

## **Intestinal Parasitosis, Haemoglobin and Eosinophil Levels of Patients Attending University of Calabar Teaching Hospital, Calabar, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author IBOB designed the study and wrote the protocol. Author IEU wrote the first draft of the manuscript. Authors IBOB and PAA managed the analyses of the study. Author PAA performed the statistical analysis. Authors IEU and PAA managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To determine the effect of intestinal parasites on the haemoglobin concentration and eosinophil levels among the patients treated at the University of Calabar Teaching Hospital (UCTH), 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.

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**Results:** Overall, 30% of the studied subjects were positive for intestinal parasites, 23% had low Hb concentration (below 110.0 g/L) while 27% had high eosinophil count ( $\geq 7\%$ ). 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 trichiura* 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 (70.0 g/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. It is recommended that stool microscopy, haemoglobin estimation and eosinophil counts be routinely performed for all patients visiting the hospital.

**Keywords:** Intestinal parasitosis; haemoglobin concentration; eosinophilia; anaemia; Calabar.

## 1. INTRODUCTION

Intestinal parasites, mainly the helminthes and protozoa, inhabit and, sometimes, infect the gastrointestinal tract of humans and other animals from where they may spread to other parts of the body [1]. The most common medically important intestinal parasites include *Necator americanus*, *Ancylostoma duodenale* (hookworm), *Ascaris lumbricoides*, *Trichuris trichiura* and *Strongyloides stercoralis* [2]. Other common intestinal parasites are: *Hymenolepis nana*, the most common parasitic cestode prevalent globally; *Giardia intestinalis* which causes giardiasis and is the most prevalent protozoan parasite worldwide with an estimated 200 million people being infected, and *Entamoeba histolytica* [3]. Many intestinal parasitic infections (IPIs) are spread through the fecal contamination of food and water and means of exposure include: ingestion of under cooked meat, drinking of contaminated water and penetration through the skin [4]. Infections most frequently occur in poverty stricken areas with inadequate sanitation, lack of portable water and education [5,6]. A hot and humid, tropical climate, as is the case in Nigeria, is an important factor which promotes infection with intestinal parasites.

Parasitic infections represent one of the most prevalent pathological phenomena in humans which cause considerable morbidity and mortality worldwide with the most impact in developing countries [7]. These parasites could bring about significant nutritional deficiencies, delayed physical and cognitive development during

childhood as well as reduced productivity in adults [8,9]. About one third of the world constituting more than two billion people, are infected with intestinal parasites [3]. These worms cause severe illness in approximately 300 million people, of whom 50% are school-age children [2]. IPIs rarely cause death but because of the size of the problem, the global number of related deaths is substantial [3]. About 39 million disability adjusted life years (DALYs) are attributed to IPIs and these infections thus represent a substantial economic burden [10].

Present in the red blood cells of all vertebrates is the iron-containing oxygen transport metalloprotein known as haemoglobin which carries oxygen from the respiratory organs to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients of the organism during the process of metabolism [11]. Eosinophils are white blood cells which constitute a part of the immune system and are responsible for combating multicellular parasites and certain infections in vertebrates. Along with mast cells, they also control mechanisms associated with allergy. Eosinophils are granulocytes and their granules contain many chemical mediators such as histamine, eosinophil peroxidases, lipase, plasminogen and major basic protein [12]. 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 [13-16] and a high prevalence of intestinal parasites (26.6% and

29%, respectively) has been reported among children as well as adults on anti-retroviral therapy in the study area (Calabar) [17,18]. There is scarcity of information on the occurrence of intestinal parasitic infection and its association with anaemia and eosinophilia in Calabar. It is the intention of this study, therefore, 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

### 2.1 Study Area

This study was carried out at the University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State situated in the South-South of Nigeria.

### 2.2 Sample Collection/ Sampling Technique

With ethical approval and informed consent of participants obtained, a total of three hundred (300) randomly selected patients attending the general out-patient clinic (GOPC) in UCTH were recruited for the study. Subjects included male and female patients of any age who reported to the GOPC within the study period (July, 2013-May, 2014) and were not on any medication. Patients who were gravely ill such that they needed to be admitted as well as those on medication that would interfere with the test results were excluded. Two millilitres of blood was collected aseptically from each patient into

ethylene diamine tetra acetic acid bottle to a final concentration of 2 mg/ml. Each subject was given a sterile screw-capped wide mouth universal container for collection of stool sample.

