Evaluation of Effect of Iron Deficiency Anemia on HbA2 Levels

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ABSTRACT

Objective: To evaluate the effect of iron deficiency anemia on HbA2 levels.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Department of Pathology, Combined Military Hospital, Lahore Pakistan, from Jan to Oct 2020.

Methodology: A total of 206 subjects were included in the study out of which 56 served as healthy controls. Five milliliters of whole blood were extracted in an EDTA tube and basic haematological parameters were performed using automated hematology analyzer (Sysmex KX-21). HbA2 levels were determined by cellulose acetate hemoglobin electrophoresis at alkaline pH (Mini Hor-8). Serum Ferritin levels were measured by chemiluminescence on Cobas e411 and Serum Iron levels by spectrophotometric method using ferrozine (Cobas c501).

Results: Mean hemoglobin in control group was 12.5±0.7 g/dL and in iron deficiency anemia group was 8.1±1.36 g/dL (p= 0.01). Mean HbA2 level in control group was 2.69±0.24% and iron deficiency anemia group was 1.76±0.25 % (p=0.001). HbA2 levels were reduced in individuals having haemoglobin levels less than 10g/dL and remained unchanged/minimally changed with hemoglobin levels reaching near normal levels.

Conclusion: There was a statistically significant association of HbA2 levels with degree of anemia and serum ferritin levels (p=0.001).

Keywords: Anemia, Beta thalassemia, Ferritin, Hemoglobin A2.

INTRODUCTION

The most common nutritional deficiency around the globe is Iron deficiency which leads to iron deficiency anemia.[1] It has major effects on the cognitive and emotional development of children and adolescent. Directly or indirectly, it may have an impact on the visual and auditory functions as well.[2] Iron deficiency basically means a reduced levels of iron storage in the body which can lead to production of red blood cells which have an abnormally small size and a reduced amount of heamoglobin (hypochromic, microcytic).[3] Various microcytic anemias present with similar microscopic picture which include thalassemia, anemia associated with the chronic diseases sideroblastic anemia, and lead poisoning.

Chronic blood loss, hookworm infections, poverty and malnutrition are among the leading causes of iron deficiency anemia.[4] Anemia caused due to the deficient levels of iron is a chronic condition which sometimes may be asymptomatic due to which it usually remains undiagnosed. Fatigue, weakness and inability to concentrate may occur due to improper supply of oxygen to the vital organs and tissues of the body. The main factor behind this is the decreased activity of iron containing enzymes.[5]

There are three types of hemoglobins present in an adult individual. Around 95% of hemoglobin is of HbA type with (α2β2 chains), 2.5% HbA2 (α2δ2 chains) and < 1% HbF (α2γ2 chains).[6] Normal levels of HbA2 ranges from 2.4-3.2%.[7] Heme and hemoglobin production require a considerable amount of iron for their production. There is a marked reduction in synthesis of all these naturally occurring hemoglobins in iron deficient patients. Some patients having beta thalassemia trait suffering from an underlying iron deficiency anemia may also yield false negative results showing normal low levels of HbA2. The objective of this study was to check the association of Hb levels with HbA2 levels and hemoglobin levels at which HbA2 levels are significantly reduced.

METHODOLOGY

The comparative cross-sectional study was conducted at the Department of Pathology Combined Military Hospital, Lahore Pakistan from January to October 2020. After taking permission from the ethical review board (Ltr no. 160/2020), written informed consent was taken from each patient. Patient

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particulars were endorsed in the proforma along with history and signs and symptoms related to anemia (presence of numbness, pica, pallor, low appetite, restlessness).

Inclusion Criteria: Patients of either gender diagnosed with iron deficiency anemia were included in the study.

Exclusion Criteria: Patients with abnormalities in the pathways of hemoglobin synthesis and other co-morbid conditions were excluded from the study.

Samples were collected using non probability consecutive sampling technique. A sample size of 206 was calculated using a mean level of HbA2 in control and iron deficient group as 2.9±0.4% and 2.7±0.6% respectively,[8] keeping Power of test 80% and alpha equal to 0.05 using Open Epi sample size calculator version 3.01. Iron deficiency anemia was defined as serum ferritin level <12ng/ml in blood samples. Anemia was defined as Hb <13g/dL in males and Hb <12g/dL in female patients. Healthy individuals having normal hemoglobin (Hb), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and serum ferritin levels reporting to the hospital for routine checkup were taken as control population.

