

Original Research Article

EFFECT OF INTESTINAL PARASITOSIS ON HAEMOGLOBIN AND EOSINOPHIL LEVELS OF PATIENTS ATTENDING UNIVERSITY OF CALABAR TEACHING HOSPITAL, CALABAR, NIGERIA

ABSTRACT

Aims: To determine the role of intestinal parasites in the reduction of haemoglobin concentration and raising of eosinophil levels and subsequent development of anemia and eosinophilia among patients of the Teaching Hospital (UCTH), Calabar, Nigeria.

Study design: This was cross sectional study. Ethical approval and patients' informed consent were sought and obtained before collection and processing of samples.

Place and Duration of Study: Outpatient clinic, UCTH, Calabar between July, 2013 and May, 2014.

Methodology: We included 300 patients (114 males, 186 females). Intestinal parasites were detected by direct stool microscopy and formol ether concentration technique. Haemoglobin (Hb) concentration was determined by Cyanmethaemoglobin method while eosinophil levels were determined by differential eosinophil count.

Results: Are presented as mean (range) and Chi square analysis was used to compare percentages. Overall, 30%, 23%, and 27% of the studied subjects were positive for intestinal parasites, had low Hb concentration (below 110.0g/L) and high eosinophil count ($\geq 7\%$), respectively. Parasite positive subjects had lower Hb concentration and higher eosinophil levels compared to their parasite negative counterpart (56.7% versus 8.6%) and (70% versus 8.6%), respectively ($P < 0.001$). Multiple infections was more related to Hb reduction and high eosinophil levels than single infections (80% versus 45%) and (90% versus 60%), respectively ($P < 0.001$). Hook worm showed the highest occurrence (26.7%) followed by *Ascaris lumbricoides* 23.3%, *Trichuris trichiuria* 10%, and *Entamoeba histolytica* 6.7%. In single infections, Hook worm infected subjects recorded more reduction in Hb and increased eosinophil count (99.0 g/L and 15%, respectively) while in multiple infections, hookworm/*Entamoeba histolytica* co-infected subjects took the lead (70.0g/L and 19%, respectively). There was no association between intestinal parasites prevalence by gender of subjects ($P = 0.2128$).

Conclusions: Intestinal parasitosis may result in anaemia and eosinophilia and the decrease in haemoglobin concentration and increase in eosinophil count is directly related to the degree of parasitosis. Diagnosis for anaemia and eosinophilia should include parasitology.

Keywords: Intestinal parasitosis, haemoglobin concentration, eosinophilia, anaemia, Calabar

1. INTRODUCTION

Parasitic infections represent one of the most prevalent pathological phenomena in humans. They cause considerable morbidity and mortality worldwide with the most impact in developing countries [1]. Intestinal

