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Haematological and Biochemical Alterations in School-aged Children Infected with *Giardia lamblia* and *Plasmodium* Spp. in Owerri West, Imo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author NJC did Conceptualization, performed methodology, wrote and prepared the original draft of the manuscript. Authors NJC and AEAA performed methodology. Author AEAA did data curation, formal analysis. Author NAA wrote, reviewed and edited the manuscript. Authors NJC and NAA investigated the work. All authors read and approved the final manuscript.

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ABSTRACT

Background: Parasitic infections such as giardiasis and malaria are endemic in many parts of sub-Saharan Africa and disproportionately affect children. It remain a significant public health challenge, particularly among school-aged children in developing countries, where access to clean water and

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healthcare is limited. This study investigated the haematological and biochemical alterations associated with infections of *Giardia lamblia*, *Plasmodium* spp. and their co-infection among pupils aged 5–15 years in Owerri West LGA, Imo State, Nigeria. A total of 108 stool and blood samples were analysed using standard parasitological, haematological and biochemical techniques. Data were expressed as mean ± standard deviation. Prevalence rates showed that 72.22% of the pupils were infected with at least one parasite: 25.64% were infected with *G. lamblia* alone, 43.5% with *Plasmodium* spp., and 30.76% had co-infections, while 27.78% were parasite-free. Haematological results showed lower values in red blood cell (RBC) count, packed cell volume (PCV) and mean corpuscular volume (MCV) among infected groups compared to the control group. RBC ranged from $1.40 \times 10^{12} \pm 0.02$ /L in co-infected pupils to $2.90 \times 10^{12} \pm 0.60$/L in *Giardia*-infected pupils, compared to $4.15 \times 10^{12} \pm 0.40$/L in controls. PCV dropped from 36.45 ± 0.21% in controls to as low as 23.12 ± 0.33% in co-infected individuals. MCV followed a similar trend, falling from 81.4 ± 0.68 fL in controls to 63.0 ± 1.04 fL in co-infected cases. Biochemical analysis revealed reductions in serum total protein and albumin among infected pupils. The total protein in the control group was 8.1 ± 0.46 g/100 mL, while the co-infected pupils had 3.40 ± 0.30 g/100 mL. Albumin levels also declined, with co-infected pupils showing 2.18 ± 0.04 g/100ml compared to 4.25 ± 0.03 g/100ml in controls. The observed alterations demonstrate the impact of parasitic infections, particularly in cases of co-infection, highlighting the clinical implications and need for targeted intervention to mitigate health risks in affected populations. The alterations observed in red blood cell indices, white blood cell counts, and protein levels collectively demonstrate the impact of parasitic infections, particularly in cases of co-infection, highlighting the clinical implications and need for targeted intervention to mitigate health risks in affected populations.

Keywords: Giardia lamblia; Plasmodium; haematological parameters; serum; albumin; pupils.

1. INTRODUCTION

Malaria is a severe vector-borne illness caused by *Plasmodium,* a protozoan parasite. The bite of infected female *Anopheles* mosquitoes facilitates the transition of the parasite to humans and other animals. (Kataria et al., 2022; Khan *et al*., 2022). Parasitic infections such as giardiasis and malaria are endemic in many parts of sub-Saharan Africa and disproportionately affect children (World Health Organization, 2022). *Giardia lamblia*, a flagellate protozoan, causes gastrointestinal infection, often linked to poor hygiene and sanitation (Choy et al., 2014). while *Plasmodium* spp., the etiological agents of malaria, are transmitted via the bite of infected *Anopheles* mosquitoes (World Health Organization, 2022). These infections compromise the nutritional and immunological health of children, leading to developmental delays and poor academic performance (Tapajós et al., 2019). Intestinal parasitic infection is one of the major health issue in developing countries, particularly in Sub-Saharan Africa. It has been estimated to affect about 3.5 billion people globally, and 450 million people are thought to be ill as a result of such infections, the majority being children (Mohammed *et al*., 2019; Danish *et al*., 2021). Although individual effects of these parasites on health are documented (Afolabi et al., 2021), co-infections may result in synergistic or antagonistic interactions that exacerbate or mitigate disease impact. This study seeks to analyse haematological parameters in children infected and co-infected with these protozoa, to provide a foundation for improved clinical and public health interventions.