Five milliliters of whole blood was drawn from patient through aseptic venipuncture in a tube containing EDTA. Basic haematological parameters (Hb, TRBC, MCV, MCH) were performed using automated analyzer (Sysmex KX-21). HbA2 levels were determined by cellulose acetate hemoglobin electrophoresis at alkaline pH (Mini Hor-8). Serum Ferritin levels were measured by chemiluminescence on Cobas e411 (Roche) and Serum Iron by spectrophotometric method using ferrozine (Cobas c501).

Data was analyzed using SPSS version 25. Data normality was assessed using Shapiro Wilk test. This showed that data was not normally distributed, hence patients were divided into 3 groups based on hemoglobin levels. Group-1 (Hb= 5-8 g/dL), Group-2 (Hb= 8.1-10 g/dL) and Group-3 (Hb=10.1-13 g/dL). Mean and SD were calculated for numerical variables. Percentage and frequency were calculated for categorical variables. Chi square test was used for establishing association between qualitative variables among various groups. Non parametric Kruskal Wallis test was used to compare mean HbA2 levels among various groups based on Hb levels. Spearman's correlation was used to assess relation between Hb and HbA2 levels. *p*-value of <0.05 was considered to be significant.

RESULTS

A total of 150 individuals with iron deficiency anemia and 56 healthy subjects were inducted into the study as controls. Mean±SD age of the patients was 26.27±8.94 years with a range of 12-54 years. Out of a total 206 cases, 94(45.6%) were males and 112(54.4%) were females. Females were more prone to iron deficiency anemia as shown by a greater number of females (n=82) being affected with iron deficiency anemia as compared to males (n=68) in the iron deficiency anemia group.

Table-I: Mean Hemoglobin level (g/dL) and Mean HbA2 percentage in Control Group versus Iron Deficiency Group (n=150)

	Mean Hemoglobin level	Mean HbA2 (%)
Control Group	12.5±0.7(g/dL)	2.69±0.24
Iron Deficiency Group	8.1±1.36(g/dL)	1.76±0.25
p-value	0.01(g/dL)	0.001

A total of 77(37.4%) patients were present in Group-1, 61(29.6%) in Group-2 and 12(5.8%) patients in Group-3. Mean HbA2 levels were compared among various groups and a statistically significant difference was seen (*p*=0.001) (Figure).
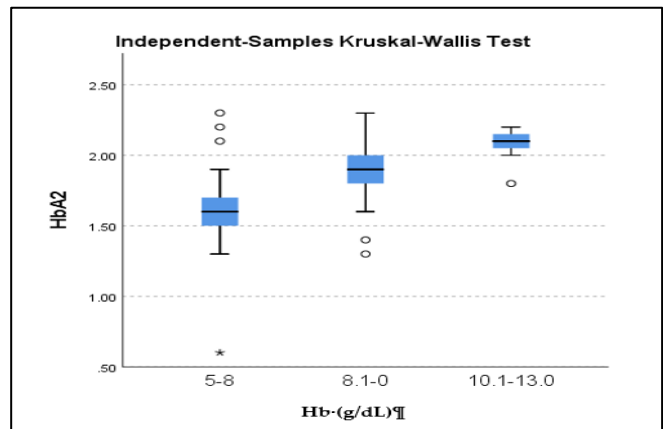
Figure: Comparison of HbA2 among various groups based on Hb (n=150)

Comparison of signs and symptoms such as pallor, pica, restlessness, low appetite and numbness also revealed statistically significant difference among the groups based on Hb levels (*p*=0.001)

Correlation between HbA2 levels and haemoglobin, age, serum ferritin and MCV was done and a strong positive relationship was observed.

Table-II: The relationship between clinical signs and symptoms and Hemoglobin levels in Iron Deficiency Anemia (n=150)

Hemoglobin level g/dL	Pica (n=150)		Pallor (n=150)		Numbness (n=150)		Restlessness (n=150)		Low appetite (n=150)	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
5-8	37(24.7%)	40(26.7%)	64(42.7%)	13(8.7%)	32(21.3%)	45(30%)	32(21.3%)	45(30%)	61(40.7%)	16(10.7%)
8.1-10	10(6.7%)	51(34%)	13(8.7%)	48(32%)	7(4.7%)	54(36%)	6(4%)	55(36.6%)	28(18.7%)	33(22%)
10.1-13	0(0%)	12(8%)	0(0%)	12(8%)	0(0%)	12(8%	0(0%)	12(8%)	0(0%)	12(8%)
p-value	0.001		0.001		0.001		0.001		0.001	

Table-III: Correlation between HbA2 levels and Hemoglobin (Hb), ferritin, Mean Corpuscular Volume (MCV) and Age (n=150)