### 2.3 Processing of Samples

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 [19]. 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 [20]. The patients' demographic data and laboratory results were fed into a computer program and analysis was hence done. Results obtained are presented as mean (range). Percentages were compared using Chi Square.

## 3. RESULTS

The results of this study are as shown in Figs. 1-2 and Tables 1-4.

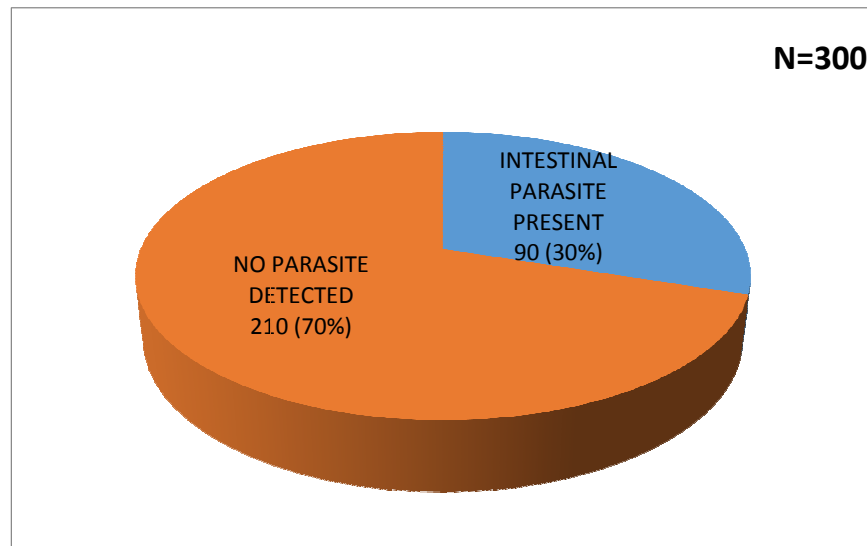
Fig. 1 shows that intestinal parasites were detected in ninety (30%) of the 300 patients screened.

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

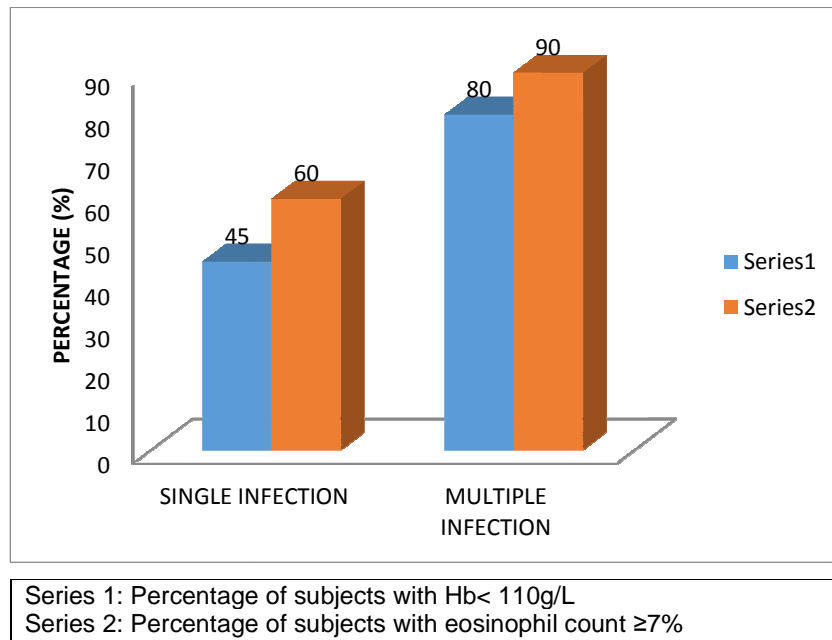
	Parasite present n=90	Parasite absent n=210	Total N=300
No. (%) with Hb<110 g/L	51(56.7)	18 (8.6)	69 (23.0)
No. (%) with Hb>110 g/L	39 (43.3)	192 (91.4)	231 (77.0)

**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 ≥7%	63 (70.0)	18 (8.6)	81 (27.0)
No. (%) with Eosinophil count <7%	27 (30.0)	192 (91.4)	219 (73.0)



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



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

Table 1 presents intestinal parasitosis and haemoglobin (Hb) concentration among the studied subjects. There was association between intestinal parasitosis and haemoglobin concentration ( $X^2 = 82.29$ ,  $df1$ ,  $P < 0.001$ ).

