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Abstract

Background: Anemia is a worldwide problem, with higher prevalence in developing countries. It is common among school-aged children and its cause is multifactorial. This study was conducted to estimate the prevalence of anemia and its possible association with IPI and the nutritional status of the schoolchildren in Gurage zone, South Central Ethiopia.

Methods: This is a cross sectional study of 680 children aged 7 to 19 years from Enemore- Ener and Abeshege District, Ethiopia. Data were collected using Hematological (Hemoglobin) and biochemical (serum ferritin) method was used to determine anemia and iron status in 680 children. The subjects were screened for intestinal parasitic infection to determine their impact on anemia. Socioeco-nomic and anthropometric data were collected from the children. Anthropometric data were entered and, processed and analyzed using WHO anthroplus software. Differences in variables between sexes were examined using t- test. Pearson's correlation coefficient (r) was used to determine the association between anemia and nutritional factors.

Results: The overall prevalence of anemia as determined by Haemoglobin level and serum ferritin was (31.3%) in Enemore-Ener, which was significantly lower than in Abeshege (39.6%) (P < 0.05).

Conclusions: The findings of this study showed prevalence of IPI to be associated with anemia. The prevalence of IPI increases anemic status schoolchildren. This survey should sensitize concerned bodies for improving primary schoolchildren health and nutrition.

Keywords: Anemia; Stunting; Schoolchildren; Nutrition; Anthropometric Measurements

Introduction

Positioning micronutrient deficiency elimination has given as a global priority in now days because many populations with micronutrient deficiency of developing nations develops blindness, anemia, goiter and are unable to achieve their full mental and physical potential due to stunted growth, low physical work capacity, reduced IQ and lower resistance to infection. Eradication of these deficiencies brought sustained economic growth and national development along with health improvement [1].

Iron deficiency with anemia can occur when a person has low values of both serum ferritin and hemoglobin, while iron deficiency without anemia can occur when a person has normal hemoglobin, but below normal serum ferritin and/or transferrin saturation, whereas nutritional iron deficiency arises when bodily necessities cannot be satisfied by iron absorption from diet [2].

Prevalence estimates of iron deficiency anemia based on hemoglobin alone are over-estimation because other causes of anemia, such as nutritional deficiencies (eg, vitamin A deficiency), infectious disorders, haemo-globinopathies, and ethnic differences are not considered in normal haemoglobin distributions [3,4]. In less developed nations, high occurrence of iron deficiency and anemia can be caused by poor living conditions of population, low socio-economic conditions, less access to food and lack of awareness for good dietary practices and personal hygiene [5]. Furthermore, intestinal parasitic infection, caused as result of poor hygienic conditions, interferes with iron absorption through reduction process, thus increase iron deficiency anemia [6].

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The prevalence of anemia by measuring a single assay alone can indicate only a very rough estimate of the prevalence of iron deficiency anemia. For accurate diagnosis either a multi-parameter analysis is required or the assay of several samples. That is why, it is an important health indicator, when it is used with other measurements of iron status, even though, the variation in the iron deficiency and anemia lacks a consistent standard for identifying iron deficiency [6]. Hemoglobin concentrations are stable and a simple and wellstandardized method ensures a relatively low day to day variation in individuals. And also, serum ferritin is the most specific biochemical test indicator of total body iron stores and is a useful indicator of iron status in absence of infection [7].

Anemia is considered as a public health problem, when the occurrence of low hemoglobin values in the population is more than 5% [8]. Iron deficiency is the most common and widespread nutritional disorder in the world [9] and affecting a large number of children and women in non-industrialized countries, and also the only nutrient deficiency which is significantly prevalent in nearly all developed nations. Even though, there is no exact current global figures for iron deficiency, using anemia as an indirect indicator, it can be estimated that more than 2 billion people are anemic in the world [8,10]. The highest prevalence of anemia exists in the developing countries (2.5 times developed nations) where its causes are multi-factorial [11]. Therefore, the aim of this study is to assess the prevalence of anemia and its association with intestinal parasitic infections and poor nutritional conditions on the well-being of primary schoolchildren.

Materials and Methods

Study area and study population: A school based cohort study was conducted from September 2014 to June 2015 to assess the magnitude of anemia among elementary schoolchildren in two purposively selected Enemore-Ener and Abeshege districts, Gurage zone. Gurage zone is found at 7044'5"-8028'5"N latitude and 37025'5"-38042'5" E longitude. Gurage zone is located 155 km from Addis Ababa on the road to Jimma, central Ethiopia. 680 primary schoolchildren participated in study. The study participants were children aged 7 - 19 years in randomly selected kebeles of Enemore-Ener and Abeshege districts. Children of the two districts live under similar socio-cultural characteristics and low socio-economic condition of rural villages.

