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Evaluation of Immunological Markers in Children Infected with Intestinal Parasites in Three Communities, Nigeria

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Abstract

Helminthiases are neglected parasites that induce changes in the host's immune response. The study assayed some immunological markers in helminth infections in children and adolescents. Stool and blood samples were collected from 276 subjects resident in three rural communities of Rivers and Imo States. Of these, 98 (35.5%) were infected with one or more of 3 parasitic species, of which *Ascaris lumbricoides* 39 (39.8%), *Ancylostoma duodenale* 30 (30.6%) and *Trichuris trichiura* 29 (29.6%) were recovered. Intestinal helminth parasites were identified using formol-ether concentration. Of the infected subjects, 66 had single species infections and 32 had multiple infections with two or three helminth species and children of the 6 - 10 years age group had the highest incidence. Sera of 60 infected subjects and 30 controls were evaluated to define immunological serum levels of IgE, IL-5, IFN-γ and C3 using the ELISA technique, while white blood cell differential counts and neutrophil-lymphocyte ratio (NLR) were evaluated using haematology auto-analyser. Elevation of IL-5, IgE, WBC and eosinophil levels were demonstrated in the infected groups ($p < 0.01$), higher in the multiple infected groups, compared to the control. Their concentrations across the different age groups were also significantly different ($p = 0.0001$), the highest in 6 - 5 years and the lowest in 0 - 5 years. Differences in IFN-γ and C3 levels among non-parasitized, single-parasitized and poly-parasitized groups were not statistically significant. Neutrophil, lymphocyte and NLR levels between control and helminth infected subjects were not significantly different. The result suggested that IgE, IL-5, total WBC and eosinophil increased in helminth infections, more in poly-parasitized and 6 - 10 years children.

Keywords

Immune Response, Parasites, Helminth, Neutrophil

1. Introduction

Helminthiases are the most common of the neglected tropical diseases affecting over two billion people worldwide and accounting for about 40% of global mortality due to infectious diseases [1]. Soil-transmitted helminths (STH) infection remains a serious public health issue globally with over 1.5 billion people infected [2]. Soil-transmitted Helminths are intestinal parasitic nematodes that significantly affect school-aged and preschool children and often harm their hosts by destroying tissue integrity, feeding on blood, and suppressing the immune response to other infectious diseases [3] [4]. They mainly affect the poorest and most deprived communities and are transmitted by eggs present in human feces which in turn contaminate soil in areas where sanitation is poor. Infection with gastrointestinal parasites has been globally considered a serious public health problem in the tropical countries of the world due to the fact that they cause iron deficiency anemia, growth retardation in children and other physical and mental health problems [5]. Poor water and sanitation practices are major risk factors for the distribution, infection, and prevalence of STH [6]. Generally, two types of cellular immune responses are important in the task of protecting the host against infection. These are the T helper cell type 1 (Th1) and type 2 (Th2) responses, with the former related to protecting against protozoans, intracellular bacteria and viruses, while the latter is more effective against helminthes and extracellular bacteria. Cytokines involved in Th1 response include interferon gamma, (IFN-γ), interleukin (IL)-6, and tumour necrosis factor (TNF)-α, while cytokines such as IL-4, IL-5 and IL-10 are involved in Th2 responses. These responses are also antagonistic, as the IFN-γ negatively modulates the Th2 response, while IL-4 and IL-10 negatively modulate the Th1 response, thereby enabling homeostasis in the immune system and a balanced immune response. The host reacts to worm infections with a dominant T-helper type 2 cell (Th2) response with the production of significant quantities of IL-4, IL-5, IL-9, IL-10, and IL-13 and consequently the development of strong immunoglobulin E (IgE), eosinophil, basophil and mast cell responses [7]. IL-5 is the most important cytokine in the transformation and development of eosinophils, and acts as an "eosinophil activator" [8]. In addition, the complement system, which is made up of a large number of distinct plasma proteins (such as C1, C2 and C3), may be important for immunity to infection with parasitic helminths, by promoting the recruitment of leukocytes to infected tissues and by modulating the function of cytotoxic effector leukocytes [9]. The complement performs its protection role by activating the attack complex at the membrane (C5-C9) and facilitating opsonization through the C3b component, which binds to the parasites and interacts at a second stage with the specific receptor existing in phagocytic cells. Studies showing the relationship between immunological response and helminths infestation among children/adolescents in Rivers State and Imo State have not been documented. Hence, the study was aimed to assay some immunological markers in children/adolescents infected with single or multiple helminths resident in Obedum and Emirikpoko Communities in Abua/Odual Local Government Area, Rivers State and Obinze Community (Mammy Market, Artillery 32 Military Base) in Owerri Local Government, Imo State. The findings will be useful for a better understanding on the immunological response to helminth infestations and subsequent identification of therapeutic targets. Also, this study will provide baseline data for future research and control programmes in this direction especially in Rivers State.

