



Original Research Article

Analysis of hookworm infection intensity and maternal haemoglobin levels in women attending antenatal clinic at Kitale, Kenya

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A B S T R A C T

Low haemoglobin level is a common public health problem in many developing countries and is mainly attributed to parasitic intestinal helminth infections. The parasite species involved and host-parasite outcomes have not been adequately studied in different specific population segments in Kenya. A six month hospital based study to assess the association between hookworm infection, spatial variation in intensity of infection and maternal haemoglobin levels was undertaken at a district hospital. A total of 153 pregnant women who consented participate were enrolled in the study. Data was analyzed using SPSS windows version 16.0. Chi-square was used to determine the association of *Necator americanus* infection and maternal haemoglobin level. 21(13.8%) out of 153, had intestinal helminth infections. *Ascaris lumbricoides* was 10 (6.5%) *Necator americanus* 6 (3.9%). *Trichuris trichiura* 2 (1.3%). A significant negative association was observed between heavy infection of *Necator americanus* and maternal low haemoglobin level (P-value 0.13). We concluded that heavy intensities of *Necator americanus* are associated with low haemoglobin levels in pregnant women. It is recommended that all women of child bearing age living in hookworm endemic areas be subject to periodic anthelmintic treatment and incorporation of de-worming in antenatal care programs.

Keywords

Pregnancy,
Hookworm,
Infection
intensity,
Haemoglobin,
Iron
deficiency

Introduction

It is well-established that human hookworm infection results in intestinal blood loss which, in turn, can contribute to low

haemoglobin levels especially in pregnant women. One third of all pregnant women in developing countries are infected with

hookworm, 56% of them suffer from anaemia, and 20% of all maternal deaths are either directly or indirectly related to anaemia (Gyorkos *et al.*, 2008). However, the extent to which hookworm parasites contribute to low haemoglobin level during pregnancy is not adequately documented thus hindering public health policy and planning for hookworm disease control and management in this important segment of subjects (Brooker *et al* 2008).

Hookworm infections are recognized as the leading cause of pathologic blood loss in tropical and subtropical countries (Crompton, 2000). The worms contribute to anaemia by causing blood loss directly through ingestion and mechanical damage of the mucosa, and indirectly, by affecting the supply of nutrients necessary for erythropoiesis (Crompton, 2000). It is estimated that on average blood losses through faeces (measured as faecal haemoglobin) increase by 0.825 mg per gram of faeces for every increment of 1,000 hookworm eggs per gram (epg) of faeces. An estimated one-third of all pregnant women in developing countries are infected with hookworm (Bundy *et al.*, 1995) majority of them in sub-Saharan Africa (Brooker *et al.*, 2008). A combination of hookworm infection and inadequate iron in the diet especially in rural and poor communities is critical during pregnancy (Stoltzfus *et al.*, 1996) and often result in potentially adverse pregnancy outcomes (Hotez *et al.*, 2005).

The impact of hookworm infection on maternal morbidity is worm-attributable anaemia, induced by deficiencies of iron, total energy, protein and possibly folate and zinc. It is a significant cause of intrauterine growth retardation and low birth weight (Stephenson *et al.*, 2000). It has been suggested that the hookworm-attributable

burden of disease during pregnancy, constitute the most important component of their global disease burden (Stephenson *et al.*, 2000).

The speed of onset of hookworm-related anaemia and its severity depend on the intensity of infection, body iron store and availability of dietary iron. The degree of anaemia is directly proportional to the worm burden. Worm loads of up to 100 worms are light and may cause no symptoms. However, high worm loads of 500 to 1000 or more cause significant blood loss and anaemia.

The worm load is indicated by the egg count of faeces. A count of less than 5 eggs per mg of faeces seldom causes clinical disease, while counts of 20 eggs or more are associated with significant anaemia. Egg counts of 50 or more represent massive infection. In hookworm disease, intestinal absorption of iron is apparently normal so that oral administration of iron can correct the anaemia. However, cure depends on elimination of the worms to reduce infection intensity as the most appropriate intervention strategy.

The absence of a widely acceptable intervention has constrained the development of evidence based understanding of the impact of hookworm infection on maternal anaemia (Stoltzfus *et al.*, 1997). The World Health Organisation guidelines suggest that pregnant women should be treated for hookworm infection after the first trimester (WHO, 2000). Whereas this provided an opportunity for further research to assess the contribution of hookworms to iron deficiency anaemia in pregnancy and the impact of treatment at country level, only Madagascar, Nepal and Sri Lanka have added de-worming to their antenatal care programs (Brooker *et al.*, 2008). Since then, there are remarkably few contemporary, specific population-based

studies of hookworm infection for sub-Saharan Africa including in Kenya (Pullan *et al.*, 2010).

