

Impact of Intestinal Helminth Infections on Malnutrition and Haematological Indices of School-Age Children in Gondar Town, Ethiopia

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Abstract

In Ethiopia stunting, underweight and wasting in school children are very common. The aim of this research was to evaluate the impact of intestinal helminthes and schistosomiasis on malnutrition, anaemia and haematological indices in the school age children of Azezo Elementary School in Gondar town. Kato Katz technique was

performed to determine infection and intensity of intestinal helminth infections. AnthroPlus software version 1.04 was used to get malnutrition indices while fully automated haematological analyzers were used to determine haematological and biochemical parameters. A total of 384 school children were recruited in this study. The overall prevalence of helminth infection was 45.8% (178/384) with the leading *Ascaris lumbricoides* infection (20.6%) compared to the second leading *Schistosoma mansoni* (17.4%) and third leading hook worm infections (13.3%). The prevalences of body mass indexes for age Z-scores (BAZ) indices were 9.6% (37) very sever thin, 17.2% (66) sever thin, 38.3% (147) thin, 34.4% (132) normal and 0.5% (2) overweight. On the other hand, the prevalences of height for age Z-scores (HAZ) indices were 12.2% (47) stunt, 87% (334) normal and 0.8% (3) over height. Of the total 384 school age children, 335 (87.2%) had normal blood glucose level (70 – 110 mg/dL) while the remaining 49 (12.8%) school age children were hypoglycemic. The number of the school children with normal total protein level (6.6 – 8 g/dL) was 259 (67.4%) while the remaining 125 (32.6%) school children were hypo-proteinaemic. The prevalence of anemia in the school age children was 33.1% (127). Intestinal

helminth infections were statistically significantly associated ($p = 0.000$) with hypo-proteinaemia, anemia and number of lymphocytes compared with non-infected school children. The likelihood of anemia in intestinal helminth infected school children, when it was compared with uninfected, was 148 times higher for both *Ascaris lumbricoides*-*Schistosoma mansoni* co-infection, 38 times for Hookworm, 20 times for *Schistosoma mansoni* and 3 times for *Ascaris lumbricoides* mono-infections. Conclusion: Intestinal helminth infections in school age children aggravate malnutrition. Prolonged malnutrition and intestinal helminth infections could result in stunting in school-age children.

Introduction

Soil-transmitted helminthiasis (STHs) and schistosomiasis are among the most common parasitic infections worldwide. They affect more than one billion of the world's poorest people [1]. Globally in 2010, 819 million roundworm (*Ascaris lumbricoides*), 464.6 million whipworm (*Trichuris trichiura*), and 438.9 hookworm (*Necator americanus*, *Ancylostoma duodenale*) were reported in Asia (70%), Sub-Saharan Africa (16%) and other part of the world [2]. Globally, there are around 200 million *Schistosoma* infected people with 160 million living in Sub-Saharan Africa (SSA) [3]. According to recent estimates, over 267 million pre-school age and 568 million school-age STH infected children are found in the world with more than 1.5 billion people infected by these infections [4]. According to food and agriculture (FAO) estimates, around 1.02 billion people are undernourished worldwide [5]. In spite of the efforts targeted at reducing malnutrition and hunger by Millennium Development Goals, there has not been any significant improvement [6, 7]. About 50% of all deaths among global children is related to malnutrition [8]. Malnutrition related with intestinal nematodes and other parasites was reported as a major factor in reducing physical, cognitive, and educational development in children while causing decreased fitness, cognition and work productivities in adults, outside the mortalities related to this complication [9-11]. The mechanisms by which these

intestinal helminth infections aggravate malnutrition (protein energy malnutrition, anaemia and other nutrient deficiencies) include anorexia (loss of appetite), malabsorption and decreased food intake due to nutrient loss through bleeding, vomiting or diarrhea [11-13]. Decreased barriers protection and impaired immune function during malnutrition can, in turn, aggravate the incidence of intestinal parasite infections [13].

In Ethiopia, intestinal parasitic infections have been reported as the cause of malnutrition, anemia and growth retardation among children under 5 years of age [14, 15]. Factors that increase the risk of intestinal parasitic infections such as swimming, bar foot walking (lack of shoes), bad hand washing habits and low education status of parents were also reports to be associated with malnutrition [16-18]. Researches describing the role of helminth infections and their intensity on malnutrition, anemia and normal haematological indices in primary school children are rare. The aims of this research was to evaluate the impact of intestinal helminthes (infections and intensities) on the malnutrition, anaemia and haematological indices of school children in Azezo primary school in Gondar town.