2. Materials and Methods

2.1. Study Area

The study area is Obedum and Emirikpoko communities in Abua/Odual Local Government Area, Rivers State. The Local Government is located geographically between Latitudes 4.5 and 6.0 degrees north of the equator and Longitude 6.0 and 7.0 degrees east of the Greenwich Meridian and has a population of 282,410 [10]. The inhabitants of these clans live in clustered homesteads of mainly brick houses and a few mud houses, reinforced with bamboo sticks. Ponds, well water, streams, rivers and recently installed borehole pumps in the communities are sources of water for both economic and domestic uses. On the other hand, the Obinze community has shanty houses or Slums with houses made of wood and zinc, clustered settlement patterns with common toilets, and often a pit latrine that is not properly closed. Some shanty houses lack toilet facilities and bushes are used for toilet purposes. They are predominantly Moslems and have distinct socio-cultural and traditional norms peculiar to them. Also, their occupational and sanitary conditions predispose them to parasitic infections. The present research was carried out at the Departments of Environmental Biology, Medical Microbiology and Parasitology, Haemotology and Chemical Pathology Department, University of Port Harcourt Teaching Hospital (UPTH), Obio/Akpor Local Government Area, Rivers State. UPTH is located at East West Road, Port Harcourt, Nigeria; 4˚53'58"N 6˚55'43"E (coordinates).

2.2. Study Design

The study was a cross-sectional descriptive survey conducted in three rural communities: Obedum and Emirikpoko in Abua/Odual Local Government Area of Rivers State and Obinze in Owerri L.G.A in Imo State to evaluate the immune status of helminth infected children/adolescents with single and multiple helminths. Subjects were selected through a simple random sampling technique to ensure that all subjects had an equal chance of being selected. Plastic containers were given to each child/ adolescent for the collection of stool sample which was analysed. From the results obtained in the stool analysis, participants were grouped into three (3) categories (G0, G1, and G2). Subjects with no parasite infection and apparently healthy were considered as G0 (control). Subjects infected with a single parasite—*Ascaris lumbricoides*, *Ancylostoma duodenale*, or *Trichuris trichiura* were categorized as G1. Subjects infected with more than one of the parasites were categorized as G2. Samples of ninety children/adolescents comprising, 30 subjects without infection (control), 30 subjects with single helminth infection and 30 subjects with multiple helminth infection (30) and sexes were males (42) and females (48). The number of subjects in the different age brackets was 0 - 5 years (7), 6 - 10 years (42) and 11 - 15 years were subjected to enzymes–linked immunosorbent assay (ELISA) to measure IgE, IL5, IFN-γ, and C3. White Blood Cell Counts (WBC) and Neutrophil Lymphocyte Ratio (NLR) of subjects were also measured.

2.3. Stool Analysis

Stool samples were processed and first examined for their consistency, colour and presence of blood, mucous and proglottides of tapeworms, with the aid of an applicator stick. Stool samples were processed and examined using direct saline, iodine, and formol ether concentration techniques. Infection intensity was detected by modified Kato-Katz thick smear technique to counting the number of eggs per gram (epg) of feaces according to the World Health Organization standard, which divides the intensity of infection to light infection (1 - 4,999 epg), moderate infection (5000 - 49,999 epg) and heavy infection (≥50,000 epg). Parasite species recovered were identified on the basis of morphological features of the parasite's eggs and cyst [11].

2.4. Analysis of Immunological Parameters and White Blood Cell (WBC) Counts

Venous blood samples were used for the analysis of lymphocyte and neutrophil counts, and NLR was calculated as a simple ratio of the absolute neutrophil count to the absolute lymphocyte count. Serum was used for the analysis of IgE, IL5, IFN-γ, and C3 using human enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, Shanghai, China, while IgE was from Calbiotech, El Cajon, CA, USA) were used according to the manufacturer's instructions.

