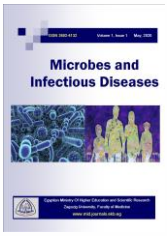
Microbes and Infectious Diseases 2024; Article-In-Press, **DOI:** 10.21608/mid.2024.254711.1709



Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Immune response and prevalence of *Entamoeba histolytica* and *Schistosoma haematobium* among young adults in Nigeria

Mathew F Olaniyan*, Tolulope TB Olaniyan, Bukhari Isah Shuaibu

Department of Medical Laboratory Science, Edo State University Uzairue Edo State.

ARTICLE INFO

Article history:
Received 11 December 2023
Received in revised form 5 February 2024
Accepted 6 February 2024

Keywords:
TNFα
IL-10
Fibrinogen
Prevalence
Entamoeba histolytica
Schistosoma haematobium
Young adults
Nigeria

ABSTRACT

Background: Abnormal immunological responses can occur due to interactions between parasites and their hosts during parasitic infections caused by protozoa and helminths. **Aim:** This study aimed to examine the immune response and prevalence of *Entamoeba histolytica* and *Schistosoma haematobium* infections among young adults in Nigeria. **Material and methods:** Initially, 325 young adults were recruited from the ETSAKO West local government area of Edo State, Nigeria. After exclusions, a total of 200 participants (100 females and 100 males, aged 18-39 years) were included in the study. Plasma levels of fibrinogen, TNFα, IL-10, HIV1p24ag+Ab, anti-HCV, and HBsAg were measured using ELISA in all participants. Microscopy was used to identify AFB (acid-fast bacilli) and parasites in wet and stained smears. **Results:** Among the 200 young adults studied, 62% (124/200) were uninfected with *E. histolytica* and *S. haematobium* (63/124 females and 61/124 males). Of the total participants, 38% (76/200) were infected with either *E. histolytica* or *S. haematobium*, with no evidence of multiple infections (37/200 females and 39/200 males). Among the infected individuals, 17% (34/200) were infected with *E. histolytica* (19/200 females and 15/200 males), while 21% (42/200) were infected with *S. haematobium* (18/200 females and 24/200 males). Young adults infected with either *E. histolytica* or *S. haematobium* had significantly higher levels of TNF-α and lower levels of IL-10 in their plasma compared to the control subjects ($p<0.05$). The proportion of young adults infected with *E. histolytica* and *S. haematobium* was significantly lower than the proportion of uninfected individuals among the 200 participants ($p<0.05$). Additionally, the total number of young adults infected with either *E. histolytica* or *S. haematobium* was significantly higher than the number of individuals infected with only *E. histolytica* or *S. haematobium* ($p<0.05$). **Conclusion:** The study found an overall prevalence of 38% (76/200) for *E. histolytica* and *S. haematobium* infections among young adults, with no evidence of multiple infections. Among the infected individuals, 17% (34/200) were infected with *E. histolytica* and 21% (42/200) were infected with *S. haematobium*. The infected young adults showed significantly higher levels of TNF-α and lower levels of IL-10 in their plasma compared to the uninfected individuals.

Introduction

Young adulthood is the healthiest time of life and individuals in this age group are generally in good health, subjected neither to diseases nor the problems of senescence. Young adults are within the age group of 18 and 39 years, highly fertile, have

DOI: 10.21608/MID.2024.254711.1709
* *Corresponding author:* Mathew F OLANIYAN
E-mail address: *olaniyanmat@yahoo.com*

strong immunity to pathogens, and are strong with the highest physical performance because in the first months of life a child depends on the maternal antibodies that the mother had previously produced against pathogens like parasites for protection. Currently, another means of protection is water sanitation and hygiene practices and the availability of vaccines [1]. The frequent infections occurring in the first years of life serve to build the pool of memory T and B cells that will prevent reinfection or the development of disease by commonly encountered pathogens. The immune system of children is prepared and fit, but diminished in adults and ineffective in elderly people aged 70 years and above [2]. Although innate immunity and T cells play a crucial role in the defense against infection, antibodies also play an important role [1].

Parasitic infections include infections of protozoa and helminth parasites [3]. Parasites stimulate immune responses due to their continuous interaction with the immune system. Body immunity to parasitic infections could be innate or adaptive involving cellular and humoral responses [4]. Innate immune responses include body-engaging secretions (lysozyme), skin, mucous membranes, soluble substances (transferrin), complements, and cytokines. The immune system produces antibodies as a form of humoral response for adaptive immunity [5].

Parasites produce glycans of structures different from those of humans and as a result, are antigenic. Parasites express glycan-binding proteins (GBPs) to invade the system by overcoming the host's first line of immunity. In addition to its involvement in the disease process glycans produced by parasites can trigger the host's innate immune system for the induction of adaptive immune responses [3].

Frequently, parasites stimulate vigorous immune response which is partially cell-mediated and humoral [6]. Protective immunity in some parasitic infections combines humoral and cellular immunity when parasites are coated with antibodies which makes them susceptible to direct cytotoxicity by macrophages, eosinophils, and neutrophils [7]. The immune response to parasitic infections could be pathogenic by inducing hypersensitivity, immunologically mediated fibrosis, or circulating immune complexes [8]. Parasites also use glycoconjugates and glycan-binding proteins (GBPs) mechanisms for host-cell attachment and invasion [9].

Initial parasitemia in man is brought under control by lytic antibodies as a form of immune response to parasitemia [10]. In *Plasmodia* infections, there is no evidence of the synthesis of specific antibodies at the pre-erythrocytic stage in the life cycle of *Plasmodium*, but acquired immunity to natural *Plasmodium* infection is directed mainly against the erythrocytic phase and circulating gametocytes will not be affected [11].

