Iron Deficiency Is a More Important Cause of Anemia than Hemoglobinopathies in Kuwaiti Adolescent Girls¹

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ABSTRACT Anemia is the most prevalent nutritional problem worldwide, due mainly to iron deficiency. Studies of anemia are less common in adolescents than in women and children. We examined anemia prevalence in adolescent Kuwaiti schoolgirls, and its association with hemoglobinopathies as well as the most common environmental cause, Fe deficiency. A cross-sectional sample of 1051 healthy adolescent schoolgirls was studied. Sample size was based on WHO criteria. Anemia, Fe deficiency and hemoglobin (Hb) variations were studied by Hb concentration, erythrocyte protoporphyrin (EP) and an HPLC quantitation, respectively. Of the subjects sampled, 30% were anemic. Mildly elevated EP values were found in 68%. Girls with high EP levels were more likely (P < 0.001) to be anemic than girls with normal EP. Up to 25% of the girls may have had Fe deficiency anemia. Hemoglobinopathies were neither prevalent nor significantly associated with anemia. These data indicate that environmental factors play a significant role in anemia among healthy, well-to-do Kuwaiti adolescent girls. J. Nutr. 130: 1212–1216, 2000.

KEY WORDS: • anemia • iron deficiency • Kuwait • adolescent girls • hemoglobinopathies

Anemia is the most prevalent nutritional problem worldwide (ACC/SCN 1991, DeMaeyer and Adiels-Tegman 1985). Iron (Fe) deficiency is responsible for most anemia. Deficiencies of other nutrients (including folic acid, protein, vitamin B-12, vitamin A and copper) may also contribute (Viteri 1998). Other causes include thalassemia, hemoglobin (Hb)³ variants, as well as infection and inflammation. However, the contribution that Hb variants make to anemia is rarely examined. Worldwide, the prevalence of anemia in adolescents is largely unknown. Few studies have been performed in the Arabian Gulf, and none in Kuwait.

Previous studies in Kuwait. It is important to know what factors are associated with anemia in the Gulf region to augment our knowledge about anemia in this part of the world. Previous Kuwaiti studies focused solely on Hb concentrations in children or women. Hemoglobinopathies and other contributors to anemia were not studied. Dawood et al. (1990) conducted a nationally representative study of 1582 pregnant women between the ages of 14 and 45 y living in Kuwait and found that anemia was present in 36.8%. Al-Awadi et al. (1995) studied 980 Kuwaiti women living in Kuwait and found that anemia was present in 1582 pregnant women between the ages of 14 and 45 y. Their results indicated that 23.2% were anemic (Hb < 120 g/L). Eid et al. (1986) examined 1208 children between the ages of 6 and 17 y. Of the 593 males studied, 12.8% were anemic; however, 26% of the 615 females were anemic. None of these studies focused on adolescents or examined etiological factors responsible for the low Hb concentrations. Consequently, we do not know the cause of the anemia in this population. Public health measures, instituted by the Kuwaiti government, coupled with a constellation of environmental factors (including extremely high ambient temperatures, aridity, paucity of vegetation and standing bodies of water) have eliminated the most common parasites as significant contributors to anemia.

The objectives of this study were to ascertain the prevalence of anemia in healthy adolescent girls attending secondary schools and to explore Fe deficiency and Hb variations as contributors to the anemia. We also wanted to identify regional variation in anemia prevalence in Kuwait.

Background on Kuwait. Kuwait is an oil-rich, Arab country with an area of 17,818 km² located at 30.27°N and 48.46°E. The country is bordered on the north and west by Iraq, on the south by Saudi Arabia and on the east by the Arabian Gulf. The total population numbers 2 million, 38% of whom are Kuwaitis (Ministry of Information 1996). Kuwait is divided into 5 administrative divisions, called governorates; all of its citizens enjoy a high standard of living that includes free education and medical care, among other amenities. The discovery of oil in the 1950s brought dramatic changes in living standards, including changes in lifestyle, diet and the transformation of the physical environment. The 1997 per capita income was ~$22,300, making it one of the wealthiest countries in the Gulf area and the world. The infant mortality rate is 10.7 and the life expectancy at birth is 76.8 y.
SUBJECTS AND METHODS

The sample was chosen to estimate a 20% prevalence of anemia, with 15% relative precision and 95% confidence. The resulting sample size of 683 was then increased due to stratification (i.e., different governorates). The sample size calculation was based on WHO criteria for sample size estimation (WHO/NUT 1996).

