CIRCULATING EOSINOPHILIA AND IMMUNOGLOBULIN A ANTIBODIES TO PARASITE ANTIGENS IN PARASITOLOGICAL AND CLINICALLY DEFINED ONCHOCERCIASIS PATIENTS

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ABSTRACT

Background: The role of eosinophils in helminthic infections is either directed against the parasite in killing and or causing pathological sequela in the host. Eosinophils are involved in determining the clinical outcome of helminthic infection including lymphatic filariasis and onchocerciasis. Eosinophilia is a marker for exposure to one or more helminths' infections particularly for travelers visiting tropical sub-Saharan Africa.

Aim: This study was to examine the cause and effect of peripheral eosinophil levels and serum IgA antibodies on the presence or absence of filarial (adult worm and microfilaria), skin dermatitis and ocular disease.

Methodology: The correlation between eosinophilia in onchocerciasis patients (n=95) aged 15 years and above with defined skin microfilarial load, palpable nodules, and in those manifesting papular onchodermatitis, chronic skin and optic nerve disease were assessed using differential white blood cell (WBC) and total leucocyte count of peripheral blood. Serum IgA antibody reactivity with *Onchocerca volvulus* sodium dodecyl sulphate adult worm extracted antigens were evaluated using direct sandwich enzyme linked immunosorbent assay.

Results: The skin microfilaria negative (0 mf/snip) individuals (n=15) had lower percentage and absolute blood eosinophil counts than the skin snip positive ones (n=52). Among the latter group, those with low skin microfilaria load, ≤4.5mf/snip (n=18), had higher eosinophilia than the group having ≥5mf/snip (n-44). The mean and standard deviation (±SD) of eosinophil count was more in the group of patients with evidence of optic nerve disease (n=12) than those with chronic skin disease such as leopard skin, hernia, oedema and lichenoid onchodermatitis (n=20). The observed insignificant rate of *in situ* de-granulation and vacuolation of eosinophils on stained slides did not attract further detail analysis. There was no correlation between serum IgA and percentage and absolute eosinophil counts with regression coefficient, $R^2 = 0.0000$ and $R^2 = 0.009$. Both serum IgA and blood eosinophil counts had strong association with decreased skin microfilaria load. Secondly, the higher IgA and eosinophil were associated with the development of chronic skin disease and optic nerve disease, respectively.

Discussion: The association of eosinophilia with skin clinical disease outcome is less likely as there was significant difference between those without overt signs (n=33) having higher percentage eosinophil count than those with papular onchodermatitis. However, the observed increase blood eosinophilia has a causal relationship with the development of optic nerve disease manifestation among the sample population.

Conclusion: This study has amply demonstrated the possible influence of antibodies on the pathology of skin clinical manifestations compared to the likely role of eosinphils in optic nerve disease. These observations are in consonant with reports of autoantibody involvement in experimental onchocercal dermatitis and cytopathology of ocular clinical lesions.

Keywords: Eosinophilia, Immunoglobulin A antibodies, Microfilaria, Onchocerciasis , Onchodermatitis, Optic nerve disease

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INTRODUCTION

Eosinopilia can develop as an immunologically mediated response in association with diverse processes. Allergic disorders (such as atopic diseases and drug-related hypersensitive reactions), collagen vascular diseases, and malignancies are known to be associated with eosinophilia, which in medical literature, is seen especially in association with helminth infections, and during the tissue-invasive stages of development in particular (Wilson *et al*., 1999; Schulte *et al*., 2002). Eosinophilia is a common feature in parasitic nematode infection and diseases including onchocerciasis. Apparently high level of eosinophilia is found in the blood and skin. Pronounced eosinophilia and strong antibody reactivity coupled with low skin microfilariae characterized the polar form of the disease, hyper-reactive onchodermatitis or sowda (Butt *et al*., 2017). Granulocytes, which comprise eosinophils, neutrophils and mast cells, do not interact directly with living microfilaria except after treatment or natural attrition. Both basophil and eosinophil are rare granulocytes under normal conditions (0.5% and 5% in peripheral blood, respectively), both are found with increased frequency in type 2 immunity, including allergy and helminth infections (Obasa-Ninomiyan *et al*.. 2020). Eosinophils are also responsible for considerable pathology in mammals because they are inevitably present in large numbers in inflammatory lesions associated with helminth infections or allergic conditions (Behm and Ovington, 2000). Only mild inflammatory lesions and large numbers of living undamaged microfilariae are seen in the generalized onchocerciasis (GEO).