Metazoan parasites (helminths) induce type 2 immune response which involves the CD4+ T helper 2 (TH2) cell, which produces a broad range of cytokines, including interleukin-4 (IL-4) and IL-13, which act on target cells expressing the IL-4 receptor α-chain. Target cells include most cells of the immune system, but also local tissue cells such as epithelial cells that line mucosal surfaces [12].

Cells of the innate immune system, such as the recently described 'innate helper cells', can also produce type 2 cytokines. These cells function not only as effectors during the early stages of infection, but additionally create an environment that favors the induction of TH2-type responses [12].

In addition to killing or expelling helminth parasites, type 2 immune responses contribute to rapid tissue repair, and this sometimes leads to fibrosis-related pathology [12]. Many facets of type 2 immunity are consistent with evolutionary origins in wound-healing pathways, a reflection of the capacity of helminth parasites to damage tissue through migration and feeding. T cell dynamics change over time, and TH2-type responses often decline during chronic helminth infection. Regulatory pathways, including regulatory T cells, restrain pathology and immune responses during infection, and some helminths can actively induce the expansion of regulatory populations [12].

Because mammals evolved in the presence of chronic infection, their immune systems may have compensated for the immune-dampening effects of helminths. If so, over-reactive responses to innocuous antigens in the absence of infection may contribute to autoimmune disease and allergy [12].

Parasites exhibit immunomodulation to suppress IFN-γ production, elimination of immune cells and soluble immune mediators, and metabolic alterations against reactive oxygen and nitrogen

species to fend off the attack from the immune system[13].

Adherence of trophozoites of *Entamoeba histolytica* to the colonic epithelial cells through a specific galactose-N-acetylgalactosamine lectin causes the death of colonic epithelial cells through cytolysis and apoptosis resulting in the release of interleukin-1α and precursor interleukin-1β(IL-1β) [14].

Interleukin-1β(IL-1β) activates NF-κB in distal cells to produce cytokines and other inflammatory mediators such as COX-2, interleukin-1, and interleukin-8. Amoebic cysteine proteinases can also convert precursor IL-1β to active IL-1β[14]. These cytokines and inflammatory mediators subsequently attract neutrophils and macrophages. Neutrophils can be damaged by direct contact with trophozoites which can cause more damage to colonic epithelial cells resulting in the release of more mediators. Macrophages release other mediators as well, such as TNFα, which further contributes to inflammation [15]. Amoebic cysteine proteinases in the trophozoites' of *Entamoeba histolytica* can suppress the host's immune response through cleavage and inactivation of anaphylatoxins C3a, C5a, IgA, and IgG. Trophozoites can reach other areas of the body, most commonly the liver, which can cause tissue necrosis and abscess formation [15].

Schistosoma haematobium is a complex multicellular parasitic worm that can cause chronic disease. Schistosomes induce dominant, distinct, polarized T helper 2 (TH2)-cell response involved in the development of many of the pathological changes associated with the infection of *Schistosoma haematobium*, but which also allows host survival while infected. The parasites can persist for many years in the immunocompetent host. However, infected individuals can develop resistance to superinfection. Balanced TH response is required for the prevention of disease progression as excessive TH1 and TH2 can lead to damaging pathology [16]. The TH2 response to schistosomes is initiated by the egg stage of the parasite, and carbohydrates on egg antigens. Dendritic cells that are exposed to schistosome egg antigens are not activated conventionally, but they can potently induce TH2 responses [16].

Fibrinogen is a positive acute phase protein that its blood levels rise in response to inflammation, tissue injury and certain other events [17]. TNFα is a pro-inflammatory cytokine that acts on the liver to induce acute phase response while IL-10 is an anti-inflammatory cytokine [18,19].

Material and methods

Study area

Etsako West is a local government area of Edo State located in South Nigeria. Its headquarters is in Auchi. Etsako West is made up of six clans which include Uzairue, Auchi, South Ibie, Anwain, Jagbe, and Aviele. The major towns in this LGA include Auchi, Jattu, Agbede, Ughiole, Odighie, Egho, Ubiane, Iyamho, Iyuku, Ayogwiri, Apana, Iyora, Afowa, Afashio, Ikabigbo, Irekpai, Ogbido, Ayaoghena, Ikholo, Uluoke, Ugbhenor, Idato, Ayua, Imeke, Elele, Sabo Iyakpi, Ibienafe, Ughieda, Iyerekhu, Egbogio, Jagbe clan (Ikhwa, Imiokono, Inhianmhen, Imogian) and Awain clan (Ewora, Idegun, Ama, Ibhioba). The Local government houses primary, secondary, and tertiary educational and healthcare institutions.

Study population

The study population included young adults aged 18 – 39 years in Etsako West a local government area of Edo State, Nigeria.

Sample size

The sample size of this study was determined [20] using this formula:

$$n = \frac{z^2pq}{d^2}$$

n = the desired sample size when the population is greater than 10,000.

z = the standard normal deviated, usually set at 1.96 (or 2.0) which corresponds to the 95% confidence limit.

p = the proportion in the target population estimated to have a particular characteristic which is 30.3% [21]. This is in line with the pooled prevalence of parasites amongst African school children which ranged between 21.2%-30.3% as reported by [21].

d = degree of accuracy desired usually set at 0.05.

