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Haematological and Inflammatory-Associated Cytokine Profiles of Gastrointestinal Parasite-Infected Individuals in The Ahafo Region of Ghana

Badiwon Charles[1], Bhavana Singh[1], Ayimbire Abonuusum[2], Patrick Feglo[1], Yusif Mubarik[3] and Sanjeev Singh*[4]

[1]Department of Clinical Microbiology, SMS, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]Bolgatanga Technical University, Upper East Region, Bolgatanga, Ghana.
[3]West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Legon, Greater Accra, Ghana.
[4]Department of Anaesthesiology and Intensive Care, SMS, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

***E. mail : drsanjeev73@rediffmail.com**

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ABSTRACT

Background: The Ahafo region of Ghana, an intestinal parasites endemic community where the primary approach for control involves annual deworming, lacks haematological and immunological epidemiological investigations. This study aimed to investigate the haematiological and immunological profiles of individuals hosting intestinal parasitic infections in the Ahafo region. **Methods:** This was a single-centre case-control cross-sectional study with convenience sampling among 200 individuals, 100 intestinal parasites infected and 100 non-infected controls. We gathered demographic and epidemiological details via a survey, identified intestinal parasites using formol-ether concentration method and collected blood samples to analyze haematological immunological indices. **Results:** A significantly higher intestinal parasite infection rates were observed among male participants than female participants. Our study revealed abnormally low levels of eosinophils, monocytes, neutrophils lymphocytes, RBCs and haemoglobin among individuals in the Ahafo region. The average IFN-γ levels in the plasma of individuals with intestinal parasites infection and the non-infected controls were (95.44 ± 51.435 ρg/mL) and (103.20 ± 67.77 ρg/mL), respectively with p-value=0.588. However, the mean TNF-α plasma level (1355.68 ± 541.54 ρg/mL) of intestinal parasites infected participants was significantly higher as compared to the non-infected controls (1570.57 ± 511.87 ρg/mL) (p-value < 0.001). Furthermore, the mean levels of the anti-inflammatory cytokine IL-10 (215.89 ± 126.16 ρg/mL) among intestinal parasites infected participants was higher as compared to that of the controls (215.89 ± 126.16 ρg/mL) (p-value < 0.001). **Conclusions:** The study revealed increased IL-10 levels and reduced TNF-α and IFN-γ levels in parasite-infected participants as compared to uninfected controls. Our study underscored the need for critical public health interventions to address the immunological and haematological alterations in intestinal parasites endemic populations.

INTRODUCTION

Soil transmitted gastrointestinal parasites impact over 2 billion individuals globally, particularly in developing nations within tropical and subtropical regions. The most prevalent species include *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms (*Necator americanus* and *Ancylostoma duodenale*). These parasitic infections contribute to approximately 40% of the worldwide disease burden caused by infectious agents (WHO, 2012). Notably, vulnerable groups to these parasites include children and women of childbearing age. The severity of illness due to intestinal parasites infection is closely associated with parasite burden. High parasites infection burden hinders physical growth, cognitive development, and result in micronutrient deficiencies, particularly iron-deficiency anemia (Hotez *et al*., 2008). Lower parasitic infection burden, due to their chronic and subtle nature, can negatively affect health outcomes. To mitigate the intestinal parasites-associated morbidity, the World Health Organization (WHO) has proposed periodic (annual or biannual) mass drug administration of benzimidazole-based drugs to high-risk groups, especially pre-school and school-age children (WHO, 2012). Besides the health impact, gastrointestinal parasite infections influence the human host in more nuanced ways. As extracellular parasites, they mostly induce a type-2 immune response driven by T-helper type 2 (Th2) cells. This response is characterized by an expansion of mast cells, eosinophils, basophils, group 2 innate lymphoid cells (ILC-2), and alternatively activated macrophages (AAMs), accompanied by increased production of Th2 cytokines (IL-4, IL-5, and IL-0) and IgE (Mishra *et al*., 2014; Díaz and Allen, 2007; Gause *et al*., 2013). Chronic helminth infections, such as those caused by STH, display a modified type-2 response where regulatory mechanisms are superimposed upon the basic response pattern. This regulation is largely achieved through the expansion and induction of regulatory T cells (Treg), accompanied by elevated levels of anti-inflammatory cytokines like IL-10 and transforming growth factor beta (TGF-β) (McSorley and Maizels, 2012; Taylor *et al*., 2012). This anti-inflammatory network is thought to contribute to the reduced prevalence of allergic diseases in populations with a skewed Th2 response (Endara *et al*., 2010; van den Biggelaar *et al*., 2004; Yazdanbakhsh *et al*., 2001). However, concerns arise in regions where STH infections co-exist with other ailments like HIV/AIDS, malaria, and tuberculosis, as effective clearance of the latter is dependent on an active and timely type-1 response (Mulu, Legesse, *et al*., 2013; Salazar-Castañon *et al*., 2014). Consequently, successful deworming treatment is proposed as a practical and cost-effective means to rebalance the immune response (Mulu *et al*., 2015; Mulu, Maier, *et al*., 2013). Recent research has observed significant decreases in eosinophil counts, IL-10 levels, and IgE concentrations following anti-helminthic treatment (Abate *et al*., 2015; Anuradha *et al*., 2016). In support, in HIV-1 infected individuals, deworming led to reduced plasma HIV RNA levels and CD8+ T cell counts (Mulu, Maier, *et al*., 2013; Anuradha *et al*., 2016). However, some studies have found no evidence of these effects (Mulu, Maier, *et al*., 2013; Abate *et al*., 2015). Therefore, the main aim this study was to characterize the haematological and immune profiles of study participants living in the Ahafo region of Ghana with gastrointestinal parasite infections.

