

Original Research Article

INTESTINAL PARASITOSIS, 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: 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 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*-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. 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

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I suggest the aim should simply be : to determine the role of intestinal parasites on the haemoglobin concentration and eosinophil levels among the patients treated at the University of Calabar Teaching Hospital (UCTH), Nigeria.

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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* (these are important species of soil transmitted helminthes (STHs))[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]. Approximately 300 million people are severely ill with these worms and of these, at least 50% are school-age children. IPIs rarely cause death but because of the size of the problem, the global number of related deaths is substantial [2]. 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

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It can also be rephrased as:
These worms cause severe illness in approximately 300 million people, of whom 50% are school-age children.

44 basic protein. These mediators are released following activation of the eosinophil and are toxic to both
45 parasitic and host tissue. In normal individuals, eosinophils make up about 1-6% of white blood cells and
46 are about 12-17 micrometers in size [12].

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47 Anaemia and eosinophilia have been associated with helminthic infection previously [13, 14, 15, 16] and
48 a high prevalence of intestinal parasites (26.6% and 29%, respectively) has been reported among
49 children as well as adults on anti-retroviral therapy in the study area (Calabar) [17, 18]. There is scarcity
50 of information on the occurrence of intestinal parasitic infection and its association with anaemia and
51 eosinophilia. It is the intention of this study, therefore, to investigate the occurrence of intestinal parasitic
52 infection and its effect on the haemoglobin and eosinophil levels among patients attending clinics in
53 University of Calabar Teaching Hospital (UCTH), Calabar, Nigeria.

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54 2. MATERIALS AND METHODS

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56 2.1 Study area: This study was carried out at the University of Calabar Teaching Hospital (UCTH),
57 Calabar, Cross River State situated in the South-South of Nigeria.

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58 2.2 Sample collection/Sampling technique: With ethical approval and informed consent of
59 participants obtained, a total of three hundred (300) randomly selected patients attending the general
60 out-patient clinic (GOPC) in UCTH were recruited for the study. Subjects included male and female
61 patients of any age who reported to the GOPC within the study period (July, 2013-May, 2014) and
62 were not on any medication. Patients who were gravely ill such that they needed to be admitted as
63 well as those on medication that would interfere with the test results were excluded. Two millilitres of
64 blood was collected aseptically from each patient into ethylene diamine tetra acetic acid bottle to a
65 final concentration of 2mg/ml. Each subject was given a sterile screw-capped wide mouth universal
66 container for collection of stool sample.

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67 2.3 Processing of samples: Blood and stool samples were processed and examined in the University of
68 Calabar Teaching Hospital laboratory. The stool samples were macroscopically examined for
69 appearance, consistency, presence of blood, mucus, worm segments and worms. Stool samples
70 were further processed by direct smear microscopy and formol ether concentration technique and
71 examined microscopically using the 10x and 40x objective

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72 for the presence of larva, cysts, and eggs of parasites [19]. Thin blood films were made, fixed and stained
73 using Leishman stain (PH 6.8). The films were viewed microscopically with oil immersion objective for
74 differential white cell count. The haemoglobin concentration was determined by
75 Cyanmethaemoglobin method [20]. The patients demographic data and the laboratory results were thus
76 fed into a computer program and analysis was hence done. Results obtained are presented as mean
77 (range). Percentages were compared using Chi Square.

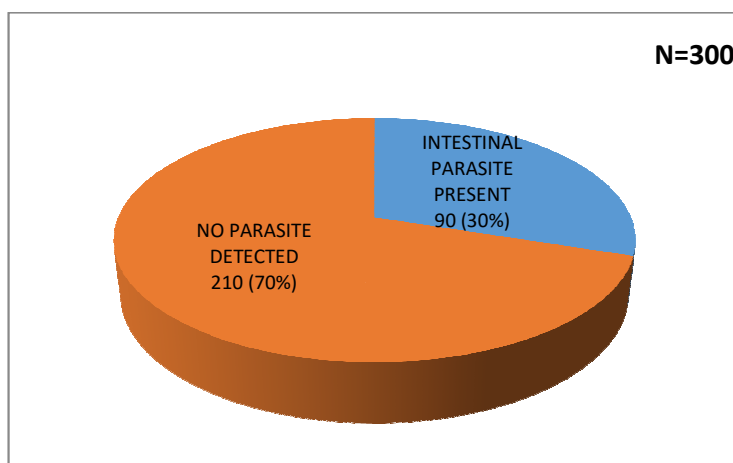
78 3. RESULTS

79 The results of this study are as shown in figures 1-2 and tables 1-4.

80 Figure 1 presents the occurrence of intestinal parasites among studied patients attending clinics in
81 University of Calabar Teaching Hospital. Intestinal parasites were detected in ninety (30%) of the 300
82 patients screened.