The study protocol was reviewed and approved by the Kuwait Ministries of Health and Education, and was conducted after obtaining written consent from the parents of the school children.

Information was collected by interview onto structured questionnaires, pretested in a small group of subjects similar in characteristics to those in the study. A variety of social, economic, health and medical data were collected. Information on infections and illnesses diagnosed by a medical doctor within the preceding month, supplement usage and menstrual history was also obtained.

Blood collection and analyses. Capillary blood was collected from the finger. Hb was assessed by the HemoCue method. HemoCue calibration was checked on a daily basis by using the control cuvette supplied with the photometer. Fe-deficient erythropoiesis was assessed by measurement of erythrocyte protoporphyrin (EP) using the zinc protoporphrin (ZPP) hematofluorometer (AVIV Biomedical, Lake-wood, NJ).

Additional blood was obtained in capillary tubes from the first 228 unrelated girls who entered the study to determine Hb variation. Hemolysates were prepared and Hb variant quantitation was carried out with cation-exchange HPLC (Waters LC Module 1, Milford, MA) to check for clinically important Hb variants (Bisse and Wie-land 1988).

The WHO definitions of anemia (Hb < 120 g/L) and severe anemia (Hb < 70 g/L) were used (WHO 1996). Normal EP concentrations were <5 μg/g Hb, a level suggested by AVIV Biomedical, the manufacturer of the ZPP hematofluorometer. Standards (high, medium and low) for EP were obtained from the manufacturer (AVIV Biomedical) and tested daily with the samples. There are only four known causes of elevated ZPP levels, i.e., elevated Phe burdens, a rare genetic disorder called erythropoietic protoporphryia, Fe deficiency and chronic inflammatory diseases (Lamola et al. 1975). EP is not elevated in thalassemia minor (Stockman et al. 1975).

Statistical analysis. Means were tested using Student’s t test and/or ANOVA, e.g., average Hb concentration among the five Kuwaiti governorates. Correlations between variables such as mean duration of menses in days and Hb concentration, and EP value and Hb concentrations, were examined using the Pearson Product Moment Correlation test. EPINFO was used to calculate odds ratio (OR), and a 95% confidence interval (CI) for the OR and for sample size calculations. Hb was regressed on EP using simple linear regression. Erythrocyte protoporphyrin (EP) values were elevated (≥ 5.0 μg/g Hb) in all but 37.1% of those with Hb concentrations ≥ 120 g/L (in the normal range). However, EP was elevated in 81.1% of those with Hb concentrations < 120 g/L. Seventy-five percent of the sample had EP ≤ 6.5 μg/g Hb. Only 4.75% of this sample had EP levels > 10 μg/g Hb. The percentage with elevated EP values varied by governorate and was significantly lower in Ahmadi than in the other regions (Table 2).

Utility of EP as the sole indicator of iron status. Most cases of anemia worldwide are due to Fe deficiency. Initially, Fe stores are depleted. This is followed by impaired Fe status, and finally the deficiency progresses to Fe deficiency anemia. A low Hb concentration is likely to be the result of Fe deficiency. However, Hb is not a sensitive indicator of Fe deficiency because reduced Hb occurs only in the later stages of iron depletion, and a low Hb concentration may be due to other causes.

Because EP was the only indicator of body Fe status measured, and an EP of 5.0 μg/g was used as the cut-off to determine the presence or absence of Fe deficiency, we examined the suitability of this cut-off value in different ways. First, we compared the Hb concentrations of the girls in three EP ranges. Group 1 contained girls with EP < 5.0 μg/g, i.e., below the 25th percentile, (normal by AVIV standard). Group 2, including the second and third quartiles (~50% of the sample), had EP levels between 5.0 and 6.5 μg/g Hb. Group 3 contained the remaining quartile with EP > 6.5 μg/g Hb. The mean Hb concentrations for these three groups are shown in Table 1. The differences in Hb concentration between Groups 1 and 2 and between Groups 2 and 3 were significant. The overall correlation between the EP and Hb values was r = −0.459, (P = 0.0001, n = 1048).