17 parasites, mainly the helminthes and protozoa, inhabit and, sometimes, infect the gastrointestinal tract of
 18 humans and other animals [2]. They
 19 live throughout the body, but most prefer the intestinal wall. Many intestinal parasitic infections (IPIs) are
 20 spread through the fecal contamination of food and water. Means of exposure include: ingestion of under
 21 cooked meat, drinking of contaminated water and penetration through the skin. Parasitic infections most
 22 frequently occur in poverty stricken areas with inadequate sanitation, lack of portable water and education
 23 [3, 4, 5]. A hot and humid tropical climate, as is
 24 the case in Nigeria, is an important factor which promotes infection with intestinal parasites. The most
 25 common medically important intestinal parasites include *Necator americanus*, *Ancylostoma duodenale*
 26 (hookworm), *Ascaris lumbricoides*, *Trichuris trichiura* and *Strongyloides stercoralis* (these are important
 27 species of soil transmitted helminthes (STHs)). Other common intestinal parasites are: *Hymenolepis*
 28 *nana*, the most common parasitic cestode prevalent globally; *Giardia intestinalis* which causes giardiasis
 29 and is the most prevalent protozoan parasite worldwide with an estimated 200 million people being
 30 infected, and *Entamoeba histolytica* [6, 7]. These parasites could bring about significant nutritional
 31 deficiencies, delayed physical and cognitive development during childhood as well as reduced
 32 productivity in adults [8, 9]. About one third of the world constituting more than two billion people, are
 33 infected with intestinal parasites. Approximately 300 million people are severely ill with these worms and
 34 of these, at least 50% are school-age children. IPIs rarely cause death but because of the size of the
 35 problem, the global number of related deaths is substantial [6]. About 39 million disability adjusted life
 36 years (DALYs) are attributed to IPIs and these infections thus represent a substantial economic burden
 37 [10].
 38 Present in the red blood cells of all vertebrates is the iron-containing oxygen transport metalloprotein
 39 known as haemoglobin. It carries oxygen from the respiratory organs to the rest of the body (i.e. the
 40 tissues) where it releases the oxygen to burn nutrients of the organism during the process of metabolism
 41 [11]. Eosinophils are white blood cells which constitute a part of the immune system and are responsible
 42 for combating multicellular parasites and certain infections in vertebrates. Along with mast cells, they also
 43 control mechanisms associated with allergy. Eosinophils are granulocytes and their granules contain
 44 many chemical mediators such as histamine, eosinophil peroxidases, lipase, plasminogen and major

basic protein. These mediators are released following activation of the eosinophil and are toxic to both parasitic and host tissue. In normal individuals, eosinophils make up about 1-6% of white blood cells and are about 12-17 micrometers in size [12]. Anaemia and eosinophilia have been associated with helminthic infection

previously [13, 14, 15, 16]. It is the intention of this study to investigate the occurrence of intestinal parasitic infection and its effect on the haemoglobin and eosinophil levels among patients attending clinics in University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria.

2. MATERIALS AND METHODS

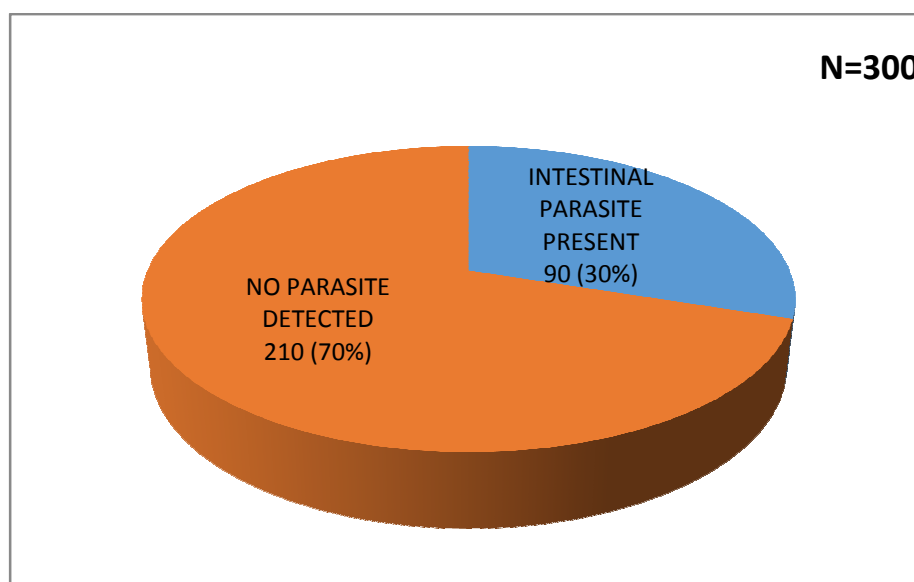
This study was carried out at the University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State situated in the South-South of Nigeria. With ethical approval from the UCTH research ethics committee and informed consent of participants, a total of three hundred (300) patients attending the general out-patient clinic in UCTH were recruited for the study. Two millilitres of blood was collected aseptically from each patient into ethylene diamine tetra acetic acid bottle to a final concentration of 2mg/ml. Each subject was given a sterile screw-capped wide mouth universal container for collection of stool sample. Blood and stool samples were processed and examined in the University of Calabar Teaching Hospital laboratory. The stool samples were macroscopically examined for appearance, consistency, presence of blood, mucus, worm segments and worms. Stool samples were further processed by direct smear microscopy and formol ether concentration technique and examined microscopically using the 10x and 40x objective for the presence of larva, cysts, and eggs of parasites [17]. Thin blood films were made, fixed and stained using Leishman stain (PH 6.8). The films were viewed microscopically with oil immersion objective for differential white cell count. The haemoglobin concentration was determined by Cyanmethaemoglobin method [18]. Results obtained are presented as mean (range). Percentages were compared using Chi square analysis.

