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The Prevalence of Anemia and Hemoglobinopathies among Students: Cross Section Study

Yousif NG[1]*, Ahmed LA[1], Sadeq AM[2], Al-Fadhel SM[2] and Altimimi A[3]

[1]College of Medicine, Al Muthanna University, Iraq
[2]Kufa Medical College, Kufa University, Ministry of Health, Iraq

Abstract

Introduction: Nutritional anemia is a worldwide problem with the highest prevalence in developing countries. It is found especially among women of child-bearing age, young children and during pregnancy and lactation. It is estimated to affect nearly two-thirds of pregnant and one-half of nonpregnant women in developing countries, so we need to shed light on the prevalence of anemia and types among Iraqi young ages, to estimate the prevalence of anemia in an Al-muthanna Medical College with abnormal CBC.

Methods: A cross-section study was carried out in the Faculty of the Medicine/University of Al- Muthanna, the participant was enrolled as a screening program for the evaluation of the spectrum of abnormal hemoglobin, for those who study in Al-Muthanna Medical College. A total of 278 patients with abnormal CBC included in this study, in the period between September2018 to January 2019 were (139) female and (139) male in the same age, by using CBC and PCR to confirm anemia cases.

Results: The results show that only 110 cases from 287cases suffer from anemia, and anemia increased among women more than man and about 57 (20.5%) cases from 110 cases suffer from iron deficiency anemia followed by 31(11.2%) minor β-thalassemia and low percentage for another type of anemia.

Conclusions: Our results conclude that the most frequent cause of hypo chromic and/or microcytic anemia in our population was iron deficiency Anemia and the minor β-thalassemia was the second because that needs to more attention in the screening program.

Keywords: Anemia; Hemoglobinpathies; PCR; Iron deficiency

Correspondence to: Nasser Ghaly Yousif, College of Medicine, Al Muthanna University, Iraq, E-mail: yousif_ghaly@yahoo.com

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Introduction

Anemia is defined as a condition in which the body has a decreased number of circulating erythrocytes, or red blood cells (RBCs). It can also be defined as a decreased hemoglobin concentration or RBC mass compared with age-matched controls with almost all human laboratory assays, the normal value is a statistical term used to define a range within which 95% of the population's values fall [1].The World Health Organization (WHO) defines anemia as a hemoglobin less than 13 g/dL in adult men and less than 12 g/dL in non-pregnant adult women.3 However, these values were chosen somewhat arbitrarily; most laboratories define anemia as the lowest 2.5% of the distribution of hemoglobin values from a normal, healthy population [2]. Erythrocytes originate in the bone marrow as hematopoietic progenitor and precursor cells. After several cell divisions, mature RBCs emerge as discoid, pliable anucleate cells, each containing 4 hemoglobin molecules. An erythrocyte typically survives for 100 to 120 days before undergoing apoptosis (programmed cell death) [3]. Erythropoiesis, or the process of RBC production, occurs in a regulated fashion under the control of the hormone erythropoietin (EPO). EPO is a glycoprotein, secreted from peritubual cells within the kidney when renal cells detect decreased oxygen in circulation available for metabolism [4]. Successful Erythropoiesis depends on 4 factors:

- A stimulus for erythrocyte production.
- The ability of precursor cells in the bone marrow to respond to the stimulus.
- The presence of essential nutrients required for erythrocyte synthesis.
- The life span of the erythrocyte [5]

Erythropoiesis should be stimulated in response to most forms of anemia, but it takes 3 to 7 days for new RBCs to appear in the blood (Toniuebari and Inusa, 2009). Hemoglobin is a tetramer made up of 2 pairs of polypeptide (globin) chains, with each chain containing an iron-containing Heme complex for oxygen binding. The structure of hemoglobin is under both genetic and environmental influence [6]. Various forms of hemoglobin are known to exist. In adults, hemoglobin A and A2 are the major and minor forms of hemoglobin, respectively. Hemoglobin F, present in uteri, should make up less than 1% to 2% of adult circulating hemoglobin but may be present in higher quantities in the setting of other hemoglobin variants [7]. Under the genetic