2.5. Statistical Analysis

Summary statistics of each variable were presented as mean ± SD and as the number of subjects (percentage) as appropriate. Continuous variables were analysed by independent sample t-tests, while categorical variables were analysed by Chi-square tests. The One-Way ANOVA was used to make comparisons in the immunological and haematological parameters between the 3 groups, while Dunn's post's test was used for multiple comparisons across the groups. The t-test statistic was used to test for differences in the haematological and immunological parameters between the two groups. All analyses were done with the GraphPad Prism software at a 95% confidence interval and a p-value less than 0.05 was considered significant.

3. Results

A total of 276 children and adolescents aged 0 - 15 years were examined (124 males and 152 females). Among the total population sampled, 98 (35.5%) were infected with one or more of the intestinal parasites: *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Trichuris trichiura*. The infection rate was the highest in the Obinze community, 59 (46.6%), followed by the Emirikpoko community, 26 (29.9%), while the Obedum community, had the least prevalence 13 (19.1%). Among the infected population, the infection rate in the 0 - 5 years age bracket was 8 (8.2%), 6 - 10 years was 54 (55.1%) and 11 - 15 years was 36 *i.e.* 36.7% (Table 1), Also, the total number of infected males was 42 (42.9), while females were 56 (57.1). Furthermore, the total number of *Ascaris lumbricoides* was 39 (39.8%), *Ancylostoma duodenale* was 30 (30.6%) and *Trichuris trichiura* was 29

Table 1. Age and sex distribution and parasitological findings of the study population.

Variable	Number of participants	Number infected (%)
Community		
Obedum (Rivers State)	68	13 (19.1)
Emirikpoko (Rivers State)	87	26 (29.9)
Obinze (Imo State)	121	59 (46.6)
Total	276	98 (35.5)
Age		
0 - 5 years	39	8 (8.2)
6 - 10 years	126	54 (55.1)
11 - 15 years	111	36 (36.7)
Gender		
Male	124	42 (42.9)
Female	152	56 (57.1)
Parasite profile		
A. lumbricoides		39 (39.8)
Ancylostoma duodenale		30 (30.6)
T. trichiura		29 (29.6)
Single infection		66 (67.3)
Multiple infection		32 (32.7)
Intensity (epg)		
Single infection		814.7 ± 99.30 epg
Multiple infection		1516 ± 856.0 epg
Serum study n = 90		
Groups		
Non-parasitized (control)		30 (33.3)
Single infection		30 (33.3)
Multiple infection		30 (33.3)
Gender		
Male		42 (46.7)
Female		48 (53.3)
Age Groups		
0 - 5 years		7 (8.9)
6 - 10 years		42 (46.7)
11- 15 years		41 (45.6)

(29.6%). In addition, the number of subjects that had single parasite infestation was 66 (67.3%) while those that had more than one multiple infection was 32 (32.7%) (Table 1). For serum analysis the number of subjects without infection (control) was 30, the number of subjects with single helminth infection was 30 and subjects with multiple helminth infection was 30. In addition, the sexes were males (42) and females (48). The number of subjects in the different age brackets was: 0 - 5 years (7), 6 - 10 years (42) and 11 - 15 years (41) Table 1. The mean serum concentrations of immunoglobulin E (IgE), interleukin-5 (IL-5), interferon gamma (IFN-γ), and complement 3 (C3) in control subjects without helminth infection were- 8.10 ± 2.83 µL, 54.4 ± 2.97 ng/mL, 1.49 ± 0.07 ng/mL and 55.36 ± 5.61 ng/mL, respectively (Table 2). IgE, IL-5, IFN-γ, and C3 of subjects with single helminth infections were: 346.65 ± 29.25 µL, 56.68 ± 3.11 ng/mL, 3.17 ± 0.98 ng/mL and 58.59 ± 10.67 ng/mL respectively, while the concentrations in subjects with multiple helminth infections were: 447.03 ± 21.52 µL, 78.03 ± 12.3 ng/mL, 1.94 ± 0.52 ng/mL, 52.03 ± 9.88 ng/mL, respectively (Table 2). IgE concentrations across the different groups of subjects were statistically significant ($p < 0.0001$). The IgE concentration in the control subjects was significantly lower ($p < 0.0001$) than the IgE concentrations in subjects with single or multiple helminth infections. Similarly, the difference in the IgE concentrations between the subjects with single and multiple infections was statistically significant ($p = 0.0020$), and the multiple helminth infected participants value was higher (Table 2). There was no significant difference in IL-5, IFN-γ and C3 across the different groups, however, the IL-5 concentration in the subjects with multiple infections was higher ($p < 0.05$) compared to control subjects as well as single infection (Table 2). The total WBC count in the control subjects was 5.11 ± 0.31 × 10^9/L, while the count in single helminth infected subjects was 5.75 ± 0.38 × 10^9/L and multiple infected subjects were 6.64 ± 0.33 × 10^9/L. The total WBC concentration was higher in subjects with multiple parasites infection (Table 3). Eosinophil concentration in the control subjects was 3.01% ± 0.28% and was significantly lower ($p < 0.0001$) than the eosinophil concentration in subjects with single helminth

