ORIGINAL ARTICLE

Evaluation of Microcytic Hypochromic Anemia by Electro-phoresis for Hemoglobinopathies in Young Population

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ABSTRACT

Aim: To study hematological indices and peripheral smear examination in microcytic hypochromic anemia and to detect hemoglobinopathies by doing hemoglobin electrophoresis in microcytic hypochromic anemia.

Study design: Cross sectional Study

Place and duration of study: Department of Medicine Ghurki Trust Teaching Hospital, Between June 2010 and June 2014.

Methods: Subjects for study obtained from the clinical cases suspected of anemia were 100 in total. All patients who presented with pallor and were detected to have microcytic hypochromic anemia on peripheral examination were included in the study. Anemic patients having cause other than microcytic hypochromic anemia and confirmed cases of Iron deficiency anemia were excluded. Investigations were done to confirm that Anemia is microcytic hypochromic anemia and to find out hemoglobinopathies as a cause MHA. Complete hemogram was performed and Hb electrophoresis was done after studying the iron profile and ruling out iron deficiency anemia by performing serum Iron level.

Results: The study group included 53-males and 47 females. Use of electrophoresis showed out of 100 cases, 21% of cases were thalassemia and 79% were iron deficiency anemia. Age distribution is not statistically associated with either Iron deficiency Anemia and Thalassemia with P=0.412. Gender is not statistically associated with either Iron deficiency Anemia and Thalassemia with P=0.358. 9 patients had history of consanguineous marriage which constituted 42.9%. The remaining 12 patients i.e. 57.1% did not give such history. Of the 21 cases of thalassemia, all (100%), presented with pallor, generalized weakness. Only 3 cases i.e.14.3% gave history of jaundice. None of the cases had any bleeding tendencies.13 cases i.e., 61.9% had iron overload 14 cases showed growth impairment, this constituted 66.7% of total.

Conclusion: Most common cause of MHA was iron deficiency anemia and 2nd being thalassemia. Differential diagnosis based on complete hemogram and peripheral smear is possible but special tests like serum iron profile and hemoglobin electrophoresis are a must for confirmation of diagnosis.

Keywords: Microcytic Hypochromic Anemia, Electrophoresis, Hb, RDW, MCV, TIBC,

INTRODUCTION

Anemia is defined as decrease in hemoglobin concentration below lower limit of normal with reference to age and gender. Anemia is not a diagnosis but a finding that requires further investigation. While investigating anemia, apart from low hemoglobin levels certain other parameters need consideration. Red cell indices, red cell count, and morphological examination of peripheral blood film are the basic tests in line of investigations for these patients. Reticulocyte count and LDH levels are very useful tests to differentiate production failure defects from hemolytic anemias. Clinically presence of jaundice along with pallor, age of appearance of pallor, hepatosplenomegaly, dietary history, history of drug intake and socioeconomic conditions all help in diagnosis[1].

Anemia constitutes world's problem of a great magnitude, and children under 5 years old represent one of the highest risk population group, although some studies have suggested a decline in prevalence of anemia[2]. The most recent reports showed an increase in its frequency among low income group and more in underdeveloped countries[3]. The causes of anemia vary by age[4]. In general, anemia can be caused by either decreased production[5] (as seen with nutritional deficiencies, bone marrow failure, pure red cell aplasia, sideroblastic anemia, congenital dyserythropoietic anemias etc) or it can be due to increased destruction (as seen with congenital