Thus, if the proportion that contains the characteristic is 0.05, the z statistic is 1.96, and the desired sample size was calculated thus:

n = **325**

Study design

This was a case-control observational study. Three hundred and twenty-five (325) young adults were initially recruited with a minimum of 50 young adults from each of the six clans that constitute ETSAKO West local government area of Edo State, Nigeria.

The 325 recruited for this work were subjected to HIV1p24, anti-HCV, HBsAg and tested for *Plasmodium*, and Acid-Fast Bacilli. Seventy-one (21.9% (71/325)) of the subjects were positive for one or more of these tests. The remaining 254 were further subjected to laboratory tests for identification of parasites, only 51 (20.1%) out of the 254 participants were infected with parasites other than *E. histolytica* and *S. haematobium*.

The remaining two hundred and three (203) were either free of parasitic infection or infected with either *E. histolytica* or *S. haematobium*.

Out of the 203, 200 (100 females, 100 males) were successfully studied. The 200 participants were first subjected to the microscopic identification for *E. histolytica* and *S. haematobium* which was used to group the participants into test participants (76) and control participants (124) aged 18-39 years. Plasma fibrinogen, TNFα, and IL-10 were measured in the test participants (76) and control participants (124).

Inclusion criteria

1. Age: Young adults aged 18-39 years.
2. Location: Participants from the ETSAKO West local government area of Edo State, Nigeria.
3. Availability of samples: Participants who provided stool, urine, sputum, and blood samples.

Exclusion criteria

1. Age: Participants younger than 18 years or older than 39 years.
2. Location: Participants who do not belong to the ETSAKO West local government area of Edo State, Nigeria.
3. Incomplete data or missing samples: Participants who did not provide the required samples for analysis (stool, urine, sputum, and blood).
4. Participants with positive HBsAg, AFB, HIVp24 Ag-Ab, or *Plasmodium*.

Sample collection

Stool, sputum, venous blood, and urine samples including terminal urine were collected from each of the 325 young adults (18 - 39 years) initially recruited for the study.

Laboratory methods

Analysis of stool, urine, sputum, and blood samples

Stool, urine, sputum, and blood samples were examined for parasites using concentration techniques, Giemsa staining, and microscopy as described by Cheesbrough [22].

Detection of Acid-Fast Bacilli (AFB) in sputum and identification of *Plasmodium* in blood

Acid-Fast bacilli (AFB) in sputum and Identification of *Plasmodium* in blood were determined in each of the subjects by the method described by Cheesbrough [22].

HBsAg ELISA

Hepatitis B envelope antigen (HBeAg) was detected in test and control subjects by ELISA using the reagent kit of DIA.PRO (Diagnostic Bioprobes Srl Via Columella, Milano, Italy). The manufacturer's instructions were strictly followed and applied.

Principle: This is an ELISA method where HBsAg if present in the serum, was captured by a specific monoclonal antibody in the microtiter plate after incubation which binds with the specific anti-HBsAg monoclonal antibodies, labeled with peroxidase, The concentration of the bound enzyme on the solid phase is proportional to the amount of HBsAg in the sample and its activity is made observable by adding the chromogen/substrate. The presence of HBsAg in the sample was determined using a cut–off value that allows for the semiquantitative detection of antigen.

HIVp24 antigen and antibodies ELISA

Genscreen ULTRA HIV antigen and antibodies is a qualitative enzyme immunoassay kit for the detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2. This kit can be used for both HIV Ag and HIV Ab screening. The manufacturer's instructions were strictly followed and applied.

Principle: The Genscreen™ ULTRA HIV Ag-Ab employs an enzyme immunoassay based on the sandwich technique to detect HIV antigen and associated antibodies related to HIV-1 and/or HIV-

2 viruses in human serum or plasma. The solid phase is coated with monoclonal antibodies against p24 HIV-1 antigen, along with purified antigens such as gp160 recombinant protein, a synthetic peptide mimicking an artificial HIV-1 group O-specific epitope, and a peptide imitating the immunodominant epitope of the HIV-2 envelope protein. Conjugates involve biotinylated polyclonal antibodies to HIV Ag (conjugate 1) and streptavidin and HIV antigens - peroxidase conjugate (gp41 and gp36 peptides mimicking immunodominant epitopes of the HIV-1 and HIV-2 envelope glycoproteins, and a synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope used for the solid phase) (conjugate 2). The assay procedure encompasses adding Conjugate 1, followed by serum samples and controls, initiating binding processes and resulting in a validated color change. After subsequent steps involving incubation, washing, and the addition of Conjugate 2, a change in color signifies the presence of the complexed conjugate. Absorbances measured on a spectrophotometer at 450/620-700 nm indicate the presence or absence of HIV Ag or HIV-1 and/or HIV-2 antibodies.

Anti-HCV ELISA

Anti–HCV test was determined in each of the subjects by a third-generation enzyme immunoassay reagent kit of DIA.PRO Diagnostic Bioprobes Srl Via Columella, Milano, Italy.

Principle: HCV antigen is attached to the microtiter plates which in turn captures HCV antibody if present in the sample, the anti-HCV was detected by the addition of anti-human immunoglobin G and M antibody labeled with peroxidase. The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HCV antibodies present in the sample. The manufacturer's instructions were strictly followed and applied.

TNF-α ELISA

Plasma TNF-α was determined in each of the subjects by ELISA using Human ABCAM ELISA Kit. The manufacturer's instructions were strictly followed and applied.