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83
84 **Fig. 1. Occurrence of intestinal parasites among patients attending clinic in University of Calabar**
85 **Teaching Hospital**

86 Table 1 presents intestinal parasitosis and haemoglobin (Hb) concentration among the studied subjects.

87 Twenty three percent (23%) of the 300 subjects had low Hb concentration (<110.0g/L). It was further
88 observed that 51(56.7%) of those with intestinal parasitosis compared to 18(8.6%) of their non-infected
89 counterpart had lower Hb levels. On the other hand, more of the parasitenegative subjects 192 (91.4%)

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recorded higher Hb levels than their parasite positive counterpart 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%, 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%. Of the 90 parasite positive persons, 63 (70.0%) compared to 18 (8.6%) of their parasite negative counterpart were positive for eosinophilia. On the other hand, more of the parasite negative subjects 192 (91.4%) compared to only 27 (30.0%) of their parasite positive counterpart had normal eosinophil count. The difference in prevalence was statistically significant ($X^2 = 120.61$, df1, $P < 0.001$).

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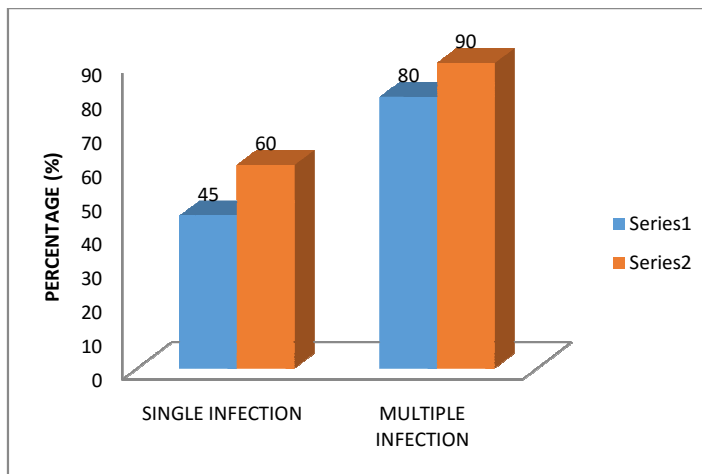
Table 2. Intestinal parasitosis and eosinophil count among patients attending clinics in UCTH

	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	27 (30.0)	192 (91.4)	219 (73.0)

count <7%

110
111 Figure 2 expresses the haemoglobin concentration and eosinophil count of parasite positive subjects with
112 single and multiple infections. Eighty percent (80%) of subjects with multiple infections had Hb
113 concentration less than 110.0g/L and this is higher when compared with forty five percent (45%) for single
114 infections. Similarly, ninety percent (90%) of those with multiple infections and sixty percent (60%) of
115 those with single infections had eosinophil counts greater than seven percent (7%).

116



117

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

118

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

121

122

123 Table 3 shows the haemoglobin and eosinophil levels of parasite positive patients by species of parasite.

124 Of the ninety parasite positive patients, 60 (66.7%) had single infections with mean Hb of 119 (60-161g/L)

125 and eosinophil count of 11 (2-25%), while 30 (33.3%) had multiple infections with mean Hb of 100 (60-

126 160) g/L and eosinophil count of 18 (2-24%), respectively. In single infections, hook worm showed the

127 highest occurrence 24 (26.7%) with mean haemoglobin level of 99.0 (60-161g/L) and mean eosinophil

128 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 *Trichuristrichiura* 9 (10.0%) with 112.0 (70-140g/L) and 13 (4-25%) as the mean haemoglobin and eosinophil values, respectively. *Entamoebahistolytica* had the least occurrence 6 (6.7%) with 140.0 g/L mean haemoglobin and 9 (2-16%) eosinophil count. For multiple infections, hookworm/*Ascaris*co-infection recorded the highest 15 (16.7%) out of 10 occurrences with haemoglobin level of 110.0 (80-160g/L) and eosinophil count of 16 (2-24%). Hookworm/*E.histolytica* co-infection was detected in 9 (10.0%) with haemoglobin level of 70.0(60-80g/L) and 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/Trichuristrichiura*co-infection was detected in 3 (3.3%) with 90.0g/L as haemoglobin concentration and 15% as eosinophil count.

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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>Ascarislumbricoides</i>	21 (23.3)	123 (90-161)	8 (2-18)
<i>Trichuristrichiuria</i>	9 (10.0)	112 (70 -140)	13 (4-25)
<i>Entamoebahistolytica</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)

142

143 Table 4 shows the prevalence of intestinal parasites, haemoglobin concentration and eosinophil count by
 144 gender and age of subjects. Of the 90 subjects positive for intestinal parasites, males constituted 39
 145 (34.2%) with haemoglobin value of 103g/L and mean eosinophil count of 14% while females were 51
 146 (27.4%) with haemoglobin value of 112g/L and mean eosinophil count of 13%. Intestinal parasites
 147 prevalence by gender of subjects was statistically insignificant ($\chi^2 = 1.55$, df1, $P = 0.2128$). Adult subjects
 148 (≥ 18 years) had an infection rate of 75 (30.5%) out of the 246 subjects examined with mean haemoglobin
 149 value of 110.0 (60-161g/L) and mean eosinophil count of 13 (12-15%) while children (<18 years) had
 150 infection rate of 15 (27.8%) out of the 54 subjects that were examined with mean haemoglobin value of
 151 100.0 (80-130g/L) and mean eosinophil count of 17 (11-21%).