Materials and Methods

Study Area

Gondar town is located in Northwest Ethiopia with $12^{\circ}35'N$ latitude and $37^{\circ}28'E$ longitude at an altitude of around 2200 meters above sea level. It has a total population of 207,044 (98,120 men and 108,924 women) based on 2007 national population census [19]. The ranges of mean maximum and minimum temperature in 2015 were $22.7^{\circ}C - 29.7^{\circ}C$ and $17.6^{\circ}C - 22^{\circ}C$ respectively with the annual rainfall of 1151 mm. The highest rainfall was registered in July (328 mm) and August (307 mm) compared to the driest January (4 mm) and February (6 mm) in the same year [20]. This study was conducted in the Azezo-Teda sub-city. The Azezo-Teda sub-city administration is located south of the central part of Gondar. Compared to the other sub-cities, the Azezo-Teda sub-city contains urban and rural populations. The Gondar town is expanding mostly in

Azezo-Teda area. The rivers that cross Azezo-Teda sub-city are Angereb, Kaha, Shenta, Demaza and Magech rivers. The Gondar Town Administration Health Bureau regularly conducts deworming in school children in around May and June. The last date for the last deworming was June, 2017. Almost 1 year has passed since the last deworming program in the study school. Infrastructure such as piped borne water, good roads and electricity are lacking in the rural areas. Inhabitants, therefore, obtain ground water for drinking and domestic purposes. By occupation, majority are farmers, with few other working in thread mills or as laborers in central Gondar town.

Study Design

This study was a cross-sectional survey, involving systematic random sampling of school aged children in Azezo-Teda sub-city, Gondar town between February and May, 2018. Azezo elementary school was selected due to high prevalence rate of intestinal helminth infections in school age children during previous study in the Azezo area. Grade level stratified random sampling by rotary method was used to select the study participants. Pre-tested questionnaires were used to obtain demographic data and data about food shortage in the family and other possible risk factors which could be the source of malnutrition or intestinal helminth infections. Using anthropometric measures, school children were grouped as stunted, wasted, obese and Normal. Kato-Katz technique was used to determine intestinal helminth infections while automated hematological and biochemical machines were used to measure haematological and biochemical values. Appropriate statistics were used to see if intestinal helminth infections are associated with malnutrition.

Sample Size Determination Technique

The prevalence of intestinal helminth and *Schistosoma mansoni* infections (72.9%) [18] during previous study in Azezo primary school was used to calculate sample size of school-age students participated in the study. A single population proportion formula at 95% confidence interval [$n = (Z \alpha/2)^2 p (1-p) / (0.05)^2$] was used to calculate the minimum 303 sample size by

using the probability of intestinal helminth and *Schistosoma mansoni* infections during previous study. Including 30% contingency (91) for possible discarded samples and drop out study participants, a total of 404 sample size was planned to include in the study. At the end of the sampling, 20 participants were excluded due to incomplete data which made the overall sample to be 384.

Sampling Procedure

Following ethical approval by research ethical review board of University of Gondar and permission letter obtained from Azezo – Teda sub-city administration to conduct this research in Azezo government elementary school, study participants were recruited for the study. This elementary school was selected purposefully as it has students coming from both urban and rural areas and high prevalence rate of helminth infections. A stratified random sampling method was used to select the study participants based on their grade level. A rotary method was used to select the total number sample required for the study from all students attending from grade 1 up to grade 8. The students were participated in the study after the purpose and a benefit of the research explained to their parents or guardians and written consent was obtained. Only students willing to participate in the research were included. The students could also withdraw from the study at any time during the execution of the sampling process

Data Collection

After pre-tested structured questionnaire prepared in English was translated exactly to Amharic Version (National language), one of the investigators interviewed the parents or guardians to obtain data about socio-demography and risk factors for possible malnutrition and intestinal helminth infections. Then, anthropometric measures, stool and blood samples were obtained from all study participants by senior laboratory science personnels. The questionnaire contained questions related to socio-demographic factors (first part) and possible risk factors for malnutrition and intestinal helminth infections. Data about residences, accessibility of food, swimming and/or washing in rivers in the study

areas, possession of shoes and proper wearing habits and hand washing habits were collected from the study participants to address possible risk factors for malnutrition and intestinal infections. Family without known income or no or low (<1500 birr/month) salary and very low annual harvest, no television, serious problem in getting enough food and clothing was grouped as family with low accessible food. The other families with no problem of food accessibility grouped as family with accessible food. Mothers who could at least read and write were grouped as literate and those who could not read and write were grouped as illiterate. Based on residence, respondents living in Gondar town were grouped as urban inhabitants, and those in surrounding rural area were grouped as rural inhabitants.