MATERIALS AND METHODS

Study Participants Selection and Ethical Clearance:

The study was carried out at the Saint Elizabeth Hospital in the Ahafo region of Ghana. Approval for the study was granted by the Committee on Human Research, Publication and Ethics (CHRPE)

at Kwame Nkrumah University of Science and Technology (KNUST) with reference number CHRPE/AP/241/20. For inclusion in the study, participants who had not received deworming treatment for more than two years were included in the study. Written informed consent was obtained from all the participants.

Determination of Sample Size:

The sample size was calculated using the formula, $n = \frac{[(z^2 \, x \, p(1-p)]}{e^2}$. Where, n = sample size, Z = t- value at 95% confidence level, P = prevalence and e = critical error limit. A report by WHO (WHO Ghana 2023 Annual Report), revealed that, the national incidence of TB among adults in Ghana was 129 per 100,000 population. Using this value to calculate the prevalence, $\frac{129}{100{,}000} \, x \, 100$ = 0.129 % (0.00129) as prevalence.

Substituting this value of prevalence, p = 0.129% (0.00129), at 95% confident level, z = 1.96,

and error, e = 0.5% (0.005)

$n = \frac{[(1.96^2 \, x \, 0.00129(1-0.00129)]}{0.005^2} = [\frac{(3.8416 \, x \, 0.00129 \, x \, 0.99871}{0.000025}]$ = 198 Hence the sample size is 198

Considering a 1% dropout rate = 1/100 × (198) = 1.98

The required sample size (n'): [estimated: 198 + (dropout: 1.98)] = [199.98] = 200.

Whole blood samples were collected from enrolled participants for full blood count (FBC) and cytokines profile analyses after centrifugation to obtain serum, also single stool sample from each participant. P -value was significant at ≤0.05.

Data Compilation:

A structured questionnaire was utilized to gather fundamental demographic and epidemiological data from the study participants. This was carried out through brief face-to-face interviews conducted in the local language "Twi".

Stool Collection and Parasite Identification:

Each participant provided a single stool sample. These samples were stored in portable coolers and examined at the laboratory facilities of the Saint Elizabeth Hospital in the Ahafo region of Ghana. The presence of intestinal parasites was assessed using the wet mount and formol-ether concentration methods. To ensure diagnostic accuracy, a different researcher reviewed 100% of negative smears and 10% of positive smears immediately after the initial reading. Formol-ether concentration was performed one month after sample collection.

Blood Collection and Haematological Analysis:

Blood samples were collected from the cubital vein using 5ml tubes each containing either K2 EDTA or serum separator and clot activator (BD Vacutainer, NJ, USA). Serum separator tubes were centrifuged within 4 hours, and serum was stored at -80°C until immunological analysis. Hematological parameters analyses were conducted within 2 hours of sample collection using the Sysmex XN-550 analyzer. Briefly, once the XN-550 haematology analyzer is powered on, a check is performed to ensure that all device switches are set to the "ON" position before the system logs in. Quality and stored patient controls were analysis and approval prior to progressing to the analysis of participant samples. Patient identification numbers (IDs) were entered and samples were mixed before being placed within the tube holder. By activating the tube holder through the start switch, the samples were aspirated and the tube holder was withdrawn to facilitate the removal of the samples.