3. RESULTS

The results of this study are as shown in figures 1-2 and tables 1-5.

73 Figure 1 presents the occurrence of intestinal parasites among studied patients attending clinics in
 74 University of Calabar Teaching Hospital. Intestinal parasites were detected in ninety patients (30%) while
 75 two hundred and ten (70%) of the patients tested negative.

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78 **Fig. 1. Occurrence of intestinal parasites among patients attending clinic in University of Calabar**
 79 **Teaching Hospital**

80 Table 1 presents intestinal parasitosis and haemoglobin (Hb) concentration among the studied subjects.
 81 Using Hb concentration of 110.0g/L as a cut-off point for healthy individuals of all age groups (WHO,
 82 2011), 23% of the 300 subjects had low Hb concentration (<110.0g/L) while 77% had Hb concentration
 83 above 110.0g/L. It was further observed that 51(56.7%) of those with intestinal parasitosis compared to
 84 18(8.6%) of their non-infected counterpart had lower Hb levels (<110g/L). On the other hand, more of the
 85 parasite negative subjects 192 (91.4%) recorded higher Hb levels than their parasite positive counterpart
 86 39 (43.3%). This difference was statistically significant ($X^2 = 82.29$, $df1$, $P < 0.001$).

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Table 1. Intestinal parasitosis and haemoglobin concentration among patients attending clinic in UCTH

	Parasite present n=90	Parasite absent n=210	Total N=300
No. (%) with Hb <110g/L	51(56.7)	18 (8.6)	69 (23.0)
No. (%) with Hb>110g/L	39 (43.3)	192 (91.4)	231 (77.0)

Intestinal parasitosis and eosinophil count among the studied subjects is shown in table 2. With respect to the reference range for eosinophil count which is 1-6% (Dacie and Lewis, 2010), 7% was used as a cut-off value for eosinophilia diagnosis. Twenty seven percent (27.0%) of the 300 subjects examined were observed to have eosinophil count greater than or equal to 7% while 73 (73.0%) had less than 7%. Of the 90 parasite positive persons, 63 (70.0%) were positive

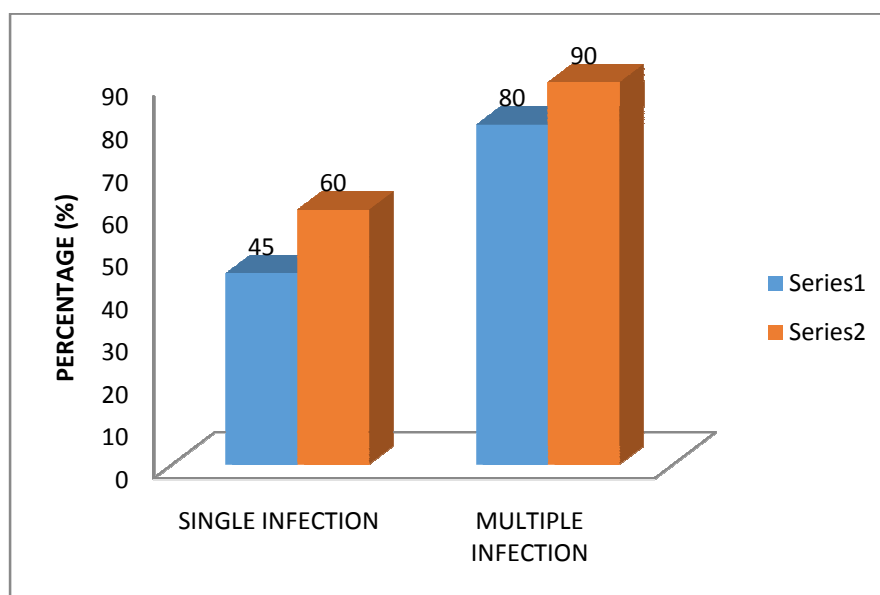
for eosinophilia while 27 (30%) were negative. For parasite negative persons, 192 (91.4%) had eosinophil count below 7% while 18 (8.6%) had eosinophilia. The difference in prevalence was statistically significant ($X^2 = 120.61$, $df1$, $P < 0.001$).