	HbA2	*p*-value
Hemoglobin	r=0.90	0.001
Ferritin	r=0.69	0.001
Mean corpuscular volume	r=0.79	0.001
Age	r= 0.60	0.001

DISCUSSION

The high prevalence of iron deficiency anemia in the developing world has substantial health and economic cost including poor pregnancy outcome, impaired school performance and decreased productivity. The prevalence of beta thalassemia trait is 5% in Pakistan.[9] The mean levels of Hb in iron deficiency anemia group was 8.1±1.36 g/dL which was significantly low as compared to the control group of our study sample i.e. 12.5±0.7 g/dL. Clinical presentation among various Hb levels was statistically significant in our study (*p*=0.001). Pallor, low appetite, numbness and pica were more prevalent in low Hb group (5-8 g/dL) as compared to higher Hb levels. Clinical signs and symptoms can be an indicator of the disease severity.

A decrease in intracellular level of iron leads to a marked reduction in synthesis of α globin chains as compared to the non α globin chains. After a reduction in the β globin chain levels, they have a more effective mechanism of competing with α chains than the δ globin chains thus reducing the HbA2 levels. A reduction of 15-36% has been observed in various studies showing the greatest decline in severely iron deficient individuals.[10] In our study mean HbA2 level in control group was 2.69±0.24% and iron deficiency anemia group was 1.76±0.25% (*p*=0.001). Similar results were shown by a study conducted by Keramati *et al.* in Iran. He concluded that HbA2 levels were 2.9±0.4% in control group and in Iron deficiency anemia patients it was 2.7±0.6%.[8] In a study by Denic *et al.* various potential factors effecting HbA2 levels were studied. They concluded that mean HbA2 levels were 0.2% lower in subjects with iron deficiency than normal subjects.[11]

A greater percentage of females suffered from iron deficiency anemia as compared to males. 54.6% of the females in our study sample had iron deficiency anemia as compared to 45.4% males. Various causes of iron deficiency include chronic blood loss (peptic ulcer, hookworm infections, heavy menstrual bleeding, post-partum hemorrhage etc.), decreased iron intake (poor diet), decreased iron absorption (atrophoic gastritis, coelic disease etc.) and increased demand (pregnancy, blood donation etc.).[12] In a study conducted by Yavarian *et al.* similar results were revealed. 66.8% females and only 33.2% males suffered from iron deficiency anemia, showing a greater prevalence in the female gender.[13]

A strong positive correlation was found between Hb, ferritin levels, MCV and age when compared to the HbA2 levels. After the establishment of normal iron levels as depicted by the serum ferritin values after iron therapy, there was a marked increase in the production of HbA2. A weak positive correlation (r=0.24) between ferritin levels and HbA2 levels was also seen in a study conducted by Hameed *et al.*[14] Mean levels of HbA2 in low and high serum ferritin groups were 2.12% and 2.46% respectively as shown by Chandrashekar *et al.* in their study.[15] There was a weak positive correlation (r=0.24) between age and HbA2 levels as depicted by Kingchaiyaphum *et al.*[16]

Microcytic hypochromic picture is linked to beta thalassemia trait and iron deficiency anemia. Keeping in mind the 5% prevalence of beta thalassemia trait in Pakistan, differentiation of above-mentioned conditions is very important due to varying impact on the patients overall well being.[17] Hemoglobin A2 level is a strong indicator of beta thalassemia trait and is used during beta thalassemia carrier screening. HbA2 level appears to be decreased in iron deficiency anemia however its level is increased in beta thalassemia trait and megaloblastic anemia. HbA2 is decreased in Iron Deficiency Anemia (IDA) due to an overall decrease in hemoglobin synthesis. This phenomenon of decreased HbA2 in IDA patients can mask Beta Thalassemia trait and may yield a false negative result.[18] Thus, diagnosis

of beta thalassemia trait should be carried out after correction of iron deficiency of the patients.

CONCLUSION

There was a statistically significant direct correlation of the HbA2 levels with degree of anemia and serum ferritin levels (p=0.001). HbA2 levels were severely affected in individuals having lower haemoglobin levels (Hb<10g/dL), and remain unchanged or minimally changed with Hb levels reaching near normal levels. So, there should not be any diagnostic difficulty related to beta thalassemia trait in patients with mild to moderate iron deficiency anemia. However, for patients having severe iron deficiency anemia, the diagnostic workup for thalassemia trait should be carried out after correction of iron deficiency.

Conflict of interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

AH & SN: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

NUDK & HMR: Data acquisition, data analysis, approval of the final version to be published.

SJ & AT: Critical review, concept, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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