Original article

Types of anaemia due to hookworm infection among the populations of Wolisso

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Abstract: A community-based cross-sectional study to investigate the association between hookworm infection and anaemia was carried out on 227 apparently healthy individuals living around Wolisso. Of these subjects, 155 were positive and 72 were negative for hookworm infections. It was found out that 32 (20.6%) of the hookworm positvie and 4 (5.6%) of the hookworm negative individuals were anaemic. Chi-square analysis showed hookworm infection was significantly associated with low haemoglobin (Hb), low transferrin saturation (TS) and low ferritin levels. Intensity of infection as expressed in eggs per gram of faeces showed a highly significant negative association with TS and serum ferritin levels ($p<0.001$) but not with Hb levels. The degree of anaemia could be considered as mild, however, the light hookworm infection could possibly lead to iron deficiency anaemia. When multiple criteria involving elevated mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and macrocytic blood picture were taken as suggestive of a macrocytic type of anaemia, 20 (13.0%) of the hookworm positive and 4 (5.6%) of the hookworm negative individuals fell into this category. However, the association between parameters suggestive of macrocytic anaemia and hookworm infection was not strong. [*Ethiop. J. Health Dev.* 1999;13(1):33-39]

Introduction

Anaemia is a major public health problem throughout the world, especially in developing countries (1). In Ethiopia, anaemia has not been considered as a serious public health problem even in the high risk groups such as pregnant women (2). Prevalence rates as high as 40.5% in the general population (3) and 47.2% in children (4) were reported from north-western Ethiopia. Higher rates of about 57% have also been reported in pregnant women in Jimma, Ethiopia (5).

Anaemia is known to have multiple etiologies. Particularly in the tropics, deficiency of essential substances resulting from the effect of blood loss due to hookworm infection is one of the most important factors (6). It has been previously reported that the type of anaemia associated with hookworm infection is iron deficiency with a typical hypochromic microcytic blood picture (7-10). There are also a few reports, though not well established, suggesting the possible associations of heavy hookworm infections with deficiencies of vit B_{12} and folic acid or megaloblastic erythropoiesis (8, 11-13). With regard to Ethiopia, despite the fact that hookworm infection is reported as an important occupational health problem in many areas (14-16) its association with anaemia is not well assessed and documented. The available information is obtained either from hospital patients (17,18) or from a community- based study with its own limitations (19). Although hospital based studies are generally regarded as unsatisfactory

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representatives of a population, in view of the scarcity of other community-based studies, they can shed some light on the existence of the problem in the country. The study of Bulto and colleagues (19) provides a better picture pertaining the relationship between hookworm infection and anaemia in a given community. However, this study did not assess the effect of other confounding factors such as malaria as stated by the authors. There was no data on intensity of infection and the nature of the anaemia was not described.

The present study describes the association of the type of anaemia due to hookworm infection among the populations living around Wolisso.

Methods

Subjects and study design: A cross-sectional study was conducted during the months of November-January 1994/95 on 227 apparently healthy individuals living in three rural villages around Wolisso, Western Ethiopia (8^0N, 38^0E) about 2100 m above sea level. These villages were surveyed by the Integrated Family Planning, Health Education and Parasite Control Project (IP) of the Ethiopian Health and Nutrition Research Institute (EHNRI) and hookworm infection was indicated as the most serious problem in the area with prevalence rate greater than 80%. The sample size was estimated by considering the odds ratio reported by Bulto and colleagues (18) that is, by taking into account the risk of anaemia in both exposed and unexposed groups using the EPI-info version 5 statcalc programme to provide a confidence interval of 95% and a power of 90%.

The study was conducted after recieving the full consent of the subjects. Based on the estimated sample size, 155 individuals positive for hookworm ova only and 72 individuals negative for all intestinal helminths participated in the study. Pregnant women, malaria positive individuals, and those with bleeding disorders were excluded to minimize the role of other confounding factors. Children under 14 years of age were also omitted on account of taking anthelmintic treatment which was distributed by IP. Hookworm infected subjects and anaemic individuals were treated at the health centre.

