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Immunomodulatory Activity of Helminth Infection in Inflammatory Bowel Disorder

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ABSTRACT

Background and Objective: The co-existence of Helminths with their human hosts over many years has created mutual benefits from each other. Helminths induce immunoregulation and immunosuppression that stop the death or expulsion of the parasite thereby enhancing its fitness, while on the side of the host, preventing inflammatory reactions and another harmless antigen thereby hindering primary and secondary pathologies produced against it. This study aims to evaluate the immunomodulatory activity of Helminth infection in inflammatory bowel disorder with the potential of a better treatment option. **Materials and Methods:** This case study was conducted on 100 patients with inflammatory bowel disorder with Helminth parasites in Jos and its environs, Plateau State, Nigeria between January and September, 2019. Stool samples collected were used to identify the parasites using the concentration method and wet preparations. About 3 mL of individual blood were put into an EDTA bottle and were used for CD4 count using cyflow and Eosinophil count using both automated method and peripheral blood film methods. The data obtained were analyzed by SPSS software version 22. **Results:** Multiple comparisons of Eosinophil and CD4 in inflammatory bowel disease and Helminths co-infections every 6 weeks for months shows significant ($p<0.001$) establishing the high presence of Helminth infections in causing immunomodulatory effect of CD4. **Conclusion:** The finding of this work shows that significant change in the immune system could take place every 6 weeks. We also established that Helminth infections' immunomodulatory activity improves the immunological system thereby diminishing the effect of inflammatory bowel disease.

KEYWORDS

Helminth parasites, inflammatory bowel disease, immunomodulator, Eosinophil, CD4

INTRODUCTION

The co-existence of Helminths with their human hosts over many years has created mutual benefits for each other. Helminths induce immunoregulation and immunosuppression that stop the death or expulsion of parasites thereby enhancing their fitness, while on the side of the host, it prevents inflammatory reactions and another harmless antigen thereby hindering primary and secondary pathologies produced against it[1].

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Furthermore, the Helminth's immunoregulatory capacities hinder the immunological reaction by the stimulation of regulatory T lymphocytes[2]. Elevated levels of IL-10 and TGF-β secretion stop Th1 and Th2 cell activity, logically safeguarding both the parasite against elimination and the host against harm to the tissues caused by the strong inflammatory reaction[3].

The intestinal lamina propria is made up of elaborate immune cell populations which stabilize the demand for physiologic immune tolerance to luminal antigens and the advantage to defend against pathogens. The feature of active Inflammatory Bowel Disease (IBD) is a remarkable mucosal infiltration by innate immune cells (mainly neutrophils, macrophages and dendritic cells) and adaptive immune cells (T and B cells). Effector $CD4^+$ T cells such as Th1, Th2, Th17 and T follicular helper Tfh are vital in the protection against pathogens, whereas regulatory T cells such as nTreg, iTreg, Tr1 and Th3 perform a remarkable function in restraining the increase and hyperactivity of $CD4^+$ effector T cells. Hyperactivation of effector T cells and modification of T cell-mediated tolerance mechanisms, through distortion in the formation of Treg or modification in their immunosuppressive properties, may likely be the cause of IBD[4].

In the pathogenesis of human IBD, $CD4^+$ T lymphocytes flood into the inflamed mucosa confirming its major function in such disease conditions and the proficiency of depleting anti-CD4 antibody drugs[5].

For years, Eosinophils have been known as a spectacular highlight of the infiltrate in inflammatory bowel diseases. The numbers of Eosinophils in the colon are significantly escalated in inflammatory bowel disease patients and exhibit an activated phenotype. The number of infiltrating Eosinophils is appreciatively connected with disease severity in IBD[6,7].

Human Inflammatory Bowel Disorder (IBD) is an instinctively relapsing, immunologically mediated disorder of the gastrointestinal tract, marked by uncontrolled inflammation resulting from perverse and ceaseless stimulation of the mucosal immune system. The two most common forms of the condition, with associated significant morbidity and mortality, are Crohn's disease (CD) and ulcerative colitis (UC)[8].

Crohn's disease is one of the most common kinds of inflammatory bowel disease globally with the features of the restricted structure, fistulas, ulcers and granulomas in the mucosa. The CD's patient gastrointestinal manifestation affects mainly the terminal ileum region and any part from the mouth to the rectum. Diarrhoea or bloody diarrhoea, malnutrition, abdominal pain and weight loss are the clinical features of CD[9,10].

Ulcerative colitis (UC) is another kind of IBD with features of surface ulcerations and granularity. Inflammation in UC is restricted to the mucosal layer of the colon in vascular nature[11,12].

The capability of Helminth infections to modify and suppress immune responses and intestinal inflammation could be useful in Inflammatory Bowel Disease (IBD)[13].

Therefore the study aimed to evaluate the immunomodulatory activity of Helminth infection in inflammatory bowel disorder with the potential of a better treatment option.

MATERIALS AND METHODS

Study area: This case study was conducted on 100 patients with Inflammatory Bowel Disorder (IBD) infected with Helminth parasites in Jos and its environs, Plateau State from January to September, 2019.

