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Effect of Microcytic Hypochromic Anemia and Parasitic Infestations on Stature in Adolescents

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ABSTRACT

Bakground: Microcytic hypochromic anemia is the commonest form of iron deficiency anemia in adolescents. The occurrence of this type of anemia among adolescents is around 27% in developing countries. Clinical management should be based on a full knowledge of the prevalence of this disease in the age group mentioned.

Subjects and Methods: The present study reported the distribution of this type of anemia across age, anthropometric guides, and parasitic infestations in a sample of 300 adolescents attending various schools in Giza region, Egypt. Red blood cell size and iron concentration were assessed by mean corpuscular volume, hemoglobin levels, serum ferritin and total iron binding capacity from a venous blood sample. The adolescent was considered to have the microcytic form of anemia when their mean corpuscular volume was below 80 femtoliters (fL). An adolescent with hypochromic anemia was defined as any subject with hemoglobin (Hb) below the WHO cutoff for age and sex: 12.0 g/dl for girls and for boys aged 12.5–14.99 years and 13.0 g/dl for boys aged ≥ 15 years. Also, hypochromic anemia included every subject having either serum iron < 50 µg/dL, or a serum total iron binding capacity (TIBC) > 400 µg/dL.

Results: The incidence of microcytic hypochromic anemia in this study was 53%. There were highly statistically significant differences between anemic and non-anemic groups as regards age and height (P <0.05). Anemic adolescents also had significantly lower values for weight (P < 0.01), BMI (P < 0.01) and hemoglobin concentration (P<0.01) compared to non-anemic adolescents. Also, microcytic hypochromic anemia was more common in adolescents who did not have lunch regularly. Adolescents with current parasitic infestations showed a higher frequency of anemia compared to those who did not. There were no statistically significant differences between adolescents with parasitic infestation and adolescents without parasitic infestation as regards age, weight, height and BMI (P >0.05). Signs of pallor were more common in adolescents suffering from microcytic hypochromic anemia. Subjects with a history of chronic conditions such as cardiac diseases, renal failure or cancer had a significantly higher incidence of anemia than adolescents who did not.

Conclusion: It was concluded that the anemic group of adolescents enrolled in the study were susceptible to growth retardation. This type of anemia is more common in adolescents who do not have lunch, have a chronic disease or a parasitic infestation.

Keywords: Stature, microcytic hypochromic anemia, parasitic Infestation, school adolescents.

Introduction

Anemia could be defined as an abnormally low hemoglobin level due to pathological conditions associated with nutritional and non-nutritional etiologies. Iron deficiency is one of the most prevalent causes of nutritional anemia. Iron deficiency anemia (IDA) is a disorder in which severe iron deficiency causes anemia [(1)]. Adolescents are one of the main risk groups for anemia[(2)]. The prevalence of the microcytic hypochromic anemia form of iron deficiency anemia amongst adolescents is 27% in developing countries and 6% in developed countries [(3)]. In the adolescence period, the iron requirements are increased due to fast growth. In order to enhance the absorption of iron, the level of ferritin decreases. In boys, increased muscular growth leads to increased demand for iron. The onset of menstruation in females and monthly loss of iron (around 30 mg/month) leads to reduced ferritin levels. Unbalanced eating habits and the lower consumption of animal food adds to the progress of anemia in this age group [(4)]. The diagnosis of iron deficiency is based primarily on laboratory measurements of biochemical iron indicators. Cut-off values for

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hemoglobin concentrations as defined by world health organization (WHO) for age and sex are 12.0 g/dl for girls and boys aged 12.5–14.99 years and 13.0 g/dl for boys aged ⩾15 years [5]. Some studies suggest that microcytic hypochromic anaemia can have detrimental effects on memory and learning processes in pre-adolescents and adolescents [6].

The aim of the study:

This study was carried out to estimate the prevalence of microcytic hypochromic anemia as a common form of iron deficiency anemia among school adolescents in Giza, Egypt, the probable etiologies and possible effects on general stature.

