

A Laboratory Study of Anemia in Children Aged 6 Months to 6 Years in Erbil City

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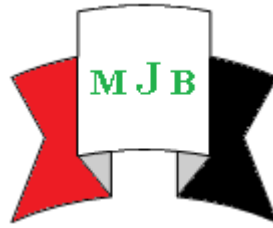
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Abstract

Background and Objectives: Anemia is one of the most common health problems in the world and one of the important clinical markers of the underlying disorder. The aim of this study was to study causes of anemia in children aged 6 months to 6 years and to define its main morphologic types through the laboratory evaluation.

Materials and Methods: The study group included 325 patients with Hb levels less than 11.0 g/dl in Erbil city. The laboratory investigations included complete blood picture, ESR, reticulocyte count, iron profile, Hb electrophoresis, G6PD screening by methemoglobin reduction test, Coombs' test and bone marrow study.

Results: Anemia was more frequent in children up to 2 years age (54%) than in children of (2–6 years) age group. Anemia was commoner in male children (62.1%) than female children (37.9%). According to MCV; types of anemia were microcytic (60.7%), normocytic (26.7%) and macrocytic (12.6%). Anemia was mild (Hb: 9-10.9 g/dl), moderate (Hb: 7-8.9 g/dl) and severe (Hb: <7 g/dl) in 55.4%, 26.7% and 17.9% of cases respectively. The underlying causes of anemia were iron deficiency anemia (42.6%), hemolytic anemia (18.6%), thalassemia syndromes (16.6%), anemia of chronic disease (particularly respiratory and gastrointestinal infections) (15.5%), acute leukemia (2.7%), liver diseases (1%), megaloblastic anemia (0.6%), acute blood loss (0.6%), aplastic anemia (0.3%) and anemia of undetermined cause (1.5%).

Conclusions: The commonest type of anemia in children (6 months to 6 years) was microcytic anemia followed by normocytic and then macrocytic anemia. Iron deficiency was the commonest cause of anemia followed by hemolytic anemia, anemia of inflammation, thalassemia syndromes and then other causes.

Keywords: anemia in children, Iron deficiency, Red cell enzyme disorder, Thalassemia, Erbil.

الخلاصة

الخلفية والأهداف: فقر الدم هو أحد المشاكل الصحية الأكثر شيوعاً في العالم، وهو من العلامات السريرية الهامة للأمراض الكامنة. كان الهدف من هذه الدراسة هو التحقيق في أسباب فقر الدم عند الأطفال الذين تتراوح أعمارهم بين ٦ أشهر إلى ٦ سنوات، وتحديد أنواعه الرئيسية حسب شكل كريات الدم الحمراء ومن خلال تقييم الفحوص المختبرية.

المواد والطرق: مجموعة الدراسة شملت 325 مريض مع مستويات الهيموجلوبين أقل من 11.0 غم / دل في مدينة أربيل. الفحوصات المختبرية شملت صورة الدم كاملة، معدل ترسب كريات الدم الحمراء (ESR)، عدد الخلايا الشبكية، فحوصات مستوى الحديد وفصل الهيموجلوبين الكهربائي، وفحص نقص الانزيم G6PD بواسطة فحص اختزال ميثيموغلوبين، اختبار كومب ودراسة نخاع العظام.

النتائج: كان فقر الدم أكثر شيوعاً في الأطفال دون سنتين (٥٤٪) من الأطفال الذين اعمارهم بين (٢-٦ سنوات). كان فقر الدم أكثر شيوعاً في الأطفال الذكور (٦٢,١٪) من الأطفال الإناث (٣٧,٩٪). وفقاً لمقياس معدل حجم كرية دموية حمراء (MCV)؛ كانت أنواع فقر الدم ذات الكريات الصغيرة ٦٠,٧٪، وذات الكريات دم الطبيعية ٢٦,٧٪ وذات الكريات دم الكبيرة ١٢,٦٪. كانت نسب فقر الدم الخفيف (خضاب الدم: ٩-١٠ غم/دل)، معتدلة (خضاب الدم: ٧-٨,٩ غم/دل) وشديدة (خضاب الدم: >٧ غم/دل) هي ٥٥,٤٪، ٢٦,٧٪ و ١٧,٩٪ من الحالات على التوالي. وكانت الأسباب الكامنة وراء فقر الدم كالتالي: فقر الدم بسبب نقص الحديد (٤٢,٦٪)، فقر الدم الانحلالي (١٨,٦٪)، متلازمات التلاسيميا (١٦,٦٪)، فقر الدم من الأمراض المزمنة (التهابات الجهاز التنفسي بشكل خاص والجهاز الهضمي) ١٥,٥٪، سرطان الدم الحاد بنسبة ٢,٧٪، أمراض الكبد ١٪، فقر الدم الضخم الأرومات (ميكالوبلاستك انيميا) ٠,٦٪، فقدان الدم الحاد بنسبة ٠,٦٪، فقر الدم اللاتنسجي (ابلاستك انيميا) ٠,٣٪ وفقر الدم غير محددة السبب ١,٥٪.