Analyses of Cytokine Levels Using ELISA:

Cytokine concentrations in serum samples were quantified using the Biolegend's Elisa Max™ Standard Sets. The cytokine analyses were conducted following the manufacturer's instructions. Briefly, serum samples from all participants enrolled in the study and stored at a temperature of -80°C. The commercially acquired ELISA test kits were employed for

the measurement of IFN-γ (with a minimum detectable level of 4 pg ml^{-1}), IL-10 (with a minimum detectable level of 2 pg ml^{-1}), and TNF-α (with a minimum detectable level of 4 pg ml^{-1}). To outline the procedure briefly, the capture antibodies were appropriately diluted in buffer A at a 1X concentration and subsequently added (100µl) to each well of a 96-well plate. After undergoing overnight incubation at a temperature of 5°C, the plates were subjected to four rounds of washing (with 300µl/well of wash buffer), and excess buffer was removed through tapping. Subsequently, 200µl of 1X assay diluent was introduced to each well. The plates were covered and left to incubate at room temperature for an hour on a shaker. After the blocking step, the plates were again subjected to four wash cycles using the wash buffer. Standards and samples (100µl/well) were introduced and subjected to a two-hour incubation at room temperature with shaking. Following this incubation, another round of four washes were performed, after which 100µl of the appropriately diluted detection antibody was added to each well. The plates were sealed and subjected to an hour of incubation with shaking. Four wash cycles were conducted using the wash buffer. Subsequently, 100µl of Avidin-HRP solution, suitably diluted, was introduced to each well. The plates were sealed and incubated for 30 minutes with shaking. Following this, the plates underwent five washes, each involving soaking for one minute. Then, 100µl of TMB substrate solution was added to each well. After a 20-minute incubation in darkness, 100µl of stop solution was added to terminate the reaction. Finally, the absorbance at 450 nm was measured within a span of 15 minutes

Statistical Analysis:

Data was analysed using Statistical Package for Social Sciences (SPSS) 23.0, IBM, Armonk, NY. Normally distributed data was presented as mean ± SD, and percentage. The study population was characterized using descriptive statistics. Point prevalence with 95% confidence intervals (CI) was calculated for overall gastrointestinal infections, individual parasite species, and mixed STH infections (involving two or more species). Given the non-Gaussian distribution of immunological markers, non-parametric methods were used. All statistical analyses with significance set at $p < 0.05$.

RESULTS

Demographics characteristics of the study population:

Table 1, outlines the proportions of frequency and distribution of demographic variables among study participants. A total of 200 study participants; 100 each of intestinal parasites and non-infected control participants were included in this study as shown in Figure (1). The average age of parasite infected participants was 35.56 ± 8.49 years, while for the controls, it was 35.56± 8.49 years. The difference in ages between parasites infected and control participants was not statistically significant (p =0.062) as shown in Table 1. The number of male participants were significantly higher than female participants (p =0.002) (Table 1).

Table 1: Characteristics of study population.

Variables		Control	Parasite-infected	Total	p-value
Age	Mean (SD)	35.56 (8.5)	35.53 (7.2)	35.54 (7.8)	0.062 ¥-
Sex	Male (%)	89 (44.5)	73 (36.5)	162 (81.0)	0.002#
	Female (%)	11 (5.5)	27(13.5)	38 (19.0)	
	Total	100	100	200	