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hemolytic anemias, autoimmune hemolytic animas, drugs or microangiopathies). Iron deficiency anemia (IDA) is the most common of nutritional deficiencies in childhood, affecting all socioeconomic levels of society. Global prevalence of iron deficiency in young children is 43%[6] but children between 1-2 years are at greatest risk and delayed weaning is the most significant risk factor at this age. Pakistan nutrition survey found that 65% of children between the ages of 7-60 years had IDA[7]. Other studies have shown different prevalence rates (e.g. 70%,78%)[8,9]. The major factors in this age group include prolonged consumption of cow's milk, delayed weaning, poor dietary habits (use of foods with low bioavailability of iron). Other factors leading to iron deficiency in children are worm infestation, increased requirements due to rapid growth and development, malabsorption, gastrointestinal or urinary losses. The complications of anemia are well known but some may go unnoticed and may have adverse effects on child's life. Many studies have reported an association between anemia and poor mental performance and behavioural abnormalities[10]. The hemoglobinopathies are a diverse group of inherited recessive disorders that include thalassemia and sickle cell disease. The incidence of β-thalassemia is reportedly high in endemic regions such as the Mediterranean, Africa, Southeast Asia, Southern China, India, and Pakistan. In these regions, β-thalassemia is associated with a hypochromic, hemolytic anemia. Thalassemia and other hereditary hemoglobin disorders are associated with considerable morbidity and mortality and require lifelong care.[2] Thalassemia is among the most common genetic disorder worldwide. 4.83% of world population carries globin gene variants which include 1.67% of population heterozygous for α and β thalassemia, with high prevalence in Asia including Pakistan comprising of nine million carriers resulting in more than 5000 transfusion dependent children per year. In addition 1.92% carry sickle Hb, 0.95% carry Hb E and 0.29% Hb C. Carrier rate of Thalassemia in Pakistan is about 6%[7,8,9].

METHODOLOGY

A total of 100 cases were included in this study that was proved to have MHA. Study subjects were recruited at Ghurki Trust Teaching Hospital Lahore and department of medicine Lahore General Hospital from June 2010 to June 2014. All patients who presented with pallor and were detected to have microcytic hypochromic anemia on peripheral examination were included in the study while anemic patients having cause other than microcytic hypochromic anemia and confirmed cases of Iron deficiency anemia were excluded. Investigations were done to confirm that Anemia is microcytic hypochromic anemia and to find out thalasemia as a cause MHA. Complete hemogram was performed on the patients and Hb electrophoresis was done after studying the iron profile and ruling out iron deficiency anemia as cause of Microcytic Hypochromic anemia. Other tests which were performed included serum Iron level, Ferritin and TIBC.

Electrophoresis was done using the Hydra gel hemoglobin (E) K 20. Electrophoresis was done for separation of normal hemoglobin (A & A2) & for detection of major Hb variants –S/D & C/E on alkaline agarose gels. The resulting electrograms are evaluated visually for pattern abnormalities. Hb molecules in an alkaline pH have a net negative charge and move towards anode in an electrophoretic system. These include Hb Barts and the two fastest, HbH & HbI,HbC is the slowest of the common Hbs.A few in the order of increasing mobility are HbA2, E=O=C, F, A, K, J, Barts, N=I and H.

The t test for paired observations under significance of 0.05 was used to evaluate significance. The descriptive statistics values are presented as mean±standard deviation. Data was processed in SPSS software version14.

RESULTS

A Cross sectional study with 100 patients of young age group was undertaken to study the role of electrophoresis in diagnosing the microcytic hypochromic anemia cases. The study group included 53-males and 47 females. Use of electrophoresis showed out of 100 microcytic hypochromic anemia cases, 21% of cases were thalassemia and 79% were iron deficiency anemia. Age distribution was not statistically associated with either Iron deficiency Anemia or Thalassemia with P=0.412. Gender was neither statistically associated with Iron deficiency Anemia nor Thalassemia with P=0.358. Nine patients had history of consanguineous marriage which constituted 42.9%. The remaining 12 patients i.e., 57.1% did not give such history. Of the 21 cases of thalassemia, all (100%), presented with pallor, generalized weakness and had history of mass per abdomen. Only 3 cases i.e.14.3% gave history of jaundice. None of the cases had any bleeding tendencies. All 21 cases had pallor, Hepatosplenomegaly which constituted 100% cases. Only 3 cases (14.3%) had jaundice and 14 cases i.e., 66.7% had Frontoparietal bossing as complications of thalassemia. Thirteen cases i.e., 61.9% had iron overload (serum iron more than 200µg /dl). 14 cases showed growth impairment, this constituted 66.7% of total. In this study no cases had endocrine

abnormalities, cardiac failure or bleeding tendencies.

Analysis of Laboratory Profile:

Mean±SD of hemoglobin concentration in iron deficiency anemia was 8.34±2.2 g/dl and in thalassemia was 8.44±1.61g/dl.