Principle: The solid-phase sandwich ELISA for human TNFα is structured to quantify the target substance captured by a complementary antibody pair. Initially, a specific antibody for the target is precoated onto the wells of a provided microplate. Subsequently, samples, standards, or controls were introduced into these wells, binding with the immobilized (capture) antibody. The formation of the sandwich is completed by introducing a second (detector) antibody. Following this, a substrate solution is applied, initiating a reaction with the enzyme-antibody-target complex, resulting in a measurable signal. The signal's intensity is directly correlated with the concentration of the target substance in the original specimen.

Detection of Acid-Fast Bacilli (AFB) in sputum and identification of *Plasmodium* in blood

Acid-fast bacilli (AFB) in sputum and identification of *Plasmodium* in blood were determined in each of the subjects by the method described by Cheesbrough [22].

Human fibrinogen using ELISA kit of ABCAM

Principle: Fibrinogen-specific antibody is precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently, a fibrinogen-specific biotinylated detection antibody is added and followed by washing with wash buffer. Streptavidin-peroxidase conjugate was added, and unbound conjugates are washed away with wash buffer. TMB is then used to visualize streptavidin-peroxidase enzymatic reaction. TMB is catalyzed by streptavidin-peroxidase to produce a blue color product that changes into yellow after adding an acidic stop solution. The density of yellow coloration is directly proportional to the amount of fibrinogen captured in the plate.

Study duration

8 months (June 2022 – January 2023).

Ethical consideration

Permission/approval was obtained from the ethical and research committee of the School of Postgraduate Studies, Edo State University, Uzairue, Nigeria. Informed consent was also obtained from each of the subjects.

Statistical analysis

Data collected was analyzed using ANOVA and a statistical package of New York IBM SPSS 20.0 to determine Chi-square, DF, P-value, and significance at the probability of 95% confidence limit (0.05).

Validity of instrument

In this study, ELISA was used to measure specific proteins and antibodies associated with immune response and infections. ELISA is a valid

technique when used correctly, as it allows the detection and quantification of specific proteins and antibodies of interest. However, it is important to ensure that the ELISA kits used are validated by the manufacturer and that proper quality control measures are implemented during testing to ensure accurate and valid results.

Microscopy was also utilized in this study to identify AFB and parasites in wet and stained smears. Microscopic examination is a commonly used method for identifying and characterizing various pathogens, including parasites. The validity of microscopic examination depends on the expertise and training of the microscopist, as well as the quality of the staining techniques and microscopy equipment used.

Reliability of instrument

Reliability refers to the consistency and stability of measurement tools. In this study, ELISA was used to measure plasma levels of fibrinogen, TNFα, IL-10, HIV1p24 Ag and Ab, anti-HCV, and HBsAg. ELISA is a widely accepted and reliable technique for detecting and quantifying specific proteins or antibodies in biological samples. To ensure reliability, standardized protocols, and quality control measures were typically employed during ELISA testing, including the use of appropriate controls and replicates.

Avoidance of bias

Randomized participant selection

The initial recruitment of young adults from the ETSAKO West local government area of Edo State, Nigeria, was done using a random sampling technique. This helped to minimize selection bias and ensured that the sample represented the target population.

Clear inclusion and exclusion criteria

Clearly defined criteria for inclusion and exclusion of participants were used in the study. This helped to ensure that the selected individuals met the specific characteristics required for the study and reduced the potential for bias.

Standardized measurement techniques

Standardized and validated methods for measuring plasma levels of fibrinogen, TNFα, IL-10, HIV1p24 Ag+Ab, anti-HCV, and HBsAg using ELISA were used. This helped to ensure the reliability and validity of the measurements and reduced measurement bias.

Blinding of researchers

Blinding techniques were also used. For example, the researchers performing laboratory analyses were blinded to the infection status of the participants to avoid any potential bias in the interpretation of results.

Quality control measures

Strict quality control measures were employed during laboratory analyses, through the use of appropriate controls, replicates, and standardized protocols. This helped to ensure the accuracy and reliability of the obtained results.

Statistical analysis

Appropriate statistical analyses were conducted to determine the significance of the findings. The statistical tests were carefully chosen and were suitable for the study design and data type. The p-values and confidence intervals were reported to provide a comprehensive understanding of the results.

Results

There was a significantly higher plasma value of TNF-α and a significantly lower plasma IL-10 in young adults infected with *E. histolytica* and *S. haematobium* than the control subjects without parasitic infection (***p***<0.05) **(Table 1)**.

There was no significant difference in the plasma value of fibrinogen in young adults infected with any of *E. histolytica* and *S. haematobium* and the control subjects (***p***<0.05) **(Table 1)**.

There was no significant difference in the plasma values of fibrinogen, IL-10, and TNF-α in young adults infected with *E. histolytica* and *S. haematobium* (***p***>0.05) **(Table 2)**.

Out of 200 young adults successfully studied, 62%(124/200) were uninfected with *E. histolytica* and *S. haematobium* (Female-50.8% (63/124); Male- 49.2% (61/124)); 38%(76/200) of the young adults were infected with either *E. histolytica* or *S. haematobium* and no evidence of multiple infection(Female-18.5% (37/200); Male-19.5% (39/200)); 17% (34/200) of young adults were infected with E. *histolytica* (Female- 9.5% (19/200); Male- 7.5% (15/200)) while 21% (42/200) of the young adults were infected with *S. haematobium* (Female - 9% (18/200); Male - 12% (24/200))**(Table 3) (Figure 1)**.