Table 2. Intestinal parasitosis and eosinophil count among patients attending clinics in UCTH

	Parasite present n =90	Parasite absent n=210	Total N=300
No. (%) with Eosinophil count $\geq 7\%$	63 (70.0)	18 (8.6)	81 (27.0)
No. (%) with Eosinophil count <7%	27 (30.0)	192 (91.4)	219 (73.0)

Figure 2 expresses the haemoglobin concentration and eosinophil count of parasite positive subjects with single and multiple infections. Eighty percent (80%) of subjects with multiple infections had Hb concentration less than 110.0g/L and this is higher when compared with forty five percent (45%) for single

infections. Similarly, ninety percent (90%) of those with multiple infections and sixty percent (60%) of those with single infections had eosinophil counts greater than seven percent (7%).



Series 1: Percentage of subjects with Hb < 110g/L
Series 2: Percentage of subjects with eosinophil count ≥ 7%

Fig. 2. Haemoglobin concentration and eosinophil count of parasite positive subjects with single and multiple infections

Table 3 shows the haemoglobin and eosinophil levels of parasite positive patients by species of parasite. Of the ninety parasite positive patients, 60 (66.7%) had single infections with mean Hb of 119 (60-161 g/L) and eosinophil count of 11 (2-25%), while 30 (33.3%) had multiple infections with mean Hb of 100 (60-160) g/L and eosinophil count of 18 (2-24%), respectively. In single infections, hook worm showed the highest occurrence of 24 (26.7%) with mean haemoglobin level of 99.0 (60-161g/L) and mean eosinophil count of 15 (4-21%). Next was *Ascaris lumbricoides* 21 (23.3%) with mean haemoglobin level of 123.0 (90-161g/L) and mean eosinophil count of 8 (2-18%), followed by *Trichuris trichiura* 9 (10.0%) with 112.0 (70-140g/L) and 13 (4-25%) as the mean haemoglobin and eosinophil values, respectively. *Entamoeba histolytica* had the least occurrence of 6 (6.7%) with 140.0 g/L as the mean haemoglobin and 9 (2-16%) as the mean eosinophil count. For multiple infections, hookworm/*Ascaris* co-infection recorded the highest, 15 (16.7%) out of 10 occurrences with 110.0 (80-160g/L) as the mean haemoglobin level and 16

(2-24%) as the mean eosinophil count. Hookworm/*E.histolytica* co-infection was detected in 9 (10.0%) with mean haemoglobin level of 70.0(60-80g/L) and mean eosinophil count of 19 (16-22%). Next was *Ascaris/E. histolytica* co-infection which recorded 3 (3.3%) with haemoglobin level of 130.0g/L and eosinophil count of 21(21%). Hookworm/*Ascaris/Trichuris trichuria* co-infection was detected in 3 (3.3%) with 90.0g/L as haemoglobin concentration and 15% as eosinophil count.