¥- One-way ANOVA, F, # Pearson Chi-Square, χ , *statistically significant

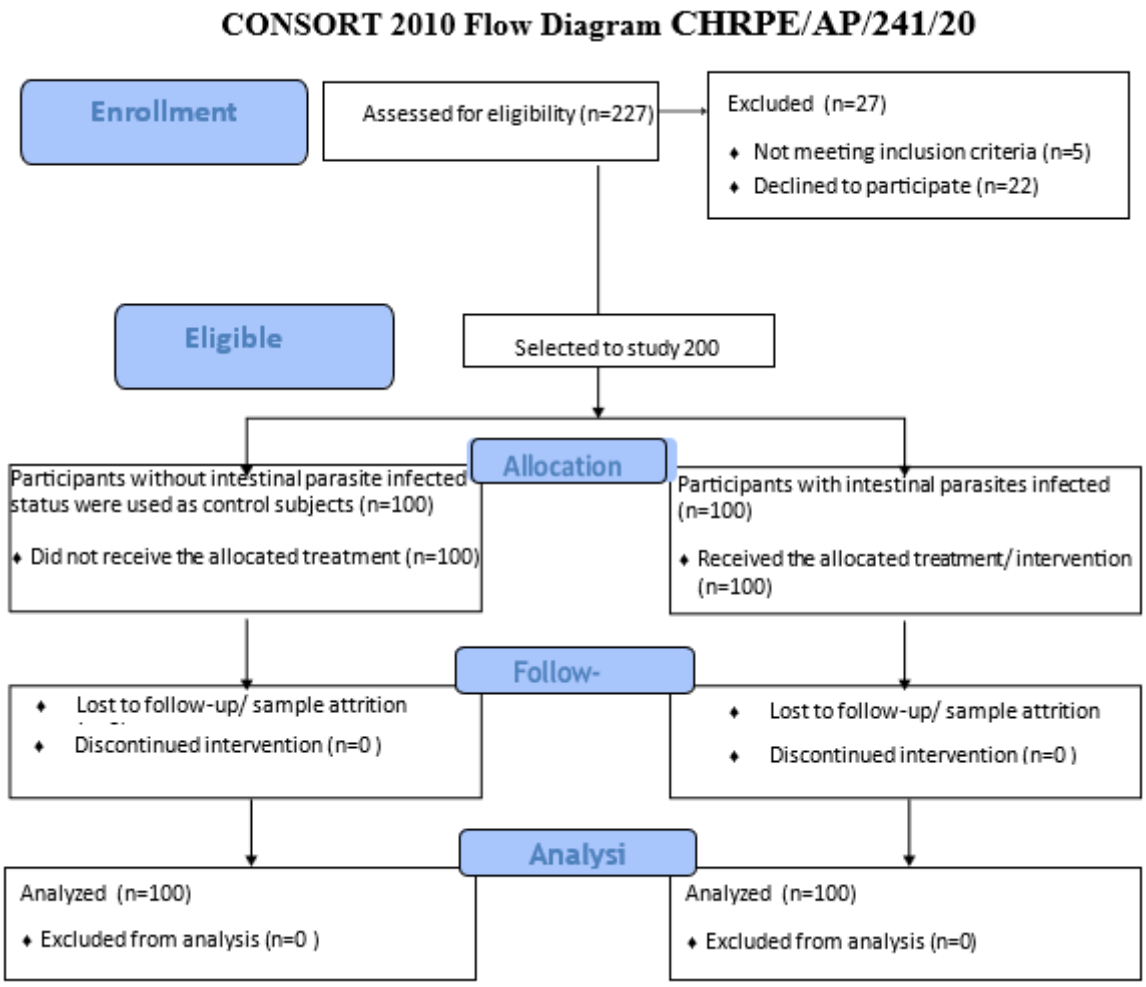

Fig. 1: Consort 2010 flow diagram for study CHRPE/AP/241/20**.**

Prevalence of intestinal parasites infections among study participants:

From Table 2, *Giardia lamblia* infection prevalence was 18.0%. Both *Ascaris lumbricoides* and *Entamoeba histolytica* infections prevalence among participants was 9.5%. In addition, the percentages of participants infected with hook worm, *Strongyloides stercoralis* and *Balantidium coli* were 7.0%, 5.5% and 1% respectively as shown Table 2.

Table 2: Prevalence of soil transmitted parasitic infections among participants.

Parasite	Number of participants infected (%)
G. lamblia	36 (18.0)
A. lumbricoides	19 (9.5)
E. histolytica	19 (9.5)
Hook worm	14 (7.0)
S. stercoralis	11 (5.5)
B. coli	1 (0.5)
Total	100

Haematological Indices of Study Participants:

Table 3, compares where applicable the medians and means of haematological parameters among parasites infected and non-infected controls. The Mean difference of RBC (4.1 ± 0.8 vs 4.0 ± 0.8; p=0.645), HgB (11.6 ± 2.1 vs 11.2 ± 2.3; p= 0.227), PLT (241.3 ± 96.0 vs 248.5 ± 108.4; p=0.346), NEUT (4.7 ± 2.9 vs 4.8 ± 3.3; p= 0.564), LYMPH (3.1 ± 2.3 vs 3.2 ± 2.5), MONO (0.8 ± 0.1 vs 0.8 ± 0.1; p=0.960), BASO (0.1 ±0.4 vs 0.04 ± 0.04; p=0.006) and IG (0.1 ±0.3 vs 0.1 ± 0.1; p=0.109) between these two groups were not significant. On the contrary, the mean differences of WBC (10.7 ± 4.9 vs 8.2 ± 3.3; p=0.004) and EO (2.1 ± 1.1 vs 0.1 ± 0.2; p=0.0001) between the parasites infected and non-infected controls were significant (Table 2).