There was a significantly lower proportion of *E. histolytica* and *S. haematobium* infections among young adults than those that were not

infected among the 2oo participants successfully recruited (***p***<0.05). The proportion of young adults infected with either *E. histolytica* or *S. haematobium* was significantly higher than the results obtained in the young adults infected with *E. histolytica* **(*p*<0.05) (Table 4)**.

However, there was no significant difference in the proportion of the total number of young adults infected with any of *E. histolytica* and *S. haematobium* compared with young adults infected with *S. haematobium* and also the proportion of young adults infected with any of *E. histolytica* compared with the young adults infected with *S. haematobium* **(*p*>0.05) (Table 4)**.

There is no significant gender difference in the frequency of *E. histolytica* and *S. haematobium* infection among young adults**(*p*>0.05) (Table 5)**.

Table 1. ANOVA results of the comparative analysis of the IL-10, TNFα, and fibrinogen values obtained in control, young adults infected with *E. histolytica* and *S. haematobium*.

	Control (n=124)	Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76)	Young adults infected with *E. histolytica* (n= 34)	Young adults infected with any of *S. haematobium* (n= 42)	F-Stat	p-value
Fibrinogen(pg/ml)	118 ± 5.0	154.0 ± 9.0	150.0 ± 8.0	155 ± 7.0	0.0763	0.9695
IL-10 (pg/ml)	4.8 ± 0.1	3.0 ± 0.2	3.6 ± 0.1	3.5 ± 0.2	23.307	0.005*
TNF-α (pg/ml)	2.2 ± 0.2	4.5 ± 0.2	4.8 ± 0.1	4.7 ± 0.2	47.2963	0.0014*

*Significant

Table 2. ANOVA results of the comparative analysis of the IL-10, TNFα, and fibrinogen values obtained in young adults infected with *E. histolytica* and *S. haematobium*.

	Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76)	Young adults infected with *E. histolytica* (n= 34)	Young adults infected with *S. haematobium* (n= 42)	F-Stat	p-value
Fibrinogen(pg/ml)	154.0 ± 9.0	150.0 ± 8.0	155 ± 7.0	0.173	0.849
IL-10 (pg/ml)	3.0 ± 0.2	3.6 ± 0.1	3.5 ± 0.2	3.4455	0.167
TNF-α (pg/ml)	4.5 ± 0.2	4.8 ± 0.1	4.7 ± 0.2	0.778	0.5343

Table 3. The frequency of *E. histolytica* and *S. haematobium* infection in young adults.

	Control (n=124)	Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76)	Young adults infected with *E. histolytica* (n= 34)	Young adults infected with *S. haematobium* (n= 42)	Young adults infected with both *E. histolytica* and *S. haematobium*
Frequency (%)	62% (124/200)	38% (76/200)	17% (34/200)	21% (42/200)	0
Female	50.8% (63/124)	18.5% (37/200)	9.5% (19/200)	9% (18/200)	0
Male	49.2% (61/124)	19.5% (39/200)	7.5% (15/200)	12% (24/200)	0

Table 4. Results of the comparative analysis of the frequency/proportions of *E. histolytica* and *S. haematobium* infection.

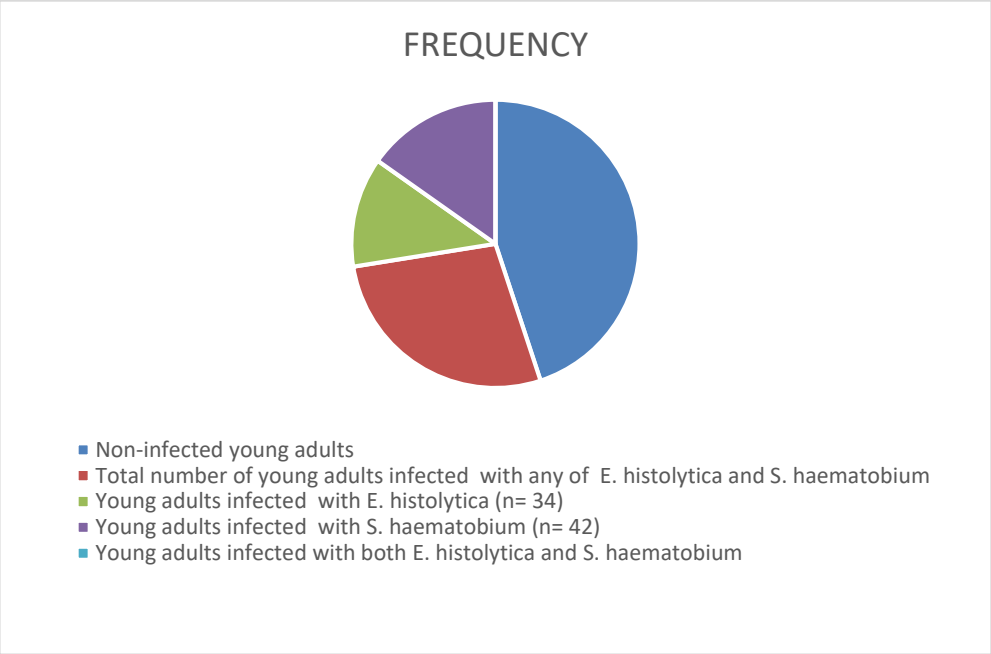
	Control (n=124) Vs Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76)	Control (n=124) Vs Total number of young adults infected with *E. histolytica* (n=34)	Control (n=124) Vs Young adults infected with *S. haematobium* (n= 42)	Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76) Vs Young adults infected with *E. histolytica* (n= 34)	Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76) Vs Young adults infected with *S. haematobium* (n= 42)	Young adults infected with any of *E. histolytica* (n= 34) Vs Young adults infected with *S. haematobium* (n= 42)
Chi-squared	10.838	21.523	20.991	4.757	3.565	0.191
95% CI	9.7360% to 36.9109%	26.9424% to 57.1406%	24.0745% to 53.5421%	2.2741% to 35.4481%	-0.6368% to 31.7870%	-14.4735% to 21.0809%
DF	1	1	1	1	1	1
P-value	0.0010**	0.0001**	0.0001*	0.0292*	0.0590	0.6620