Ethical approval: Ethical approval and patient consent statements were obtained and the study was performed in the Medical Laboratory Department of DEE Medical Centre and Jos University Teaching Hospital, Jos Plateau State, Nigeria.

Research protocol: Samples were collected from asymptomatic individuals confirmed with Helminths parasitic infection and inflammatory bowel disorder every 6 weeks knowing that significant change in the immune system takes place every 6 weeks[14]. Individuals who took antihelminth drugs were excluded from the study.

Stool samples collected were analysed to identify Helminth parasites using the concentration method and wet preparations[15]. About 3 mL of individual blood were put into an EDTA bottle and were used to determine CD4 counts using cyflow and Eosinophil count as both automated and peripheral blood film methods[16].

Statistical analysis: The data obtained were analyzed by SPSS software version 22.

RESULTS

Inflammatory bowel disorder patients infected with Helminth parasites were studied with 35% male and 65% female and the mean age was 36.32±13.3 years as shown in Table 1.

This is supposed that Helminth infections are not immunomodulators and therapeutic agents against rheumatoid arthritis. The null hypothesis is based on the results and accepts the alternative hypothesis that Helminth infection is an immunomodulator and therapeutic agent against rheumatoid arthritis.

Table 2 shows the mean of eggs per gram of faeces and the percentage (%) of Helminthic parasites are as follows: *Strongyloides stercoralis*: 316.67±180.07 (12), *Ascaris lumbricoides* 411.11±242.32 (26), Hookworm (*Necator americanus*): 344.44±247.87 (17), *Trichuris trichiura*: 344.44±200.69 (9), *Schistosoma mansoni*: 420.00±226.18 (20), *Taenia* species: 550.00±289.83 (16).

Table 3 shows a highly significant difference (p = 0.000, 0.054) of the great effect of inflammatory bowel disease and Helminth co-infections on CD4 count every 6 weekly for 9 months indicating the relevance of Helminth infection improving the immunological system thereby diminishing the effect of inflammatory bowel disorder.

Table 1: Demographic and clinical characteristics of Inflammatory Bowel Disease (IBD) patients with Helminth parasitic infestations

Characteristics	Number of patients	Percentage (%)
Number of patients	100	
Ages (years)		
18-30	40	40
31-43	50	50
44-55	10	10
Mean age		
36.32±13.3 years		
Gender		
Male	35	35
Female	65	65

Table 2: Mean and percentage distribution of Helminth parasites in the study population

Helminth parasites	Mean±SD (eggs per gram of faeces)	Percentage (%)
Strongyloides stercoralis	316.67±180.07	12
Ascaris lumbricoides	411.11±242.32	26
Hookworm (*Necator americanus*)	344.44±247.87	17
Trichuris trichiura	344.44±200.69	9
Schistosoma mansoni	420.00±226.18	20
Taenia species	550.00±289.83	16

Table 3: General effect of Inflammatory Bowel Disease (IBD) and Helminth co-infections on CD4 count every 6 weekly for 9 months

Source	Type III sum of squares	df	Mean square	F	p-value
Corrected model	26363429.822[a]	10	2636342.982	676.357	0.000
Intercept	254745815.540	1	254745815.540	65355.377	0.000
Parasites	42619.716	5	8523.943	2.187	0.054
Weeks	26305590.395	5	5261118.079	1349.747	0.000
Error	2342611.137	601	3897.855		
Total	317371103.000	612			
Corrected total	28706040.959	611			

[a]R Squared = 0.918 (Adjusted R squared = 0.917)

Table 4: Multiple comparisons of CD4 in Inflammatory Bowel Disease (IBD) and Helminth co-infections every 6 weekly for 9 months

Weeks	Mean difference	Standard error	p-value
1-2	-133.324*	8.785	0.000
1-3	-262.853*	8.785	0.000
1-4	-380.127*	8.785	0.000
1-5	-484.235*	8.785	0.000
1-6	-615.412*	8.785	0.000
2-3	-129.529*	8.785	0.000
2-4	-246.804*	8.785	0.000
2-5	-350.912*	8.785	0.000
2-6	-482.088	8.785	0.000
3-4	-117.275*	8.785	0.000
3-5	-221.382*	8.785	0.000
3-6	-352.559*	8.785	0.000
4-5	-104.108*	8.785	0.000
4-6	-235.284*	8.785	0.000
5-6	-131.176*	8.785	0.000

*Significant p<0.001

Table 5: General effect of Inflammatory Bowel Disease (IBD) and Helminth co-infections on Eosinophil count every 6 weeks for 9 months

Source	Type III sum of squares	df	Mean square	F	p-value
Corrected model	1983.680[a]	10	198.3680	180.553	0.000
Intercept	19105.190	1	19105.190	17389.420	0.000
Parasites	5.515	5	1.103	1.004	0.415
Weeks	1978.503	5	395.701	360.164	0.000
Error	660.299	601	1.099		
Total	24377.000	612			
Corrected total	2643.979	611			

[a]R Squared = 0.750 (Adjusted R squared = 0.746)

Multiple comparisons of CD4 in inflammatory bowel disease and Helminth co-infections every 6 weekly for 9 months are highly significant (p = 0.000) as shown in Table 4 establishing the immunomodulatory effect of CD4 as a result of Helminth infections.