2. MATERIALS AND METHODS

2.1 Study Area and Population

This research was conducted in Owerri West Local Government Area of Imo State, Nigeria. The region includes rural, semi-urban, and urban communities. A total of 20 primary schools were randomly selected across these settings. The study targeted pupils aged 5–15 years, and ethical approval was obtained from the relevant institutional review board.

2.2 Sample Collection

Stool and blood samples were collected from 108 pupils using sterile containers and Vacutainer tubes, respectively. Pupils were classified into test and control groups based on the presence or absence of infection.

2.3 Parasitological Analysis

- ***Giardia lamblia*** **Detection:** Faecal samples were processed using saline and

iodine wet mounts and examined microscopically for the presence of *Giardia* cysts and trophozoites.

- ***Plasmodium* spp. Detection:** Thick and thin blood smears were prepared, air-dried, and stained using Giemsa stain. Parasite identification and quantification were performed under oil immersion (100x objective lens). Parasitemia was calculated as the number of parasites per microliter of blood.

2.4 Haematological Analysis

- **Red Blood Cell (RBC) Count:** Measured using an automated haematology analyser calibrated before use.
- **Packed Cell Volume (PCV):** Determined using the microhematocrit method. Blood was centrifuged in capillary tubes at 12,000 rpm for 5 minutes, and the PCV was read using a hematocrit reader.
- **Mean Corpuscular Volume (MCV):** Calculated using the formula: MCV (fL) = (PCV × 10) / RBC count.
- **White Blood Cell (WBC) Count and Differentials:** Total and differential counts were done using the same haematology analyser.Manual verification was performed using Leishman-stained blood films.

2.5 Biochemical Analysis

Total Protein: Test tubes were labelled as reagent blank, standard or sample. 0.02 mL each of distilled water, standard (66g/L bovine serum albumin), and serum sample was pipetted into the test tubes. Then,1.0 mL of Biuret Reagent solution (6mmol/L Copper (II) acetate, 12mmol/L potassium iodide, 1.15mmol/L sodium hydroxide, and detergent) was added to each of the test tubes and mixed thoroughly. The tubes were incubated for 30 minutes at room temperature, and the absorbance of the sample and standard was read against the reagent blank at 500nm wavelength in a spectrophotometer (Anyalogbu, 2022). The total protein content [TP] of the serum sample was calculated using the formula:

$$[TP] = \frac{Abs\ of\ sample}{Abs\ of\ std}\ x\ conc.of\ std$$

Albumin: The method employing bromocresol green (BCG)was used (Anyalogbu, 2022). The absorbance of the albumin–BCG complex at 630nm wavelength is proportional to the albumin concentration in the sample. In an acidic medium (pH 4.1), BCG reacts with albumin to form an intense green complex.

$$Albumin + Bromocresol \xrightarrow{pH\ 4.1} Green\ complex$$

Based on the protocol, test tubes were labelled blank, standard or sample. Albumin standard and serum samples (10µl each) were pipetted into the standard and sample test tubes, respectively. Then 1.0 mL of Bromocresol reagent (100mmol/L acetate buffer (pH 4.1), 0.27mmol/L bromocresol green, detergent) was added to all the test tubes, mixed thoroughly and incubated for 1 min at room temperature. The absorbance of the sample (and standard) was read against the blank at 630nm wavelength in a spectrophotometer. The albumin content [A] of the sample was calculated using the formula.

$$[A] = \frac{Abs\ of\ sample}{Abs\ of\ std}\ x\ conc.of\ std$$

Globulin: Globulin levels were calculated by subtracting the albumin concentration from the total protein concentration (Savory & Hammond, 1980).