Sample size and sampling method

The study populations were residents of the two purposively selected districts. Then sample size was estimated using Daniel's formula $n = Z^2 P (1-P)/d^2 (27)$. Where P = prevalence of intestinal parasites from previous studies, d = margin of error and Z = standard score corresponds to 1.96. This would give a sample size of 349. The prevalence rate (p) of anemia from previous similar study was 34.8% in children [12], a 95% confidence interval and a 5% margin of error were added. Due to the use of multistage sampling of which cluster sampling was used, a design effect of 2 was added. Therefore, a total population of 698 children was included in the study however 18 children did not give a complete data during the survey. The study participants were selected from the school lists using stratified random sampling methods from the eleven schools of two districts after informed consent/assent.

Sampling method

A multistage sampling was used to select the sample. It was a two-stage sampling. The first stage was stratified cluster sampling with proportional allocation to size, and the second stage was simple random sampling.

Fecal collection and examination

Kato-katz method

From each child, about 2 grams of fresh stool samples were collected using sterilized cups. A portion of the specimen was processed using Kato technique [13]. It was examined for *Ascaris lumbricoides, Trichuris trichiura,* hookworm and other intestinal helminths. The number of eggs per slide were counted and multiplied by 24 to obtain the number of eggs per gram (epg) of feces [14].

Formol-ether concentration method

Stool samples of approximately one gram from all subjects were collected into test tubes containing 8 ml of 10% formol-ether and transported to Aklilu Lemma Institute of Pathobiology, Addis Ababa University. A portion of each fresh stool sample was processed as described by Ritchie [15]. Infected cases were treated by medical personnel from respective health institution.

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Blood sampling and Biochemical and hematological measurements

From each student finger prick 5 ml venous blood was collected into an airtight vial, allow the blood to clot by leaving it undisturbed at room temperature for 15 - 30 minutes. The clot was removed by centrifugation at 5,000 rpm for 5 - 10 minutes. Following centrifugation, the serum was immediately transferred to polypropylene tubes using a Pasteur pipette. The serum sample was maintained at -20°C and transported to EPHI laboratory once a week on dry ice, and stored at -20°C until the analysis was done for ferritin. The serum ferritin concentration was determined with Elegance Amplified Enzyme Linked Immuno Sorbent Assay (ELISA) system using kits from Bioclone Australia Pty. Ltd at EPHI Diagnostic Laboratory within a month. Iron deficiency was defined as serum ferritin level below 12 ug/dl as a cut off. Haemoglobin concentration was determined from finger prick blood sample using "Hemocue globinometer (HemoCue, Angelholm HB, Sweden). This apparatus is a portable battery-operated Hb meter and was used for haemoglobin determination in the field.

Statistical Analysis

Data entry and management were done using SPSS statistical software version 16.0. The data was then exported to SPSS version 16 program for analysis. Descriptive statistics of means, standard deviations and percentages were calculated. Differences in variables between sexes were examined using student's t- test. Pearson's correlation coefficient (r) was used to determine the association between anemia and nutritional and health factors. Level of significance for acceptance was p-value < 0.05.

Ethical Consideration

The study was reviewed and approved, and ethical clearance was obtained from the Ethical Committee of College of Natural Sciences, through the Department of Microbial Cellular and Molecular Biology, Addis Ababa University. Informed verbal consent or permission was also obtained from Southern Nation and Nationalities of People Regional State Health Bureau, Gurage zone Education and Health Departments. Each of these zonal departments was passed the message of permission to their respective districts including schools and health centers. Participation in the study was on a voluntary basis. Privacy and confidentiality of the information was ensured. The ethical considerations were addressed by treating positive children using standard drugs under the supervision of a local nurse. The objective of the study was explained to school communities, kebele leaders and parents; and written consent was obtained from every participant's parent or guardians of the selected children before conducting the survey and the children also gave their assent before collecting the samples.

Results

A total of six hundred eighty (680) students, from grade 1 to 8, were enrolled in the study. The mean age of the total study population was 14.2 (range: 7 - 19 years) and the male to female proportion was 51.2% and 48.8% of Abeshege, and 57% and 43% Enemore-Ener district respectively (Table 1). Stool samples were collected and examined from 680 children (Table 1).

Variable	Reside				
	Enemore-Ener (%)	(%) Abeshege (%) Tota		Total No	P-value
Sex					
Male	247 (54.9%)	12 (48.7 %)	359 (52.8)	680	0.03
Female	203 (45.1%)	118 (51.3%)	321 (47.2)		
Age					
6 - 9yr	44 (9.8%)	33 (14.3%)	77 (11.3)	680	0.002
10 - 14yr	264 (58.7%)	142 (61.7%)	406 (59.7)		
≥ 15	142 (31.6%)	55 (23.9%)	197 (29)		
Family size					
2 - 5	165 (36.7%)	126 (54.8%)	291 (42.8)	680	<0.001
> 5	285 (63.3%)	104 (45.2%)	389 (57.2)		

 Table 1: Socio-demographic characteristics of the Primary Schoolchildren in Enemore-Ener and Abeshege districts,

 Sep 2014 -Jun 2015.