influence, other forms of hemoglobin may make up the minority or most of the circulating hemoglobin, affecting the overall RBC oxygen-carrying capacity. Hemoglobin S is the predominant hemoglobin in sickle cell disease. Other hemoglobin variants also include hemoglobin C and E as well as thalassemia.4 Hemoglobin variants generally have altered oxygen affinity, a shorter life span, and are more unstable leading to increased hemolysis [8]. Abnormalities in the production of erythrocytes can be caused by insufficient cofactors, such as vitamin B12 and foliate, or by genetic abnormalities, such as congenital Hemoglobinpathies or membranopathies. Hemoglobinopathies is a clinical disease result from inherited mutation occurs in genes that coded for the synthesis of hemoglobin. Hemoglobin consists of two part; first portion Heme protein chain usually not affected in genes abnormality while second molecules or portion named globin affected by inherited genes disorder [9]. Globin chain abnormality usually can be either quantitative or qualitative; the qualitative abnormality form arises from a genetic mutation in the globin coding genes either deletion or insertion of nucleotides lead to forming amino acid that form globin chain protein differ from normal globin like sickle cell anemia [10]. Another form of globin gene abnormality is the quantitative disorder that occurs from various genetic defects that reduced in the synthesis of the amino acid chain leading to defects in globin protein like thalassemia [11]. Anemia can be classified in several different ways. This classification can be identified based on clinical presentation as well as laboratory investigations. The common causes of acute anemia include hemorrhage secondary to trauma, gastrointestinal (GI) blood loss, ruptured aneurysm, or genitourinary bleeding including postpartum hemorrhage and ruptured ectopic pregnancy. Less often, rapid hemolytic from aplastic crisis or acute splenic sequestration in sickle cell disease can be a cause of acute anemia. The autoimmune hemolytic anemia's and disseminated intravascular coagulation (DIC) [12]. If the anemia is not caused by acute RBC loss, it can be characterized by its cause:

- Destruction of RBCs
- Decreased production of RBCs

A concomitant approach using RBC size (mean corpuscular volume [MCV]) can help further describe the anemia (Tables 1 and 2). The most common type of anemia is iron deficiency anemia, followed by anemia of chronic disease in the older adult population. A significant percentage of those with iron deficiency anemia are found to have a GI source of bleeding [13].

The study aims to assess the prevalence of anemia types among cases with CBC abnormal through the following objective: CBC; PCR

Materials and Methods

Study Design

A cross-section study was carried out in the Faculty of the Medicine/University of Al-Muthanna, the participant was enrolled as a screening program for the evaluation of the spectrum of abnormal hemoglobin, for those who study in Al-Muthanna Medical College. A total of 278 patients with abnormal CBC included in this study, in the period between September 2018 to January 2019 were 139 female and 139 male in same age group.

Sample size

The sample size was calculated using the

$$N = \frac{t^2 pq}{d^2} = \frac{(1.96)^2 \times 24.3 \times 75.7}{(0.06)^2} = 278$$

The formula for unknown population sizes, taking 24.3% as the prevalence for anemia among CBC abnormal patients [*N*: sample, p: the prevalence for anemia in Iraq, q: cases without anemia in Iraq (1- p), t: theoretical t value, d: standard error]. All cases are diagnosed by CBC, cellulose acetate gel electrophoresis was performed for all students and the abnormalband were identified by citrate agar gel electrophoresis and PCR. Study protocol approved by Faculty of Medicine/University of Al muthana ethical committee. All cases excluded from patients

Table 1: Anemia characterized by destruction and decreased the production of RBCs.

Destruction/Loss	Decreased Production
Intrinsic Hemolysis: Spherocytosis, Elliptocytosis, Sickle cell, Pyruvate kinase deficiency, G6PD deficiency	Abnormal Hemoglobin Synthesis: Iron deficiency, Thalassemia, Anemia of chronic disease, Megaloblastic
Extrinsic Hemolysis: Immune, microangiopathic, infectious, hypersplenism	Hematopoietic stem cell lesions: Aplastic anemia, leukemia Bone marrow infiltration: Lymphoma, Carcinoma Immune-mediated: Aplastic anemia, Pure red cell aplasia

Where: G6PD: Glucose-6-phosphate dehydrogenase

Table 2: Typical causes of chronic anemia.