Statistical Analysis: Results were expressed as mean ± standard deviation.

3. RESULTS

3.1 Prevalence of Infection

Fig. 1 shows the distribution of the parasites among the pupils in the selected schools. Out of the total of 78 samples infected, 20 (25.64%) had *Giardia spp.*, 34(43.5%) had *Plasmodium* spp. while 24(30.76%) samples had mixed infections of *Giardia* and *Plasmodium* spp.

3.2 Haematological Parameters

The mean values of the haematological parameters assayed are shown in Table 1, and the percentage effect of the presence of the parasites on these parameters is depicted in Fig. 2. Analysis of the data showed that the RBC of the control ($4.15x10^{12}$±0.40/L) was higher than that of the tests, which ranged from $1.40x10^{12}$±0.02 to $2.90x10^{12}$± 0.6/L. The PCV (36.45±0.21%) and mean cell volume (81.40±3.29 fL) of the control were higher than

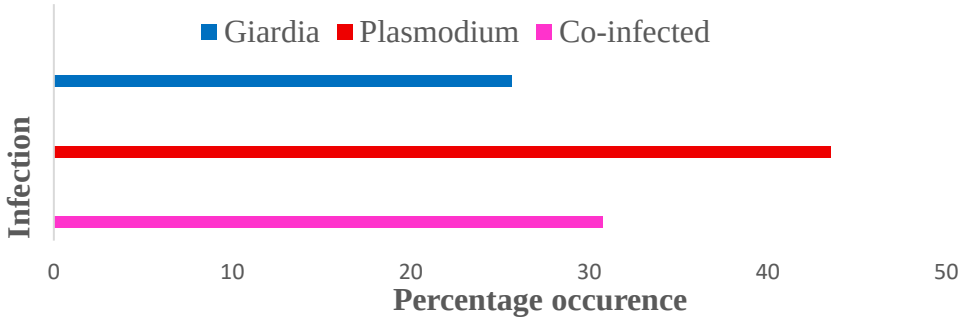

Fig. 1. Frequency of infection in sampled population

Table 1. Mean values of the Haematological Indices assayed

Group	RBC (×10^12/L)	PCV (%)	Reticulocyte Proportion (%)	MCV (fL)
Control	4.15 ± 0.40	36.45 ± 0.21	0.68±0.05	81.40 ± 3.29
Giardia	2.90 ± 0.60	28.40 ± 0.30	1.40±0.20	72.1 ± 0.72
Plasmodium	2.10 ± 0.20	25.12 ± 0.76	1.20±0.05	78.71 ± 1.12
Co-infected	1.40 ± 0.02	23.12 ± 0.33	0.93±0.05	63.0 ± 1.04

Values are means ± standard deviations of triplicate determinations

those of the tests, which gave the ranges: 23.12±0.33 - 28.40±0.03% and 63.0±1.04 - 78.70±01.12fL, respectively. On the other hand, the reticulocyte proportion of the control (0.68%) was lower than that of the tests, 0.93±0.08 - 1.40±0.20%. Relative to the control, the presence of the parasites has a decreasing effect on the mean values of RBC, PVC, and MCV, while a converse effect was seen in the reticulocyte proportion.

3.3 Biochemical Parameters

Table 2 presents the mean serum levels of Total Proteins, Albumin, and Globulin (g/100ml) for the test groups (*Giardia, Plasmodium*, and *Plasmodium* + *Giardia*) and the control group. Analysis of the data showed that the total protein level in the control group (8.10±0.46 g/100ml) was higher than that of the test groups, which ranged from 3.40±0.31 to 6.13±0.01 g/100ml. The albumin level in the control group (4.25±0.03 g/100ml) was also higher compared to the test groups, with the range 2.18±0.04 to 4.16±0.10 g/100ml. For globulin, the control group's value (1.15±0.06 g/100ml) was comparable to those of the test groups, which ranged from 1.22±0.02 to 1.97±0.02 g/100ml. Fig. 3 indicates that while total proteins and albumin were variably decreased, globulin was increased.