Parasitological analysis: Faecal samples from all subjects were collected at the field laboratory. On the same day of collection, hookworm ova were counted for each sample following the improved Kato thick smear method of Martin and Beaver (20). Ova counts were expressed as eggs per gram (epg).

Haematological analysis: Venous blood was collected from each subject into two tubes using the vacutainer system; one of the tubes containing ethylene diamine tetraacetic acid (EDTA) for haematological analysis and a plane tube to separate sera for the biochemical analysis. Blood smears were prepared from fresh whole blood samples and fixed with methanol. The sera and whole blood samples were transported to EHNRI laboratory on the same day of collection.

Coulter counter T-540, which was standardized against a 4C plus blood control, was used for the whole blood analysis giving results for white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), haematocrit (Hct), and platelets (Plts). The red cell indices mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentraiton (MCHC) were calculated using standard formulae (21). The slides were stained with May-Grunwald Giemsa stain and inspected for any abnormality and the percentages composition of the different white cells were determined by counting 200 cells per slide.

Biochemical analysis: Ferritin concentration was determined by an enzyme linked immunosorbent assay on ES 300, a fully automated multibatch immunoanalyser. The procedure discussed in Evatt et al. (22) was adopted for serum iron determination and that of Piccardi et al. (23) for the Total Iron Binding Capacity (TIBC). Transferrin saturation (TS) was calculated for each subject from the values of the total serum iron and the TIBC and expressed as percentage (24).

Data processing and analysis: Data entry and analysis were done using a D base III$^+$ and SPSS/PC$^+$ programmes, respectively. Statistical methods employed include: Chi-squared (χ^2) test, one way analysis of variance (ANOVA), Student t-test, F-test, and correlation analysis. A p-value less than 0.05 was taken as statistically significant. As the distribution of ova count was very skewed, the mean egg count was calculated after logarithmic transformation; however, the results are presented in the original units.

Ethical considerations: The project protocol was evaluated and accepted by the ethical committee of EHNRI.

Results

The study populations were of similar socio-economic group with ages ranging between 15 and 75 years. They were composed of 101 (44.5%) hookworm positive and 56 (24.7%) of hookworm negative individuals between the ages of 15 and 45 years, and 54 (23.8%) hookworm positive and 16 (7.1%) hookworm negative individuals above 45 years. The overall male to female ratio was 1.18 : 1.

The findings of quantitative analysis of stool showed that 67 (43.2%) had counts between 1 - 100, 59 (38.1%) had counts of 101 - 500, 14 (9.0%) had counts of 501 - 1000, and the remaining 15 (9.7%) had counts above 1000 per gram of faeces, including the 3.2% with counts above 2000. The maximum ova count encountered was 6785 epg in one subject. The mean ova count with the standard deviation was 156 ± 3.67 epg.

Table 1: **Mean and standard deviation values for haematological measurements of the study population of Wolisso in 1994/95.**

Parameters[a]	HK (+)[b] (n=155)		HK (-) [c] (n=72)		p[d]
RBC	4.62	(0.09)	4.85	(0.42)	<0.01
Hb	14.33	(1.41)	14.87	(1.09)	<0.01
Hct	42.99	(4.27)	44.60	(3.37)	<0.01
MCV	93.08	(4.47)	103.75	(99.17)	NS
MCH	31.06	(1.77)	30.28	(3.81)	<0.05
MCHC	33.36	(0.87)	33.35	(0.67)	NS
Plt	313. 42	(95.81)	297.15	(85.38)	NS
WBC	7.83	(2.91)	7.65	(2.45)	NS

[a] The units for haematological parameters are as follows:
RBC = x 10^{12}/L, Hb in g/dl, Hct in %,
MCV = femto litre (fl) MCH = pico gram (pg), MCH = g/dl,
WBC = x 10^{9}/L. Figrures in parentheses are standard deviations.
[b] Hookworm positive [c] Hookworm negative [d] t-test for significant difference between means. NS for non-significant difference.