Table 3. Hematological profile comparison of study participants, categorized into Mtb infection and controls.

Parameters	Groups	Mean (SD)	p-value ¥-
RBC (10^6/uL)	Control	4.0 (0.8)	0.645
	Infected	4.1 (0.8)	
	Total		
HgB(g/dL)	Control	11.2 (2.3)	0.227
	Infected	11.6 (2.1)	
	Total		
PLT (10^3/uL)	Control	248.5 (108.4)	
	Infected	241.3 (96.0)	0.346
	Total		
WBC (10^3/uL)	Control	8.2 (3.3)	0.004*
	Infected	10.7 (4.9)	
	Total		
NEUT (10^3/uL)	Control	4.8 (3.3)	0.564
	Infected	4.7 (2.9)	
	Total		
LYMPH (10^3/uL)	Control	3.2 (2.5)	0.399
	Infected	3.1 (2.3)	
	Total		
MONO (10^3/uL)	Control	0.8 (0.1)	0.960
	Infected	0.8 (0.1)	
	Total		
EO (10^3/uL)	Control	0.1 (0.2)	0.0001*
	Infected	2.1 (1.1)	
	Total		
BASO#(10^3/uL)	Control	0.04 (0.04)	0.006*
	Infected	0.1 (0.4)	
	Total		
IG (10^3/uL)	Control	0.1 (0.1)	0.109
	Infected	0.1 (0.3)	
	Total		
¥- Student T test, *statistically significant			

Table 3. RBC: Red blood cell, HgB: Hemoglobin, LYM: Absolute lymphocyte, WBC: Total white blood cell, PLT: Platelet, MON: Absolute monocyte, NEUT: absolute neutrophil, EO: absolute eosinophil, BASO: absolute basophil, IG: absolute immunoglobulins. Independent samples t-test and MannWhitney U test were used appropriately to compare parametric and non-parametric distributions respectively, between parasites infected patients and controls, p-value was significant at ≤0.05.

Comparison of haematological indices among study participants:

Figure 1, displays the comparison of the frequencies of the various hematological parameters between parasites infected and the control study participants. PLT, WBC and BASO levels of majority of the participants were normal and revealed no significant associations between these parameters and intestinal parasites infection (p= 0.780, p=0.159 and p= 0.246 respectively). However, HgB and RBC levels among majority of the participants were low with no significant associations between HgB and RBC levels and the parasites infection (p=0.372, p=0.593 respectively). Interestingly, all the study participants recorded low NEU, LYM, MONO and EO levels (FIGURE 2f, 2g, 2h, 2i respectively).