This paper presents a comprehensive analysis of hookworm infection intensity as a risk factor for low haemoglobin levels among pregnant women. The study findings provide a basis that can help public health decision makers in formulating intervention strategies for hookworm disease in expectant women.

Materials and Methods

Study area

The study was conducted at Kitale District Hospital located in Kitale town, with an estimated population of 20,000 people as per 2009 census. Kitale is located between latitude 10°01'58" North and longitude 35°00'02" East. It consists of slum settlements such as Kipsongo, Folk lands and Shimo la Tewa. The residents can be categorised as town residents and rural set-ups such as Bikeke, Cherangany, Kiminini, and Moi's Bridge.

Study design and subject inclusion and exclusion criteria

A hospital based survey was carried out for six months (January-June 2013). Consecutive sampling was used to recruit participants that met required criteria.

The target population were pregnant women. Pregnant women of age between 18-45 years, seeking antenatal services at the hospital were considered. Pregnant women who were residents of the study area and had not received anthelmintics treatment in the last 3 months preceding the study were recruited. Only those who consented were recruited in the study. Those with ova of

Schistosoma mansoni in their stool and malaria parasites in their peripheral blood films were excluded from the study. Because these are blood parasites, and are also associated with low haemoglobin concentration. Pregnant women who did not consent were excluded from the study.

Study sample size determination

The required sample size for this study was calculated based on the prevalence rate of 11.2% of Hookworm (Luoba *et al.*, 2005). The 95% confidence level and 5% marginal error, sample size (n) was determined using the formula (Mugenda and Mugenda, 1999).

$$n = \frac{Z^2 P (1 - P)}{D^2}$$

Whereby:

D is margin of error (0.05)

n is the minimum sample size

P is the estimated prevalence (11.2%)

Z is the standard normal deviate that corresponds to 95% confidence interval (1.96)

$$n = \frac{(1.96)^2 \times 0.112 (1 - 0.112)}{0.05^2} = 152.828$$

rounded up to 153

153 pregnant women were recruited in the study. Consecutive sampling was done at the health facility whereby every pregnant woman who fitted the inclusion criteria and consented was recruited into the study.

Ethical considerations

Prior ethical clearance from Egerton University ethics review committee was obtained as well as consent from Kitale District Hospital. A written informed consent was sought from pregnant women who accepted to participate in the study. A special code for each subject was used to conceal subject identity and maintain confidentiality. Pregnant women infected

with intestinal helminths were referred for appropriate treatment and management at the hospital.

Specimen Collection, Processing and Data Analysis

Stool specimen

A sample of fresh stool specimen was collected from all the 153 participants who consented. Subjects were provided with a labelled leak proof stool container (polypots), toilet paper, and applicator stick. Approximately 5gm of stool specimens was collected in to each polypot, using applicator stick. The stool specimens were examined microscopically within 24 hours of collection using the Kato-Katz technique. The procedure measures the prevalence and intensity of infection since it provides an accurate measure of the number of eggs present per gram of stool (Katz *et al.*, 1972). Each stool specimen was prepared using a sieve and a calibrated template to contain 47.1mg of stool. The preparation on the glass slide was covered with glycerin/malachite green impregnated cellophane.

The preparation was then turned upside down on a flat surface and pressed gently to spread the stool sample ready for reading. The slides were examined within one hour to avoid over clearing of hookworm eggs by glycerine. All eggs in each preparation were counted to determine the number of eggs per gram. For each stool sample two Kato slides were made and the average of the total number of eggs was taken. The magnifications of x10 and x40 were used respectively to visualize and identify the ova/eggs of Hookworm. The egg counts were classified as Light infection, Moderate infection, and Heavy infection, (WHO, 1987). Light hookworm infection,(1-999

eggs/gram), Moderate infection (2000-3999 eggs/gram), and Heavy infection (>4000 eggs/gram).

Faecal culture procedure for isolation and identification of hookworm larvae

Harratamori technique (Harata and Mori, 1955) was used to culture and hatch hookworm eggs to differentiate the two human hookworms, *Necator americanus* filariform larvae from those of *Ancylostoma duodenale*.