Table 2. Serum levels of immunological parameters in non-parasitized (control) and parasitized children/adolescents resident in selected rural communities in Rivers State and Imo State.

Immunological parameters	Control (Non-parasitized) n = 30	Single helminth Infection n = 30	Multiple helminth infection n = 30	ANOVA	Dunn's Multiple Comparison Test		
					Control vs Single	Control vs Multiple	Single vs Multiple
IgE (µL/mL)	8.10 ± 2.83	346.65 ± 29.25	447.03 ± 21.52	0.0001	0.0001***	0.0001***	0.0020**
IL-5 (ng/L)	54.48 ± 2.97	56.68 ± 3.11	78.03 ± 12.32	0.0657	0.8448	0.0424*	0.0463*
IFN-γ (ng/L)	1.49 ± 0.07	3.17 ± 0.98	1.94 ± 0.52	0.2305	0.1085	0.6727	0.2105
C3 (ng/L)	55.36 ± 5.61	58.59 ± 10.67	52.03 ± 9.88	0.8804	0.8152	0.8133	0.6148

Data are presented as Mean ± Standard Error of Mean, n = 90. *Significant at $p < 0.05$, **Significant at $p < 0.01$, ***Significant at $p < 0.0001$.

Table 3. Blood levels of WBC counts and NLR in non-parasitized (control) and parasitized children/adolescents resident in selected rural communities in Rivers State and Imo State.

Parameters	Control (Non parasitized)	Single infection	Multiple infection	ANOVA	Dunn's Multiple Comparisons		
					Control vs Single	Control vs Multiple	Single vs Multiple
Total WBC (10^9/L)	5.11 ± 0.31	5.75 ± 0.38	6.64 ± 0.33	0.0156	0.2177	0.0045**	0.0697
Eosinophil (%)	3.01 ± 0.28	8.14 ± 0.26	15.62 ± 0.97	0.0001	0.0001***	0.0001***	0.0001***
Basophil (10^9/L)	0.01 ± 0.002	0.03 ± 0.01	0.04 ± 0.01	0.2344	0.3174	0.0895	0.4306
Neutrophils (10^9/L)	1.88 ± 0.23	1.62 ± 0.10	2.83 ± 0.81	0.1890	0.7196	0.2011	0.0792
Lymphocytes (10^9/L)	2.37 ± 0.19	2.72 ± 0.16	4.32 ± 1.62	0.3339	0.8041	0.1795	0.2330
NLR	0.89 ± 0.14	0.65 ± 0.05	0.78 ± 0.06	0.1637	0.0593	0.3668	0.2987

Data are presented as Mean ± Standard Error of Mean, n = 90. *Significant at p < 0.01; **Significant at p < 0.001. WBC: Total white blood cell count; NLR: Neutrophil-lymphocyte ratio.

