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## Original Research Article

# Determinants of Hemoglobin Level in Adolescence Students at Gaza Strip, Palestine

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### Abstract

**Purpose:** Hemoglobin (Hb) level could be affected by different interfering factors that include nutritional, socio-demographical, economical, and habitual determinant factors. The present study was designed to identify possible factors that could affect the hemoglobin level in adolescence students (14-22 years) at Gaza Strip, Palestine.

**Methods:** Complete blood count (CBC), serum iron (SI), total iron binding capacity (TIBC), and HbA<sub>2</sub> were performed on blood samples of 1171 students from both sexes (548 M & 623 F). Socioeconomic, demographic, habitual and nutritional information were collected through close-ended questionnaire.

**Results:** Iron deficiency and beta thalassemia minor are prevalent in 9.6% and 4.2%, respectively, of the adolescent students. At all categorizations, except in carriers for beta thalassemia gene, significantly higher Hb levels were reported in males than females. The determinant factors for Hb level of the overall subjects were student education (school vs university), parents consanguinity, geographical locality (urban, rural, refugee camp), owning house, smoking, family size/income, tea drinking, consumption of citrus, meat, and green leafy vegetables.

**Conclusions:** It was concluded that different socio-demographic variables and nutritional factors are significant detriments for Hb levels in Palestinian adolescents (14-22 yr). Interventional educational programs directed toward improving the dietary and nutritional issues among the adolescents is recommended.

**Keywords:** Hemoglobin; Determinant factors, Socioeconomic; Demographic; Refugees.

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## Introduction

Iron is the most abundant heavy metal in the earth's crust<sup>1</sup>, which is required by almost all organisms<sup>2</sup>. In the human body, iron is present in all cells and has several vital functions<sup>3</sup>. Most of the iron is utilized for the synthesis of hemoglobin. Careful recycling of iron derived from hemoglobin and its re-utilization in the synthesis of new red blood cells provide emphases of one of the most carefully controlled systems in the human body<sup>4</sup>. Thus, it seems that hemoglobin levels are determined and affected by the factors that mainly related to the existence and bioavailability of iron in diets<sup>5</sup>. Other determinant factors include the presence of genetic disorders like hemoglobinopathies and hemochromatosis<sup>6,7</sup>.

Iron that is present in food is classified as heme iron (heme-protein complexes) or as non-heme iron (ferric hydroxides and ferric-protein complexes). A smaller of heme iron (heme proteins, hemoglobin and myoglobin), is present in meat<sup>1</sup>. The non-heme iron represents the larger contribution to the body iron pool.

The bioavailability of iron decreases in the following order: heme > Fe<sup>2+</sup> > Fe<sup>3+</sup>. Heme iron and non-heme iron differ markedly in their bioavailability and absorption by the intestinal cells. Heme iron is absorbed more efficiently (two to three times) than non-heme iron and in different manner. In addition to its lower absorption rate, the absorption of non-heme iron is markedly influenced by concomitantly consumed dietary components. However, consumed dietary components, have little or no effect on the absorption of heme iron<sup>8</sup>. Some dietary components diminish or decrease iron (mainly non-heme) absorption mainly by the formation of insoluble compounds, most of which are phytates, tannates, polyphenols<sup>9,10</sup>, oxalates, phosphates<sup>11</sup>, calcium<sup>12</sup>, lectins<sup>13</sup>, and alkali antacids<sup>14</sup>. On the other hand, other dietary compounds enhance iron absorption by forming readily absorbed compounds, such as ascorbic acid<sup>15</sup>,

chelators like Na<sub>2</sub>EDTA<sup>16</sup>, vitamin A<sup>17</sup>, fructose, and citrate<sup>11</sup>.

The amount of iron required per day to compensate for iron losses from the body and afford growth needs varies with age and sex; this amount is highest during infancy, early childhood, adolescence, pregnancy, and menstruating females<sup>18</sup>.