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T-test analysis of anemia (normal vs anemic) as dependent variable and intestinal parasitic infections as risk factors in Enemore-Ener and Abeshege districts showed that IPIs were strongly (P = 0.001) associated with an increased risk for anemia in the schoolchildren in Enemore-Ener district whereas IPIs were not significantly associated with anemia in Abeshege district (Table 2).

Risk factors			95% C.I.		P value
	B*	EXP(B)**	Lower	Upper	
IPI Enemore-Ener	-0.71	0.49	0.33	0.74	0.001
IPI Abeshege Two districts	-0.19 -5.21	0.83 0.59	0.49 0.43	1.4 0.82	0.48 0.002

Table 2: Intestinal parasitic infections as risk factors for anemia, among schoolchil-dren in Enemore-Ener and Abeshege districts Gurage zone, South Central Ethiopia,2014-2015.*Coefficient of regression**Odds Ratio

Comparison of anemic status

Differences in anemic status due to measurements of haemoglobin and serum ferritin between two different districts of Gurage zone were examined using student's t test. The t-test analysis showed that the mean haemoglobin and the mean serum ferritin values among schoolchildren in Enemore-Ener district were higher than Abeshege district (p < 0.05) (Table 3). That means the two district populations had different anemic status.

				95% CI		
Anemic status measurement	N	Mean Hb/Sf	t*	Lower	Upper	Р
Hb (g/dl)						
Enemore-Ener	450	12.08	2.146	0.007	0.158	0.032
Abeshege	230	11.96				
Serum ferritin(ug/dl)						
Enemore-Ener	450	58.29	2.612	0.029	0.208	0.009
Abeshege	230	41.42				

 Table 3: Comparison of anemic status among Enemore-Ener and Abeshege district schoolchildren (n=680) in
 Gurage zone, South central Ethiopia, 2014-2015.

t*=t-test for equality of means

Gender and infection status based comparison of haemoglobin levels

There was no significant difference between mean haemoglobin levels in male and female in the two districts. The mean distribution of haemoglobin between genders was similar (p > 0.05). There was no significant difference between the mean of haemoglobin in ascaris positive and ascaris negative children (p > 0.05) (Table 4).

Therefore, the independent sample t-test mean comparison showed that except for ascaris positive and gender, there were significant difference between IPI positive and IPI negative children, hookworm positive and hookworm negative children, Giardia positive and Giardia negative children (p < 0.05) (Table 4).

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				95% CI		
Independent variables	N	Mean Hb(g/dl)	t*	Lower	Upper	Р
Sex						
Male	359	12.06	0.725	-0.045 0.098	0.000	0.460
Female	321	12.02	0.725		0.098	0.468
Infection status						
IPI positive	269	11.88	2 1 0 0	0.100	0.045	0.001
IPI negative	411	12.15	-3.199	-0.190	-0.045	0.001
Hookworm positive	63	11.82	2.270	0.272	026	0.010
Hookworm negative	617	12.06	-2.379	-0.272		0.018
Giardia positive	60	11.76	2.02	0.210	-0.318 -0.067	0.003
Giardia negative	620	12.07	-3.02	-0.318		
Ascaris positive	63	11.85	1 257	-0.202 -0.044	0.209	
Ascaris negative	617	12.06	-1.257			

Table 4: Gender and IPI status based comparison of haemoglobin levels among primary schoolchildren study participants (n = 680) in Enemore-Ener and Abeshege districts, Gurage zone, South Central Ethiopia, 2014. $t^*=t$ -test for equality of means

Nutritional conditions and association with anemia

Assessment of nutritional conditions and association with anemia showed underweight to have significant association with anemia (Table 5).

	Prevalence of nutritional condition			
Nutritional condition	(%)	r	P-value	
Underweight	47.7	0.237	0.003	
Stunted	40.3	0.06	0.118	
Thin	41.7	0.047	0.084	

Table 5: Overall Nutritional condition and its association with anemia in Enemore-Ener and Abeshege districts, Gurage zone, South Central Ethiopia, 2014-2015.