Anthropometric Measurements

Body weight and height of the school aged children were measured using digital weighing balance and tape ruler respectively. The students were weighed without shoes and the instruments were calibrated to measure weight with 0.1 gram scale while their heights were measured with vertical length measurement with 0.1 cm scales. Measurements were taken twice to use the average for each student. Height-for-age Z-score (for stunting) and body mass-index-for-age Z-score (for wasting and thinness) were calculated using the WHO AnthroPlus software version 1.04 (WHO, Geneva, Switzerland) (available <http://www.who.int//en/>) [21]. Weight for age (WAZ) has not been used as it is not recommended for age group above 10 years. The zscores were classified based on WHO Child growth standards as stunting or wasting/thinness. Stunting is defined as insufficient height for age or when height-for-age Z score (HAZ) is less than -2 SD (standard deviation). Underweight was defined as insufficient weight for age or when weight for age Z - scores (WAZ) is less than -2 SD from standard median scores. Wasting or sever thinness is defined as insufficient mass for height (low body mass index (BMI) for age) or when body mass index for age Z - score is less than -2 SD [22, 23].

Collection of Stool Samples and Parasitological Examination

Sterile sample bottles were distributed to consenting school-aged children. Collected specimens were analysed using the Kato-Katz technique to determine the presence of intestinal helminths ova [30, 31]. The Kato Katz technique was performed on the same date to determine infection and intensity of infections. Kato-Katz slides thick stool smears prepared from 40.7 milligram stool filtrate were examined microscopically within 30 to 60 minutes after preparation to count helminthes eggs and multiply by 24 .6 to get egg per gram stool (epg).

Collection of Blood Samples and Haematological Analysis

About 3 ml venous blood was collected from consenting school-aged children in a sterile tube containing anti-coagulant. The tube was transported quickly to the University of Gondar before the blood was analysed using fully-automated haematological cell counter (Mindray BC-3200). The parameters assessed include total white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hgb), packed cell volume (PCV), hematocrit value; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes and neutrophils. The haematological values were classified as normal, below normal and above normal according to standard normal range values [24]. In addition, 2 mm of venous blood sample were collected using sterile syringe from the arm of the consenting school -aged children. The blood samples were placed in a separator test tube and centrifuged at 3000 rpm for 5 minutes. The separated serum was analysed for glucose and total protein concentrations by using Mindray BS-200E chemistry analyser to determine their normal concentration in the blood according to standard reference values [25].

Ethical Consideration

Ethical approval was obtained from the University of Gondar ethical review committee. Written consent was obtained from study participant's parent/guardian after permission was obtained from Teda - Azezo district administration and school administration.

Laboratory results were kept confidential and; children who were infected with helminth infections and with abnormal hematological test were linked to the Gondar district health centre for free treatment.

Statistical Analysis

Data about socio-demography and the possible risk factors were collected using questionnaire before the results for malnutritional indices, intestinal helminth infection and haematological tests obtained. The data collected was cleaned in an Excel spread sheet before transferred to statistical package for social sciences (SPSS) version 20 for statistical analysis. Descriptive statistics was used to analyze the prevalence of the different outcome variables. Mann whitney test (non-parametric analysis of variance (ANOVA)) was used to determine the existence of gender or age group related statistically significant difference for intestinal helminth infection, intensity of infection, co-infections, infecting species, anaemia, stunting, wasting and haematological categories in the school children. Binary logistic regression test was used to see association between intestinal helminth infection, anemia, stunting, wasting, age groups, malnutrition indices and haematological categories. Multiple logistic regression analysis was used to determine the likelihood of anemia or odd ratio (OR) in school children, when it was compared with uninfected, mono-infected, coinfecting and species of intestinal helminth. For the statistical analysis, p-value < 0.05 was considered as significant different for 95% confidence interval.