The procedure involved the use of filter paper strips of about 5 inches slightly tapered at one end for each stool specimen suspected and or confirmed to contain hookworm eggs. One gram of faecal sample was smeared at the centre of the strip. Four millilitre of distil water was added to 15 millilitre conical centrifuge tube. The paper strips were inserted into the tube such that the tapered end was near the bottom of the tube with water level slightly below the faecal point. The tube was plugged cotton wool and allowed to stand upright in a rack at 25⁰ C for 10 days. Small amount of the fluid was withdrawn from the bottom of the tube and a smear prepared on a glass slide. The preparation was cover slipped and examined microscopically using 10X objective for the presence of filariform stages. Any filariform larvae present were identified based on morphological features to differentiate between the two species of human hookworms.

Blood sample collection and processing

Blood samples were collected using sterile syringe and needle, after sterilizing the cubital vein on the arm using 70% methylated spirit. The blood was transferred to heperanized vials to prevent clotting. Hemoglobin levels were estimated using

coulter counter machine—by analysing heparanised blood sample in an automated haemoglobin machine. Low haemoglobin may result from other causes apart from intestinal helminths. To differentiate this from other causes, white blood cell (eosinophils) count was done using coulter counter machine. Raised eosinophil of more than 6% was considered a positive indicator of parasitic infection (Cheesbrough, 2002).

Iron deficiency anaemia was assessed by making peripheral blood films from collected blood, air dried and fixed using 70% methanol. Staining was done using 10% Leishman stain for 10 minutes and the preparation examined using oil immersion with x100 objective. The presence of anisocytosis, poikilocytosis and microcytic, hypochromic red cells suggested iron deficiency anaemia (Cheesbrough, 2002). Presence of malaria parasites also known to cause low maternal haemoglobin levels was determined from the blood film. Subjects positive for malaria were excluded from the study and referred for appropriate treatment.

Data Analysis

Chi-square test was employed to measure the association between, *Necator americanus* infection and maternal haemoglobin level. The infection intensity was categorised as low, moderate and heavy as recommended by world and applied in related studies (WHO, 1987; Wekesa *et al.*, 2014).

Results and Discussion

A total of 153 pregnant women were recruited in the study. 21 (13.7%), were positive for intestinal helminth infection. *Ascaris lumbricoides* accounted for 10 (6.5%), *Necator americanus* 6 (3.9%), *Trichuris trichiura* 2 (1.3%), *Enterobius vermicularis* 1(0.7%), and, multiple

infection of *A. lumbricoides* and *T. trichiura* 2 (1.3%). A high percentage of 132 (86%) samples were negative.

Morphological identification of *Necator americanus*

Of the two known human hookworms, *Necator americanus* was the only species associated with hookworm disease in the study subjects. *Necator americanus* filariform larvae were identified basing on the following morphological features: average larval length of 590 μ m, head and tail pointed, oesophagus with thistle-funnel shape (oesophageal bulb), presence of gap between the oesophagus and intestine, oesophagus length approximately 1/3 in relation to entire body length. Other intestinal helminth parasite species encountered and their association with maternal haemoglobin levels are indicated in Table 1. Heavy and moderate intensities of *Necator americanus* infections were associated with low haemoglobin levels. There was an association between increasing *N. americanus* egg counts and decreasing haemoglobin levels. Five (83%) out of 6 women infected with *N. americanus* had low haemoglobin levels whereas all subjects positive for ascariasis 10 (100%) had normal haemoglobin levels. Hookworm egg threshold count associated with low haemoglobin was at > 2,000 epg. The eosinophil counts in intestinal helminth positive cases and the types of anaemia are indicated in Table 2. The results of peripheral blood film (Table 2) indicate that pregnant women with high intensity of infection with *N. americanus* had microcytic hypochromic red blood cells at (P- 0.015). An accompanying high eosinophilia (90%) was evident in subjects positive for ascariasis as compared to those with moderate and or heavy hookworm disease/infection (66.7%).

Table.1 Association between type of intestinal helminth and maternal haemoglobin levels

VARIABLE		HB LEVELS		P-value
		< 11g% No. (%)	>11g% No. (%)	
Helminth infection	Infected	7 (28%)	14 (10.9%)	.032
	Not infected	18 (72%)	114 (89.1%)	
Type of helminth infection	<i>A. lumbricoides</i>	0 (0%)	10 (100%)	.013
	<i>N. americanus</i>	5 (83.3%)	1 (16.7%)	
	<i>T. trichiura</i>	1 (50%)	1 (50%)	
	<i>E.vermicularis</i>	0 (0%)	1 (100%)	
	Multiple(Ascaris/ <i>Trichuris</i>) infection	1 (50%)	1 (50%)	

Significance level, (p<0.05)