Studies on the obligatory losses from the healthy body show a loss of 14 µg/kg body weight per day for men, or a total of 1 mg / day for an adult male<sup>19</sup>. For women, in addition to the basal loss of 14 µg/kg body weight per day, there is a considerable loss of iron in menstruation. Although menstrual blood losses are relatively constant from one period to another, a very wide range of individual variations exist. When basal losses are added, the total iron loss for menstruating women is about 1.40 – 1.60 mg per day<sup>20</sup>.

The present study was designed and aimed at the identification of the determinant factors that may negatively or positively influence the hemoglobin level in adolescent students (14-22 years old) living at the Gaza Strip, Palestine.

## Materials and Methods

This study was conducted in the Gaza Strip in Palestine which is a narrow piece (365 km<sup>2</sup>) of land along the Mediterranean coast. Forty kilometers long and 10 km wide, it is divided into five governorates, namely, Gaza, Khan Younis, Rafah, Midzone, and North governorate. Gaza strip is considered as one of the most populated areas in the world (3890 persons/km<sup>2</sup>) with an estimated population of about 1.42 million according to February 2008 records. Reargues constitute more than 71% of the total population of the Gaza Strip and they are living in eight major refugee camps distributed along the Gaza Strip. The UNRWA is the main responsible

agency for all the living and logistic issues of the refugees<sup>21</sup>.

Blood samples and questionnaires for the present study were collected from 1171 university and secondary school students (14-22 years old, 548 males and 623 females), representing the geographical localities (urban, rural, refugee camps) of the Gaza strip. Age, education level, locality, no previous history of chronic health problems and the health appearance of the students formed the basis for inclusion. Venous blood samples (5 ml) from the antecubital vein were collected in EDTA tubes (2 ml) and plain tubes (3 ml). The study was performed in accordance with the ethical standards laid down in the 1964 and 1975 Declarations of Helsinki, and the modifications of 1996.

The sampling was performed at 8.00 am. Blood collected in the EDTA tubes was used within 2-3 hr of collection to perform complete blood count (CBC) analysis using Cell Dyne 1700 electronic counter (Sequoia-Turner corporation, California, USA), and then stored at 2-4 °C for 3 days for HbA<sub>2</sub> quantification using the DEAE cellulose anionic exchange microcolumn (BioSystems Ltd, Spain). The serum was separated from blood samples 4-5 hr after sampling by centrifugation at 1200-1500 g for 15 min. The separated serum was placed in new plain tubes, sealed, and stored at 2-4 °C for the analysis of serum iron and TIBC the following day. Iron and TIBC values were determined using the colorimetric kits of Randox (UK).

The diagnosis of  $\beta$ -thalassemia trait was based on the elevation of HbA<sub>2</sub> > 3.5% of the total hemoglobin<sup>22</sup>, while for Iron deficiency the diagnosis was defined as transferrin saturation (SI/TIBC) < 16%<sup>23</sup>. Iron deficiency anemia was considered when transferrin saturation was less than 16% in combination with low Hb [ $<13\text{g/dl}$  for males,  $<12\text{g/dl}$  for females]<sup>11,24</sup>.

Every student who freely agreed to be involved in the present study was requested to fill a questionnaire that contained

personal, socioeconomic demographic, and dietary information.

### **Statistical data analysis**

The CBC, serum iron TIBC and data from the questionnaire were tabulated, encoded and statistically analyzed using the Statistical Package for the Social Sciences (SPSS) version 13. Statistical comparison of data was carried out using the Student's t-test, Z-test, and ANOVA, while Spearman rank correlation coefficient (*rs*) analysis was performed to establish any association between the hemoglobin level and other variables. At 95% confidence interval, any 2-tailed p value less than 0.05 was considered to be statistically significant.

### **Results**

The CBC results revealed that microcytosis and/or hypochromasia (MCV  $\leq 80\text{ fl}$  and/or MCH  $\leq 26\text{ pg}$ ) were prevalent in 15.1% (55 M & 122 F) of the students and they were considered as suspects of being iron deficient or having minor thalassemia. Laboratory diagnosis was achieved in about 91.0 % (N=161) of the microcytic and /or hypochromic cases, with iron deficiency being the major (63.3 %) diagnosis of those suspicious students, while carriers for beta thalassemia constituted 27.7 % of the suspicious students. There was significant correlation between the concentration of hemoglobin and the transferrin saturation ( $rs = 0.497$ ,  $p = 0.0001$ ).