Subjects and Methods

This study was conducted in Giza region as a typical cross-sectional study. Three hundred adolescent pupils were enrolled from the local schools in Giza district aged between 12 and 18 years old. All subjects were enlisted for this study with parents' agreement. Demographic data was collected using surveys filled out by the parents. The socio-economic status was defined with the following considerations in mind: the family income, the parental educational ranking and the employment status of the parents.

All adolesents included in this study were subjected to:

1) Full History taking; paying special attention to:
- Nutritional history; especially regular daily consumption of lunch.
- History of parasitic infestation (by asking about affection with parasites or previous intake of any anti-helminthic treatment).
- Any affection by chronic diseases (such as cardiac diseases, diabetes mellitus, pulmonary diseases, renal diseases, rheumatic fever or cancer).

2) Thorough clinical examination; paying special attention to:
- Anthropometric measures including: Height, weight, and BMI; all the results were compared to the standard curves for age according to the world health organization criteria[7].
- Body weight was measured using the platform weighing Scale (770 alpha; SECA, Hamburg, Germany) to the nearest 0.1 kg and body height was also measured using Microtoise tape (Microta Type Height Measure; Chasmors limited) to the nearest 0.1cm.
- Pulse, respiratory rate and blood pressure measurement were described as normal or abnormal for age according to standards described by Robert [8].
- Significant clinical findings such as: Pallor, jaundice, general weakness, weight loss and short stature were recorded.

3) Laboratory investigations:

Sample collection :
- Three samples of stool were taken from different parts of each stool specimen obtained.
- For each adolescent, 2 ml. of venous blood were obtained for the measurement of hemoglobin concentration, mean corpuscular volume (MCV), serum iron and total iron binding capacity (TIBC).

1 - Hemoglobin concentration and MCV:
- Were measured using an electronic Coulter counter (Cobas, Roch Inc. Switzerland). In addition, a complete blood picture was carried out for each subject.

2- Stool examination:

This test was carried out using light microscopy in order to detect any parasitic ova which could be a sign of parasitic infestations.

3 – Serum iron and total iron binding capacity (TIBC):

Serum iron level assessment: This was carried out using colorimetric kits produced by BQKITS Diagnostics Company (United States of America)

TIBC assessment: transferrin was measured first in order to calculate the TIBC of transferrin using the formula TIBC (µg/ml) = transferrin x24. The level of serum transferrin was estimated in all subjects joining in the study by using serum immune-reactive transferrin that was measured in duplicates by a radioimmunoassay method that utilizes $_{125}$I-labeled transferrin and a sheep anti-human transferrin antiserum to determine the level of transferrin in serum by the double antibody/PEG technique. The transferrin standards were prepared using recombinant human transferrin and were used to determine the circulating levels of transferrin in human serum samples. This was done according to the method described by Feldkamp and Smith [9].

Statistical analysis

Statistical analysis was performed using Statistica v. 10 software. Quantitative results were expressed as mean ± SD. T-test or the Mann-Whitney U test was used to compare

quantitative variables in the two qualitative groups [10].

Results

The results are shown in the following tables. 300 secondary school adolescents were studied over the period from June 2009 to January 2010: girls (60%) and boys (40%). Table 1 shows the range, mean value and standard deviation (SD) for tested parameters in the study population.

Table (1): The clinical and laboratory parameters of the adolescents enrolled in the study (Mean±SD).

Factors	Mean ±SD
Age (years)	15.0 ±3.2
Weight (Kg)	45.1 ± 5.3
Height (cm)	156± 18.5
BMI (Kg/m^2)	22.5 ± 8.6
Hemoglobin (g/dl)	10.2 ± 2.4
MCV (fL)	75±5.6
Serum iron (µg/dl)	63.4 ± 12.9
Serum TIBC (µg/dl)	450.3 ± 20.5

(n=300)

A history of parasitic infestation was present in 36% of the current study population, while the presence of a parasite in stools was found in 44 % of subjects enrolled. The majority of the study population did not show signs of pallor (65%). These results are shown in table 2.

Table (2): The occurrence of the several factors investigated in the adolescents enlisted in the study.