Table 3. Haemoglobin concentration and eosinophil count of subjects positive for intestinal parasites by species of parasite

Parasite species	No (%) Detected	Hb (g/L) Mean (Range)	Eosinophil (%) Mean (Range)
SINGLE INFECTIONS			
Hookworm	24 (26.7)	99 (60-161)	15 (4-21)
<i>Ascaris lumbricoides</i>	21 (23.3)	123 (90-161)	8 (2-18)
<i>Trichuris trichiuria</i>	9 (10.0)	112 (70 -140)	13 (4-25)
<i>Entamoeba histolytica</i>	6 (6.7)	140 (140-140)	9 (2-16)
Sub Total	60 (66.7)	119 (60-161)	11 (2-25)
MULTIPLE INFECTIONS			
Hookworm/ <i>Ascaris</i>	15 (16.7)	110 (80-160)	16 (2-24)
Hookworm/ <i>E. histolytica</i>	9 (10.0)	70 (60 – 80)	19 (16-22)
<i>Ascaris/E.histolytica</i>	3 (3.3)	130 (130)	21 (21)
Hookworm/ <i>Ascaris/T.trichuria</i>	3 (3.3)	90 (90)	15 (15)
Sub Total	30(33.3)	100 (60-160)	18 (2-24)
Total	90 (100.0)	110 (60- 161)	15 (2-25)

Table 4 shows the prevalence of intestinal parasites, haemoglobin concentration and eosinophil count by gender of subjects. Of the 90 subjects positive for intestinal parasites, males constituted 39 (34.2%) with haemoglobin value of 103g/L and mean eosinophil count of 14% while females were 51 (27.4%) with haemoglobin value of 112g/L and mean eosinophil count of 13%. Intestinal parasites prevalence by gender of subjects was statistically insignificant ($\chi^2 = 1.55$, $df=1$, $P = 0.2128$).

Table 4. Intestinal parasites, haemoglobin concentration and eosinophil count of male and female subjects

Gender	No. Examined	No. (%) positive for parasites	Hb (g/L) Mean (Range)	Eosinophils (%) Mean (Range)
Males	114	39 (34.2)	103 (60-150)	14 (2-22)
Females	186	51 (27.4)	112 (60-161)	13 (2-25)
Total	300	90 (30.0)	108 (60-161)	14 (2-25)

In table 5, intestinal parasites, haemoglobin concentration and eosinophil count of subjects based on age is presented. Adult subjects (≥ 18 years) had an infection rate of 75 (30.5%) out of the 246 subjects examined with mean haemoglobin value of 110.0 (60-161g/L) and mean eosinophil count of 13 (12-15%) while children (< 18 years) had infection rate of 15 (27.8%) out of the 54 subjects that were examined with mean haemoglobin value of 100.0 (80-130g/L) and mean eosinophil count of 17 (11-21%).

Table 5. Intestinal Parasites, haemoglobin concentration and eosinophil count of subjects by age

Age (Years)	No. Examined	No. (%) positive for parasites	<u>Hb (g/L)</u> Mean (Range)	<u>Eosinophils (%)</u> Mean (Range)
Children (< 18)	54	15 (27.8)	100 (80-130)	17 (11-21)
Adults (≥ 18)	246	75 (30.5)	110 (60-161)	12 (2-25)
Total	300	90 (30.0)	105 (60-161)	15 (2-25)

4. DISCUSSION

In this study, the occurrence of intestinal parasitic infection and its effect on haemoglobin and eosinophil levels among three hundred patients in University of Calabar Teaching Hospital were investigated. Intestinal parasites were detected in thirty percent of the subjects studied (Fig.1). This finding differs from previous report (17.5%) in a study conducted among patients in University of Maiduguri Teaching Hospital [19] located in Northern Nigeria and implies that attendance to clinics in UCTH is not a determinant of the infection status (with reference to intestinal parasites) of the subjects studied. Ehiaghe *et al.*, 2013 [15] reported 50% intestinal parasite infection in a study conducted in Okada, Edo State, Nigeria. The variance with the present study may be due to the fact that their study population consisted of children both in urban and rural areas.