الاستنتاجات: كان أشيع نوع من فقر الدم في الأطفال من عمر (٦ أشهر إلى ٦ سنوات) فقر الدم ذات كريات الدم الصغيرة تليها فقر الدم ذات كريات دم الطبيعية ثم ذات كريات الدم الكبيرة. وكان نقص الحديد السبب الأكثر شيوعاً لفقر الدم يليه فقر الدم الانحلالي وفقر الدم المصاحب للالتهاب، متلازمات التلاسيميا ثم أسباب أخرى.

Introduction

The World Health Organization (WHO) defines anemia as a reduction in the hemoglobin concentration of blood appropriate for the age and gender. For preschool children the WHO lower cutoff level is (11.0 g/dl) [1].

Anemia is one of the most common health problems in the world and one of the important clinical markers of the underlying disorder. Prevalence of anemia in preschool children, particularly between 6 months and 5 years varies in different countries e.g. 20% of children in the United States and 80% of children in developing countries have anemia [2,3].

Causes of anemia are many, but with a thorough clinical information and laboratory evaluation, a specific diagnosis can usually be established. The use of the mean red cell volume (MCV) is a standard diagnostic approach, to classify the anemia into microcytic, normocytic or macrocytic [3].

The commonest type in children is microcytic anemia which is mostly due to iron deficiency that is usually caused by reduced dietary intake and increased demands in children. Therefore anemia in children is easily

treatable with supplemental iron and early intervention may prevent later loss of cognitive function (4). Less common causes of microcytic anemia in children are thalassemia, and sometimes anemia of chronic disease [1]. In normocytic anemia, the reticulocyte count will help to narrow the differential diagnosis; however, additional investigations may be necessary to rule out anemia of chronic diseases, bone marrow diseases (e.g. acute leukemia and aplastic anemia), hemolytic anemia (e.g. red cell membrane defects and enzymopathies), and hemoglobinopathies [3]. Macrocytic anemia is uncommon in children unless it is caused by reticulocytosis and it may be caused by folate or vitamin B12 deficiency, hypothyroidism or liver disease [5].

Anemia in children is regarded as one of the biggest issues in the primary children health care centers and pediatric hospitals. It deserves to be illustrated by a series of studies especially in our locality and for this reason we designed this study.

The objective of this study is to determine the underlying causes of anemia in children aged 6 months to 6 years, their frequencies and laboratory features in Erbil city and also to

determine the significance of complete blood count and blood film morphology in the diagnostic approach to childhood anemia.

Materials and Methods

The design of the study is descriptive case series and the study population included a group of 325 children aged 6 months to 6 years presented with anemia. The study was conducted during the period between September 2009 and August 2010 in Raparin Pediatric Hospital, Erbil, Iraq. Patients and controls were classified into two age groups; G1: 6 months to 2 years and G2: 2 to 6 years. Complete blood picture was done for all the patients and control group.

Anemic children were classified according to their MCV (Mean Cell Volume) values into microcytic, normocytic and macrocytic. Also they were classified into 3 groups according to the Hb values: mild = 9 – 10.9 g/dl, moderate = 7 – 8.9 g/dl and severe < 7 g/dl.

Further investigations for microcytic anemia included iron profile (serum iron, TIBC, transferrin saturation and serum ferritin) to identify iron deficiency anemia and Hb electrophoresis to identify thalassemia syndromes and hemoglobinopathies.

Investigations of normocytic anemia have been performed according to reticulocyte count. If it was associated with reticulocytosis then proceed to the investigations of hemolytic anemia by total and indirect serum bilirubin, direct antiglobulin test (Coombs' test) (Core Diagnostics LTD, UK) and screening for G6PD deficiency by methemoglobin reduction test (Inverness Medical, C/O Biosite International, Switzerland).