Results

A total of 384 school children (179 (46.7%) males and 205 (53.3%) females) were recruited in this study with age ranged from 7 to 15 years. Of the total school children, 39.3% (151) were in 7-10 years age group while 60.7 % (233) were in 11-15 year age group (Table 1). Mean \pm St.D (Median) (range) for height and weight for the all ages were 140.93 cm \pm 11.4 (140) (114-178) and 30.6 kg \pm 7.3 (29.50) (15-55) respectively. Linear increase in height and weight for different age from age 7 to 15

were observed in statistically significant different manner for mean (P=0.000) and median (P=0.000) values.

The overall prevalence of helminthes infections in the school children was 45.8% (178/384) with 56 light, 47 moderate and 75 heavy infections (Table 2). *Ascaris* infection (20.6% or 79/384) was the leading infection. The second and third leading infections were *Schistosoma mansoni* and hookworm infections with prevalence of 17.4% (67/384) and 13.3% (51/384) respectively. The prevalent of the remaining *Tricuris trichura*, *Hymenolopis nana* and *Taenia* spp were 3.4%, 1% and 0.3% respectively. Of the total 178 school children found infected, 37(9.6%) were with double infections (15 *Ascaris* - *Schistosoma*, 12 *Ascaris* - *Hookworm*, 4 *Schistosoma* - *Hookworm* and 6 *Ascaris* and others) and 5 (2.8%) were with triple infections (4 *Ascaris* - *Schistosoma* - others and 1 *Ascaris* - *Hook worm* - *Tricuris trichura*).

Of the total school children analyzed for body mass index for age Z scores (BAZ), 37(9.6%) were very sever thin (sever wasted) (Z scores < -3SD), 66 (17.2%) sever thin (wasted) (-3 SD < Z < -2SD), 147 (38.3%) thin (-2 SD < Z < -1SD), 132 (34.4%) normal (-1SD < Z 2SD). Only 34.4% were included with in the 25th and 75th percentiles of the WHO normal distribution curve. Of all heights for age analyzed, 7(1.8%) were severely stunted (< - 3 SD), 40 (10.4%), stunted (< -2SD), 334 (87 %), normal height for age (-2SD > Z < 2SD) and 3(0.8%) more height for age (> 2SD). Only 87 % were included in the 15th and 85th normal curve range. Gender showed no statistically significant differences (p>0.05) for BAZ and HAZ scores (table 3). The prevalence of overall stunting in 7-10 age group was 0.7% (1/146) compared to 19.5% (46/236) in 11-15 age group. Stunting was statistically significantly different in 7-10 and 11-15 age group intervals (p=0.000). Of the total 47 school children with stunted growth, 25(53%) were male while the 22 (47%) were female. Statistically significant difference was not observed (p=0.7) for gender. Of total 47 stunted school children, the percentage with light, moderate, heavy and none infections were 34%, 4.3% 12.8% and 48.9% respectively. Of 337 school children who were not stunted,

Table 1. Socio-demographic characteristics of study participants in Azezo primary school, Gondar Town, Northwest Ethiopia.

variables	category	Frequency	Percentage (%)
Sex	Male	179	46.6
	Female	205	53.4
	Total	384	100
Age group	7-10 years	151	39.5
	11-15 years	233	60.5
	Total	384	100
Residence	Urban	153	39.8
	Rural	231	60.2
	Total	384	100
Educational level of mother	illiterate	198	51.6
	Primary education	110	28.6
	Secondary education	60	15.6
	Diploma and above	16	4.2
	Total	384	100

Table 2. Prevalence and Intensity of intestinal helminth infections in Azezo primary school, Gondar town, Northwest Ethiopia. May, 2018.

	Light infection	Moderate Infection	Heavy infection	None infection	Total(%)
Ascarislumbercoides	1	11	30		42(10.9%)
Schistosomamansoni	18	11	11		40(10.4%)
Hookworm	21	13	0		34(8.9%)
Trichuristrichura	10	2	2		14(3.6%)
Hymenolopis nana	3	1	0		4(1%)
Taenia species	1	0	0		1(0.3%)
Ascaris - Schistosoma	0	2	13		15(3.9%)
Ascaris - Hookworm	0	2	10		12(3.1%)
Schistosoma - Hookworm	1	2	1		4(1%)
Ascaris - others	0	2	4		6(1.6%)
Ascaris -Schistosoma - others	1	2	1		4(1%)
Ascaris - hookworm - others	0	0	1		1(0.3%)
Schistosoma - Hookworm - others	0	0	1		1(0.3%)
None infected	-	-	-	-	206(53.6%)
Total	56	48	75	206	384(100%)

Table 3. Prevalence of malnutrition indices in Azezo primary school, Gondar town, Northwest Ethiopia. 2018.