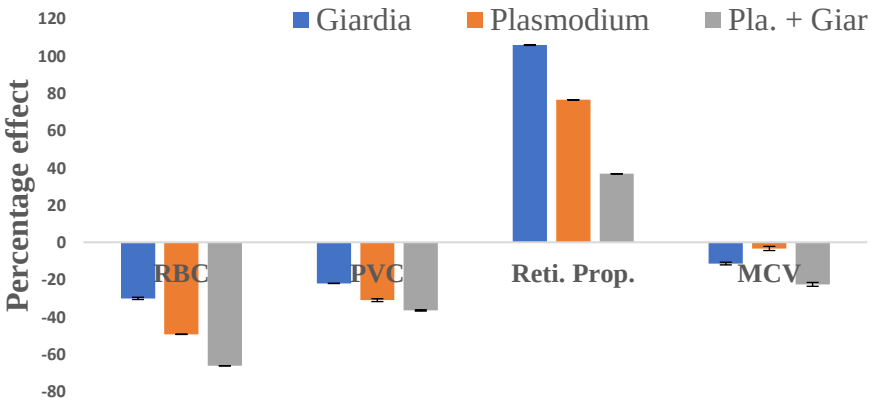

Fig. 2. Percentage effect of the parasites on the Haematological Indices

Table 2. Mean Serum levels of Total Proteins, Albumin and Globulin (g/100ml)

Group	Total Protein (g/100ml)	Albumin (g/100ml)	Globulin (g/100ml)
Control	8.14 ± 0.46	4.25 ± 0.03	1.15 ± 0.06
Giardia only	4.50 ± 0.10	3.21 ± 0.08	1.29 ± 0.04
Plasmodium	6.13 ± 0.01	4.16 ± 0.10	1.97 ± 0.02
Co-infected	3.40 ± 0.31	2.18 ± 0.04	1.22 ± 0.02

Values are Means ± Std deviations of triplicate determinations

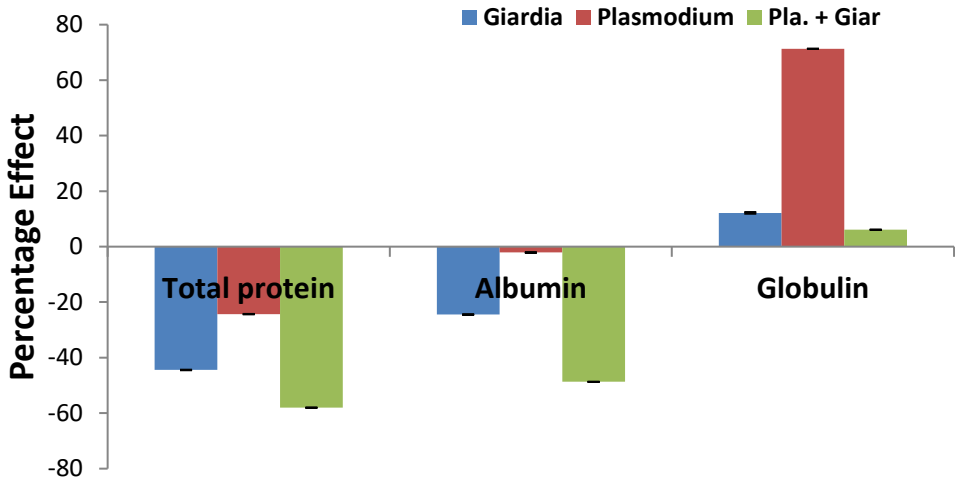

Fig. 3. Percentage effect of parasites on Serum levels of Total Proteins, Albumin and Globulin

4. DISCUSSION

This study demonstrates the considerable effects of infections with *G. lamblia* and *Plasmodium spp*. on haemotological and biochemical parameters among school-aged children in Owerri West, Nigeria. The prevalence of infection (72.22%) among pupils reflects the endemicity of these protozoan parasites in sub-Saharan Africa. This finding is consistent with similar studies that have reported high parasitic burdens in children from resource-poor environments (World Health Organization, 2022; Choy et al., 2014).