The overall means and standard deviations of haematological and biochemical measurements are presented in Tables 1 and 2, respectively. The mean RBC, Hb and Hct values of hookworm positive individuals were significantly lower ($p<0.01$) than the hookworm negative individuals. The differences were found to be highly significant ($p<0.001$) for the iron status parameters. Correlation analysis revealed that TS correlated positively with Hb, Hct, and the serum iron levels (TS with Hb, Hct, serum iron; $r = 0.408$, $r = 0.420$, $r = 0.840$, $p<0.001$, respectively) and ferritin correlated positively with Hb and Hct ($r = 0.259$, $r = 0.251$, $p<0.001$, respectively) and negatively with TIBC ($r = - 0.168$, $p<0.05$). A very strong positive correlation was found between haemoglobin and haematocrit ($r = 0.977$, $p<0.001$); hence haemoglobin values were taken to classify individuals as anaemic and non-anaemic.

Table 2: **Mean and standard deviation values for the biochemical assays of the study population of Wolisso in 1994/95.**

Parameters	HK (+)[a] (n=155) SD	HK (-) [b] (n=72) SD	p [c]
Serum iron (mg/L)	0.84 (0.32)	1.05 (0.34)	<0.01
TIBC (mg/L)	3.58 (0.79)	3.21 (0.56)	<0.001
TS (%)	24.45 (10.25)	33.39 (10.81)	<0.001
Ferritin (ng/ml)	42.38 (24.98)	66.13 (45.78)	<0.001

[a] Hookworm positive [b] Hookworm negative [c] t-test for significant difference between means.

When judged by the altitude-adjusted World Health Organization (WHO) (25) cutoff levels for haemoglobin, 32 (20.6%) of the hookworm positive and 4 (5.6%) of the hookworm negative individuals were anaemic (Table 3). Haemoglobin values less than 12.8 for females and 13.9 for males are regarded as the altitude-adjusted "equivalent" cutoffs levels (WHO recommendation + 7%) to characterize anaemia prevalence in the Ethiopian highlands between 2000 - 2500 metres (2). The results in Table 3 also show a statistical association between hookworm infection and the three parameters of progressive stages of iron deficiency anaemia.

Intensity of infection as expressed in epg showed a highly significant negative relationship with transferrin saturation and serum ferritin levels ($p<0.001$) but not with haemoglobin levels ($p>0.05$) (Table 4). As determined by the serum ferritin levels,

Table 3: **Association of anaemia parameters with hookworm infection of the study population of Wolisso in 1994/95.**

Parameters	HK (+)[a]		HK (=)[b]		Odds ratio	X^2	P[c]<
	n	%	n	%			
Ferritin Normal (NR)	128	82.6	71	98.6	14.98	10.25	0.01
below NR	27	17.4	1	1.4			
TS normal	125	80.5	71	98.6	17.18	12.08	0.001
below NR	30	19.5	1	1.4			
Hb normal	123	79.4	68	94.4	4.42	7.30	0.05
below NR	32	20.6	4	5.6			

[a] Hookworm positive [b] Hookworm negative [c] Chi-square (X^2) analysis for testing significant associations between anaemia parameters and hookworm infection.

relatively higher proportions of the hookworm positive individuals had precarious iron stores than the hookworm negative individuals.

When multiple criteria involving elevated MCV (>97 fl), MCH (>31 pg) (26) and macrocytic blood picture were taken as suggestive of a macrocytic type of anaemia, 20 (13.0%) of the hookworm positive and 4 (5.6%) of the hookworm negative individuals fell into this category. However, Chi-square analysis revealed that the association was not statistically significant (odds ratio = 2.54, X^2 = 2.13, $p>0.05$).