A research study was initiated with the aim to study the relationship between blood eosinophilia and infection status and clinical manifestations in onchocerciasis patients. Unlike in the case of lymphatic filariasis caused by *Wuchereria bancrofti*, eosinophilia is associated with defined clinical syndrome called tropical pulmonary eosinophilia (TPE) that results from a hypersensitivity reaction to lymphatic filarial parasites found in endemic regions (Vijayan, 2007, Rothenberg *et al*., 2008)). There is evidence that it is more likely to occur in non-immune individuals, *ie*, visitors to endemic regions, than in individuals of endemic populations who have developed immunity to filarial infections (Ong and Doyle, 1998, Rosenberg *et al*., 2013). Also, eosinophil is well documented to be associated with protection of parasites survival when in balance with neutrophils against *O. ochengi* in cattle (Huang *et al*., 20015a, Hasen *et al*., 2016). And at the same time causing tissue or organ damage (Huang et al., 2015b). An eosinophil is 12 to 17 µm in diameter, has segmented nucleus and abundant cytoplasmic granules containing proteolytic enzymes consisting of four major proteins: the major basic protein (MBP1), eosinophilic cationic protein (ECP), eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EOP). Eosinophil is one of the granulocytes cells stain red-orange with Romanowsky stains (Larsen and Savage, 2019).

The rationale behind this investigation is to ascertain if blood eosinophil levels show correlation with the parasitological and clinical trend (Kaifi *et al*., 2000). Eosinophilia induced by parasitic infection is dependent on interleukin-5 produced by Th2 lymphocytes. Eosinophilia has been a subject of investigation in travelers returning and migrant from tropics who have stayed in onchocerciasis, other filariasis and parasitic disease endemic areas (Specht *et al*., 2006, Checkley *et al*., 2010). For this study, Butt *et al*. (2017) definition of eosinophilia as an elevation of the eosinophil count above levels observed in healthy subjects, usually taken as above 0.5×10^9/l will be adopted. According to this guideline, the eosinophil counts are higher in neonates than in adults and the values gradually fall in the elderly. There is no sex or ethnic variation in the eosinophil count.

MATERIALS AND METHODS

Study area and population: Before the commencement of mass drug administration with ivermectin (Mectizan) in the study communities, precontrol samples were collected from persons living within an onchocerciasis meso-endemic area in Kachia Local Government area of Kaduna State, Nigeria. The study area falls within the geographical savannah ecological zone and the Gurara river basin known to be breeding ground for *Similium damnosum* complex described by (Osue *et al*., 2008). Both male and female of varying ages of 15 years and above were screened for nodule presence and skin snipped for microscopic examination for emerged microfilaria. Participants were clinically examined for onchocercal skin and ocular manifestation as described by (Mudoch *et al*. 2001).

Experimental design: Only those individuals (n=95) comprising males (n=42) and females (n=53) who voluntarily consented to participate in the study were enlisted for screening and sampling. Among them are those having palpable nodule, skin microfilariae and or onchocerciasis related skin and ocular signs (n=80) and those with no palpable nodule and have no skin microfilaria (n=15) were selected for haematology and serology.

Blood sample collection and analysis: About 3ml of venous blood was collected from participants into a tube containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant. Thin blood films were prepared and air dried in the field. The films were stained with Leishman stain, washed in PBS pH/7.2, and allowed to dry in slanting position on a wooden slide rack. Films were stored in a slide box till when examined under the light microscope (x 100 objectives) with oil immersion. For each film, 200 white blood cells (WBC) were enumerated using differential counter. A 1:20 (20µl/380µl) dilution of anticoagulant blood in WBC diluent (3ml of glacial acetic acid, 2ml methylene blue and 95ml distill water was made. Total WBC count was performed using improved Neubeur haemocytometer chamber under x40 objective enumerating with a tally counter. For purpose of this study, eosinophilia is as defined by Wilson *et al*. (1999) as the presence of >500 eosinophils per µl of blood or as a WBC count in which >7% of the WBCs are eosinophilic leukocytes. Under normal homeostatic conditions, eosinophils level is 120/mm^3 to 450/mm^3 (Hartl *et al*., 2020). We adopted the definition of eosinophilia as an increase of circulating eosinophils >500 /mm^3 (Montgomery *et al*., 2013). Severity of eosinophilia will be categorized as mild (500 and 1500/mm^3), moderate (150 to 5000/mm^3), and severe (>5000/mm^3) (Gotlib 2014, Butt *et al*., 2017). Hypereosinophilic syndrome is defined as an absolute eosinophil count greater than 1500/mm3 on two occasions at least one month apart or marked tissue eosinophilia (Klion, 2015) as cited by Kanuru and Sapra, (2020).