Table 1 summarizes the socioeconomic and demographic determining factors of hemoglobin concentration. Gender was found to be the major determining factor for hemoglobin concentration in the adolescents. Except in carriers for beta thalassemia gene, significantly higher hemoglobin levels were reported by males than females. For normocytic and/or normochromic students (MCV > 80 fl and/or MCH > 26 pg), the males showed significantly higher levels of hemoglobin (approximately 15.7%) when compared to

females. Among the males, the university students and students of unconsanguineous parents significantly exhibited higher hemoglobin levels ( $14.90 \pm 0.95$  g/dl and  $14.62 \pm 1.07$ , respectively) than the school students ( $14.31 \pm 1.45$  g/dl) and those of first ( $14.31 \pm 1.28$  g/dl) or second ( $14.28 \pm 1.97$  g/dl) degree consanguineous parents respectively. No significant differences were observed among the females.

For both males and females, students living in rural areas, those that owed houses, and smokers showed significantly higher hemoglobin concentrations when compared to their counterparts who are living in refugee camps, renting a house, or nonsmokers, respectively (Table 1).

The effects of family size and family income on hemoglobin levels were observed only on females. Correlation coefficient analysis revealed a significant correlation between hemoglobin level in females and family size ( $r_s = -0.213$ ,  $p = 0.003$ ), and family income ( $r_s = 0.512$ ,  $p = 0.001$ ). Small family size and higher incomes were associated with significantly higher hemoglobin levels than large family size and low income (Table 1).

The possible dietary and biochemical determining factors affecting hemoglobin concentrations are provided in Table 2. None of the students in this present study were found to be pure vegetarian. All the students were omnivorous. For the overall students (both sexes), higher hemoglobin levels were observed among students who frequently ate meat ( $\geq$  twice per week) as compared to those who infrequently ate meat (2-3 three times per month). The frequent consumption of citrus fruits and green leafy vegetables were also associated with significantly higher hemoglobin concentrations as compared to the infrequent consumption of these determinants. However, consumption of milk or dairy products was not associated with any significant differences in hemoglobin levels.

Importantly is the habit of tea drinking which prevails in 90.6% of the screened students. Correlation coefficient analysis revealed a significant correlation between hemoglobin level of the overall students and the daily consumption of tea ( $r_s = -0.349$ ,  $p = 0.001$ ). Tea drinking was found to be associated with lower hemoglobin levels among the students. About 11.5% reduction in hemoglobin concentration was observed in heavy tea drinkers ( $>5$  cups per day) as compared to non-tea drinkers. The effect is more pronounced in males than females. Moreover, the students who drank tea independently of meals exhibited higher hemoglobin levels ( $14.48 \pm 1.17$  g/dl) than those who drank tea with or directly after meals ( $13.03 \pm 1.53$  g/dl).

## Discussion

The results of the present study showed that microcytosis and /or hypochromasia are prevalent in the Gaza Strip with iron deficiency and  $\beta$ -thalassemia trait as the common causes that encountered in 9.6% and 4.2% of the over all screened students respectively. The presence of Hemoglobinopathies and reduced iron stores are primary determining factors for hemoglobin concentration. The coexistence of both disorders in a developing country like Palestine could aggravate the hemoglobin level of the people, therefore, proper intervention for the correction and replenishing of iron is justified especially for the females of the child-bearing age.