Discussion

The present work has attempted to examine the association between hookworm infection and anaemia among the population living in villages around Wolisso. The significant association between hookworm infection and anaemia is in agreement with the findings of

Bulto and colleagues (19) who reported about three times the risk of developing anaemia in their hookworm positive adult subjects. The results of biochemical analysis show iron deficiency as the major cause of anaemia in the infected subjects. Hookworm infected individuals were at greater risk of being iron deficient than those who were not infected (Table 3). The observed significant association of intensity of infection with serum ferritin levels and TS but not with haemoglobin levels (Table 4) emphasizes the effect of such low variation in intensity of infection on the iron status of the subjects. However, the variation in intensity was not as such remarkable enough to affect the haemoglobin values at the various intensity categories. This finding is generally in line with the general concept that iron deficiency first affects iron stores, then transferrin saturation, and lastly haemoglobin production (27). Pritchard and co-workers (28)

Table 4: **Anaemia parameters and their relationship to hookworm ova load.**

egg count (epg)	n[c]	Haemoglobin (g/dl)[a]	TS (%)[a]	Ferritin (ng/ml)[a]
0	72	14.66 ± 2.05	32.93 ± 11.43	65.22 ± 46.12
1 - 100	67	14.44 ± 1.18	26.13 ± 9.32	51.62 ± 27.52
101 - 500	59	14.41 ± 1.69	24.59 ± 11.13	39. 17 ± 20.86
501 - 1000	14	13.91 ± 1.24	19.50 ± 10.62	28.02 ± 21.38
> 1000	15	13.39 ± 1.28	20.93 ± 8.78	27.13 ± 12.62
		$P^b > 0.05$	$P^b < 0.001$	$P^b < 0.001$

[a] Mean ± standard deviation. [b] One way analysis of variance testing a null hypothesis of no segnificant difference between group means. [c] Number of subjects.

reported a similar finding, though they determined intensity of infection by worm burden. The finding of the association between hookworm infection and serum ferritin levels in the present study contrasts with that of Herceberg et al (29) and Eager et al (30), though no information regarding intensity of infection was provided in the latter case.

When the degree of anaemia was graded into mild, moderate, and severe (24), all individuals had what would be described as mild anaemia (Hb between 10 g/dl and the cut-off value). This could be attributed to the low intensity of infection. Our data supports previous findings in lightly infected subjects (31,32). On the othe hand, this result is in contrast to other studies, which failed to demonstrate such associations at lower intensities (33,34). One of the possible explanations for the observed disagreement with these results could be variations in the duration of infection which could not be established. Moreover, Roche and Layrisse (8) have pointed out that infections which are light at the time of examination might have been much heavier previously. Perhaps, the subjects of the present study could have been heavily infected previously. Thus, the mild infection was found to have a highly significant association with iron status parameters (transferrin saturation and ferritin) but not with parameters suggestive of macrocytic anaemia.

Even though in Ethiopia, no report is available for the existence of the problem in the community at large, Shamebo (35) reported that about 9.3% of the haematological abnormalities in hospitalized patients were due to megaloblastic anaemia. Prior to Shamebo (35), Abdulkadir (36) also suggested that folic acid deficiency, the major cause of megaloblastic anaemia, would be more common than appears to be the case otherwise in Ethiopia. This is so because the consumption of fresh vegetables is very low in many communities; and folates are known to be destroyed by prolonged cooking (37).

A much better information about the role of hookworm infection in the genesis of megaloblastic anaemia could have been obtained if analysis of serum folic acid and vitamin B_{12} levels had been made. This is a limitation of the present study. Nevertheless, the finding of macrocytic cells in the peripheral blood combined with the red cell indices values could be considered as a possible morphological evidence of either folic acid or vitamin B_{12} deficiency, or deficiencies of both (22,38).

In conclusion, the light hookworm infection could possibly lead to iron deficiency anaemia unless measures for both deworming and correction of the iron levels are taken. Despite the weak association between parameters suggestive of macrocytic anaemia and hookworm infection, macrocytic anaemia as a problem may not be ruled out in the hookworm positive individuals.

Acknowledgements

This work was part of an MSc thesis of the first author financially supported by the Swedish Agency for Research Cooperation with developing countries (SAREC) and the Ethiopian Health and Nutrition Research Institute (EHNRI). All the laboratory work was done at EHNRI. We are grateful to all those who made this study possible.

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