*Significant

Table 5. Results of the comparative analysis of the frequency/proportions of *E. histolytica* and *S. haematobium* infection with *E. histolytica* and *S. haematobium among* the young adults

	Control (uninfected subjects) (n=124)	Total number of young adults infected with any of *E. histolytica* and *S. haematobium* (n=76)	Young adults infected with *E. histolytica* (n= 34)	Young adults infected with *S. haematobium* (n= 42)	Young adults infected with both *E. histolytica* and *S. haematobium*
Female	50.8% (63/124)	18.5% (37/200)	9.5% (19/200)	9% (18/200)	0
Male	49.2% (61/124)	19.5% (39/200)	7.5% (15/200)	12% (24/200)	0
95% CI	-15.5458% to 18.6137%	-16.9223% to 18.5773%	-22.4078% to 23.4997%	-19.6419% to 22.6162%	0
Chi-squared	0.031	0.012	0.041	0.094	0
DF	1	1	1	1	0
p-value	0.8592	0.9122	0.8389	0.7586	0

Figure 1. Frequency of parasitic infection of *E. Entamoeba* and *S. haematobium* in young adults.



Discussion

Out of 200 young adults successfully studied, 62%(124/200) were uninfected with *E. histolytica* and *S. haematobium* (Female-50.8% (63/124); Male- 49.2% (61/124)); 38% (76/200) of the young adults were infected with either *E. histolytica* or *S. haematobium* and no evidence of multiple infections (Female-18.5% (37/200); Male-19.5% (39/200)); 17% (34/200) of young adults were infected with E. *histolytica* (Female- 9.5% (19/200); Male- 7.5% (15/200)) while 21% (42/200) of the young adults were infected with *S. haematobium* (Female - 9% (18/200); Male - 12% (24/200)).

The overall prevalence of parasitic infection of *E. histolytica* and *S. haematobium* was 38% (76/200) in young adults with no evidence of multiple infections.

In addition, the frequency of parasitic infection in the young adults includes 17% (34/200) *E. histolytica* (Female- 9.5% (19/200); Male- 7.5% (15/200)) and 21% (42/200) *S. haematobium* (Female - 9% (18/200); Male - 12% (24/200)). There was a significantly lower proportion of *E. histolytica* and *S. haematobium* infections among young adults than those that were not infected among the 200 participants successfully recruited for the work. The proportion of the total number of young adults infected with either *E. histolytica* or *S. haematobium* was significantly higher than the results obtained in those young adults infected with *E. histolytica* and also than those infected with *S. haematobium*.

Dawet *et al.* [23] reported that 17.0 % (54) of the children were infected with *E. histolytica / E. dispar* among school children in Jos North L.G.A., Plateau State, Nigeria which is consistent with the 17% (34/200) *E. histolytica* obtained in this work in young adults. *E. histolytica* is contracted by drinking contaminated water and eating contaminated food including vegetables which could happen in both adults and children.

There were several reports on urinary schistosomiasis from some Nigerian states as **Charles *et al.*** [24] reported that Ogun State has the highest prevalence, followed by Ekiti State, while the lowest prevalence was recorded in Adamawa. No incidence of *Schistosoma haematobium* was recorded for states such as Akwa Ibom, Bayelsa, Nasarawa, Jigawa, and Gombe.

Shuaibu *et al.* [25] also reported in 2017 that *S. haematobium* infection is prevalent among school age and significantly associated with water contact with a prevalence of 32.09%, with male pupils having the highest prevalence among primary school children in Kebbi State, Nigeria. This prevalence is higher than 21% (42/200) *S. haematobium* (Female - 9% (18/200); Male - 12% (24/200)) reported in this study probably due to the differences in the frequency of water contacts between children and young adults.

However, the prevalence of 21% (42/200) of *S. haematobium* (Female - 9% (18/200); Male - 12% (24/200)). Obtained in this work was higher than the reports of **Ogundeji *et al.*** [26] who reported a prevalence of *S. haematobium* infection at Kuje General Hospital as 13.4% whilst at Zionness Medical Centre the prevalence was 7.9%. with a higher prevalence among males (69.7%) than females (30.3 %) from both hospitals in Kuje Village, Federal Capital Territory, Nigeria. This difference might be explained as it was hospital-based as only individuals manifesting symptoms of ailment which may include urinary schistosomiasis because some infected individuals may harbor the infection without any life-threatening symptom. Furthermore, normal *S. haematobium* infection in adults does not produce symptoms [26]. The difference could also be due to geographical/environmental differences because in the review of **Charles *et al.*** [24] reported in 2019 that Ogun State has the highest prevalence, followed by Ekiti state, while the lowest prevalence was recorded in Adamawa. No incidence of *Schistosoma haematobium* was recorded for states such as Akwa Ibom, Bayelsa, Nasarawa, Jigawa, and Gombe.

The results obtained showed a significantly higher plasma value of TNF-α and a significantly lower plasma IL-10 in young adults infected with *E. histolytica* and *S. haematobium* than the results obtained in the non-infected control volunteers.