Gender was found also to be a primary determining factor for hemoglobin concentration. This could be explained on the basis of the physiological effects of androgens on erythropoiesis. Gender as a determinant of hemoglobin concentration was has only be reported in humans. In a study by Rushton et al., there was no significant difference between male's and female's hemoglobin concentrations<sup>25</sup>. Losses of iron during normal menstruation intensify the degree of hemoglobin level decrease in females who lose considerable

**Table 1:** Socioeconomic and demographic determining factors of hemoglobin concentration

Category (Number)	Overall N= 1171	Hemoglobin concentration g/dl	
		Males <sup>§</sup> N=548	Females N=623
<b>Sex</b>	13.39 ± 1.57	14.47 ± 1.35	12.44 ± 1.06
<b>Laboratory diagnosis</b>			
Normal (1010)	13.69 ± 1.40 *	14.69 ± 1.09 *	12.70 ± 0.86*
Iron deficiency or depletion (112)	11.53 ± 1.13	12.54 ± 0.92	11.24 ± 1.02
Thalassemia minor (49)	11.42 ± 1.45	11.37 ± 1.84	11.46 ± 1.14
<b>Student education level</b>			
School (853)	13.31 ± 1.55	14.31 ± 1.45	12.45 ± 1.04
University (318)	13.61 ± 1.61 *	14.90 ± 0.95 *	12.40 ± 1.09
<b>Parents consanguinity</b>			
1 <sup>st</sup> degree (307)	13.25 ± 1.48	14.31 ± 1.28	12.41 ± 1.08
2 <sup>nd</sup> degree (249)	13.31 ± 1.81	14.28 ± 1.97	12.49 ± 1.13
Non relatives (615)	13.49 ± 1.51 *	14.62 ± 1.07 *	12.43 ± 1.01
<b>Locality</b>			
Urban (837)	13.37 ± 1.63	14.52 ± 1.41	12.41 ± 1.09
Rural (90)	13.83 ± 1.44 *	14.63 ± 1.31 *	12.82 ± 0.83 *
Refugee camp (244)	13.30 ± 1.41	14.25 ± 1.17	12.43 ± 1.06
<b>Housing</b>			
Own (552)	13.59 ± 1.82 *	15.10 ± 1.11*	12.89 ± 1.11*
Rent (375)	13.15 ± 1.21	13.52 ± 1.25	12.08 ± 0.89
UNRWA camp (244)	13.29 ± 1.41	14.25 ± 1.17	12.43 ± 1.06
<b>Smoking</b>			
Smokers (197)	14.74 ± 0.97 *	14.83 ± 0.92*	13.25 ± 1.30*
Nonsmokers (974)	13.5 ± 1.53	14.29 ± 1.49	12.42 ± 1.05
<b>Family size</b>			
1-3 (36)	14.37 ± 1.21 *	14.67 ± 1.01	12.83 ± 0.96*
4-8 (264)	13.35 ± 1.47	14.58 ± 1.05	12.45 ± 1.01
9-12 (657)	13.36 ± 1.65	14.40 ± 1.15	12.42 ± 1.09
≥ 13 (214)	13.31 ± 1.48	14.47 ± 1.35	12.47 ± 1.02
<b>Family income US \$</b>			
< 350 (502)	13.09 ± 1.64	13.45 ± 1.89	11.83 ± 0.96
350-700 (246)	12.93 ± 1.45	14.68 ± 1.56	12.15 ± 0.86
701-1050 (292)	13.95 ± 0.87	14.30 ± 0.47	12.68 ± 1.09
≥ 1051 (131)	14.10 ± 1.89 *	15.41 ± 0.65*	13.27 ± 1.07*

\* significantly higher within same column values (between male's values, or female's values, or overall's values); § for all categorization, except for the Hb values in thalassemia minor, males showed significantly higher than females