Table 5 shows the highly significant difference (p = 0.000) of the great effect of inflammatory bowel disorder and Helminth co-infections on Eosinophil count every 6 weekly for 9 months indicating a high presence of Helminth infections in all the patients used for the study while Eosinophil count among the parasites are not significant (p = 0.415)

Multiple comparisons of Eosinophil in inflammatory bowel disease and Helminth co-infections every 6 weekly for 9 months is significant (p = 0.000), it is only 1-5 weeks (p = 0.463) and 2-4 weeks (p = 0.504) that show no significance as shown in Table 6 establishing the presence of Helminth infections in causing immunomodulatory effects.

Table 6: Multiple comparisons of Eosinophil in Inflammatory Bowel Disease (IBD) and Helminths co-infection every 6 weekly for 9 months

Weeks	Mean difference	Standard error	p-value
1-2	-1.775*	0.147	0.000
1-3	-3.873*	0.147	0.000
1-4	-1.676*	0.147	0.001
1-5	0.108	0.147	0.463
1-6	1.814*	0.147	0.000
2-3	-2.098*	0.147	0.000
2-4	0.098	0.147	0.504
2-5	1.882*	0.147	0.000
2-6	3.588*	0.147	0.000
3-4	2.196*	0.147	0.000
3-5	3.980*	0.147	0.000
3-6	5.686*	0.147	0.000
4-5	1.784*	0.147	0.000
4-6	3.490*	0.147	0.000
5-6	1.706*	0.147	0.000

*Significant p<0.001 and 0.01

DISCUSSION

This study shows that Helminth parasites improve the function of the immune systems and can also be used as an anti-inflammatory agent in the treatment of inflammatory bowel disease. This is in an agreement with some findings showing the negative impacts of antihelminthic drugs, since the Helminths are capable to downregulate specific immune responses, modulating autoimmune and allergic inflammatory responses and providing metabolic homeostasis[17].

The findings of this study are also in agreement with immune modulation theories which illustrate the Helminths and immunity relationships involving these two theories namely: Co-evolution and hygiene[18,19].

Co-evolution theory postulated that "humans adapted to Helminth parasitic infections over a long period of global underdevelopment with the human body maintaining an asymptomatic stance from immune tolerance of the Helminths infections by allowing the antigens regulate the immune system and manifested as a protective effect on allergies, asthma, autoimmune conditions such as arthritis, inflammatory bowel disease, multiple sclerosis etc."[20].

This indicates that parasitic Helminths developing with the mammalian immune system will enhance their survival by modifying host immune responses[21].

Another study that supports the immune modulation co-evolution theory was studied by Anuradha *et al.*[22], in which he observed that human infection with *Strongyloides stercoralis* reduces the functional Th1 and Th17 cells and increases functional Th2 cells, compared to uninfected individuals demonstrating the role of $CD4^+$ T cells in expressing Th1, Th2 and Th17 cytokines in such a subject[22].

The hygiene theory pointed to "the higher prevalence of allergy and autoimmune diseases in more developed nations than their developing counterparts which it attributes to a higher standard of living which favoured a reduced prevalence of Helminth infections and a higher allergenic propensity than the lesser hygienic nations which have a higher Helminth infections propensity from a lower living standard"[18,23,24].

The study that supports this hygiene theory was the one that indicated the Helminth parasites possessed an immunomodulatory effect on the innate immunity which "impedes the development of aberrant immunity" and that a decrease in Helminth infections often observed in more developed countries has resulted in an increased prevalence of inflammatory bowel diseases[25].

Nigeria, where the study was carried out is still a developing country. In summary, Helminths are complex organisms that have a diversity of immunomodulatory substances called Excretory-Secretory (ES) products comprised of lipids, carbohydrates and proteins. The identification of these products can represent the whole parasite's biological in the research industry. Excretory-Secretory (ES) product capabilities have been observed to inhibit intestinal inflammation in colitis models in animal studies[26]. This implies that excretory-secretory products of Helminths organism can be used to develop immunotherapy for the treatment of inflammatory bowel disease and other immunological disorders. However, this study could not develop the immunotherapy of Helminths organism due to lack of facility. It is therefore recommended that more research should be carried out to explore this excretory-secretory product of Helminths organism.

CONCLUSION

The finding shows that significant change in the immune system takes place every 6 weeks. We also found that Helminth infection is an immunomodulatory activity that improves the immunological system thereby diminishing the effect of inflammatory bowel diseases.

SIGNIFICANCE STATEMENT

This study established that Helminth parasites can be used as an immunomodulator and suggest a potential way to discover new drugs for Inflammatory Bowel Disease (IBD). Therefore, we will advocate that Helminth organisms should not be called parasites but symbiotic organisms. Further research could harvest the Excretory-Secretory (ES) products of these Helminth organisms for the development of new immunotherapy for the management of inflammatory bowel disease and other immunological disorders.

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