152 **Table 4. Intestinal Parasites, haemoglobin concentration and eosinophil count of**
 153 **subjects by gender and age**

	Gender		Total	Age (years)		Total
	Males	Females		Children (<18)	Adults (≥ 18)	
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)						

154

155

156

Comment [k11]: Comment [k8] applies here also. If the author wants to include the text, make it a summary of the findings.

157 4. DISCUSSION

158
159 In this study, the occurrence of intestinal parasitic infection and its effect on haemoglobin and eosinophil
160 levels among three hundred patients in University of Calabar Teaching Hospital ~~were was~~ investigated.

161 Intestinal parasites were detected in thirty percent of the subjects studied (Fig-1). This finding differs from
162 previous report (17.5%) in a study conducted

163 among patients in University of Maiduguri Teaching Hospital [21] located in Northern Nigeria and. In a
164 study conducted in Okada, Edo State,

165 Nigeria [15], 50% intestinal parasite infection was reported. The variance with the present study may be
166 due to the fact that their study population consisted of children both in urban and rural areas.

167
168 As presented in (Table 1), in this study, it was observed that most (56.7%) of the patients who tested
169 positive for intestinal parasites, had haemoglobin levels < 110g/L. According to WHO report [22], Hb
170 concentration below 110g/L for all age groups and gender is suggestive of anaemia. Several studies have
171 reported the presence of anaemia in helminthic infection [23, 24] mostly in children. Eighty two percent of
172 the study population in the present study were mostly adults (≥18 years), thus implying that anaemia is
173 associated with intestinal parasitosis irrespective of age. The low haemoglobin concentration observed in
174 intestinal parasitosis has been attributed to reduced iron absorption at the duodenum and jejunum since
175 the parasites tend to colonize these sites, as well as chronic blood loss, especially in hookworm infection
176 [15].

177 Twenty seven percent of the 300 subjects examined were observed to have eosinophil count greater than
178 7% (Table 2) while seventy three percent had less than 7%. Furthermore, seventy percent of the parasite
179 positive subjects were positive for eosinophilia with the remaining thirty percent being negative. Helminthic
180 infection is usually the first suspicion

181
182 when eosinophilia is observed in our locality and this study confirms that intestinal parasitosis is
183 associated with eosinophilia. Interestingly, 8.6% of the parasite negative persons had eosinophilia. This
184 could most likely be due to other causes including allergic reactions and drug sensitivity. Previous reports
185 [25, 26] support this finding. As part of host defenses against helminthic infestation, helper 2 T cells (Th-
186 2) produce interleukin 5 which in turn stimulates an increase in the production of eosinophils.

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187 Eosinophils attach to the surface of the parasite through IgE and secrete cytotoxic enzymes contained in
188 their granules [20, 27].

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

204
205 Although the difference in Hb concentration between males and females has been firmly established, in
206 this study the Hb concentration of male subjects was lower than that of female subjects as presented in
207 Table 4. This is probably due to the fact that the female subjects were more in number than their male
208 counterparts who may also have been more affected by the infection. No gender difference was observed
209 with respect to eosinophil count. Similarly, the adult subjects enrolled in the present study outnumber the
210 children, hence the differences observed are not statistically comparable.

211 A line of the significance of this study will be appreciated.

212

213 CONCLUSION

214 This study has shown that 30% of patients attending clinic at University of Calabar Teaching Hospital are
215 positive for intestinal parasitosis with resultant anaemia and eosinophilia. The decrease in haemoglobin
216 concentration and increase in eosinophil count is directly related to the degree of parasitosis with
217 reference to single and multiple infections.

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218 Intestinal parasitosis results in anaemia and eosinophilia. ~~The decrease in haemoglobin concentration~~
219 ~~and increase in eosinophil count is directly related to the degree of parasitosis with reference to single~~
220 ~~and multiple infections.~~ Although other diseases may be associated with haemoglobinaemia and
221 eosinophilia, it is recommended that stool microscopy, haemoglobin estimation and eosinophil counts be
222 routinely performed for all patients visiting the hospital

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223 **CONSENT**

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

226 **INCLUSION AND EXCLUSION CRITERIA**

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

232 **ETHICAL ISSUE**

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

235 **CONFLICT OF INTERESTS:** None declared.

AUTHORS' CONTRIBUTIONS

'Author A' designed the study and wrote the protocol. 'Author B' wrote the first draft of the manuscript. 'Author A' and 'Author C' managed the analyses of the study. 'Author C' performed the statistical analysis. 'Author B' and 'Author C' managed the literature searches. All authors read and approved the final manuscript."

ACKNOWLEDGEMENTS

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