This can be explained as follows and as explained earlier. Adherence of trophozoites of *Entamoeba histolytica* to the colonic epithelial cells through a specific galactose-N-acetylgalactosamine lectin causes the death of colonic epithelial cells through cytolysis and apoptosis resulting in the release of interleukin-1α and precursor interleukin-1β (IL-1β) [14]. Interleukin-1β activates NF-κB in distal cells to produce cytokines and other inflammatory mediators such as COX-2, interleukin-1, and interleukin-8. Amoebic cysteine proteinases can also convert precursor IL-1β to active IL-1β[14]. These cytokines and inflammatory mediators subsequently attract neutrophils and macrophages. Neutrophils can be damaged by direct contact with trophozoites which can cause more damage to colonic epithelial cells resulting in the release of more mediators. Macrophages release other mediators as well, such as TNFα, which further contributes to inflammation [15]. Amoebic cysteine proteinases in the trophozoites' of *Entamoeba histolytica* can suppress the host's immune response through cleavage and inactivation of anaphylatoxins C3a, C5a, IgA, and IgG. Trophozoites can reach other areas of the body, most commonly the liver, which can cause tissue necrosis and abscess formation [15]. *Schistosoma haematobium* is a complex multicellular parasitic worm that can cause chronic disease. Schistosomes induce dominant, distinct, polarized T helper 2 (TH2)-cell response involved in the development of many of the pathological changes associated with the infection, of *Schistosoma haematobium*, but which also allows host survival while infected. The parasites can persist for many years in the immunocompetent host. However, infected individuals can develop resistance to superinfection. Balanced TH response is required for the prevention of disease progression as excessive TH1 and TH2 can lead to damaging pathology [16]. The TH2 response to schistosomes is initiated by the egg stage of the parasite, and carbohydrates on egg antigens. Dendritic cells that are exposed to schistosome egg antigens are not activated conventionally, but they can potently induce TH2 responses [16].

Conclusion

This work revealed that a total of 38% (76/200) of the young adults were infected with either *E. histolytica* or *S. haematobium* and no evidence of multiple infections; 17% (34/200) of young adults were infected with E. *histolytica* and 21% (42/200) of the young adults were infected with *S. haematobium* with a significantly higher plasma value of TNF-α and a significantly lower plasma IL-10 in young adults infected with *E. histolytica* and *S. haematobium* This study provides insights into the plasma levels of TNF-α, IL-10, and fibrinogen in young adults infected with *E. histolytica* and *S. haematobium* in Etsako West, Nigeria. The findings suggest a dysregulation of inflammatory cytokines in parasitic infections, with increased TNF-α and decreased IL-10 levels. Further research is warranted to investigate the underlying mechanisms and implications of these findings.

Contribution to knowledge

This study contributes to the understanding of the immune response and infection status among young adults in the study area, specifically regarding *E. histolytica* and *S. haematobium* infections. The findings provide valuable insights into the prevalence and associated plasma markers, contributing to the existing knowledge on parasitic infections and immune responses in the region.

Authors' contributions

a. Mathew Folaranmi OLANIYAN: Conceptualized the idea, research design, recruitment of participants, sample collection and analysis, statistical analysis, literature search, review, synthesis, and preparation of the manuscript.

b. Tolulope Busayo OLANIYAN: recruitment of participants, sample collection and analysis, literature search, review, synthesis, and preparation of the manuscript.

c. Bukhari Isah Shuaibu: recruitment of participants, sample collection and analysis, statistical analysis, literature search, and preparation of manuscript.

Ethics approval and consent to participate

Ethical approval and consent of participants were obtained

Consent for publication

Not applicable.

Availability of data and material

Available.

Competing interests

Nil.

Funding

Nil.

References

1- **Carsetti R, Quintarelli C, Quinti I, Piano Mortari E, Zumla A, Ippolito G, et al**. The immune system of children: the key to understanding SARS-CoV-2 susceptibility? Lancet Child Adolesc Health 2020;4(6):414-416. DOI: 10.1016/S2352-4642(20)30135-8.

2- **Fyfe-Johnson AL, Hazlehurst MF, Perrins SP, Bratman GN, Thomas R, Garrett KA, et al.** Nature and Children's Health: A Systematic Review. Pediatrics 2021;148(4):e2020049155. DOI: 10.1542/ peds.2020-049155.

3- **Bunte MJM, Schots A, Kammenga JE, Wilbers RHP.** Helminth Glycans at the Host-Parasite Interface and Their Potential for Developing Novel Therapeutics. Front Mol Biosci 2022;8:807821. DOI: 10.3389/ fmolb.2021.807821.

4- **Cummings RD, van Die I, Varki A, Cummings RD, Esko JD, Stanley P, et al.** Parasitic Infections. In: Essentials of Glycobiology [Internet]. 3rd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2015. Chapter 43; 2017.

5- **Kuipers ME, Nolte-'t Hoen ENM, van der Ham AJ, Ozir-Fazalalikhan A, Nguyen DL, de Korne CM,** Konig RI, Tomes JJ, Hoffmann KF, Smits HH, Hokke CH. DC-SIGN mediated internalisation of glycosylated extracellular vesicles from Schistosoma mansoni increases activation of monocyte-derived dendritic cells. J Extracell Vesicles 2020;9:1753420. DOI: 10.1080/ 20013078.2020.1753420.

