-14.5 g/dL. The mean hemoglobin level of normal non-diabetic controls was 12.5+1.86 g/dL with a range of 10.1-17.1g/dL.4 (8%) cases had low hemoglobin levels for given age and gender while 46 (92%) had normal hemoglobin levels. None had high hemoglobin levels. Of the 50 controls, 07 (14%) had low hemoglobin for given age and gender while 42 (84%) had normal hemoglobin levels. One control (2%) had a hemoglobin level higher than normal. Difference in mean hemoglobin levels of cases and controls was statistically insignificant (p value>0.005). The Mean Corpuscular Volume (MCV) of red blood cells of β-thalassemia traits i.e size of red blood cells was 61.74+7.25 fL. Of the total cases, 47 (94%) had low MCV while 3 (6%) had normal MCV. Range of mean corpuscular volume among β-thalassemia traits was 52.2-93.9 Fl. Mean corpuscular volume of controls was 74.4+7.6 fL ranging from 61.1 to 92.6 fL. Of total controls 6 (12%) had low MCV while 44 (88%) had normal MCV. None of the cases and controls had high MCV. This difference in the mean corpuscular volume of red blood cells of cases and controls as measured by an automated hematology analyzer was statistically insignificant (p-value >0.005). Red cell distribution width which is the measurement of the degree of variation in size of red blood cells among cases and controls was 40.54+4.27 fl and 42.38+4.24 fl.3 (6%) cases and 2 (4%) controls had low red cell distribution width 4 (8%) cases and 5 (10%) controls had high and rest i.e 43 (86%) cases and controls had normal red cell distribution width. The range of red cell distribution width among cases and controls was 32.7-57.9 and 30.5-53.7 fl, the statistical difference being insignificant (p-value >0.005). Mean red cell count of β-thalassemia traits was 6.23+0.80 X 10^6/million with a range of 4.5-8 × 10^6/million. 45 (90%) of β-thalassemics had raised red cell count while 5 (10%) had normal red cell count. None of the β-thalassemics had a low total red cell count. Mean red cell count of cases was 5.14+0.80 X 10^6/million ranging from 3-6.5 × 10^6/million (p-value>0.005). Of total controls 2 (4%), 44 (88%), and 4 (8%) had low, normal, and elevated total red cell count respectively. The mean value of glycosylated hemoglobin A1C in non-diabetics i.e controls and β-thalassemia traits i.e cases was 5% and 5.04% (p-value>0.005) i.e mean level of glycosylated hemoglobin A1C were comparable in both cases and controls. None of the cases and controls had raised hemoglobin A1C (Table 1).

Variables	Mean		Standard Deviation		Range	
	Cases	Controls	Cases	Controls	Cases	Controls
Hemoglobin (g/dL)	11.37	12.5	1.56	1.86	8.3-14.5	10.1-17.1
Mean Corposcular Volume (fL)	61.74	75.84	7.25	7.4	52.2-93.9	61.1-92.6
Red cell distribution with (fL)	40.54	42.33	4.27	3.75	32.7-57.9	35.7-53.7
Red cell count (*10^6/mil)	6.23	5.27	0.8	0.67	4.5-8	3.7-6.5

Table 1: Raise of hemoglobin in the different cases.

Discussion

Diabetes mellitus is one of the most common non-communicable diseases globally, affecting an astounding 462 million people worldwide with the prevalence rate of 6059 cases per 100,000 in 2017 and estimated to increase to 592 million people by 2035 [8,9]. The increase in popularity of Hb A1C as a marker for glycemic control came with the publication of Diabetes Control and Complications Trial (DCCT) and UK Prospective Diabetes Study (UKPDS) [10]. Hb A1C deviation of 1% reflects a change of 1.4-1.9 mmol/L in average blood glucose concentration [11]. In clinical practice, clinicians often consider a change in the value of 5 mmol/L (0.5%) in successive samples as a clinically significant change in patients' glycemic control. In certain situations in which hemoglobin variants or derivatives are present, method-specific interferences may occur; therefore, Hb A1C measurement could be unreliable [6,12,13] Therefore, a falsely high or low Hb A1C value is caused by the presence of clinically silent variant may result in erroneous levels [14]. Cation exchange high-performance liquid chromatography is one of the methods vulnerable to the effect of hemoglobin variants on Hb A1C measurements [15]. As the prevalence of β thalassemia trait in Pakistan is 5%-7% [16], the effect of β thalassemia mutation on the determination of Hb A1C by high-performance liquid chromatography needs to be ascertained.

Our study noted that the mean hemoglobin level of cases i.e known β-thalassemia traits was 11.37+1.56 g/dL ranging from 8.3-14.5 g/dL. A study carried out in Peshawar noted that the mean Hb level of hemoglobin of beta-thalassemia traits was 10.7 ± 1.67 g/dl while minimum and maximum were 5.2 and 15.5 g/l respectively [17]. Similarly, a study conducted in Lahore reported the mean hemoglobin level of β-thalassemia traits to be 10.40 ± 1.77 gm/dl [18]. Thus, our study noted slightly higher hemoglobin levels than Peshawar and Lahore study. However, the results of our study with regards to the mean hemoglobin level of the Pakistani population are comparable to those of a study conducted in Nawabshah city wherein the mean hemoglobin level of the population was 11.9 g/dl [19]. Similarly, the results of the mean corpuscular volume of the Peshawar study are different from our study as mean corpuscular volume was 59.1 fl ± 4.86 with no subject having mean corpuscular volume within normal limits while our study noted normal mean corpuscular volume in 3 (6%) β thalassemic traits. Reasons for normal mean corpuscular volume may be inherited nature of underlying mutation or acquired e.g megaloblastic anemia, liver disease, etc [20]. Reasons for low mean corpuscular volume in controls may be many but iron deficiency anemia is most common [21]. Anisochromia as measured by red cell distribution width by automated hematology analyzer is characteristic of iron deficiency anemia and is usually not a feature of uncomplicated β thalassemia trait. As iron deficiency may co-exist with β-thalassemia traits therefore 3 (6%) cases had raised red cell distribution width. The mean red cell count of β-thalassemia traits was higher as compared to controls i.e 6.23+0.80 × 10^6/million while the mean red cell count of cases was 5.14+0.80 × 10^6/million. The mean value of glycosylated hemoglobin A1C in non-diabetics i.e controls and β-thalassemia traits i.e cases was 5% and 5.04% (p-value>0.005) i.e mean level of glycosylated hemoglobin A1C were comparable in both cases and controls. These results are comparable to another study that determined the effect of heterozygous beta-thalassemia on HbA1c levels [7].

Conclusion

Hemoglobin A1C can be used to monitor glycemic control in β-thalassemia traits. Therefore, glycosylated hemoglobin levels are reliable in patients with β thalassemia traits when measured by high-performance liquid chromatography. Thus, glycosylated hemoglobin may be used to monitor glycemic control regardless of β-thalassemia status.

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