**Table 2:** Dietary and biochemical determinant factors of hemoglobin concentration

Category (Number)	Overall N= 1171	Hemoglobin concentration g/dl	
		Males <sup>§</sup> N=548	Females N=623
<b>Eating meat /omnivorous</b>			
≥ 2 times per week (290)	14.30 ± 1.87 *	15.62 ± 1.52 *	13.19 ± 0.85 *
Once a week (421)	13.75 ± 1.02	14.66 ± 1.61	12.51 ± 1.36
2-3 times per month (460)	12.49 ± 1.31	13.62 ± 1.41	11.98 ± 1.05
<b>Eating green leaf vegetables</b>			
Frequently (866)	13.60 ± 1.01 *	14.97 ± 1.25 *	12.95 ± 0.97 *
Sometimes (176)	12.77 ± 2.07	14.51 ± 1.07	12.33 ± 1.23
Infrequently (129)	12.73 ± 2.91	14.30 ± 0.87	12.38 ± 1.14
<b>Eating citrus fruits</b>			
Frequently (597)	13.63 ± 1.34 *	14.60 ± 1.75 *	12.72 ± 1.20 *
Sometimes (307)	13.17 ± 2.15	14.43 ± 1.04	12.70 ± 1.03
Infrequently (267)	13.10 ± 1.11	14.34 ± 1.54	11.47 ± 1.13
<b>Consumption of milk and diary products</b>			
Frequently (914)	13.29 ± 1.10	14.48 ± 1.49	12.54 ± 1.30
Sometimes (81)	13.45 ± 1.77	14.43 ± 1.09	12.22 ± 0.98
Infrequently (176)	13.23 ± 1.04	14.51 ± 1.35	12.27 ± 1.21
<b>Tea cups</b>			
0 (110)	14.29 ± 2.24 *	15.86 ± 0.62 *	12.60 ± 1.04 *
1 (280)	13.78 ± 1.22	14.48 ± 0.44	12.30 ± 1.02
2 (352)	13.64 ± 1.56	14.73 ± 1.54	12.58 ± 0.52
3 (54)	13.18 ± 1.22	13.89 ± 1.01	12.47 ± 0.98
4 (166)	12.61 ± 1.09	12.66 ± 1.41	12.42 ± 1.4
≥ 5 (209)	12.64 ± 1.43	13.11 ± 1.35	11.5 ± 1.11
<b>Tea and meal</b>			
With or directly after meal (883)	13.03 ± 1.53	14.32 ± 1.55	12.39 ± 1.02
Independently of meals (288)	14.48 ± 1.17 *	14.65 ± 1.04 *	13.26 ± 1.32 *

\* significantly higher within same column values (between male's values, or female's values, or overall's values); <sup>§</sup> significantly higher within same raw values (between males and females)

quantities of blood during their menstrual cycles <sup>26</sup> Each milliliter of blood contains approximately 0.4 mg of iron so that the monthly menstrual loss of approximately 60 ml creates the need for an additional 20 to 30 mg of iron absorption each month or as much as 2.2 mg Fe or more per day in heavily menstruating young women <sup>27,28</sup>.

The educational level of students and their parents consanguinity have been reported in this study to be determinants of hemoglobin levels in males. The higher levels exhibited by the males as compared to the females

can be attributed to the increase awareness of the university student about the nutritive diets and the factors that enhance their health status in order to face academic challenges for better achievements<sup>29</sup>. Unlike the males, hemoglobin concentration in females was not affected by the education level or parental consanguinity. This may be explained based on the magnitude of menstruation effect and female physiological conditions that diminish or lessen other minor factors.

Different genes are responsible for iron metabolism in human beings which include the processes of absorption, transportation utilization and recycling. Single copies of defective genes that affect iron absorption or utilization can accumulate or cluster in the diploid embryo resulting in poor iron pool in that individual and hence low hemoglobin concentration<sup>30-32</sup>. The probability of defective genes clustering are higher in consanguineous marriages<sup>33,34</sup>.

Subjects from refugee camps in different settings have been investigated and data revealed childhood anemia that are associated with factors reflecting poor socioeconomic status, diarrheal and febrile illnesses in refugee camps<sup>35</sup>. This may provide some explanation why the males students from urban or rural and female students from rural areas in our study have significantly higher hemoglobin values when compared to their counterparts in refugee camps.