EP values were elevated (≥ 5.0 μg/g Hb) in all but 37.1% of those with Hb concentrations ≥ 120 g/L (in the normal range). However, EP was elevated in 81.1% of those with Hb concentrations < 120 g/L. Seventy-five percent of the sample had EP ≤ 6.5 μg/g Hb. Only 4.75% of this sample had EP levels > 10 μg/g Hb. The percentage with elevated EP values varied by governorate and was significantly lower in Ahmadi than in the other regions (Table 2).

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TABLE 2

Hemoglobin (Hb) concentration, erythrocyte protoporphyrin (EP) and anemia in Kuwaiti adolescent girls by governorate

<table>
<thead>
<tr>
<th>Governorate</th>
<th>n</th>
<th>Hb1 (g/L)</th>
<th>Hb2 (g/L)</th>
<th>Anemic (% )</th>
<th>High EP (&gt;5.0 µg/g Hb)</th>
<th>FE deficient + Anemic (% )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capital</td>
<td>148</td>
<td>12.8 ± 1.6</td>
<td>8.4–17.0</td>
<td>22.2</td>
<td>80.3</td>
<td>31</td>
</tr>
<tr>
<td>Hawalli</td>
<td>308</td>
<td>12.7 ± 1.8</td>
<td>7.1–17.0</td>
<td>29.9</td>
<td>71.0</td>
<td>86</td>
</tr>
<tr>
<td>Farwania</td>
<td>251</td>
<td>12.7 ± 1.7</td>
<td>6.8–16.7</td>
<td>32.2</td>
<td>77.7</td>
<td>67</td>
</tr>
<tr>
<td>Ahmadi</td>
<td>224</td>
<td>12.5 ± 1.6</td>
<td>5.7–17.4</td>
<td>34.4</td>
<td>41.1</td>
<td>42</td>
</tr>
<tr>
<td>Jaha</td>
<td>118</td>
<td>13.0 ± 1.3</td>
<td>8.4–15.8</td>
<td>23.7</td>
<td>76.7</td>
<td>25</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1049</td>
<td>12.7 ± 1.7</td>
<td>5.7–17.4</td>
<td>29.6</td>
<td>68.1</td>
<td>251</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.
2 Range.
3 Percent of total per governorate.

< 12.0 g/L was present in only 18.2% of those with EP < 5 µg/g Hb. However, anemia was present in 34.9% of those with EP ≥ 5.0 µg/g Hb. The mean Hb concentration of the subjects with EP < 5.0 µg/g Hb was 132 g/L (reference group) compared with 125 g/L for those with EP values ≥ 5.0 µg/g Hb. We also examined the ZPP hematoiliometer cut-off point recommended by the manufacturer using OR. The odds of being Fe deficient (given EP ≥ 5.0 µg/g Hb) was 2.44 times higher (95%, CI: 1.75, 3.39) in anemic girls than in nonanemic girls. Figure 1 shows the scatter plot of EP and Hb values and the regression of Hb on EP. The model was significant (P < 0.0001) and the regression coefficient, b = −0.309, was significantly different from zero.

Classification of Fe deficiency and anemia. Table 2 provides the breakdown of Fe and anemia status by governorate and shows that less than a third (26.2%) of the sample had simultaneously normal Hb (≥ 120 g/L) and EP (<5.0 µg/g Hb). Forty-four percent of the total sample had elevated EP values, possibly due to Fe deficiency, but had normal Hb concentrations. Over half of the girls with elevated EP values resided in two governorates, Hawalli and Farwania. Nearly one quarter (23.9%) of the sample had elevated EP values, possibly due to Fe deficiency, but had normal Hb concentrations. Over half of the latter were from Ahmadi, and their anemia may be due to reasons other than Fe deficiency.

Hemoglobinopathies and anemia. A number of Hb variants prevalent in this part of the world are associated with anemia. In our study, screening for hemoglobinopathies was conducted on the first 228 nonrelated subjects. Clinically important Hb variants were present in nine of the 228 individuals; of these, only three had Hb < 120 g/L. One had β-thalassemia trait and two had sickle cell trait (Table 3).