Mean±SD of PCV in iron deficiency anemia was 23.5±6.91% and in thalassemia was 17.16±4.11%.

Mean±SD of RBC count in iron deficiency anemia was 3.53±1.09 million/dl and in thalassemia was 2.77±1.28 million/dl.

Mean±SD of MCV in iron deficiency anemia was 68.66±11.23fl and in thalassemia was 51.22±11.89fl.

Mean±SD of MCH in iron deficiency anemia was18.42±4.39 pg and in thalassemia was 16.23±5.21 pg.

Mean±SD of MCHC in iron deficiency anemia was 27.74±10.34g/dl and in thalassemia was 26.05±5.66 g/dl.

Mean±SD of RDW in iron deficiency anemia was 18.27±2.83% and in thalassemia was 13.06±1.11%.

Mean±SD of serum iron in iron deficiency anemia was 46.08±12.92µg/dl and in thalassemia was 195.52±49.83 µg/dl

Mean±SD of total iron binding capacity in iron deficiency anemia was 416.95±51.77 µg/dl and in thalassemia was226.67±37.28 µg/dl

Mean ± SD of transferrin saturation in iron deficiency anemia was 10.38±4.44% and in thalassemia was74.71±17.72%.

Hemoglobin Electrophoresis in Thalassemia cases:

HbA: Present study had 1 case with HbA between 0 & 4%, 7 cases between 4 & 24% and 5 cases between 24 & 76%. In the study 13 out of 21 patients (61.90%) fall into β^{o}/β^{+} thalassemia group and 8 out of 21 (38.09%) fall into β^{+}/β^{+} thalassemia group.

HbF: In our present study 14 out 21 cases (66.66%). Only one case had HbF of 14.6%, one case had 25% and one case had 41.6%

HbA2: In the present study 20 out of 21 patients (90.47%) had HbA2 0.3 - 3.9% & only one case had HbA2 = 6.1%.

DISCUSSION

According to Astaldi et al. several grades of the disease were Recognized[11]. A severe form causing anemia early in infancy and often resulting in death in 1st year. A slightly less severe form of the disease usually first becoming manifest in 2nd half of 1st year, the child often surviving until school age. A milder form usually diagnosed in 2nd year of life and compatible with survival until adult age. The cases, which were diagnosed within 6 months of life, represented severe form of β thalassemia major and cases diagnosed after 6 months represented less severe forms. In the present study only one case was less than 6 months. Mohan N.et al. studied the hematological status of β thalassemia in the ethnic group particularly from Tamil Nadu, Southern India, who are still practicing a high degree of consanguinity. In the present study of 21 cases 9 cases (42.9%) had parents with history of consanguineous marriage[12].

Swaroop Mitra et al study of clinical and hematological profile of thalassemia had pallor in 100% cases, generalized weakness in 100% cases, mass per abdomen in 100% cases, jaundice in 0% and epistaxis in 4.08% cases[13]. In the present study pallor, generalized weakness and mass per abdomen was seen in 100% cases in concordance with Swaroop Mitra study. Jaundice was seen in14.3% cases and none of the cases presented with epistaxis. Thirteen cases (61.9%) had serum iron more than 200µg/dl. correlate with Cartwright and coworker who showed iron overload in 80%, Smith et al had 82% showing iron overload. Erlandson et al 66.66 %[14]. In thalassemia, iron loading is frequent & most important complication, more so in patients receiving multiple transfusions. Iron overloading is the result of multiple transfusions and also due to increased absorption of iron from intestines. Diabetes mellitus and delay in puberty are common endocrinal dysfunctions seen in β thalassemia major. Ellis et al showed 1 out of 13 cases (7.69%) and Kattamis et al[15] showed 82 out of 405 (20.24%) cases with endocrinal dysfunction. But in our present study none of the 21 cases had any endocrinal abnormality. According to Canak[16] and coworkers, the pituitary, adrenal, thyroid and gonadal functions are normal in most patients with thalassemia.

Our study had hemoglobin concentration between 5.6 & 10.2G/dl with a mean of 8.44±1.61G/dl. Majority i.e.12 out of 21 (57.14%) had hemoglobin between4-6G/dl[13]. Swaroop Mitra et al also showed hemoglobin percentage between 4 & 6G/dl. Our study closely correlates with the above study.