Iron profile and Erythrocyte Sedimentation Rate (ESR) by Westergren tube method have been performed to determine anemia of inflammation or anemia of chronic disease (AOI/ACD) secondary to infection, inflammation or malignancy.

Bone marrow study (aspiration and biopsy) was performed for the cases of pancytopenia and abnormal WBC morphology to exclude malignant blood diseases e.g. acute leukemia or hypoplastic marrow e.g. aplastic anemia.

Investigations of macrocytic anemia included reticulocyte count to detect hemolytic anemia, serum folate and serum vitamin B₁₂ using Cobas 8000 modular analyzer series (Roche Diagnostics, UK) to detect megaloblastic anemia and liver function tests to detect liver disease.

Statistical analysis:

The study design is a descriptive case series and the data has been enrolled and analyzed using computer software Statistical Package of Social Sciences (SPSS) version (18). Means of continuous variables and proportions of categorical variables have been analyzed using Student t-test and Chi-square tests respectively and *P* values less than 0.05 was regarded as significant.

Results

The study group consisted of 325 cases, 176 (54.1%) of them were of G1 age group and 149 (45.9%) of them were of G2 age group. Of them 202 (62.1%) were males and 123 (37.9%) of them were females (Table 1).

Table 1 Gender and age distribution of the patients

Age groups	Male	Female	Total	%	P values
G1	99	77	176	54.1	< 0.001
G2	103	46	149	45.9	< 0.001
Total	202	123	325	100	< 0.001
%	62.1	37.9	100	----	----
P values	0.134	< 0.001	< 0.001	----	----

The main causes of anemia were; iron deficiency anemia (IDA) which was present in 138 (42.6%) of the cases, hemolytic anemia (HA) in 61 (18.6%), thalassemia [including both β -thalassemia trait (BTT) and homozygous β -thalassemia (HBT)] in 54 (16.6%) and anemia of inflammation or infection or anemia of chronic disease (AOI/ACD) that was present in 50 (15.5%) of the cases.

Other causes of anemia were acute leukemia (AL) which was present in 9 (2.7%) cases, liver disease in 3 (1%), megaloblastic anemia (MA) and anemia of acute bleeding (AAB) due to thrombocytopenia in 2 (0.6%). Only 1 case (0.3%) was diagnosed as aplastic anemia (AA) and the remaining 5 cases (1.5%) were anemia of undetermined cause (AUC) (Figure 1).

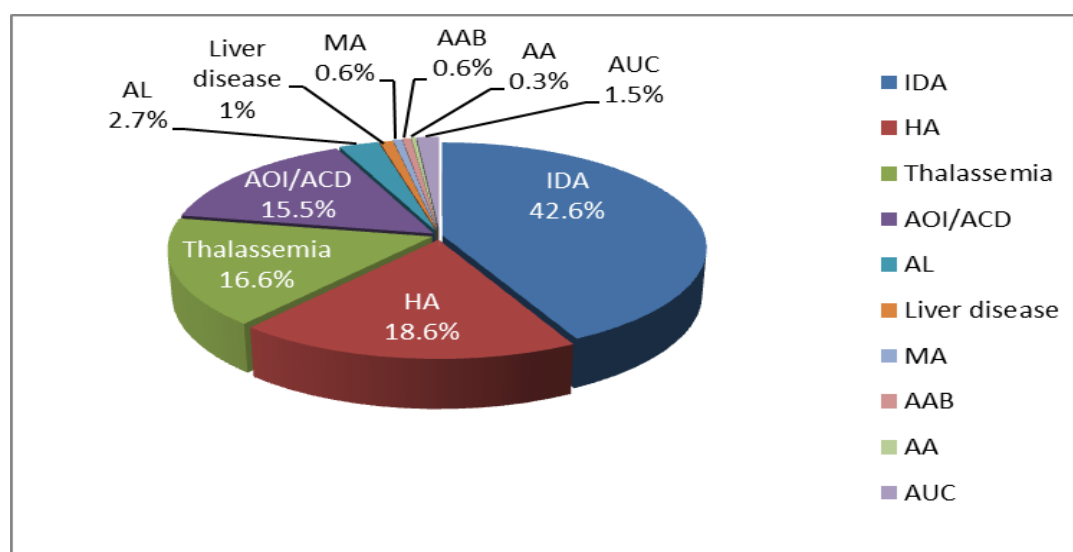


Figure 1 Distribution of underlying disorders of anemia in 325 cases

There were significant differences between the age groups regarding IDA, HA, BTT, HBT, AOI/ACD and ALL cases ($P < 0.001$). In 138 IDA cases, 88 (64%) of them were within G1 age group while 50 (36%) of them were within G2 age group. Considering 61 cases of HA, 38 (62.3%) of them were within G1 age group and 23 (37.7%) of them were within G2 age group. In contrast in 38 BTT cases, 15 (39.5%)