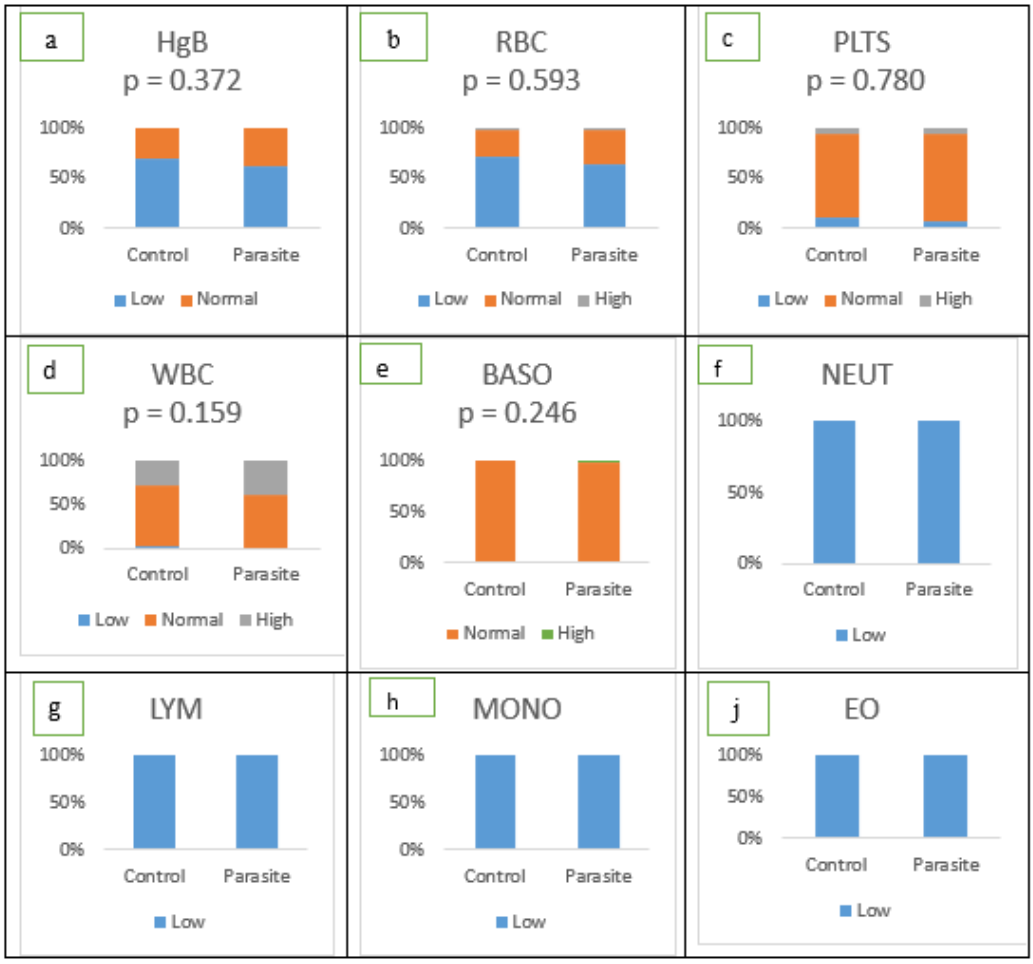

Fig. 2. RBC: Red blood cell, HgB: Hemoglobin, LYM: Absolute lymphocyte, WBC: Total white blood cell, PLT: Platelet, MON: Absolute monocyte, NEUT: absolute neutrophil, EO: absolute eosinophil, BASO: absolute basophil, IG: absolute immunoglobulins. Chi-square test was used appropriately to determine the association between these parameters and intestinal parasites infection, p-value was significant at ≤0.05.

Comparison of Serum Inflammatory-Related Cytokine Profiles Among Study Participants:

According to Figure 3a, the average IFN-γ levels in the plasma of individuals with intestinal parasites infection and the non-infected controls were determined to be 95.44± 51.435 ρg/mL and 103.20±67.77 ρg/mL respectively with no significant difference between them (p-value=0.588). However, the mean TNF-α plasma level (1355.68±541.54 ρg/mL) of intestinal parasites infected participants was significantly higher as compared to the non-infected controls (1570.57 ±511.87 ρg/mL) (p-value < 0.001) (FIGURE 3b). Furthermore, the mean levels of the anti-inflammatory cytokine IL-10 (215.89 ±126.16 ρg/mL) among intestinal parasites infected participants was significantly higher as compared to that of the controls (215.89 ±126.16 ρg/mL) (p-value < 0.001) (FIGURE 3c).