As presented in (Table 1), it was observed that most (56.7%) of the patients who tested positive for intestinal parasites, had haemoglobin levels < 110g/L. According to WHO, 2011 [20] report, Hb concentration below 110g/L for all age groups and gender is suggestive of anaemia. Several studies have reported the presence of anaemia in helminthic infection [21, 22] mostly in children. Eighty two percent of the study population in the present study were mostly adults (≥ 18 years), thus implying that anaemia is associated with intestinal parasitosis irrespective of age. The low haemoglobin concentration observed in intestinal parasitosis has been attributed to reduced iron absorption at the duodenum and jejunum since the parasites tend to colonize these sites, as well as chronic blood loss, especially in hookworm infection [15].

Twenty seven percent of the 300 subjects examined were observed to have eosinophil count greater than 7% (Table 2) while seventy three percent had less than 7%. Furthermore, seventy percent of the parasite positive subjects were positive for eosinophilia with the remaining thirty percent being negative. Helminthic infection is usually the first suspicion

when eosinophilia is observed in our locality and this study confirms that intestinal parasitosis is associated with eosinophilia. Interestingly, 8.6% of the parasite negative persons had eosinophilia. This could most likely be due to allergic reactions and drug sensitivity. Previous reports support this finding. As

part of host defenses against helminthic infestation, helper 2 T cells (Th-2) produce interleukin 5 which in turn stimulates an increase in the production of eosinophils. Eosinophils attach to the surface of the parasite through IgE and secrete cytotoxic enzymes contained in their granules [23, 18].

When the haemoglobin concentration and eosinophil count of parasite positive subjects with single and multiple infections were compared (Fig. 2), it was observed that 80% of subjects with multiple infections had Hb concentration less than 110.0g/L while it was 45% for single infections. Also, 90% of those with multiple infections had eosinophil counts greater than seven percent (7%) as compared to sixty percent for single infections. The highest single infection was hookworm infection with the affected subjects having lowest haemoglobin value compared to others (Table 3). This can be linked to chronic blood loss observed in hookworm infection. It has also been suggested that ascariasis which was the second highest single infection, reduces the gastric juices that enhance iron absorption hence contributing significantly to reduced Hb levels. Indeed, the anaemia observed in multiple infections in this study was largely due to Hookworm/*Ascaris* co-infection which recorded fifteen out of thirty occurrences. Haemoglobin concentration of subjects with multiple infections has been reported to be lower than the value obtained for single infections [15]. Interestingly, while the mean Hb of subjects with multiple infections was lower, their mean eosinophil count was higher when compared with values for subjects with single infections. These findings suggest that the degree of parasitosis directly reduces the haemoglobin concentration and increases the eosinophil count of infected subjects.

Although the difference in Hb concentration between males and females has been firmly established, in this study the Hb concentration of male subjects was lower than that of female subjects as presented in Table 4. This is probably due to the fact that the female subjects were more in number than their male counterparts who may also have been more affected by the infection. No gender difference was observed with respect to eosinophil count. Similarly, the adult subjects enrolled in the present study outnumber the children, hence the differences observed (Table 5) are not statistically comparable.

CONCLUSION

This study has shown that intestinal parasitosis results in anaemia and eosinophilia. The decrease in haemoglobin concentration and increase in eosinophil count is directly related to the degree of parasitosis with reference to single and multiple infections. Although other diseases may be associated with haemoglobinaemia and eosinophillia, stool microscopy, haemoglobin and eosinophil tests should be included in medical examination and routine medical checkup.

CONSENT

Patients/guardian written informed consent was sought and obtained, after details of the study was explained to them, before enrollment into the study. Participation in the study was strictly voluntary.

ETHICAL APPROVAL

Ethical approval was obtained from the Committee on Research Ethics of the University of Calabar Teaching Hospital (UCTH), Calabar.

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