of them were within G1 age group and 23 (60.5%) of them were within G2 age group i.e. all HBT cases were within G1 age group and all ALL cases were within G2 age group and 2 AML cases were within G1 age group.. Regarding 50 cases of AOI/ACD, 27 (54%) of them were within G1 age group and 23 (46%) of them were within G2 age group (Table 2).

Table 2 Distribution of underlying causes of anemia according to the age

Underlying anemias	G1	G2	Total	P values
IDA	88 (64%)	50 (36%)	138	< 0.001
HA	23 (37.7%)	38 (62.3%)	61	< 0.001
BTT	15 (39.5%)	23 (60.5%)	38	< 0.001
HBT	14	0	14	< 0.001
HbH disease	0	2	2	NS
AOI/ACD	27 (54%)	23 (46%)	50	< 0.001
ALL	0	7	7	< 0.001
AML	2	0	2	NS
Liver disease	0	3	3	NS
Megaloblastic anemia	2	0	2	NS
AAB	1	1	2	NS
AA	0	1	1	NS
AUC	2	3	5	NS
Total	176 (54%)	149 (46%)	325	< 0.001
P values	< 0.001	< 0.001	< 0.001	

There was significant male predominance in IDA and HA cases ($P < 0.001$). There was no significant difference between males and females regarding other types of anemia ($P >$

0.05). There was a significant decline in the frequency of IDA in females and significant rise in the frequency of G6PD deficiency in males at G2 age group ($P < 0.001$) (Table 3).

Table 3 Distribution of underlying causes of anemia according to the gender

Underlying anemias	Male	Female	Total	P values
IDA	91 (66%)	47 (34%)	138	< 0.001
HA	45 (74%)	16 (26%)	61	< 0.001
Thalassemia	25	29	54	NS
AOI/ACD	28	22	50	NS
AL	6	3	9	< 0.001
Liver disease	1	2	3	NS
Megaloblastic anemia	1	1	2	NS
AAB	1	1	2	NS
AA	1	0	1	NS
AUC	3	2	5	NS
Total	202(62.1%)	123(37.9%)	325	< 0.001
P values	< 0.001	< 0.001	< 0.001	

The hematological profiles of most frequent causes of anemia in this study (IDA, BTT, AOI/ACD, HA and HBT) are shown in table (4).

Table 4 Hematological profiles of the most frequent causes of anemia in this study

Parameter (Mean $\pm$ SD)	IDA (N = 138)	BTT (N = 38)	AOI/ACD (N = 50)	HA (N = 61)	HBT (N = 14)	Control group (N = 164)
Hb (g/dl)	9 $\pm$ 1.26	9.7 $\pm$ 0.6	9.7 $\pm$ 0.5	6.5 $\pm$ 1.7	6.7 $\pm$ 1.4	12.43 $\pm$ 0.78
Hct (%)	30 $\pm$ 3.7	32.6 $\pm$ 2.1	31.35 $\pm$ 2	20.7 $\pm$ 5.39	16.5 $\pm$ 2.4	0.36 $\pm$ 0.018
RBC count ($\times 10^{12}/L$)	4.6 $\pm$ 0.48	5.5 $\pm$ 0.27	4 $\pm$ 0.36	2.36 $\pm$ 0.6	3.7 $\pm$ 1.2	4.5 $\pm$ 0.28
MCV (fl)	63.9 $\pm$ 7.7	59.37 $\pm$ 3.9	77.25 $\pm$ 4.4	88.48 $\pm$ 7.4	59.16 $\pm$ 8.7	78.3 $\pm$ 3
MCH (pg)	19.3 $\pm$ 3	17.8 $\pm$ 1.18	23.9 $\pm$ 2	27.7 $\pm$ 2.2	18.48 $\pm$ 3	26.5 $\pm$ 1.37
MCHC (g/dl)	29.9 $\pm$ 2	30 $\pm$ 1.57	30.9 $\pm$ 1.5	31.5 $\pm$ 1.9	30.2 $\pm$ 2	33.3 $\pm$ 1.3
RDW (%)	17.3 $\pm$ 2.6	15.7 $\pm$ 3.7	14.3 $\pm$ 2.18	14.2 $\pm$ 2.9	27.08 $\pm$ 8.5	13.16 $\pm$ 0.9
WBC count ($\times 10^9/L$)	10.7 $\pm$ 3.3	10.8 $\pm$ 4.17	13 $\pm$ 8.07	16 $\pm$ 8.7	14.19 $\pm$ 12.1	10.83 $\pm$ 2.58
Platelet count ($\times 10^9/L$)	435 $\pm$ 146	387 $\pm$ 130	375 $\pm$ 152	359 $\pm$ 130	358 $\pm$ 248	355 $\pm$ 77.5
Reticulocyte count (%)	0.8 $\pm$ 0.7	1.6 $\pm$ 0.8	0.7 $\pm$ 0.3	7.6 $\pm$ 4.8	4.2 $\pm$ 2.8	1.46 $\pm$ 0.4