Prevalence of Body mass indexes for Age Z-scores (BAZ) indices				Prevalence of Body mass indexes for Age Z-scores (BAZ) indices			
Indices	Male	Female	Total(%)	Indices	Male	Female	Total(%)
Wasting (< -3 SD)	23(12.8%)	14(6.8%)	37(9.6%)	Stunt ($3SD < Z < 2SD$)	25(14%)	22(10.7%)	47(12.2%)
Sever thinness ($-3SD < Z < -2SD$)	33(18.4%)	33(16.1%)	66 (17.2%)	Normal ($-2SD < Z < 2SD$)	153 (85.5%)	181 (88.3%)	334(87%)
Thinness ($-2SD < Z < -1SD$)	57(31.8%)	90(44.4%)	147 (38.3%)	Over height	1(0.6%)	2(1%)	3(0.8%)
Normal ($-1SD < Z < 1SD$)	64(35.8%)	68(32.7%)	132 (34.1%)				
Overweight ($1SD < Z < 2S$)	2(1.1%)	0(0%)	2(0.5%)				
Total	179 (100%)	205 (100%)	384 (100%)	Total	179 (100%)	205 (100%)	384 (100%)

Table 4. Prevalence of haematological parameters in Azezo primary school, Gondar town, Northwest Ethiopia. 2018.

d	Normal	Below normal	Above normal
1. Glucose concentration	335(87.2%)	49(12.8%)	0(0%)
2. Protein concentration	259(67.4%)	125(32.6%)	0(0%)
3. Red Blood Cells	366(95.3%)	18(4.7%)	0(0%)
4. White blood cells	31.3(81.5%)	32(8.3%)	39(10.2%)
5. Haemoglobin concentration	361(94%)	23(6%)	0(0%)
6. Hematocrit values or packed cell volume (PCV)	329(85.7%)	55(14.3%)	0(0%)
7. Mean corpuscular volume (MCV)	368(95.8%)	16(4.2%)	0(0%)
8. Mean corpuscular hemoglobin (MCH)	309(80.5%)	75(19.5%)	0(0%)
9. Mean corpuscular hemoglobin concentration(MCHC)	240(62.5%)	143(37.2%)	1(0.3%)
10. Lymphocytes count	260(67.7%)	0(0%)	124(32.3%)
11. Neutrophil count	203(52.9%)	0(0%)	181(47.1%)

Table 5. Association between intestinal helminth infection, malnutrition indices and haematological parameters. May 2019.