infection which was 8.14% ± 0.26%. Also, the concentration of eosinophil in the control subjects was lower ($p < 0.0001$) than in subjects with multiple helminth infections, 15.62% ± 0.97% (Table 3). Similarly, the difference in the eosinophil concentration between the subjects with single and multiple infections was to be statistically significant ($p < 0.0001$). Eosinophil concentration was higher in subjects with multiple parasite infections (Table 3). Also, the control group had a basophil count of 0.01 ± 0.002 × 10^9/L, while single helminth infected subjects had 0.03 ± 0.01 × 10^9/L and multiple infected subjects had 0.04 ± 0.01 × 10^9/L, the values were not significantly different (Table 3). Neutrophils and lymphocytes counts of control subjects were 1.88 ± 0.23 × 10^9/L and 2.37 ± 0.19 × 10^9/L respectively; the counts of subjects with single helminth infection were 1.62 ± 0.10 × 10^9/L and 2.72 ± 0.16 × 10^9/L respectively; while the counts of subjects with multiple infections were 2.83 ± 0.81 × 10^9/L and 4.32 ± 1.62 × 10^9/L respectively (Table 3). NLR of the control, single and multiple helminth infected subjects were 0.89 ± 0.14, 0.65 ± 0.05 and 0.78 ± 0.06, respectively (Table 3). The values of neutrophil, lymphocytes and NLR in helminth infected subjects were not significantly different compared to the control (Table 3). All the age groups (0 - 5 years, 6 - 10 years and 11 - 15 years) had single helminth infections, but only subjects of 6 - 10 years and 11 - 15 years age groups had multiple helminth infections (Table 3). The serum concentrations of IgE obtained in the different age brackets of the control group were not statistically significant. IgE concentration in subjects with single infection across the different age groups was significantly different ($p < 0.0001$), the highest in 6 - 10 years and the least in 0 - 5 years (Table 4). Similarly, the IgE concentrations of the 6 - 10 years and 11 - 15 years age groups were significantly different ($p < 0.05$), higher in the 6 - 10 years age group (Table 4). Concentrations of IgE, IL-5, IFN-γ or C3 of all age groups in control subjects were not significantly different from each other (Table 4). Concentrations of IL-5, IFN-γ and C3 of all age groups in subjects with single helminth infection were also not significantly different when compared

Table 4. Serum levels of immunological parameters based on age in non-parasitized (control) and parasitized children/adolescents resident in selected rural communities in Rivers State and Imo State.

Age Groups (years)	IgE (μL/mL)			IL-5 (ng/L)			IFN-γ (ng/L)			C3 (ng/L)		
	Control	Single	Multiple	Control	Single	Multiple	Control	Single	Multiple	Control	Single	Multiple
0 - 5 (n = 7)	4.93 ± 0.21	154.00 ± 10.5	-	52.50 ± 10.85	55.00 ± 7.8	-	1.47 ± 0.45	1.38 ± 0.08	-	56.25 ± 11.98	37.00 ± 6.00	-
6 - 10 (n = 42)	5.52 ± 0.90	375.74 ± 1.68	459.13 ± 10.7	51.83 ± 1.25	56.84 ± 1.04	85.94 ± 7.37	1.38 ± 0.25	3.54 ± 0.67	2.65 ± 0.04	46.83 ± 6.55	63.53 ± 6.70	67.81 ± 4.07
11 - 15 (n = 41)	9.97 ± 3.30	348.75 ± 4.7	434.13 ± 22.87	56.06 ± 9.37	56.83 ± 2.61	69.60 ± 4.57	1.54 ± 0.37	3.03 ± 0.47	1.18 ± 0.10	58.53 ± 2.78	56.17 ± 6.10	35.20 ± 5.49
ANOVA	0.3701	0.0001	0.0252	0.9816	0.9863	0.0001	0.9355	0.3310	0.0001	0.2597	0.2580	0.0001

Data are presented as Mean ± Standard Error of Mean, n = 90, Number multiple infection in 0 - 5 years subjects.