Microcytic (MCV<80)	Iron deficiency Thalassemia Anemia of chronic disease (eg, rheumatoid arthritis, congestiveheart failure, chronic renal failure) Sideroblastic anemia Lead poisoning
Normocytic	Kidney disease Hemolytic anemia (Spherocytosis, elliptocytosis, sickle cell disease,G6PD) No thyroid endocrine gland failure Autoimmune (drug, viral, idiopathic) Microangiopathic Infection (malaria, parvovirus) A mild form of most acquired forms of anemia
Microcytic (MCV>100)	Megaloblastic Vitamin B12 deficiency Foliate deficiency DNA synthesis inhibitors (nonmegaloblastic) Myelodysplasia Liver disease Reticulocytosis Hypothyroidism Bone marrow failure states (aplastic anemia)

with any type of cancer under chemotherapy treatments courses and patients who had more than one determination of hemoglobin during the study period to avoid repeated measurements in the same patient, the definitions of anemia according to a recommendation from the WHO [14].

Sample Collection

Blood samples were collected by venipuncture into ethylene diamine tetraacetic acid (EDTA) anti-coagulated tubes used for the determination of hemoglobin variants, complete blood count (CBC) and for gel electrophoresis and PCR technique.

Laboratory Methods

Hemoglobin variants were determined using cationic-exchange HPLC method (BIO-RAD 'VARIANT', BIO-RAD Laboratories, USA). At first, prior to sample application, the instruments had been primed and calibrated followed by use of controls and then prepared hemolytic from EDTA blood was run by that instrument. CBC was assessed by cell counter (Sysmax Kx-21, Sysmax Corporation, Japan) and by used (AccuPrep® Genomic DNA extraction kit Bioneer/Korea) for DNA extraction and (AccuPowerTM PCRPre MixBioneer/Korea) for PCR. Patient history and family history were recorded by the counseling for the confirmation of the diagnosis of hemoglobinopathy.

Statistical Analysis

Collected data were analyzed by SPSS version 23.0 software. Chi-squared (χ^2) and Fisher's exact tests were used for the comparison of categorical data, while the Kruskal Wallis test, the Mann Whitney U test, Odd RATIO also calculated to measure the risk of anemia. Logistic regression analysis was conducted to identify explanatory variables. Data were expressed as "mean (standard deviation; SD)" and percent (%) where appropriate. $p<0.05$ was considered statistically significant.

Results

A total of 278 participants were screened for abnormal hemoglobin variants. Of these, 139 were male (50%) and 139 (50%) were female subjects. This study showed the overall prevalence of Hemoglobinpathies was 110 cases (39.56%) and 168(60%) without hemoglobinopathies (Figure 1).

Among gender, hemoglobinopathiesprevalence occurs in female (43%) from all female cases 139 (100%) while in the male the prevalence about (36%) from 139(100%), and this percentage in female more than male, figure 2**.** Different Hemoglobinpathies detected during the study are presented in Table 1 and figure 3 where iron deficiency anemia was presented in 57 of cases and its represented the highest percentage among other types of anemia among male and female (20.5%), followed by minor β- thalassemia, it was appears in 31 of cases (11.2%), sickle cell trait in 7 (2.5%), Hb. H disease in 6 (2.2%), α-Thalassemia trait was (1.4%), hemoglobin G in 3 (1.1%) and hemoglobin C 2(0.72%) from all cases.

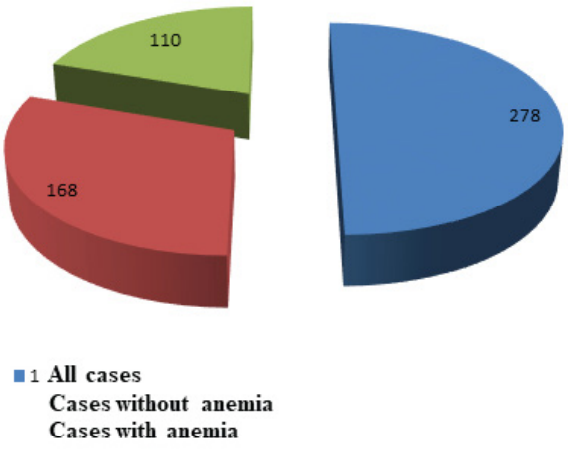

Figure 1: Description of cases.