Variables (Number)	Age group			Helminthes infection:			Stunted:			Wasted:			Anaemic:		
	N(%)	P-values	OR	N(%)	P-values	OR	N(%)	P-values	OR	N(%)	P-values	OR	N(%)	P-values	OR
Hypo-glycemic - (49)	19(38.8%)	0.9	1.1	41(23.03)	0.28	0.6	4(8.5%)	0.71	1.3	9(13.6%)	0.85	1.1	37(29.1)	0.21	0.4
Normal (335)	132(39.4%) 203(60.6%)			137 (76.97)			43(91.5%)			57 (86.4%)			88(69.3)		
Hypo-proteinemic- yes (125)	54(43.2) 71(56.8)	0.059	1.7	93(52.2)	0	3.4	10(21.3)	0.237	0.6	23(34.8)	0.99	1	178 (61.4)	0.007	2.4
Normal (259)	97(37.5)			85(47.8)			37(78.7)			43(65.2)			49(38.6)		
RBC count - Normal(366)	146(96.7) 220(94.4)	0.61	1.4	162 (91.01)	0.84	1.2	45(95.7)	0.84	1.2	62(93.9)	0.455	0.6	112 (88.2)	0.15	0.3
RBC count - Below Normal (18)	5(3.3) 13(5.6)			16(8.99)			2(4.3)			4(6.1)			15(11.8)		
WBC count - Normal (352)	137(90.7)	0.94	1	11(6.2)	0.24	1.8	1(2.1)	0.11	5.6	6(9.1)	0.43	0.7	10(7.9)	0.7	1.3
WBC - below Normal(32)	14(9.3) 18(7.7)			167(93.8)			46(97.9)			60(90.9)			117 (92.1)		
MCH - normal(309)	125(82.9)	0.57	1.2	50(28.1)	0.91	1	11(23.4)	0.16	0.5	8(12.1)	0.02	2.9	48(37.8)	0.01	2.4
MCH - below normal(75)	26(17.2)			128(71.9)			36(76.6)			58(87.9)			79(2.2)		
MCHC - normal(241)	93(61.6) 148(63.5)	0.27	0.8	75(42.1)	0.75	1.1	18(24.3)	0.77	0.9	27 (40.91)	0.25	0.7	67(52.8)	0.01	0.4
MCHC - below normal(143)	58(38.4)			102(57.3)			29(61.7)			39(59.1)			60(47.2)		
Lymphocytes -Normal (260)	105(69.5) 215(92.3)	0.39	1	69(38.8)	0.01	1	15(31.9)	0.62	1	15(22.7)	0.77	1	47(37)	0.79	1
Lymphocytes - above Normal (124)	46(30.5) 18(7.7)			109(61.2)			32(68.1)			51(77.3)			80(63)		
Neutrophils - normal (203)	82(54.3) 121(51.9)	0.58	1.1	87(48.9)	0.93	1	21(44.7)	0.59	1.2	27(40.9)	0.33	1.3	63(49.6)	0.86	0.9
Neutrophils - above normal (181)	69(45.7) 112(48.1)			91(51.1)			26(55.3)			39(59.1)			64(50.4)		
No Helminth inf. (206)	97(64.2) 109(46.8)	0	0.4	-			24(51.1)	0.198	1.7	36(54.5)	0.39	1.4	110 (86.6)	0	12
Helminth infection (178)	54(35.8) 124(53.2)			-			23(48.9)			30(45.5)			17(13.4)		
No Stunted(337)	150(99.3) 187(80.3)	0.001	0.03	154(86.5)	0.39	1.4	-			59(89.4)	0.49	0.7	116 (91.3)	0.19	0.5
Stunted(47)	1(0.7) 46(19.7)			24(13.5)			7(10.4)			11(8.7)					
Wasted	66	0.225	0.7	36(20.2)	0.489	1.3	7(14.9)	0.59	0.8	-			28 (22.05)	0.399	1.4
Not Wasted	318			142(79.8)			40(85.1)			99 (77.95)					
Anaemic - yes	47(31.1)	0.394	1.3	108(60.7)	0	13	11(23.4)	0.135	0.5	40(60.6)	0.349	1.4	-		
Not anaemic(257)	104(68.9) 155(66.5)			70(39.3)			36(76.6)			26(39.4)					

and the percentage with light, moderate, heavy and none infections were 11.9%, 13.4%, 20.5% and 54.3% respectively.

Ascaris lumbricoides, *Schistosoma mansoni* and Hookworm mono-infection or *Ascaris lumbricoides* – *Schistosoma mansoni* and *Ascaris lumbricoides* – Hookworm co-infections were statistically significantly associated ($P \leq 0.001$) with protein malnutrition (hypoproteinemia) and anaemia compared to non-infected school children. The overall infection was also statistically significantly associated with hypoglycemia, hypoproteinaemia and anemia ($p = 0.000$) (Table 4). Intestinal helminth infections, intensity, mono or coinfections between stunted and normal school children were not statistically significantly different ($P > 0.05$). Mann – Whitney analysis of variance indicated intestinal helminth infections were not statistically significantly different ($p > 0.05$) for different gender. Intestinal helminth infections were highly prevalent in rural (57.1%) compared to urban residence (30.1%) in statistically significant different ($p = 0.000$) manner. Swimming or washing in the rivers were also more common for rural residences ($p = 0.04$). But, Swimming or washing in the rivers were not associated with stunting, wasting and anaemia ($p > 0.05$). The prevalence of anemia in females was 32.6% compared to 29.1% in males. Anemia was not statistically significantly different for gender ($p = 0.17$). The prevalence of hypo-glycemia and hypo-proteinaemia were high in anemic school children compared to normal in statistically significantly different manner ($p < 0.05$). The prevalence of anemia in intestinal helminthes infected school children was 60% compared to 8.3% in non-infected children and the difference was statistically significant ($p = 0.000$). The incidence of anemia was 12 times higher in intestinal helminthes infected school children compared to non-infected. In school children with below normal MCH values, 61.3 % were anaemic compared to only 25.6% in school children with normal MCH values (27 – 30 pg). In school children with below normal MCHC values, 44.8% were anaemic compared with only 25% in school children with normal MCHC values (33 -38.5 g/dL). Statistically