(Table 4). IgE concentrations of the age brackets in the single helminth infected group were, however, significantly ($p < 0.0001$) different, the highest in the 6 - 10 years and the lowest in the 0 - 5 years age group (Table 4). Concentrations of IgE of 6 - 10 years and 11 - 15 years age groups in subjects with multiple helminth infections were significantly ($P = 0.0252$) different, higher in the 6 - 10 years age group (Table 4). The concentrations of IL-5, IFN-γ and C3 of the two age groups were equally significantly ($p < 0.0001$) different in the subjects with multiple helminth infections, the values of these parameters were also higher in the 6 - 10 years age bracket (Table 4). In the control group, eosinophil, neutrophil, lymphocytes and Neutrophil Lymphocyte Ratio (NLR) levels were different among the varied age brackets ($p < 0.0001$), lowest in 11 - 15 years (Table 5). In the single helminth infected group, neutrophil and NLR levels were significantly ($p < 0.0001$) different among the different age groups, lowest in 11 - 15 and 0 - 5, respectively; but total WBC, eosinophil, basophil and lymphocyte levels were not altered (Table 5). In the multiple helminth infected group total WBC, eosinophil, basophil, neutrophil and lymphocyte levels of 6 - 10 years and 11 - 15 years were significantly different ($p < 0.0001$), but NLR was not (Table 5).

4. Discussion

A total of three parasitic infections were observed in the study. These parasites were *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Trichuris trichiuria*. The occurrence of these parasites varied, *Ascaris lumbricoides* had the highest occurrence (60.0%), followed by *Ancylostoma duodenale* (46.2%) and the least was *T. trichiura* (44.6%). The presence of different worms among the infected population is a usual and common feature of the epidemiology of intestinal helminth parasites in the rural areas of the tropics. It also shows that parasitic infections constitute a major public health problem in the country [12]. The recovery of these intestinal parasites in these study areas indicates the level of hygiene practices exhibited by these children since these do not require an intermediate

Table 5. Blood levels of WBC counts and NLR in non-parasitized (control) and parasitized children/adolescents resident in selected rural communities in Rivers State and Imo State.

Age-Groups (Years)	Eosinophil (%)			Basophil (10^9/L)			Neutrophil (10^9/L)			Lymphocyte (10^9/L)		
	Control	Single	Multiple	Control	Single	Multiple	Control	Single	Multiple	Control	Single	Multiple
0-5 (n = 7)	3.55 ± 0.97	7.77 ± 1.88	-	0.02 ± 0.01	0.02 ± 0.001	-	1.56 ± 0.07	2.15 ± 0.06	-	2.25 ± 0.08	2.35 ± 0.06	-
6 - 10 (n = 42)	3.21 ± 0.05	8.23 ± 1.65	16.02 ± 0.08	0.02 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	1.48 ± 0.05	1.91 ± 0.04	2.22 ± 0.03	2.49 ± 0.02	2.70 ± 0.14	2.67 ± 0.09
11 - 15 (n = 41)	2.15 ± 0.05	8.15 ± 1.46	15.25 ± 0.05	0.01 ± 0.001	0.02 ± 0.01	0.02 ± 0.01	1.25 ± 0.03	1.72 ± 0.07	3.69 ± 0.06	2.18 ± 0.07	2.86 ± 0.14	5.88 ± 0.05
ANOVA	0.0001	0.9933	0.0001	0.5815	0.3185	0.0365	0.0001	0.0001	0.0001	0.0001	0.3269	0.0001

Age groups (years)	NLR			Total WBC (10^9/L)		
	Control	Single	Multiple	Control	Single	Multiple
0 - 5 (n = 7)	0.70 ± 0.04	0.62 ± 0.04	-	5.59 ± 0.09	5.76 ± 1.40	-
6 - 10 (n = 42)	1.06 ± 0.08	0.73 ± 0.03	0.78 ± 0.05	5.48 ± 0.14	5.91 ± 1.26	7.07 ± 0.02
11 - 15 (n = 41)	0.62 ± 0.02	0.65 ± 0.03	0.78 ± 0.09	3.90 ± 0.07	5.50 ± 1.90	6.18 ± 0.03
ANOVA	0.0001	0.0001	0.9999	0.0001	0.9827	0.0001

Data are presented as Mean ± Standard Error of Mean (n = 90), WBC: White Blood Cell.