The study findings showed that anemia was associated with a young ages and the odd ratio used to determine the risk of anemia development among young age group who has CBC abnormal, the iron deficiency anemia have the harmful effects between other types of anemia where odd ratio higher than 1%, compared with other types of anemia has the lowest effect as in table 3.

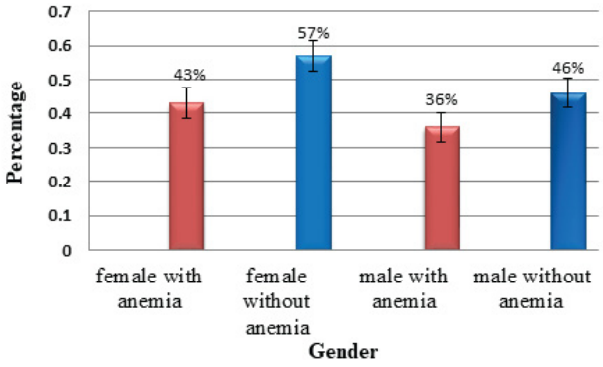

Figure 2: Prevalence of anemia among gender.

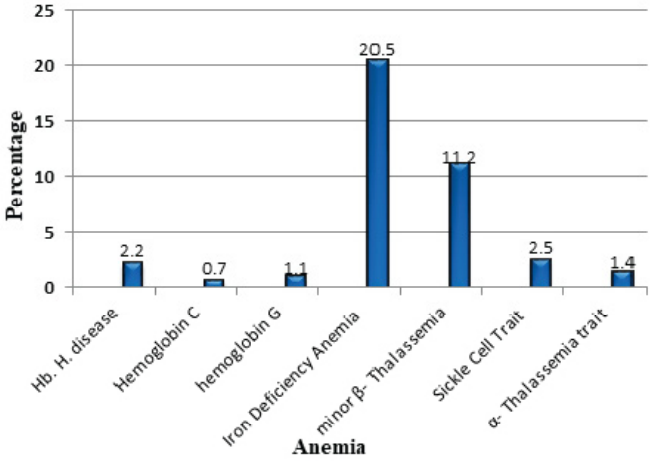

Figure 3: Distribution of anemia types among cases.

Table 3: Anemia Types Distributions among Cases.

Anemia types	***N***	**%**
Iron Deficiency Anemia	57	20.5
Minor B-Thalassemia	31	11.2
Sickle Cell Trait	7	2.5
Hb. H. Disease	6	2.2
A-Thalassemia Trait	4	1.4
Hemoglobin G	3	1.1
Hemoglobin C	2	0.72

Where: N:Number of cases, %: Percentage.

Table 4: Prevalence of risk associated anemia types.

Anemia types	*N*	Odd ratio	Confidence interval (95%)	*p*
Iron Deficiency Anemia	57	1.166	(0.8033-1.6932)	0.418
Minor B-Thalassemia	31	0.463	(0.2997-0.7257)	0.007
Sickle Cell Trait	7	0.1302	(0.1128-0.2663)	0.002
Hb. H. Disease	6	0.1153	(0.0479-0.2315)	0.001
A-Thalassemia Trait	4	0.0903	(0.0388-0.2099)	0.001
Hemoglobin G	3	0.0451	(0.0141-0.144)	0.001
Hemoglobin C	2	0.0301	(0.0073-0.124)	0.001

Discussion

Hemoglobinopathies are of worldwide occurrence, though some geographical areas have a high prevalence of these disorders. The inherited disorders of hemoglobin synthesis are one of the important public health problems in various part of Iraq according to WHO [14]. The result shows that anemia occurs in women more than man and the result agrees with other study [15] who observed that anemia was detected among women who were 15-49 years of age (p<0.05), menstruating (p<0.05), had a history of Cesarean section (p<0.05). The present study revealed that moderate numbers of people were encountered with Hemoglobinpathies in Al-Muthana. In our study, iron deficiency anemia was the most frequently encountered quantitative Hemoglobinpathies among cases with CBC abnormality and these results consistent with other published data followed by other types of anemia. This compares with previous studies [12] who show that iron deficiency anemia increased among women in America.

Conclusions

Our results conclude that the most frequent cause of hypo chromic and/or microcytic anemia in our population was iron deficiency Anemia and the minor β-thalassemia was the second cause that needs to more attention in the screening program.

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