Table.2 Peripheral blood film eosinophil count and helminth infection

Variables		<i>A.lumbricoides</i>	<i>N.americanus</i>	<i>T- trichura</i>	<i>E.vermicularis</i>	Mixed infection (<i>Ascaris/Trichuris</i>)	p-value
Peripheral Blood Film	Microcytic hypochromic	0 (0%)	4 (66.7%)	0 (0%)	0 (0%)	0 (0%)	.015
	Normocytic normochromic	10 (100%)	2 (33.3%)	2 (100%)	1 (100%)	2 (100%)	
Eosinophil count	>6 Raised	9 (90%)	4 (66.7%)	0 (0%)	0 (0%)	5 (50%)	.06
	1-6 Normal	1 (10%)	2 (33.3%)	2 (100%)	1 (100%)	5 (50%)	

Significance level, (p<0.05)

The most important clinical manifestations of hookworm disease are associated with the adult worms in the intestine. They attach to the gut mucosa and sustain their life by blood sucking, a process that ruptures the host capillaries and arterioles followed by the release of a battery of pharmacologically active polypeptides which induces chronic intestinal blood loss (Stoltzfus *et al.*, 1997; Hotez and Pritchard, 1995).

Adult *N. americanus* worm sucks approximately 0.05 ml/dl of blood and *A. duodenale* approximately 0.25 ml/dl of blood per day (Huddle *et al.*, 1999). The pumping action of the oesophagus facilitates the blood feeding process and blood sometimes may pass out of worm intestines undigested and unutilised. The worms frequently leave one site and attach themselves to another site in human intestines thus creating multiple bleeding intestinal ulcers.

This long term blood loss can manifest itself physically through facial and peripheral oedema and eosinophilia, resulting in microcytic hypochromic type of iron deficiency anaemia. The speed of onset of anaemia and its severity depend on the intensity of infection, body iron store and availability of dietary iron. Light infections may cause no symptoms, however high worm loads of 1000-2000 or more cause significant blood loss and anaemia. The results of our study confirm that high intensities of *Necator americanus* infection are associated with lower levels of haemoglobin than light infection intensities (P-value 0.013). These findings are comparable to related to studies (Brooker *et al.*, 2008) and show an association between hookworm infection and low haemoglobin levels in pregnancy. The 83.3% of hookworm positive cases infected with *Necator americanus* had haemoglobin below

11gm% compared to non infected pregnant women. Related studies in Kenya and Zanzibar (Mary and Akanmori, 2005); Ethiopia (Balachew and Yusef, 2006) found a significant association between heavy intensity of hookworm infection and low haemoglobin among pregnant women attending antenatal clinics. Larocque *et al.*, 2005 established a relationship between intensity of hookworm infections, and low haemoglobin levels during pregnancy in Peru. In this study, a significant association between increasing hookworm egg counts and decreasing haemoglobin levels was established. A study carried in Ghana among expectant mothers ~~out~~ (Ayoya *et al.*, 2006; Godwin *et al.*, 2010) also indicated that hookworm infections have greater negative impact on the haemoglobin levels of pregnant mothers. (Stoltzfus *et al.*, 1997) in a related study identified hookworms as an important contributor to low haemoglobin levels in many resource limited settings. Elsewhere, similar studies confirm that heavy intensities of hookworm infection are inversely related to haemoglobin concentrations (Bondevik *et al.*, 2000; Brooker *et al.*, 2008; van Eijk *et al.*, 2009).

The observed threshold at 2000 epg is consistent with world health organization category of moderate hookworm infection intensity even though WHO calculation was based data obtained in children (WHO, 1987). The association of egg output, infection intensity and anaemia has previously been demonstrated in Kenya (Shulman *et al.*, 1996) and Nepal (Dreyfuss *et al.*, 2000). In both studies, it was apparent that intensity was associated with anemia after a threshold of infection had been reached at 1000 and 2000 epg respectively. The discrepancy in egg threshold is attributed to difference in egg output and lifespan of the human hookworm species. The most prevalent species *Necator*

americanus has a longer lifespan (4-20 yrs) compared to *Ancylostoma duodenale* (2-7 yrs). Also, unlike the present studies, no hookworm species identification is reported and therefore it is not possible to link threshold with specific hookworm. Therefore, although the WHO categories of hookworm intensity are based on data obtained in child populations, our data suggest that the same categories may be applicable in pregnant populations.

Based on our findings it is concluded that heavy intensities of *N americanus* infection was associated with low maternal haemoglobin levels. Hookworm infections are chronic and many women enter pregnancy with these pre-existing conditions that may have adverse effects on pregnancy outcomes. Therefore there is need to incorporate de-worming in antenatal care programs and educate expectant women and those planning to become pregnant on the adverse effects of hookworm infection in pregnancy.

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