host. The level of personal hygiene and environmental sanitary conditions in these communities was low. Low parasite intensity was detected in all the communities investigated. This probably could be a result of deworming program in our rural communities in the country. Human *ascariasis* is spread through fecal pollution of soil, and so the intensity of infection depends on the degree of soil pollution [13]. Infection is spread through eggs, which are swallowed as a result of ingestion of contaminated soil or contact between the mouth and the various objects carrying the adherent eggs. Variations in the prevalence rate of intestinal parasites observed in the study from the different communities could be related to several factors including, standard of personal/ environmental hygiene and perhaps poor habits. More so, some ecological factors such as temperature, relative humidity, and rainfall could be responsible for observed differences in prevalence between the communities. The high prevalence of helminth infections recorded Obinze community could be attributed to the exposure of subjects to predisposing factors to parasitic infections, poor sanitary/environmental conditions, inadequate methods of disposal of human waste (excreta); poor personal hygiene, overcrowding, low economic status, poor housing, lack of safe water supply. Indiscriminate defecation from the animals was a common practice seen around their community. Moreover, through the practice of ablution, they can be re-infected, hence they are mostly Muslims and the feet of animals could serve as transport agents. Preschool and school-aged children tend to harbor the greatest numbers of intestinal worms and schistosomes and as a result experience growth stunting and diminished physical fitness, as well as impaired memory and cognition [14]. A high prevalence of helminths infection observed among children could be a result of indiscriminate and carefree habits. High prevalence among the age bracket 6 - 10 years could be as a result of low immunity, increased fecal-oral transmission experience, exposure, ignorance, poor personal hygiene and poor environmental conditions. They are usually active, adventurous and mindless of hygiene habits. For children less than 6 years old, no multiple helminth infections were identified among this group. The subjects may contract the infection during intense activities and adventures such as crawling or walking on bare ground, sand or grass. Transmission of infection can also be during outdoor play with no slippers or shoes on soil contaminated with feces. Some of the children might have contracted infection through ingestion of helminth eggs in contaminated food or drinking water and eating with or licking unwashed contaminated hands/fingers. Defecating in open spaces, playing in soil and the geophagus habit of children could be a good source of high intestinal infections [15]. The human immune response to helminth infections is associated with elevated levels of IgE, eosinophilia and mastocytosis, and the elevation of CD4+ T cells that preferentially produce IL-4, IL-5, and IL-13 [16]. Individuals exposed to helminth infections may have allergic inflammatory responses to parasites and parasite antigens. The result of this study showed that IgE concentration was markedly elevated by single or multiple helminth infestation and this elevation was more in the latter. This agrees with the study by [17] which recorded a 2-fold elevation in serum IgE level of children infected with the intestinal parasite in Okada, Nigeria. Also, [12] recorded that only the serum level of IgE was significantly elevated in helminth infected pregnant women compared with helminth negative pregnant women. This may be an adaptive defense mechanism to get rid of intestinal parasites by the immune system. Immunoglobulin E (IgE) participates in immunity to parasites. It binds to the parasites (acting as opsonin), while eosinophil bind to the opsonized organisms via the IgE Fc receptors. Eosinophils are stimulated to release granular contents, resulting in the lysis of the parasite. Antigen-induced cross-linkage of receptor-bound IgE initiates a process that culminates in the release of histamine and heparin, which increase vascular permeability and promote contraction of smooth muscle. In addition to a role in allergic reactions, IgE plays a critical role in the immune response to parasites. Histamine induces muscular contractions in the intestine, which aid in the expulsion of parasites.

This is the first study providing an immune profile in children/adolescents infected with helminths in Rivers State and Imo State. This study equally noted that parasitic infection caused changes in serum IL-5 concentration as the levels in subjects with parasite infection were higher than the level in non-parasitized subjects. This implies that IL-5 is elevated when there is a parasitic infection. It showed that elevation would be higher during multiple helminth infections than single infection in children. This observation was in agreement with [18] who revealed higher serum levels of IL-5 among Egyptian children with *Ascaris lumbricoides*. This result with regards to IL-5 is attested valid, considering the major role of IL-5 in immunity against helminth infection. Protection against intestinal helminths relies on the activation of type 2 immunity involving IL-4, IL-13, IL-5, and IL-9 [19]. The binding of IL-4 and IL-13 to the IL-4 receptor alpha chain (IL4Ra) on the surface of several immune and nonimmune cells activates effector mechanisms, leading to parasite expulsion [19]. Although T helper (Th2) mediated mechanisms for intestinal worm expulsion are well established, the immune mechanisms behind susceptibility to chronic helminth infections are poorly understood. Moreover, previous studies have documented that in most of the gastrointestinal helminthic infections, the immune responses tend toward the Th2-like responses [20] [21] such responses are also associated with significant production of IL-5, IL-4, IL-10 and IL-13 and with consequent strong IgE development, mast cell, and eosinophil responses. This may explain the elevation of IL-5 and IgE in the parasitized subjects in this study.