Intestinal parasitosis and eosinophil count among the studied subjects is shown in Table 2. Intestinal parasitosis was significantly related to eosinophilia ( $X^2 = 120.61$ ,  $df1$ ,  $P < 0.001$ ).

Fig. 2 expresses the haemoglobin concentration and eosinophil count of parasite positive subjects with single and multiple infections. Multiple Parasite infection was seen to be more related to low Hb concentration and high eosinophil counts than single infection.

In single infections, Hook worm showed the highest occurrence 24 (26.7%) with mean haemoglobin level of 99.0 (60-161 g/L) and mean

**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)
<b>Single infections</b>			
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)
<b>Sub total</b>	<b>60 (66.7)</b>	<b>119 (60-161)</b>	<b>11 (2-25)</b>
<b>Multiple infections</b>			
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</i> / <i>E. histolytica</i>	3 (3.3)	130 (130)	21 (21)
Hookworm/ <i>Ascaris</i> / <i>T. trichiuria</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. Intestinal Parasites, haemoglobin concentration and eosinophil count of subjects by gender and age**

	Gender			Age (years)		
	Males	Females	Total	Children (<18)	Adults (≥18)	Total
No. Examined	114	186	300	54	246	300
No. (%) positive for parasites	39 (34.2)	51 (27.4)	90 (30.0)	15 (27.8)	75 (30.5)	90 (30.0)
Hb (g/L)	103	112	108	100	110	105
Mean (range)	(60-150)	(60-161)	(60-161)	(80-130)	(60-161)	(60-161)
Eosinophils (%)	14 (2-22)	13 (2-25)	14 (2-25)	17 (11-21)	12 (2-25)	15 (2-25)
Mean (range)						

eosinophil count of 15 (4-21%) while *Entamoeba histolytica* had the least occurrence 6 (6.7%) with 140.0 g/L mean Hb and 9 (2-16%) eosinophil count. For multiple infections, Hookworm/*Ascaris* co-infection was the most detected (16.7%) with Hb level of 110.0(80-160 g/L) and eosinophil count of 16 (2-24%) followed by Hookworm/*E. histolytica* co-infection (10.0%) with 70 (60 – 80 g/L) Hb level and 19 (16-22%) eosinophil count (Table 3).

Table 4 shows the prevalence of intestinal parasites, haemoglobin concentration and eosinophil count by gender and age of subjects. Intestinal parasites prevalence by gender of subjects was not statistically significant ( $\chi^2 = 1.55$ , df1,  $P = 0.2128$ ). The differences observed in the prevalence of intestinal parasites, Hb, and eosinophil levels by age of subjects enrolled in the present study was not statistically comparable as adults outnumbered the children.

#### 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 [21] located in Northern Nigeria and in a study conducted in Okada, Edo State, Nigeria [15], 50% intestinal parasite infection was reported. 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 < 110 g/L. According to WHO report [22], Hb

concentration below 110 g/L for all age groups and gender is suggestive of anaemia. Several studies have reported the presence of anaemia in helminthic infection [23,24] mostly in children. Eighty two percent of the study population in the present study were mostly adults ( $\geq 18$  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 other causes including allergic reactions and drug sensitivity. Previous reports [25,26] 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 [20,27].

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 110.0 g/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 are not statistically comparable.

## 5. CONCLUSION

This study has shown that 30% of patients attending clinic at University of Calabar Teaching Hospital are positive for intestinal parasitosis with resultant 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 eosinophilia, it is recommended that stool microscopy, haemoglobin estimation and eosinophil counts be routinely performed for all patients visiting the hospital.

## 6. SIGNIFICANCE OF THE STUDY

The findings of this study will improve the care and management of patients treated at UCTH as intestinal parasitosis would be considered during clinical and laboratory diagnosis.

## CONSENT

Written consent of the participants was obtained after details of the study were explained to them, before enrollment into the study.

## INCLUSION AND EXCLUSION CRITERIA

Participation in the study was strictly voluntary. Only those subjects who gave their consent and were not on any anti parasitic and haematinic medication were included in the study. On the other hand, those who refused to give their consent, pregnant women, those diagnosed with cancer, on certain medications, such as anti-retroviral drugs for HIV infection and chemotherapy drugs for cancer, asthmatic, and drug allergy were left out of the study.

## ETHICAL ISSUE

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

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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