Considering the morphologic types of anemia, microcytic anemia was present in 197 (60.7%) cases, of which 138 (70%) were IDA, while 54 (27.4%) cases were thalassemia and only 5 (2.6%) cases were AOI/ACD. Normocytic anemia was present in 87 (26.7%) cases, 45 (51.72%) of them were AOI/ACD, 29 (33.33%) were

HA, 6 (6.9%) were AL, 2 (2.3%) of them had AAB and 5 (5.75%) of them had AUC. Macrocytic anemia was present in 41 (12.6%) cases, of which 32 (78%) were HA, while AL and liver disease were present in 3 (7.4%) cases, 2 (4.8%) of them were MA and 1(2.4%) of them was AA (Table 5).

Table 5 Distribution of the morphologic types of anemia

Underlying cause	Microcytic	Normocytic	Macrocytic	Total	%	P values
IDA	138(70%)	0	0	138	42.6	< 0.001
HA	0	29(33.3%)	32(78%)	61	18.6	< 0.001
Thalassemia	54(27.4%)	0	0	54	16.6	< 0.001
AOI/ACD	5	45(51.7%)	0	50	15.5	< 0.001
AL	0	6	3	9	2.7	< 0.001
Liver disease	0	0	3	3	1	NS
MA	0	0	2	2	0.6	NS
AAB	0	2	0	2	0.6	NS
AA	0	0	1	1	0.3	NS
AUC	0	5	0	5	1.5	NS
Total	197	87	41	325	100	< 0.001
%	60.7	26.7	12.6	100		
P values	< 0.001	< 0.001	< 0.001			

Regarding the severity classification, mild anemia (Hb: 9-10.9 g/dl) was noticed in 180 (55.4%) of cases, most were due to IDA 82 (45.5%), AOI/ACD 46 (25.5%) and BTT 34 (19%). Moderate anemia (Hb: 7-8.9 g/dl) was noticed in 87 (26.8%) of the cases in which the most frequent causes were IDA 46 (52.8%) and HA 19 (21.8%) and less frequently HBT 5 (5.7%). Severe anemia (Hb: <7 g/dl) was observed in 58 (17.8%) of the cases in which the most frequent cause was HA 36 (62%) and less frequently IDA 10 (17.2%) and HBT 9 (15.5%) (Table 6).

In 54 cases of thalassemia, 38 (70.4%) were BTT, 14 (25.9%) of

them were Homozygous β -thalassemia (HBT) and only 2 (3.7%) of them were HbH disease. Out of 38 BTT cases, 4 of them had moderate anemia (Hb = 8 – 9 g/dl). Considering 61 cases of hemolytic anemia, 60 (98.4%) were diagnosed as G6PD deficiency by G6PD screening test (12 of them had false normal G6PD screening test at presentation to hospital but after 2 weeks they showed deficient results), only 1(1.6%) of them was diagnosed as autoimmune hemolytic anemia (AIHA). In 60 cases of G6PD deficiency, the estimated male to female ratio was 2.75:1.