It was equally observed in the present study that no significant differences in the concentration level of IFN-γ among the control, single-infection and multiple-infection groups [22], recorded a decreased level of IFN-γ among Honduran school children with intestinal parasites. IFN-γ has a negative effect on Th2 response besides its positive role in stimulation of macrophages and IFN-γ is down-regulated by type-2 cytokines.

The results of this study showed no difference in concentration of C3 among the control, single-infection and multiple-infection groups. This was unexpected, as studies have shown that the complement system plays a crucial role in aiding the rapid recruitment of leukocytes to infected tissues and attachment to the parasite in order to damage it, which is an important early step in the host defense mechanism against parasitic helminths, especially in the absence of parasite-specific antibody [9]. The complement system also contributes to host defense by inducing cytolysis and osmotic lysis, opsonization, and clearance of the immune complexes, and the most significant and abundant component of the complement system is the C3 component [23].

Previous studies on the serum levels of complements in helminth infections are very sparse. However, a study by [24] evaluating the complement component levels in sera of leishmaniasis cases in Turkey showed that there was a difference in the C3 levels among healthy controls and subjects infected with cutaneous and visceral leishmaniasis. It remains unknown why no difference in C3 levels was observed among the different groups in the present study, but it is probable that this is related to the mechanisms employed by the parasite to evade complement recognition in the early stages of the infection. Studies have shown that following entry into the host, some parasites become resistant to serum-dependent complement killing by forming factors that destroy the activity of complement proteins and evolving surface structures that fail to bind, facilitate degradation of, or shed, complement proteins [25] [26]. Hence, even though the parasite persists in the host, the serum levels of C3 remain the same as those in healthy individuals, regardless of the type or intensity of infection. The eosinophil counts in the blood of subjects with parasite infection were increased, but more in those with multiple infections. This is expected because eosinophilia is most commonly induced by tissue-invasive parasites, particularly helminths [27]. The association between eosinophilia and helminth infection in the present study is consistent with results from previous works conducted in other populations [28] [29] [30]. Helminth infections have been largely associated with eosinophilia, especially in the early stages of infection, when larval migration occurs. Eosinophils, basophils and mast cells can produce Th2-type cytokines including IL-4 and IL-13, and thereby amplify the Th2-type response. Neutrophil, lymphocyte ratio (NLR) has recently been shown to be superior to other leukocyte parameters (e.g., neutrophil, lymphocyte, and total leukocyte counts) due to its better stability compared with the other parameters that can be altered by various physiological, pathological, and physical factors [31]. Hence, it has been suggested as a favourable indicator of inflammatory status. It has been shown to be a potential biomarker of inflammation in tumors, cardiovascular conditions, diabetes and its complications [32]. However, no previous work has been done on the predictive ability of NLR in helminthic infection. NLR represents a combination of two markers where neutrophils constitute the active nonspecific inflammatory mediator that initiates the first line of defense, while lymphocytes reflect the regulatory or protective component of inflammation [33]. In this study, no difference was observed in NLR among the different groups based on the type of infection.

5. Conclusion

This study provides the first-time data on the relationship between helminth infections and serum levels of some immunological parameters (IgE, IL-5, IFN-γ and C3) in children and adolescents in Rivers and Imo States. Three parasites were identified *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Trichuris trichiura* and children of 6 - 10 years age group had the highest prevalence rate. IgE, IL-5, total WBC and eosinophil increased in helminth infections, more in poly-parasitized and 6 - 10 years children. However, IFN-γ, C3 serum concentrations as well as basophil, neutrophil, lymphocyte and NLR levels may not be altered during these parasites infection in the population studied.

Ethical Consideration

The study protocol was approved by the Research and Ethical Committee of the University of Port Harcourt. Also, before the commencement of the survey, advocacy visits were made to the Military Hospital, Obinze Owerri, ethnic leaders "Seriki", Village Chiefs, Community Development Chairmen and Youth leaders, community heads and the village health worker mobilized the residents through the town crier. Permission from the traditional heads of each community and authorities of the Military Hospital were also obtained.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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