Factors	Yes (positive)	No (negative)
Lunch consumption	120 (40%)	180 (60%)
History of chronic diseases or intake of medications	36 (12%)	264 (88%)
History of parasitic infestations	108 (36%)	192 (64%)
Findings of parasitic infestations in stool analysis	132 (44 %)	168 (56%)
Signs of Pallor	105 (35%)	195 (65 %)

(n=300)

The prevalence of microcytic hypochromic anemia in the current study was 53%. An adolescent was considered to have the microcytic form of iron deficiency anemia when their mean corpuscular volume was below 80 femtoliters (fL). An adolescent with hypochromic anaemia was defined as any subject with haemoglobin (Hb) below the WHO cut off for age and sex [11]: 12.0 g/dl for girls and for boys aged 12.5–14.99 years and 13.0 g/dl for boys aged ⩾15 years.Also hypochromic anemia included every subject enrolled having either serum iron less than 50 µg/dL, or serum TIBC more than 400 µg/dL. These results are illustrated in table 3.

Table (3): The occurrence of microcytic hypochromic anemia in the adolescents enrolled in the study.

	Number of subjects	Percentage (%)
Microcytic hypochromic anemia	159	53
Normal Blood Picture	141	47

(n=300)

Microcytic hypochromic anemia was found in 159 (53%) adolescents participating in this study. There were statistically significant differences between anemic and non-anemic groups as regards age and height ($P < 0.05$). Anemic adolescents also had highly significant lower values for weight ($P < 0.01$), BMI ($P < 0.01$) and hemoglobin concentration ($P<0.01$) compared to non-anemic adolescents. These results are demonstrated in table 4.

Table (4): Comparison of the various clinical and laboratory parameters between adolescents with microcytic hypochromic anemia and normal subjects enrolled (Mean±SD).

Parameters	Subjects with microcytic hypochromic anemia	Subjects with Normal Blood Picture	P- value
Age (years)	13.0 ± 4.7	15.9 ± 5.6	<0.05
Weight (kg)	35.8 ±4.8	47.0± 4.3	<0.05
Height (cm)	147.3 ± 10.2	154.4± 17.6	<0.001
BMI (Kg/m^2)	21.2±4.6	25.5± 6.1	<0.05
Hemoglobin (g/dl)	9.6 ± 1.2	12.5± 2.1	<0.01
MCV (fL)	72.3±3.1	81.0±2.3	<0.01
Serum iron(µg/dl)	38.4 ± 8.2	91.5±16.4	<0.001
Serum TIBC (µg/dl)	480.3± 69.7	351.9± 72.4	<0.001

(n=300).

Table 5 shows that microcytic hypochromic was more common in adolescents who did not usually have lunch as opposed to those who did. This difference was statistically highly significant (P < 0.01). There was a highly significant statistical difference between subjects with microcytic hypochromic anemia and normal subjects as regards the history of parasitic infestations (P < 0.01).

Adolescents with a history of cardiac diseases, diabetes mellitus, pulmonary diseases, rheumatic fever, renal failure or cancers had a significantly higher frequency of microcytic hypochromic anemia than subjects who did not (P < 0.01). Adolescents with current parasitic infestations showed a higher frequency of microcytic hypochromic anemia compared to those free of parasitic infestations. This difference was highly statistically significant (P < 0.001).

Table (5): Comparison between adolescents with microcytic hypochromic anemia and normal subjects as regards possible contributing factors to iron deficiency.

Factors	Microcytic Hypochromic anemia		Subjects with Normal Blood Picture		P value
	Number	%	Number	%	
Lunch consumption	30	10	90	30	<0.01
No lunch consumption	129	43	51	17	
History of parasitic infestation	39	13	69	23	<0.01
No history of parasitic infestation	120	40	72	24	
History of chronic conditions or medications	24	8	12	4	<0.001
No history of chronic conditions or medications	135	45	129	43	
Presence of parasitic infestation in stool analysis	84	28	48	16	<0.001
Lack of parasitic infestation in stool analysis	75	25	93	31	

(n=300)

Clinical signs of pallor occurred more commonly in adolescents with microcytic hypochromic anemia. The difference between subjects with anemia and normal subjects was statistically significant (P < 0.01). This result is obvious in table 6.