Malaria, caused by *Plasmodium* parasites and transmitted via Anopheles mosquitoes, was observed in 43.50% of the infected individuals. This prevalence aligns with global trends where *Plasmodium* remains a leading cause of morbidity and mortality in tropical regions (World Health Organization, 2023).

Co-infections with *Giardia* and *Plasmodium* were observed in 30.76% of the infected population, highlighting frequent exposure to multiple parasitic species in this environment. Synergistic interactions between co-infecting parasites may lead to immunosuppressive effects, increased parasite load, and more severe clinical outcomesb (Griffiths, 2011). Giardia infection, though primarily a gastrointestinal parasite, can lead to anaemia through mechanisms like malabsorption of essential nutrients for erythropoiesis, such as iron (Calvao et al., 2011). Giardia infections have also been linked to iron-deficiency anaemia (Olivares et al., 2004). Fig. 2 shows that Giardia infection decreased the RBC count by 30.12% compared to the control group, indicating its impact on red blood cell production.

Malaria, caused by *Plasmodium* parasites, is well-known for directly destroying red blood cells as the parasite invades and ruptures them. Additionally, the immune response to *Plasmodium* leads to the destruction of infected and uninfected RBCs, further worsening anaemia (White, 2018). *Plasmodium* infection reduced RBC counts by 49.39%, as shown in Fig. 2. The reduction of this magnitude highlights malaria's profound effect on erythropoiesis and its association with anaemia, a common complication of the infection (Singh & Singh, 2022).

The co-infected group showed the most pronounced effect, with a 66.27% reduction in RBC count compared to the control group. The lowest mean RBC count was observed in this

group, suggesting a synergistic negative impact on RBC production or survival (Afolabi et al., 2021). Co-infection may exacerbate mechanisms such as RBC destruction by *Plasmodium* and nutrient malabsorption by Giardia, compounding the risk of anaemia (Tanih et al., 2023). In comparison, the control group exhibited the highest RBC count, underscoring the high reductions caused by parasitic infections. These findings emphasise the compounded effects of Giardia and *Plasmodium* on anaemia, with the co-infection group showing the most severe impact.

Packed cell volume (PVC), or hematocrit, is the percentage of blood volume occupied by RBCs and a critical indicator of anaemia and overall blood health. In Giardia infections, a decrease in PVC may be linked to chronic malabsorption and nutrient deficiencies (Haque et al., 2003). Giardia infection reduced PVC by 21.11% (2) compared to the control group, with a mean value of 28.4±1.0% (Table 1).

Plasmodium infection resulted in a more pronounced reduction in PVC (25.12±4.2%) as shown in Table 1, representing a 30.22% (Fig. 2) decrease compared to the control group. A decrease in PVC is a common finding in *Plasmodium* due to RBC destruction, further exacerbated by immune responses targeting both infected and uninfected RBCs.

The lowest PVC value was observed in the co-infected group (23.12±3.3%), reflecting a 35.77% reduction compared to the control group. This more pronounced decrease suggests the combined effects of haemolysis from *Plasmodium* and nutrient malabsorption from *Giardia*.

The control group exhibited the highest PVC (36.45±2.2%), emphasising the significant reductions caused by parasitic infections. Lower PVC levels in school-aged children can reduce the oxygen-carrying capacity of blood, leading to fatigue, impaired concentration, and diminished academic performance (Black et al., 2013).

Giardia-infected individuals exhibited an increased reticulocyte proportion (1.4%) (Fig. 2), indicating a compensatory response to potential anaemia associated with giardiasis (Anyalogbu et al., 2020). *Giardia* may inhibit bone marrow response due to nutrient deficiencies, which are critical for erythropoiesis. This aligns with the findings of (Koukounari et al., 2008), who noted lower haemoglobin levels in individuals with parasitic infections.