Correlates of hematologic status. Ninety percent of the girls did not use hematinic supplements. Among those who did use supplements, roughly half used Fe tablets. The mean Hb values for supplement users (127 g/L) and nonusers (126 g/L) were not different (P > 0.05). Mean EP values of users and nonusers were not different.

There were no significant differences between either the Hb or EP values of those who had been diagnosed as having a cold or flu by a physician within the last month (n = 270) and those who had not been “sick” (n = 780).

Ninety-two percent of the sample characterized their monthly menstrual blood losses as “normal,” whereas 8.3% described them as “heavy.” Neither the Hb (nor the EP) concentrations of those with “normal” (127 g/L) and “heavy” (125 g/L) blood flow were different (P > 0.05).

DISCUSSION

Although anemia has been studied extensively in most parts of the world, there is a dearth of information on the epidemiology of anemia in the Arabian Gulf States, especially Kuwait. Thus, to our knowledge, this report provides new information on adolescent anemia and its causes in this region of the world.

The mean Hb concentration of the entire sample was 127 g/L. After excluding those with EP < 5.0 µg/g Hb, the mean Hb was 132 g/L. Thirty percent of the girls were anemic by WHO criteria. Severe anemia was present in only three girls (0.3%).

TABLE 3

Phenotypic and genotypic frequencies of hemoglobin (Hb) variants in Kuwaiti adolescent girls

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>Phenotypic frequency</th>
<th>Gene frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Thalassemia trait</td>
<td>1</td>
<td>0.009</td>
<td>0.0042</td>
</tr>
<tr>
<td>Sickle cell trait</td>
<td>2</td>
<td>0.026</td>
<td>0.013</td>
</tr>
<tr>
<td>Hemoglobin E trait</td>
<td>1</td>
<td>0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>HPFH3</td>
<td>5</td>
<td>0.083</td>
<td>Not calculated</td>
</tr>
</tbody>
</table>

1 First 228 unrelated girls studied.
2 Estimated.
3 HPFH, hereditary persistence of fetal hemoglobin.
EP level is a measure of Fe-deficient erythropoiesis, the second stage of Fe deficiency. During the second stage of Fe depletion, the Hb concentration remains normal, but falls below normal levels during the third stage, known as Fe deficiency anemia. Overall, 68% of adolescents had elevated EP values, but very few had severely elevated levels. Only 25% of the girls had EP > 6.5 μg/g Hb; <5% had EP > 10 μg/g Hb. Of anemic adolescents, 81% had elevated EP levels.

One explanation for the mildly elevated EP values would be high blood Pb concentrations or chronic infection. However, a substantial majority of the schoolgirls reported being healthy and without infection or other illness. Furthermore, the Hb and EP concentrations of those who reported sickness and those who were not sick were not different. Previous studies investigated Pb concentrations in the tissues (blood and teeth) of Kuwaitis, but did not find them to be a public health problem (Bu-Olayan and Thomas 1999, Shaltout et al. 1989). In our study, <5% of adolescents had EP > 10 μg/g Hb, a figure once used as a diagnostic cutoff for high blood Pb concentrations.

Nine girls (3.9%) had a Hb variant. However, of those, only three were anemic. The high prevalence of elevated EP values in this sample is more consistent with Fe deficiency than β-thalassemia because EP values have not been shown to be elevated in the latter (Poh-Fitzpatrick and Lamola 1976, Stockman et al. 1975). The methodology used in this study could not identify the α-thalassemia trait, but it is clear that anemia in this sample was not due to α-thalassemia. α-Thalassemia is relatively mild in Kuwait and is thus unlikely to cause significant decrements in Hb or other hematologic values.

The earliest report of hemoglobinopathies among Kuwaitis dates to 1969 (Ali 1969) in a study of Hb H. The following year, Ali (1970) reported on the mildness of sickle cell disease (SCD) in Kuwait and suggested that this was associated with an unusually high level of fetal Hb. Subsequent clinical studies (Al-Salem and Ismail 1990) confirmed the mild course and infrequent pathology of SCD among Kuwaiti children. A similarly benign sickle cell anemia was also found in the eastern provinces of Saudi Arabia (El-Hazmi and Warsi 1993), the geographic origin of several important Kuwaiti lineages (Ministry of Information 1990).