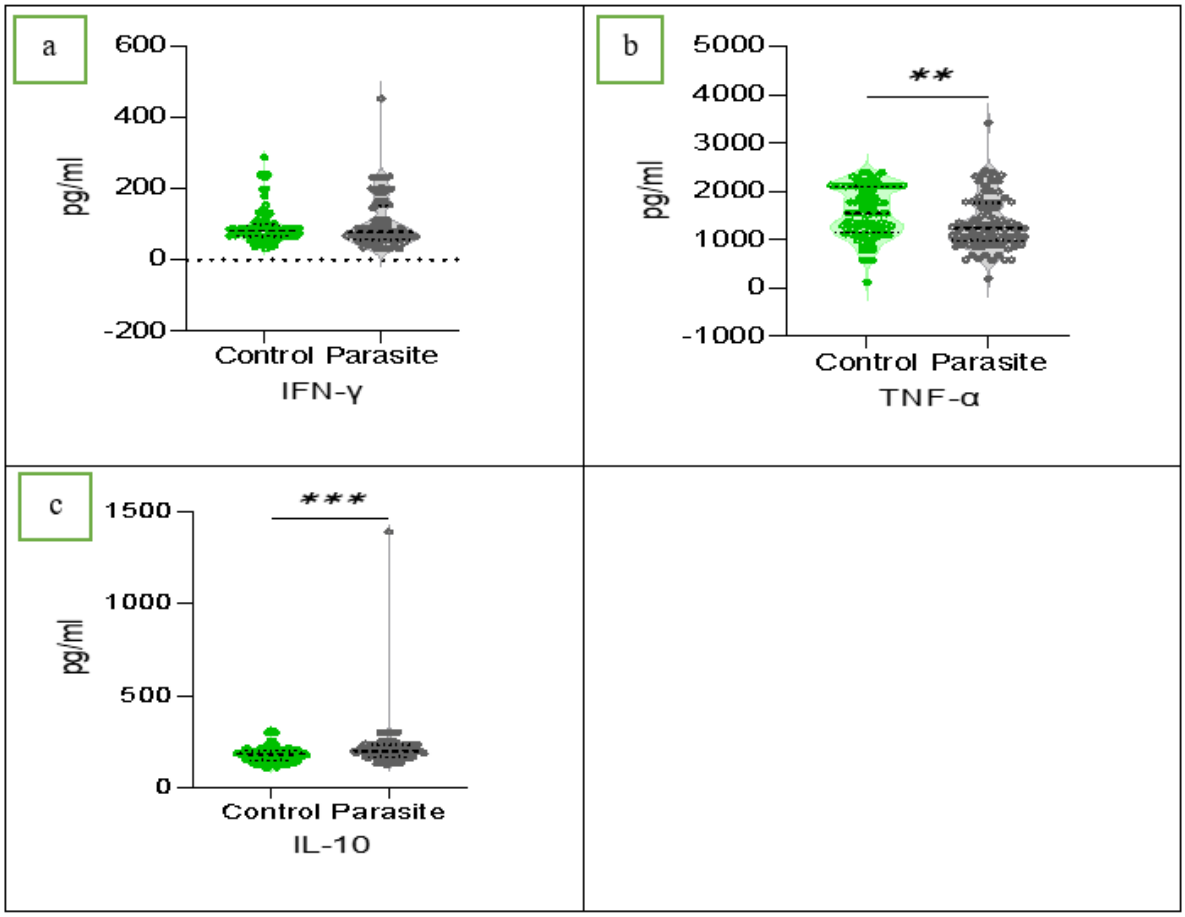

Fig. 3: Average plasma levels of **a)** TNF-α, **b)** IL-10 and **c)** IFN-γ among intestinal parasites infected patients and healthy controls.

DISCUSSION

Prevalence of Gastro-Intestinal Parasites Infection:

The results of this study revealed gastro-intestinal parasites prevalence of 36.5% and 13.5% among males and females respectively. This gender-based parasitic infection prevalence differences can be due attributed to differences in exposure rate, as males are highly exposed to parasitic infections as compared to females (Forson *et al*., 2017; Duedu *et al*., 2015). Our investigation revealed 7.0% hookworm prevalence which is lower as compared to other studies conducted in Accra, the capital city of Ghana (Forson *et*

al., 2017; Abaka-Yawson *et al*., 2020). Similarly, lower *G. lamdblia prevalence of* 18.0% was observed in our study as compared to previous study conducted in Northern and Southern Ghana (Abaka-Yawson *et al*., 2020; Sisu *et al*., 2021). The lower prevalence of hookworm and *G. lamdblia* infection observed in this current study among participants living in the Ahafo region could be attributed to the effectiveness of mass drug administration (MDA) control measure organized in the study communities. Nevertheless, our investigation revealed higher infection rates of 9.5% *A. lumbricoides*, 9.5% *E. histolystica*, 5.5% *S. stercolaris* and 0.5% *B. coli* as compared to previous studies conducted in the Southern (Forson *et al*., 2017) and middle belt (Adu-Gyasi *et al*., 2018) of Ghana, a scenario which demand further investigations.