In malaria, reticulocyte proportions are elevated as a response to anaemia caused by haemolysis of RBCs by the *Plasmodium* parasite. The *Plasmodium* group showed a 76.47% increase (Fig. 2) in reticulocyte count compared to the control group, reflecting an intensified erythropoietic response to replenish depleted RBCs.

In the co-infection group, the reticulocyte proportion was lower (0.93%) compared to single-infection groups, suggesting suppressed erythropoiesis. *Plasmodium* causes anaemia through haemolysis, while Giardia contributes via malabsorption and nutrient deficiencies. Together, they may have a synergistic effect on suppressing bone marrow activity. The smaller reticulocyte increase (36.76%) compared to single infections highlights the overwhelmed compensatory capacity of the bone marrow in co-infected individuals, which could result in chronic fatigue and developmental delays in school-aged children (Okell et al., 2019). The control group had a normal reticulocyte proportion (0.68%). Compared to the control, Giardia infection increased reticulocyte count by 105.88%, *Plasmodium* by 76.47%, and co-infection by 36.76% (Fig. 2). These findings confirm that parasitic infections hugely impact bone marrow activity, with co-infection posing the greatest challenge to maintaining adequate RBC production

Giardia-infected individuals showed a substantial reduction in MCV (72.1 fL), indicating potential iron deficiency anaemia caused by malabsorption and blood loss associated with *Giardiasis* (Hotez et al., 2008). The decrease in MCV by 11.43% (Fig. 2) compared to the control group highlights the pronounced impact of *Giardia* infection on iron metabolism and erythropoiesis. Such reductions in MCV are commonly linked to microcytic anaemia, a condition associated with iron deficiency (Rogawski et al., 2017). Individuals infected with *Plasmodium* exhibited a slightly lower MCV (78.7 fL), a 3.32% decrease compared to the control group. A study by Mohandas & An (2012) supports this observation, noting that *Plasmodium*-induced inflammatory responses impair RBC maturation in the bone marrow, leading to smaller RBCs and reduced MCV.

The co-infected group had the lowest MCV (63.0 fL), reflecting a 22.6% decrease compared to the

control group. This severe reduction suggests more pronounced microcytic anaemia due to the combined effects of both parasitic infections on iron metabolism and erythropoiesis (Afolabi et al., 2021).

The more marked decline in haematological parameters in the co-infected children suggests a possible synergism with both parasites, causing compounded damage to the host's erythropoiesis and increased red cell destruction (Afolabi et al., 2021).

The control group had an MCV of 81.4 ± 6.2 fL, representing normal RBC size. Compared to this group, Giardia and *Plasmodium* infections, as well as co-infection, showed significant reductions in MCV, indicative of microcytic anaemia.

The mean serum total protein level in Giardia-infected subjects was 4.50 ± 0.10 g/100ml, which was much lower than the control group's mean of 8.1 ± 0.04 g/100ml (Table 2). This reduction indicates possible protein malabsorption or increased protein loss due to *G. lamblia* infection, which impairs fat, fat-soluble vitamins, and protein absorption.

Plasmodium-infected subjects had a mean total protein level of 6.13 ± 0.01 g/100ml, higher than Giardia-infected individuals but still lower than the control group. This reduction may have resulted from inflammatory responses induced by *Plasmodium* species, which are known to cause mild hypoalbuminemia (Smith & Brooker, 2010).

The co-infected group exhibited the lowest total protein level of 3.40 ± 2.3 g/100ml, reflecting a 58.02% decrease compared to the control. This severe reduction likely results from the compounded effects of both parasites, causing malabsorption, impaired protein synthesis, and heightened protein catabolism (Anyalogbu et al., 2020).

The control group had a mean total protein level of 8.1 ± 0.64 g/100ml, representing normal levels. Giardia infection caused a 44.44% reduction, *Plasmodium* a 24.32% reduction, and co-infection caused the largest decrease (Fig. 3). Low total protein levels, especially in co-infected children, highlight the need for nutritional interventions to prevent complications like muscle wasting, impaired wound healing, and susceptibility to infections (Friedman et al., 2020; Black et al., 2013).