No field studies have been done to determine the frequencies of hemoglobinopathies in Kuwait. However, several studies of hospitalized populations offer insight into the course of the disease and its prevalence in clinical settings. In Farwania Hospital, hemoglobinopathies were encountered in 20% of a subsample of 1289 confined individuals (Ghosh et al. 1993). Nearly all of the common hemoglobinopathies were encountered, but α-thalassemia was surmised to be the most common hemoglobinopathy in Kuwait, as it is in eastern Arabia in general (Pembrey et al. 1980). Adekile and Haider (1996) used polymerase chain reaction to amplify and sequence α-thalassemia and β S haplotypes among a group of hospitalized Kuwaitis. They found considerable homozygosity in α-thalassemia molecular variants and β S haplotypes but considerable heterozygosity in β-thalassemia alleles. Adekile and Haider (1996) reported that the high frequencies of β S haplotype 31 (Saudi Arabia/India variant) and α-thalassemia in Kuwait contribute to the mild nature of the SCD among Kuwaiti Arabs. A 30-y study of 129 Kuwaiti children hospitalized with β-thalassemia major indicated that consanguinity may account in large part for the frequency of this hemoglobinopathy in Kuwait (Al-Fuzae et al. 1998).

The β-thalassemias are heterogeneous with respect to molecular pathogenesis (El-Hazmi et al. 1995a). Among Arabs, the predominate mutations differ by ethnic group (El-Hazmi and Warsi 1996, El-Hazmi et al. 1995b). Only one individual had the β-thalassemia trait in this study. Our results (Table 3), as one would expect, showed a lower frequency of these variants than previous studies in which hospitalized patients were studied. Thus, we conclude that hemoglobinopathies were not significant contributors to anemia or to elevated EP values in this sample.

Kamal and Martinez (1984) conducted a study of 500 adult males and females to describe food preparation methods, dietary habits and nutrient intakes of Kuwaitis. They found that cereals (wheat and rice) were important staples in the diet. Most (81%) of those studied consumed bread once or more daily. Ninety percent of the sample drank tea; 24% of the women drank three or more cups (710 mL) of tea per day. That study also found that for women, the intakes of vitamin A, Fe, Ca and Zn were below the U.S. recommended daily allowance (RDA) and that the food preparation methods employed involved prolonged boiling of meat and vegetable dishes. They surmised that prolonged boiling of vegetables may decrease the vitamin content (especially, folic acid) of foods consumed. In a more recent diet study of 203 female college students using 3-d diet record analyses, Al-Shawi (1992) found that the intakes of Fe, Zn, folacin and vitamin D were below the RDA. The diets of these women were also high in caffeine.

Despite one of the highest per capita incomes in the world, anemia and probably Fe deficiency remain important public health problems in Kuwait. The 30% of adolescent Kuwaiti schoolgirls who were anemic by WHO epidemiologic criteria for the classification of countries (WHO 1996) by anemia prevalence, places Kuwait in the moderate prevalence category (10–39.9%). Twenty-four percent of the girls had Hb and EP values suggestive of Fe deficiency anemia.

Hemoglobinopathies associated with anemia exist but at very low frequencies in this sample. On the other hand, anemia is unexpectedly high and exceeds levels that can be accounted for by the frequency of hemoglobinopathies or blood Pb. Thus, Fe deficiency is strongly suspected, given the large percentage of subjects with slightly elevated EP values and the significant differences between the mean Hb concentrations of those with normal (132 g/L) and high (125 g/L) EP values. Although we surmise that much of the anemia is due to Fe deficiency, other factors (such as deficiencies of folic acid) likely also contribute to the anemia of these subjects. Caution should be used in interpreting the EP results because no single test can identify impaired Fe status unequivocally (Expert Scientific Working Group 1985), and other causes of nutritional anemia were not investigated. Further studies using several Fe status indicators to elucidate the causes of anemia and Fe deficiency in particular are warranted.

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