significant differences were observed in prevalence of anemia between school children with below normal and normal MCH and MCHC vales ($P \leq 0.01$) (Table 5).

Discussion

In school children, chronic schistosomiasis contributes to anemia and under-nutrition, which, in turn, can lead to growth stunting, poor school performance, poor work productivity, and continued poverty while blood loss due to hook worm infection can cause iron deficiency anemia and hypo-proteinaemia [26]. Heavy *S. mansoni* infected children in Brazil (above 400 eggs/g of stool) showed 2.74 fold higher risk of stunting compared to uninfected children [27]. Ascariasis cause malnutrition in addition to pathology associated the worm migration in the body. Chronic dysentery associated with trichuriasis is also a major problem in malnutrition and health of school children [28]. Ethiopia, the second populous nation in Africa, has been classified as low income countries with 20% of people living under poverty in both urban and rural areas [29]. Low socioeconomic status or low accessibility of food (poverty) in Ethiopia could be the main cause of malnutrition [30,31] similar to this study result. Factors that increase the risk of intestinal parasitic infections in Ethiopia such as swimming, bar foot walking (lack of shoes), bad hand washing habits and low education status of parents were also reports to be associated with malnutrition [18]. In Ethiopia, intestinal parasitic infections in children are reported to be associated with malnutrition, anaemia, and growth retardation [32].

The prevalence of stunting related to malnutrition in under 5 years in Ethiopia reduced from 64% in 1990 to 47% in 2008 before further reduction to 40.4% in 2014 [33-35]. The prevalence of intestinal helminthes infections in this study (45.8%) is lower than the previous report (2008) in another Azezo primary school (72.9%) [18]. The reduction in the intestinal helminth infecions could be due to the recent regular deworming program in the primary schools.

Ascaris lumbricoides, *Schistosoma mansoni* and

hookworm mono-infection in addition to *Ascaris lumbricoides* – *Schistosoma mansoni*, *Ascaris lumbricoides*–Hookworm co-infections and triple infections were statistically significantly associated with protein malnutrition (hypo-proteinemia) and anaemia compared with none infected school children ($P \leq 0.001$). This type of association in which intestinal helminthes infections aggravate the situation of malnutrition was reported to be common in children [36]. Prevalence of stunting and anaemia were reported higher in male than in female in Kenya [37]. But, gender did not show any difference ($p>0.05$) for prevalence of stunting or anaemia in the school children studied (Table 4). The prevalence of anaemia was affected by the species and intensity of infections of intestinal helminthes in statistically significant ways ($p=0.000$) compared with none infected school children. The likelihood of anemia in school children, when it was compared with uninfected, increased 148 times for both *Ascaris lumbricoides*–*Schistosoma mansoni* co-infection, 38 times for Hook worm, 20 times for *Schistosoma mansoni* and 3 times for *Ascaris lumbricoides* mono-infection. But in Kenya *S. mansoni* mono-infection was reported to associate with anaemia and the likelihood of anemia in *Schistosoma* infection was 3.68 times compared with non- infected children [37].

Except for one school child (0.3%), there was no problem of overweight in the current study in school age children. Only 34.9% of the school children were with in WHO normal range based on BAZ scores. Majority (65%) were in state of thinness or underweight (thin, wasted or sever wasted). Thinness (underweight) were not statistically significantly associated ($p>0.05$) with age and sex. All these results could show malnutrition was very common in all children and might be related with the low socio-economic background of the community in Azezo areas and high prevalence of intestinal helminth infections. Poverty related malnutrition, most probably, have additional effect on intestinal helminth and *Schistosoma mansoni* infections development of school age children in Ethiopia as opposed to previous report which

stated the widespread prevalence of intestinal parasitosis and malnutrition among school children in Ethiopia were not associated with anaemia and/or stunting [38]. According the study conducted in school age children in Fincha'a sugar estate in western Ethiopia where children regularly dewormed and get relatively good health facilities, poverty (lack of job) was the source of stunting compared to *Schistosoma mansoni* infection which is the most important problem in the areas [39]. The lack of any effect of intestinal helminth and *Schistosoma mansoni* infections pus malnutrition on stunting and/or underweight in Zegie Peninsula villages (northwestern Ethiopia) located on or near Lake Tana [40], most probably, related to the effective regular deworming process and easy access to get fish to use as food.