Albumin levels in Giardia-infected individuals were markedly reduced (3.21 ± 0.8 g/100ml) compared to the control (4.25 ± 0.03 g/100ml) (Table 2). This reduction likely reflects nutrient malabsorption, particularly of amino acids needed for albumin synthesis, or hepatic dysfunction. Giardia-induced hypoalbuminemia, as noted by Khan et al. (2022) is primarily due to malabsorption and impaired liver function. This aligns with the findings of Mabbott (2018) who observed significantly lower albumin levels in children with Giardia infections compared to healthy controls.

The *Plasmodium* group showed a mean albumin level of 4.16 ± 0.10 g/100ml, slightly lower than the control. This minimal reduction could result from the acute-phase response, where the liver prioritises the synthesis of other proteins to combat infection.

The co-infected group exhibited the most severe reduction in albumin levels (2.18 ± 2.4 g/100ml), representing a 48.71% decrease compared to the control (Fig. 3). This marked reduction suggests compounded effects of malabsorption, inflammation, and liver dysfunction. Co-infection likely exacerbates gastrointestinal losses of albumin and suppresses hepatic albumin synthesis through the inflammatory release of cytokines like IL-6 and TNF-α (Rogawski et al., 2017). Such severe reductions align with the findings of Mohandas & An (2012); Anyalogbu et al., (2020) who emphasised the synergistic impact of dual infections on malnutrition.

The control group's albumin level was 4.25 ± 0.03 g/100ml, representing normal physiological levels. Giardia infection reduced albumin by 24.47%, *Plasmodium* by 2.11%, and co-infection by 48.71% (Fig. 3). Reduced albumin levels, particularly in co-infected children, have serious implications, including oedema, impaired nutrient transport, and malnutrition.

The mean globulin level in Giardia-infected individuals was 1.29 ± 0.04 g/100ml. Globulin proteins, including immunoglobulins, are critical for the immune response. Giardia infections result in a moderate increase in globulin levels (12.17% above control) (Fig. 3), likely reflecting a mild immune activation. Studies by Ajibaye et al., (2024); Perlmann & Troye-Blomberg, (2000) highlight that Giardia infections stimulate a humoral immune response, though to a lesser degree compared to *Plasmodium*.

In malaria patients, the globulin level was 1.97 ± 0.02 g/100ml, indicating a pronounced immune response with a 71.3% increase from the control. *Plasmodium* typically elicits a strong immunoglobulin response, with elevated levels of antibodies and acute-phase proteins like CRP (Farthing, 1996; Odunukwe et al., 2000).

The globulin level in co-infected patients was 1.22 ± 0.04 g/100ml, showing a smaller increase (6.08% above control) and a highly variable immune response, as indicated by the wide standard deviation. The control group's globulin level was 1.15 ± 0.06 g/100ml, representing baseline values. The observed increase in globulin levels with *Plasmodium* and Giardia infections reflects the body's immune activation and inflammatory response. Chronic inflammation and malnutrition, commonly associated with elevated globulin levels, can exacerbate health challenges and negatively impact school children's growth and performance (Rogawski et al., 2017; Ajibaye et al., 2024).

These findings emphasise the need for integrated parasite control programs, which should include regular screening, adequate treatment and locally appropriate sanitation and hygiene education in school health programs (Mauel, 2024).

5. CONCLUSION

The alterations observed in red blood cell indices, white blood cell counts, and protein levels collectively demonstrate the impact of parasitic infections, particularly in cases of co-infection, highlighting the clinical implications and need for targeted intervention to mitigate health risks in affected populations.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

As per international standards, parental written consent has been collected and preserved by the author(s).

ETHICS APPROVAL

Approved by the Institutional Review Board, Federal University of Technology Owerri. Approval Number: FUTO/SOBS/REC/2025/0017

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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