Discussion

The differences in the prevalence of anemia among school age children in different regions of the country might be related to causes of anemia that are multifactorial. Ethiopia is the country with diverse cultures with different communities with different lifestyles, feeding habits, personal hygiene and environmental sanitation and socio-demographic/economic conditions that may variously contribute to different levels of anemia prevalence. As a result, the risk factors of anemia may not be similar in all areas as reported by [8]. Similarly, the results of this study have shown that the prevalence of anemia in the two districts are different. The higher prevalence in Abeshege district may be explained by the low iron bioavailable content of foods the study participants consumed. For instance, they consume teff as staple food which has high iron content but low bioavailable (not more than 10% iron constitutes are bioavailable for the consumer). In Ethiopia like other developing nations, children mainly consume plant-based foods like cereals that contain non-haem iron that are not bioavailable in the presence of iron inhibitors (phytates, phenols, calcium and tannin) and also, they are the high-risk groups for parasitic

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infections that also need iron for growth and reproduction. Thus, as children also need iron for growth and development, there is potential for competition between the host and the parasites. The plant- based food like cereals, have phytates that reacts with non-haem iron and form non-soluble polymers which are not bioavailable for the consumer [16].

As the present study showed 31.3% of the children in Enemore-Ener and 39.6% of the children in Abeshege were anemic, anemia is a health problem among the children in this area. The differences in anemia prevalence between the two districts might be due to the difference in the types of staple food consumed.

Investigators such as [17], had shown that cereals, legumes, roots and tubers contain high content of iron inhibitors such as polyphenols and phytates that provide low amounts of bioavailable iron, while this is closer to the staple food situation(teff) in Abeshege district which has high iron content but low bioavailable, a relatively better iron status in Enemore-Ener children can be accounted for by the presence of high level of bioavailable iron in Kocho that is obtained from soil through contamination during fermentation process and storage.

Even though the iron content of teff was higher than bulla/kocho, because of its lower bioavailability and the presence of phytates as iron bio availability inhibitor, children in Abeshege had high anemic status. In line with this poor nutritional knowledge and practices of rural households did not seem allow consumption of fresh vegetables and fruits that will provide vitamin C that would enhance iron bio-availability among these children as shown by [18].

Children in Enemore-Ener have lower anemic status because iron found in Kocho is readily bioavailable without any influences of iron inhibitor and because of Kocho's poor nutritional content (fat and protein) and fermented taste, kocho is seldom consumed on its own. It is often consumed with cabbage, pulses and beans, and on special occasions with meat, cheese and eggs. Thus, vitamin c in fruits and vegetables and other supplemented foods enhances the bioavailability of iron in the food they consumed may increases the iron status of children.

The present study indicated that anemia was significantly associated with IPI in Enemore-Ener district. This was a similar finding with study done among Côte d'Ivoire children [19], 45.3% of anemic children were infested by IPI infections. The mechanisms by which IPIs might cause anemia can be through blood loss, malabsorption and appetite reduction. Evidence for this has been provided by studies from Tanzanian school age children [20] and from Western Kenya [21].

Iron status measurement through two or more methods is more reliable than a single method, particularly for latent anemia which can be best assessed by determining serum ferritin levels. Accurate measurement of iron deficiency is an important health indicator, when it is used with two or more measurements of iron status [6]. Similar results reported by [8], iron deficiency with anemia can occur when a person has low values of both serum ferritin and hemoglobin, while iron deficiency without anemia can occur when a person has normal hemoglobin, but below normal serum ferritin and/or transferrin saturation. The present study also indicated that the prevalence of anemia among school age children to be similar to the studies done among school age children in different regions of Ethiopia [22,23] with a prevalence of anemia ranging from 27 to 37.6%.

Variations that might have occurred due to gender and infection status differences in relation to anemia were also examined in this study. The mean concentration of haemoglobin had a significant association with total IPI, hookworm and giardia infections could be due to nutritional disturbances by parasites resulting in low food intake, nutritional competition and iron malabsorption [24-26].

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Conclusions

Anemia was a common health problem of the school age children and strongly correlated with both prevalence of IPIs and undernutrition. Difference in the magnitude of anemic status was observed between the two districts with higher anemia in Abeshege district than in Enemore-Ener district and this correlate well with the bioavailable iron in kocho and other iron enhancers like vitamin c in foods supplemented to Kocho. An intervention strategy should be designed and implemented on current and local health problems and nutrition needs that could be important to reduce the problem of IPI, anemia, stunting and underweight among school age children in the Gurage zone.

Declarations

Consent for Publication

All authors confirm that they have agreed to publish this manuscript in ECNU journal.

Competing of Interests

The authors declare that they have no competing interests.

Authors' Contributions

MB conceived the project idea, designed the study protocol, collected the data, analyzed the data, interpreted and drafted the manuscript; BP designed the study protocol, collected the data and manuscript reviewing; KU collected the data and manuscript reviewing. All authors read and approved the final paper.

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