Table 6 Distribution of the underlying causes of anemia according to the severity

Underlying cause	Mild (Hb: 9-10.9 g/dl)	Moderate (Hb: 7-8.9 g/dl)	Severe (Hb < 7 g/dl)	Total	P values
IDA	82 (45.5%)	46 (52.8%)	10 (17.2%)	138	< 0.001
HA	6	19 (21.8%)	36 (62%)	61	< 0.001
AOI/ACD	46 (25.5%)	4	0	50	< 0.001
BTT	34 (18.9%)	4	0	38	< 0.001
HBT	0	5	9 (15.5%)	14	< 0.001
HbH disease	0	2	0	2	NS
AL	4	3	2	9	NS
Liver disease	2	1	0	3	NS
MA	0	1	1	2	NS
AAB	2	0	0	2	NS
AA	0	1	0	1	NS
AUC	4	1	0	5	NS
Total	180 (55.4%)	87 (26.8%)	58 (17.8%)	325	< 0.001
P values	< 0.001	< 0.001	< 0.001		

Discussion

Anemia is one of the most common health problems in the world and one of the important clinical markers of the underlying disorder. Prevalence of anemia in preschool children, particularly between 6 months and 5 years varies in different countries e.g. 20% of children in the United States and 80% of children in developing countries have anemia [2,3]. Anemia was significantly more frequent in age group 1 (G1) (54%) than in age group 2 (G2) (46%) and this could be attributed to the higher frequency of IDA during early childhood (G1) (64%) than late childhood (G2) (36%) owing to the rapid growth spurt in the infancy and early childhood periods. These results were consistent with other studies [6,7,8]. Anemia was significantly more frequent in males than females and these results were consistent with that

of Musaiger (1996) but inconsistent with that of Dallman (1984) who showed that the frequency of anemia in baby girls is more than that of baby boys [9,10].

As expected, IDA was the most frequent cause of anemia in this study (42.6%) and this could be attributed to the increased demand of iron in early childhood period due to growth spurts. IDA was the major cause of anemia in all age groups and in both sexes [11], however; in neonates, hemorrhage and hemolytic anemia were the major causes of anemia [5]. While in elderly, AOI/ACD was the major cause of anemia [12,13]. Hereditary anemia was frequent in this study (35%) in particular G6PD deficiency (18.4%) and to a lesser extent, thalassemia syndromes (16.6%) and this could be attributed to the relatively high prevalence of both diseases in our locality [14,15].

Morphological classification of anemia in this study revealed that hypochromic microcytic anemia was the predominant class (60.7%) and to lesser extent normochromic normocytic anemia (26.7%) and the least frequent class was normochromic macrocytic (12.6%). These results were nearly identical to those of other related studies [16,17]. IDA was the major cause of hypochromic microcytic anemia (70%) and to lesser extent the thalassemia syndromes (27.4%) and these results were approximate to that of other related studies [(9,18,19]. AOI/ACD was the most frequent cause of normochromic normocytic anemia (51.7%) and to lesser extent hemolytic anemia mainly G6PD deficiency (33.3%) and these results were similar to that of other studies [20,21]. Hemolytic anemia, particularly G6PD deficiency was the major cause of macrocytic anemia (78%) as they are almost always associated with increased peripheral blood reticulocytes which have greater MCV than the mature red cells [5].

Mild anemia was the predominant form of anemia (55.4%) and to lesser extent moderate (26.8%) and severe anemia (17.8%) and these results were approximate to that of other studies [4,22]. It has been noticed that IDA was the major cause of mild (45.5%) and moderate anemia (52.8%) while hemolytic anemia was the predominant cause of severe anemia (62%). These results could be attributed to the fact that IDA in children is mostly caused by nutritional deficiency rather than blood loss (only 4 of 138 cases of IDA had clinical evidence of blood loss) so that IDA in children is usually milder than in adults. While in hemolytic anemia, there will be rapidly accelerated breakdown of RBCs which cannot be replaced soon until several days by the bone marrow [23].

There were 4 of 38 BTT cases who had moderately severe anemia. These cases are probably BTT of unusual severity, which is a form of β -thalassemia intermedia resulting either from a dominant form of inheritance which expresses clinically more severe than the usual recessive heterozygous β -thalassemia genotype or from coinheritance of α -thalassemia (24,25). Finally, there were 5 cases of anemia with undetermined etiology, all of them had mild degree of anemia which could be attributed to that the reference ranges of Hb values have a confidence interval of 95%, i.e. 5% of the population could normally have values below or above the reference ranges i.e. 2.5% above and 2.5% below the reference ranges [26]. Also these cases might have an underlying pathology which cannot be determined by the available laboratory facilities e.g. α -thalassemia trait which is diagnosed by DNA analysis via PCR technology [25].

In conclusion, anemia was prominently frequent in infancy and early childhood period and the majority were hypochromic microcytic mainly caused by iron deficiency. Hereditary anemia syndromes in children particularly G6PD deficiency and β -thalassemia were relatively common in this locality.

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