Table (6): The relationship between microcytic hypochromic anemia and pallor.

Items	Microcytic Anemia		Normal Blood Picture		P value
	Number	%	Number	%	
Signs of pallor	90	30	15	5	<0.001
No signs of pallor	69	23	126	42	

(n=300)

There were no statistically significant differences between adolescents with parasitic infestation and adolescents without parasitic infestation as regards age, weight, height and BMI (P >0.05). These results are illustrated in table 7.

Table (7): Comparison between adolescents with and without parasitic infestation as regards various clinical and laboratory parameters (Mean±SD).

Parameters	Parasitic infestations	Absence of parasitic infestations	P value
Age (years)	15.5± 3.2	16.2±2.4	>0.05
Weight (Kg)	54.6±4.5	55.0±3.8	>0.05
Height (cm)	150.6±14.5	151.1±15.8	>0.05
BMI (kg/m^2)	24.6±6.7	25.1±6.9	>0.05
Hemoglobin (g/dl)	8.8±1.4	11.7±2.4	<0.001
Serum iron (µg/dl)	47.3±12.6	98.4±13.9	<0.001
Serum TIBC (µg/dl)	475.8±91.6	344.9±77.6	<0.001

(n=300)

Discussion

Iron deficiency anemia is classically described as a microcytic anemia. Microcytic hypochromic anemia is one of the most common types of anemia in children and adolescents. It is a very diverse group of diseases that may be either acquired (mostly due to iron deficiency) or inherited. Red cells with lowered mean corpuscular volume (MCV) denote a pathologically decreased rate of hemoglobin synthesis, which could have several etiologies. It can occur from errors either in iron acquisition from diet or due to malabsorption of iron from the small intestine (e.g. in caeliac disease) or defects in heme or globin production in the bone marrow (i.e. haemoglobinopathies or thalassaemias). Experimental data in iron deprived mice confirmed that early mitotic divisions are associated with no reduction of MCV, whereas during differentiation mitotic events are accompanied with a significant reduction of MCV [(12)].

Total daily iron requirements are largely dependent on heme biosynthesis in bone marrow erythroblasts. Absorption of dietary iron by the duodenum, as well as iron recycling by the macrophage, is regulated by several different physiological signals including iron load, erythropoiesis, hypoxia and intestinal mucosa inflammation. Intestinal iron absorption and iron recycling by macrophages following erythrophagocytosis and heme catabolism are the two major processes that fulfill the iron needs of mammalian organisms [(13)]. This process allows the recycling of about 20–25 mg of iron per day, corresponding mostly to what is needed daily for bone marrow erythropoiesis. In normal conditions, iron absorbed from the diet by duodenal enterocytes (1–2 mg) is required to compensate for daily losses, resulting from desquamation of epithelial cells and minor blood losses [(14)].

Recommendations for iron are provided in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine of the National Academy of Sciences (USA) [(15)]. The daily requirements for adolescents (aged 14 to 18 years) are 11 mg/day for males and 15 mg/day for females.

Iron deficiency anemia (including microcytic hypochromic anemia) continues to be one of the most recognized types of anaemia caused by dietary deficiencies in the

world, especially` in developing countries. World Health Organization (WHO) estimated that microcytic hypochromic anaemia occurs in about 66–80% of the world's population [16]. Microcytic hypochromic anemia causes general weakness, poor stature, and a debilitated immune system that increases the morbidity of this disease. It is also thought to damage mental performance and postpone psychomotor development. Microcytic hypochromic anemia is the most common form of iron deficiency anemia in adolescence, affecting all socio-economic levels of society [17].

Adolescents (age 10-19 years) are at high risk of iron deficiency and anaemia due to accelerated increase in requirements for iron, poor dietary intake of iron, high rate of infection , worm infestation heavy menses as well as the adolescent pregnancy and lactation. It is therefore a key community health fear in adolescents and pregnant women in the developing countries. Many studies have examined these risk groups [18]. However; there is currently a lack of data on anemia in school adolescents living in rural and urban areas of developing countries [19].