Haematological Parameters:

The majority of hematological parameters examined in our study exhibited significant abnormalities in both the parasites infected and uninfected controls participants. Eosinophils, monocytes, neutrophils and lymphocytes levels were consistently low across all participant groups. Also, majority of the study participants recorded low red blood cell (RBC) count and hemoglobin (HgB) levels. Similar findings have been reported in Ghana (Addai-Mensah *et al*., 2019), Uganda (Eller *et al*., 2008), Kenya (Kibaya *et al*., 2008) and Botswana (Mine *et al*., 2012). This suggest that a proportion of the inhabitants of the study communities could be mistakenly be categorized as having anaemia, erythrocytopaenia, neutropenia, monocytopenia and lymphocytopenia using the most often quoted reference ranges. The lower RBCs and levels among our study participants as compared to the Caucasians can be attributed to the relatively lower transferrin and ferritin saturation among the African population (Beutler and West, 2005) and the poor nutritional status among the general Ghanaian population (Mutapi *et al*., 2007). Additionally, the lower absolute neutrophil levels across all participants groups in our study has been associated with asymptomatic or benign reductions in neutrophils (BEN) among individuals with African ancestry (Grann *et al*., 2008). Substantial evidence for the genetic control BEN has been reported (Charles *et al*., 2018). Similarly, Goswami et al., has reported lower neutrophil levels among healthy African population. Nevertheless, normal platelets, basophils and total WBC levels were observed among majority of our study participants. Critically, the inconsistencies in the haematological profiles, particular between the individual WBCs (neutrophils, basophils, eosinophils, monocytes, lymphocytes) profiles and the total WBCs profile could be attributed to inter-regional, intra-regional and international reference ranges for haematological profiles within the studied population as observed by (Addai-Mensah *et al*., 2019).

Inflammation-related cytokines profile:

In our study, a significant elevated levels of IL-10 were observed among the parasite infected participants as compared to the non-infected controls. Similarly, high serum IL-10 levels among parasites infected individuals have been reported (Resende *et al*., 2019; Rodrigues *et al*., 2018). In support, helminth infections in mice promoted high IL-10 levels secretion in intestinal tissues (Webster *et al*., 2022). Our study results also revealed significantly lower TNF-α and IFN-γ among the parasite infected participants as compared to the non-infected controls. This suggest that, the high levels of parasites mediated IL-10 secretion may be exhibiting its regulatory functions by suppressing the secretion of TNF-α and IFN-γ among the parasites infected participants as reported by (Mutapi *et al*., 2007; Kiflie *et al*., 2023). In in-vivo intestinal parasites infection models, IL-10 ability to suppress IFN-γ secretion by inducing elevated expression of IL-10 receptors on Th1 cells has been reported (Webster *et al*., 2022). In an agreement, IL-10 blockade in-vivo during intestinal

parasites infections mediated Tbet^{+} and IFN-γ Th1 cells expansion (Webster *et al*., 2022). Thus, elucidating the observed high and reduced IL-10 and pro-inflammatory cytokines (TNF-α and IFN-γ) respectively among the parasites infected participants as compared to the controls.

CONCLUSION

Our study highlights a significant gender disparity in relation to intestinal parasites infection. A significantly higher intestinal parasite infection rates were observed among male participants than female participants. This study revealed abnormal low levels of eosinophils, monocytes, neutrophils lymphocytes, RBCs and hemoglobin among individuals in the Ahafo region. The study also revealed increased IL-10 levels and reduced TNF-α and IFN-γ levels in parasite-infected participants, suggesting that IL-10 may play a regulatory role in suppressing pro-inflammatory cytokines, indicating the complex interactions between host immune system and intestinal parasites. Our study underscored the need for critical public health interventions to address the immunological and haematological alterations in intestinal parasites endemic populations. Further studies are needed to elucidate these dynamics and improve health outcomes.

DECLARATIONS:

Ethical Approval: The study was approved by the Committee on Human Research Publication and Ethics (CHRPE) at Kwame Nkrumah University of Science and Technology (KNUST) with reference number CHRPE/AP/241/20 and performed at the Saint Elizabeth Hospital in the Ahafo region, Ghana.

Authors Contributions: BC, BS, AA, PF and SS participated in the conceptualization and the design of the research protocol as well as participated in the laboratory work and writing of the manuscript. BC, AA and YM participated in the collection of samples.

Consent for publication: All authors agreed with the content and that all gave explicit consent to submit and that they obtained consent from the responsible authorities at the institute/organization where the work has been carried out, before the work is submitted.

Conflict of interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability Statement: All data used for the study are available in the manuscript and also upon request from the Principal Investigator.

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