In this study, analysis of HAZ scores showed 97.9% of the stunting in the school age children was found in 10 - 15 years age group compared to 2.1% in 7-10 years age group ($p=0.000$). Similar statistically significantly different ($p=0.000$) for high stunting prevalence (56.4%) in 10-15 age group compared to lower (33.6%) in 5-10 age group was reported in school children in Macha district in Northwest Ethiopia [41]. A total of 50.1% stunting in 12-14 age group school children compared to 36.9% in 6-11 age group ($p=0.000$) was also reported in Arbaminch town (Southern Ethiopia) [42]. Frequent stunting in children above 10 years compared to those under 10 was reported in Angola due to prolonged problem of food shortage during previous war time [40]. From the fact that anemia prevalence, glucose or protein malnutrition were not different between the age groups ($p>0.05$) (almost the same probability) in addition to lack of difference in incidence of intestinal helminth infections between stunted and normal school children ($P=0.49$), most probably, stunting was the result of prolonged malnutrition related to poverty and parasitic infection and re-infection during early childhood. Stunting may not be restricted to areas with war and prolonged shortage of food as indicated by Olivera et al [43]. Prolonged malnutrition and infection and re-infection of intestinal

helminth in developing nation could be the main source of stunting.

Probably, swimming habits of school children in the rivers in the study areas, unhygienic and bare foot walking habits mentioned during previous study [18] could be the important risk factor for high prevalence of intestinal helminth infections and anemia in Azezo school children. High incidence of *Ascaris lumbricoides*, *Schistosoma mansoni* and hookworm infections were statistically significantly associated with lower MCH values ($P \leq 0.001$). Similarly, *Schistosoma mansoni* and hookworm infections were associated with below normal MCHC level ($p = .000$). Below normal concentration of MCH and MCHC were statistically significantly associated with anaemia (Table 5). Similar study in Thailand has indicated statistically significantly lower ($P < 0.000$) MCH, and MCHC levels in helminthes infected group compared to the helminth-free group [40][44].

Conclusions

Helminth infection is associated with anaemia and hypo-proteinaemia, lower MCH and MCHC levels. The likelihood of anemia is very high in mono-and co-infected compared to uninfected school children. Stunting is the product of prolonged malnutrition and repeated reinfection of intestinal helminth infections in school children. Intestinal helminth infections most probably aggravate the malnutrition in school children with low accessibility of food supply. Regular monitoring of nutritional status of school children and screening and treating intestinal helminthes are required including school feeding, deworming, clean water supply and public health awareness.

Limitation of the Study

Normal ranges of haematological and biochemical values obtained from literatures were used as reference to classify the results of this study due to lack of normal range values specific for school –age children in Ethiopia.

Abbreviations

BAZ – Body mass index for age Z-score

EPG – Egg per gram

HAZ – Height for age Z-score

SD – Standard Deviation

WBC - white blood cells RBC - red blood cells

Hgb- hemoglobin concentration

PCV - packed cell volume

MCV-mean corpuscular volume

MCH-mean corpuscular hemoglobin

MCHC-mean corpuscular haemoglobin concentration

Declarations

Ethics Approval and Consent to Participate

Ethical clearance was obtained from Gondar University after proposal was reviewed by ethical review board of the University.

Consent for Publication

The author consents to Editorial Board to publish the paper. The author(s) accept responsibility for publishing this material in his own name, if any.

Availability of Data and Materials

The data analysed is available in the corresponding author and could be available on reasonable request.

Competing Interests

The authors declare that he has no competing interests.

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Authors' Contributions

MB, DT and LW designed the research and participated in the research. DT collected the samples and processed them. MB and WL supervised the processes. DT and WL analyzed the data. WL prepared this manuscript.

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