The aim of this work was to assess the prevalence of iron deficiency in school adolescents Giza district, its probable causes and its effect on general growth.

In order to achieve these objectives, this work was conducted on 300 adolescents between 12-18 years in schools in Giza district, chosen as successive random samples. All adolescents were subjected to full history taking, anthropometric parameters, stool analysis, estimation of mean corpuscular volume, hemoglobin concentration, serum iron, and serum iron binding capacity.

In this study the prevalence of microcytic hypochromic anemia was 53% among the school adolescents in Giza district.

The study results are similar to other studies carried out in developing countries that conducted surveys on the prevalence of anemia in adolescents living in those regions.

A survey on iron deficiency anemia incidence in Gujarat district in India found the prevalence to be around 74.7% for the 12-19 age group investigated which was improved to 53.2 % after a week's iron supplementation [20]. Haidar [21] selected 970 Ethiopian female adolescents aged 15-19 years for hematological and iron deficiency parameters. The overall prevalence of microcytic hypochromic anemia was 30.4% in the adolescents studied.

These findings are similar to the results of the survey conducted on 355 Egyptian young males employed in private workshops (aged 7-19) in Alexandria governorate, which showed that about 44.5% of them were anemic [22].

Another survey investigated the prevalence of microcytic hypochromic anemia among adolescent female college students attending the University of Sharjah, United Arab Emirates. Results showed that the distribution of microcytic anemia among them was 26.7% [23].

The majority of these studies are in line with the results of this work, since all of them were conducted in developing countries with a similar environment and etiologies of iron deficiency. The dissimilarity between studies could be ascribed to the different age groups studied and method of assessment of microcytic hypochromic anemia.

The high occurrence of microcytic hypochromic anemia among the adolescent age group is explained by the increased requirements for dietary iron due to fast growth, heavy menstruation in girls, low intake of required daily dietary iron, skipping meals, low dietary variety and parasitic infestations.

The adolescents in this study were classified according to the results into a group suffering from microcytic hypochromic anemia and another one with normal blood pictures.

This study showed that anemic adolescents had a significantly lower weight and BMI than adolescents without anemia. In this study there were also highly statistically significant differences between anemic and non-anemic adolescents as regards height. Allen [24] stated that the etiology of linear growth retardation is generally multi-factorial but could be clarified in part by poor nutrition which may involve lack of the intake of some important trace elements such as iron, copper and zinc which may in turn lead to the associated development of microcytic hypochromic anemia. Trace elements are dietary minerals that are present in very minute quantities (less than 0.01%) of the mass of the organism. They are useful for proper growth, development, maintaining and recovering the health of the organism. They

have several roles in living organisms. Some are essential components of enzymes as they attract substrate molecules and facilitate their conversion to specific end products. Some donate or accept electrons in reactions of reduction and oxidation, which results in the generation and utilization of metabolic energy. Some trace elements impart structural stability to important biological molecules. Finally, some trace elements control important biological processes through such actions as facilitating the binding of molecules to receptor sites on cell membranes.

Copper is required for the proper function of vitamin C and iron absorption. Zinc is needed for cell division (including red blood cell precursor cells and white blood cells) hair, tissue, nails, skin and muscles growth. Iron is essential to all cells. Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis, and protein synthesis [24, 25].

However; these results are in contrast with the findings of Sachdev *et al* [26] who reported that there were no statistically significant effects of iron supplementation on height-for-age in children and adolescents.

This discrepancy is clarified by Beckett *et al* [27] who reported that observational studies have assumed a positive effect on physical growth due to indirect effects of iron supplementation on the improvement in immunity leading to a decreased incidence of infections, and improvement in appetite and consequently eating habits. Most of these studies are carried out in developing countries, which have marginal food availability and poor feeding, where improvement in the appetite and activity levels of the adolescent as well as giving supplements may not necessarily translate in to increased energy intake and therefore enhanced height and weight gain.

In this study parasitic infestation was present in 44 % of the adolescents; Ascaris Lumbricoids, Oxyuris, Entamoeba Histolytica, and Giardia Lamblia were the most frequent parasites found in stool analysis. Adolescents with microcytic anemia had a significantly higher frequency of parasitic infestation than normal subjects.