The MCV in our study ranged from 33 - 78 fl. With a mean of 51.22±11.89 fl. Majority i.e.17 out of 21 (80.95 %) cases had MCV less than 67 fl. Deborah Rund et al showed that MCV was in the range of 56.3- 87.3 fl. In almost all cases carriers of β^{0} mutations MCV < 67 fl, whereas all but a few β^{+} heterozygotes had MCV >67fl. This showed that MCV of heterozygotes for β thalassemia correlates with the severity of mutations[17] Cartwright and coworker had 4 out of 5 patients with serum iron> than 200µg/dl. and transferrin saturation =100%. Erlandson & coworker[15] showed that 10 out of 20 patients had serum iron of 170 - 230µg/dl. and transferrin saturation of 100%. Present study had 61.90 % of patients with serum iron > 200µg/dl and transferrin saturation of > 50% in 90.47%.

The extent to which β chain production is impaired varies in β thalassemia from severe impairment resulting in no recognizable production of HbA as in homozygous β^{0} thalassemia to moderate impairment only, allowing the formation of significant amount of HbA in β+ thalassemia. Kattamis study

showed 13 cases with HbA in the range of 0 4%, 17 cases in the range of 4 -24 % and 6 cases in the range of24 - 76%[18]. Present study had 1 case with HbA between 0 & 4%, 7 cases between 4 & 24% and 5 cases between 24 & 76%. In the study 13 out of 21 patients (61.90%) fall into β^{o}/β^{+} thalassemia group and 8 out of 21 (38.09%) fall into β^{+}/β^{+} thalassemia group. In a study done by J David Bessman and Donald Feinstein to assess the importance of quantitative anisocytosis as a discriminant factor between iron deficiency anemia and thalassemia minor, 22 of 25 patients with β thalassemia and MCV <70f1, RDW was <14%. In our study 20 out of 21 patients with β thalassemia & MCV <70 fl. RDW was < 14%[19]. In iron deficiency anemia 53 patients with MCV <70 fl., RDW was always >14%. In our study 50 of 79 patients with MCV <70 f1. RDW was > 14% in 75 patients. In our study differentiating iron deficiency anemia and thalassemia from RBC indices especially MCV and RDW was useful. The p value as calculated by students T test was significant for both MCV ($p<0.001$) and for RDW ($p<0.001$). Two reasons were suggested in the Bessman study for this anisocytosis as measured by RDW. First, iron deficiency results in abnormal erythropoeisis leading to hypochromia, microcytosis, increased variations in size and shape leading to poikilocytosis and anisocytosis. Second, without iron replacement iron deficiency is progressive rather than stable[17]. But in thalassemia minor, the RBC abnormality is not progressive. Therefore they are exempt from this anisocytosis. A normal RDW with decreased MCV should give an important clue to the diagnosis of thalassemia minor. Similarly study done by Bessman et al classified anemia based on MCV and RDW together. Low MCV and normal RDW are seen in heterozygous thalassemia. Low MCV and increased RDW is seen in iron deficiency anemia. It was shown that classification by MCV alone was less than 90% sensitive. And classification by RDW alone was less than 90% sensitive. But both MCV and RDW together accurately predict the normal subjects.

CONCLUSION

Microcytic Hypochromic Anemia is a very common problem in clinical practice, early detection of which helps in the correct treatment. Most common cause of Microcytic Hypochromic Anemia in this study was iron deficiency anemia and 2nd being thalassemia.

Diagnosis of the Microcytic Hypochromic Anemia was achieved easily with the help of complete hemogram and peripheral blood picture. Differential diagnosis based on complete hemogram and peripheral smear is possible but special tests like serum iron profile and hemoglobin electrophoresis are a must for confirmation of diagnosis. Iron deficiency anemia shows decreased serum iron and transferrin saturation with increased TIBC. Hemoglobin electrophoresis is a must in the diagnosis of thalassemia. Early diagnosis of which helps to start transfusion therapy and yield better prognosis. Iron overload being a major complication in thalassemia showed increased serum iron levels.

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