Infancy and early childhood nutritional surveys among Palestinians living in refugee camps under the auspices of the United Nations Relief and Works Agency (UNRWA), showed an overall prevalence of anemia of 67%. Surveys carried out in Middle Eastern countries have showed the prevalence of anemia in childhood to range from 19 to 71%<sup>35,36</sup>. However, rural areas in some countries, where people depend on farming and agricultural activities, usually characterized by healthy and traditional meals that are produced from local and private farms and ranches where varieties of animals, bird meats and products are available. The life style and modernization of the urban areas may play a significant effects on the reduction of female hemoglobin concentration as compared to rural areas. In urban areas, and despite of losses of iron through menstruation, females in Palestine often follow specific weight loss regimen where they eat smaller amount of varied modern foods that are characterized by low nutritional and caloric values. The urban

lifestyle and weight loss regimen are infrequent in males<sup>37,38</sup>.

Smoking has been associated with higher levels of RBC mass and total hemoglobin concentration. Hemoglobin levels increase as a compensatory mechanism in smokers because the inhaled carbon monoxide of the burning cigarette results in increased carboxyhemoglobin, which has no oxygen-binding affinity. Consequently, different international health parties and centers developed smoking-specific hemoglobin adjustments to define the cut-off values of hemoglobin and hematocrit in smokers<sup>39</sup>.

The socioeconomic determinants of hemoglobin have been reported in females by other researchers<sup>40-43</sup>. Family size and income may exaggerate and increase the gap in hemoglobin level between males and females due to the physiological nature and lifestyle of females.

Significant and potential determinants of hemoglobin concentrations reported in the present study and previous studies are the consumed dietary meals and dietary-related habits. Pure vegetarians were not encountered in the screened students. Omnivorous and meat eating could be considered as a positive factor that ameliorate or reorganize the possible negative effects of other interfering factors on hemoglobin concentrations. The significantly higher hemoglobin concentrations were reported in students who are frequently eating meat, frequently consuming citrus fruits and green leafy vegetables are in agreement with previously reports. However, the consumption of leafy vegetables has been investigated and the results from different experimental researches have revealed contradictory, and confused conclusions, especially with regard to the green leaves of spinach (*Spinacea oleracea* L.), which is a very common and widely eaten vegetable among Palestinian families. The results of Zhang et al., Agte et al. and Clark et al. supported the general idea about the positive effects of green leafy vegetables

on the bioavailability and bioaccessibility of iron and hence hemoglobin concentration<sup>44-46</sup>, while other study mentioned the negative effects<sup>47</sup>.

There is conflicting report about the effect of milk and dietary product on iron absorption<sup>48-53</sup>. The work of Galan and coworkers in 1991 suggested that, under real-life conditions, consumption of normal or increased amount of dairy products may not have any effect on iron absorption from meals containing appreciable amounts of dairy products<sup>51</sup>. This is in support of the present study which has indicated no significant differences in iron levels among the students due to consumption of milk and dairy products. Since citrus fruits contain ascorbic acid, the consumption of citrus fruits by 77.2% of the students may have counteracted the effects of substances which inhibit iron absorption. Ascorbic acid is known to improve the absorption of non-heme (plant-derived) iron<sup>54,55</sup>. It is to be noted that the nutritional benefits of milk and dairy products far outweigh any inhibitory effect it has on iron absorption<sup>52</sup>. Individuals may improve their iron nutrition by supplementing their daily intake of milk and dairy products to the meals with iron content, for example, at breakfast and the evening meal<sup>50</sup>.

Tea drinking has been proven to significantly affect the absorption of iron probably due to the binding of iron in the gut by polyphenols/tannins in tea<sup>9,47,55,56</sup>. This supports the reduction in hemoglobin concentration in our study. Therefore, educational programs should be implemented in order to inform people, especially those living in refugee camps, people with the low education levels and females of child bearing age, about the negative effects of tea drinking on hemoglobin levels<sup>57,58</sup>.

## Conclusions

Factors affecting the hemoglobin concentration among adolescent students in Gaza

strip in Palestine have been identified. These include educational level, parents consanguinity, geographical locality (urban, rural, refugee camp), owning house, smoking, family size, income, tea drinking, and consumption of citrus fruits, meat, and green leafy vegetables. The results of the present study justify the necessity for a national educational programs directed toward improving the dietary and nutritional issues, especially among people living in refugee camps, those with low levels of education, and females of the child bearing age.

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