This agrees with the results of a study conducted by Hafiz Uddin and Khanum [28] on 106 male school adolescents in two villages in the district Dahaka, Bangladesh in 2002. It revealed that the prevalence of intestinal parasites was 49.01 %; Ascaris lumbricoides, Trichuris Trichiura, Entamoeba histolytica, Diphybothrium latum and Giardia lamblia were the most frequent forms. They found a significant association between intestinal parasitic infestations and iron deficiency anemia in school adolescents.

These results also agree with the results of a survey conducted by Shipala et al. [29] on 384 pregnant adolescents attending antenatal care at two facilities in western Kenya. It was found that almost half of those who had microcytic anemia had hookworm infestation as evident in stool examination.

These results are explained by Binay and Lubna [30] who state that occult blood loss, reduced appetite, decreased digestion, and malabsorption that are usually associated with gastrointestinal tract parasite infestation may be the cause of poor iron status and iron deficiency anemia that are frequently observed in adolescents suffering from intestinal parasitic infestations.

This study demonstrated that there were no statistically significant differences between adolescents with parasitic infestation and adolescents without parasitic infestation as regards age, weight, height and BMI ($P > 0.05$). This result was in agreement with a study carried out by Sanchez et al. [31] who could not demonstrate a significant relationship between parasitic infestation in adolescents and stunted growth or reduced BMI. This could be explained by the fact that stunted growth is a multifactorial disease [24] and in the case of parasitic infestation it may require a heavy parasitic load for it to occur in addition to other factors such as severe malnutrition.

This study showed that microcytic hypochromic anemia was more common in adolescents who did not usually have lunch as opposed to those who did. These results are similar to those observed in a study carried out in Serbia which found a threefold risk of developing microcytic anemia in adolescents who skipped lunch compared to those who did not [32]. Another study carried out on Indonesian adolescent girls also found a strong link between lunch skipping and the

prevalence of anemia in that study population [33]. These results illustrate the importance of consuming regular meals especially the midday one in order to obtain the necessary dietary requirements including trace elements thus avoiding the development of this type of anemia.

Serum iron is a laboratory test that measures the amount of circulating iron that is bound to transferrin **(**Normal values of men: 65 to 176 µg/dL and women: 50 to170 µg/dL). Total iron-binding capacity is a laboratory test that measures the extent to which iron-binding sites in the serum can be saturated. (Normal TIBC for men and women: 240-450 µg/dL) [34].

In this study serum iron levels were significantly lower in adolescents with microcytic hypochromic anemia compared to normal subjects ($p<0.001$). TIBC was also found to be significantly higher in anemic adolescents enrolled compared to normal subjects ($p<0.001$).

These results are similar to those presented by Ahmed et al [35] who investigated serum iron levels and TIBC in 548 adolescent girls and found a significant positive correlation between hemoglobin level and serum iron, while there was a negative correlation with serum TIBC.

The present study found a significant difference between normal subjects and adolescents suffering from microcytic hypochromic anemia as regards the presence of history of affection by a chronic disease ($p<0.001$). Weiss [36] stated that anemia related to chronic disease can be caused by chronic infections or an inflammatory process. Increased levels of cytokines accompanying chronic diseases cause a reduction in erythropoietin production, a decreased response to erythropoietin, and interference with iron metabolism.

In this study pallor was found to occur more commonly in anemic adolescents compared to the normal subjects enrolled in this study. This difference was statistically significant ($P < 0.01$).

Conclusion

It could be concluded from this study that adolescents suffering from microcytic hypochromic anemia are more susceptible to physical growth retardation and consequent reduction of stature. Microcytic hypochromic anemia is more common in adolescents who do not consume lunch, have a chronic disease or are diagnosed with parasitic infestations.

Recommendations:

The following is recommended:

a) Regular screening of adolescents for the early diagnosis and treatment of parasitic infestations.

b) Education of adolescents at home and school to consume a regular, healthy, mixed and well balanced diet paying special attention to vegetables and fruits.

c) Regular health checkups for adolescents at schools and regular follow up of blood picture on a yearly basis.

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