6- **Malik A, Steinbeis F, Carillo MA, Seeberger PH, Lepenies B, Varón Silva D.** Immunological evaluation of synthetic glycosylphosphatidylinositol glycoconjugates as vaccine candidates against malaria. ACS Chem Biol 2020;15:171–178. DOI: 10.1021/ acschembio.9b00739.

7- **Murphy N, Rooney B, Bhattacharyya T, Triana-Chavez O, Krueger A, Haslam SM, et al.** Glycosylation of Trypanosoma cruzi TcI antigen reveals recognition by chagasic sera. Sci Rep. 2020;10:16395. DOI: 10.1038/s41598-020-73390-9.

8- **Ryan SM, Eichenberger RM, Ruscher R, Giacomin PR, Loukas A.** Harnessing helminth-driven immunoregulation in the search for novel therapeutic modalities. PLoS Pathog 2020;16:e1008508. DOI: 10.1371/ journal.ppat.1008508.

9- **Xing M, Yang N, Jiang N, Wang D, Sang X, Feng Y, et al.** A sialic acid-binding protein SABP1 of Toxoplasma gondii mediates host

cell attachment and invasion. J Infect Dis 2020;222:126–135. DOI: 10.1093/ infdis/jiaa072.

10- **Cavalcante T, Medeiros MM, Mule SN, Palmisano G, Stolf BS.** The role of sialic acids in the establishment of infections by pathogens, with special focus on Leishmania. Front Cell Infect Microbiol 2021;11:671913. DOI: 10.3389/fcimb.2021.671913.

11- **West CM, Malzl D, Hykollari A, Wilson IBH.** Glycomics, glycoproteomics, and glycogenomics: an inter-taxa evolutionary perspective. Mol Cell Proteom 2021;20:100024. DOI: 10.1074/ mcp.r120.002263.**Allen J, Maizels R.** Diversity and dialogue in immunity to helminths. Nat Rev Immunol 2011: 11, 375–388. https://doi.org/10.1038/nri2992

12- **Nakada-Tsukui K, Nozaki T.** Immune Response of Amebiasis and Immune Evasion by Entamoeba histolytica. Front Immunol 2016;7:175. DOI: 10.3389/ fimmu.2016.00175..

13- **Stanley SL.** Amoebiasis. Lancet 2003;361(9362):1025-34.

14- **Haque R, Huston CD, Hughes M, Houpt E, Petri WA.** Amebiasis. N Engl J Med 2003;348(16):1565-73.

15- **Pearce E, MacDonald A.** The immunobiology of schistosomiasis. Nat Rev Immunol 2002;2(7):499–511. DOI: 10.1038/ nri843.

16- **Davalos D, Akassoglou K.** Fibrinogen as a key regulator of inflammation in disease. Semin Immunopathol 2012;34(1):43–62. DOI: 10.1007/s00281-011-0290-8.

17- **Liu CY, Tam SS, Huang Y, Dubé PE, Alhosh R, Girish N, et al.** TNF Receptor 1 Promotes Early-Life Immunity and Protects against Colitis in Mice. Cell Rep 2020;33(3):108275. DOI: 10.1016/ j.celrep. 2020.108275.

18- **Sethi JK, Hotamisligil GS.** Metabolic Messengers: tumour necrosis factor. Nat Metab 2021;3(10):1302–1312. DOI: 10.1038/ s42255-021-00470-z.

19- **Johnston KM, Lakzadeh P, Donato BM, Szabo SM.** Methods of sample size calculation in descriptive retrospective burden of illness studies. BMC Med Res Methodol 2019;19:9.

20- **Khalid Hajissa, Md Asiful Islam, Abdoulie M Sanyang, Zeehaida Mohamed.** Prevalence of intestinal protozoan parasites among school children in Africa: A systematic review and meta-analysis. PLoS Negl Trop Dis 2022;16(2):e0009971. DOI: 10.1371/ journal.pntd.0009971.

21- **Monica Cheesbrough.** District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge University Press; 2006.

22- **Dawet A, Yakubu DP, Remkyes MS, Daburum YH.** Prevalence of Entamoeba histolytica and Entamoeba dispar among School Children in Jos North L.G.A., Plateau State, Nigeria. Niger J Parasitol 2012;33(1):77-83.

23- **Charles O E, Kenechukwu C O, Olaoluwa P A, Lv S, Hu W.** Urinary schistosomiasis in Nigeria: a 50-year review of prevalence, distribution and disease burden. Parasite 2019;26:19. DOI: 10.1051/parasite/ 2019020.

24- **Shuaibu U, Saadatu H S, Shuaibu AH, Vasanthakumari N, Kumar S, Syafinaz A N, et al.** Prevalence and molecular characterization of *Schistosoma haematobium* among primary school children in Kebbi State, Nigeria. Ann Parasitol. 2017;63(2):133-139. DOI: 10.17420/ap6302.97.

25- **Ogundeji A, A Salami, Ogundeji OE, Ajobiewe J, Akinsola OM.** Prevalence of

Schistosoma Haematobium Infection in Nigeria: A Retrospective Case Study in Kuje Village, Federal Capital Territory, Nigeria. Texila Int J Public Health 2019;7(2):1-7.

26- **Pearce EJ, MacDonald AS.** The immunobiology of schistosomiasis. Nat Rev Immunol 2002;2(7):499–511. DOI: 10.1038/ nri843.

Olaniyan MF, Olaniyan TTB, Shuaibu BI. Immune response and prevalence of *Entamoeba histolytica* and *Schistosoma haematobium* among young adults in Nigeria. Microbes Infect Dis 2024; Article-In-Press, **DOI:** 10.21608/mid.2024.254711.1709.