Serology: Sera were extracted from blood samples collected in clean anti-coagulant free tubes have been allowed to clot at ambient room temperature at 27-30°C. The sera were subjected to ELISA test reactivity of parasite-specific IgA antibodies to adult worm sodium dodecyl-sulphate extracted antigens essentially as described by (Osue *et al*. 2008). Briefly, after blocking with 200µl of 2% bovine serum albumin, serum was added at 1:80 for IgA reactivity to SDS extract. Horseradish peroxidase conjugated to rabbit immunoglobulin anti-human IgG (Dako, Denmark) for IgA (P216) was applied at 1:500 dilutions. Antigen and antibody reactions were detected by adding freshly prepared substrate solution containing 200µl orthophenylene diamine (OPD) (from Sigma) in 20µl hydrogen peroxide, 0.1M citric acid and 0.2M Na_2HPo_4 buffer and allowed reacting for 15 minutes. The enzyme reaction was terminated with 30µl per well 2M H_2SO_4 for 5 minutes. Optical densities (OD) values of wells of microtitre plates were measured in an ELISA reader (model MR4000) at 492nm-test filter and 630nm-reference filter.

The mean and standard deviation (SD) from the mean OD values of ELISA results were analyzed according to parasitological and clinical status of patients.

RESULTS

Eosinophilia which is a common feature in parasitic helminth diseases appeared to be of moderate levels commensurate to the prevailing parasitological and clinical manifestations. An overall mean ±SD percentage eosinophil count of 10.6±6.9 and absolute eosinophil count of 604±472 were recorded for the sampled population (n=95). There was significant difference between endemic normal (n=8) with 13.6±10.3 compared to non-endemic normal with 2.1±3.8 percentage eosinophil count. There was no significant difference between male (n=42) with 10.9±7.5 and 628.7±507.2 and female (n=53) with 11.6±7.0 and 678.9±472.1 as in Table 1.

Table 1: Effect of IgA antibody and blood eosinophil levels on infection status

Description	Sample size	ELISA OD values >Cut-off Points	Eosinophil count Percentage
Sample population (n=95)	73 (76.8)	0.15±0.11	10.6±6.9
Male (n=43)	34 (75.5)	0.16±0.11	10.9±7.5
Female (n=53)	40 (81.0)	0.15±0.11	11.6±7.0
N-mf- (n=15)	13 (86.7)	0.14±0.10	7.8±6.8
N-mf+ (n=30)	18 (60.0)	0.13±0.11	12.9±7.4
N+mf- (n=18)	15 (83.3)	0.16±0.10	8.9±6.6
N+mf+ (n=32)	26 (81.3)	0.16±0.12	10.7±6.0

*Cut-off point is mean plus 2 standard deviation of non-endemic control. EN=endemic control, mf=microfilaria, n=sample size, N= nodule, ND=not done, SD= standard deviation, - = negative, + = positive

Similarly, the slight difference among the age groups 15-19 year, (n=24) with 10.0±7.8 and 603.7±590.9; 30-49 year (n=41) with 11.5±6.5 and 661.0±427.6; ≥50 years (n=30) with 9.9±6.8 and 529.1v427.1 were not statistically significant (p>0.05). The nodule negative and microfilaria positive (N-mf+) group had the highest with 12.9±7.4% and 760±541 eosinophil cells per millimeter and both nodule and microfilarial negative (N+mf-) had the least percentage eosinophil count of 7.8±6.8%, while nodule positive and microfilarial negative (N+MF-) had the least absolute eosinophil count of 400±343. Eosinophil counts were generally higher in microfilarial positive and decreased from 14.4±7.9% and 896±590 for low (0.5-4.5mf/skin snip) to 10.9±6.1% and 581±527.1 for high (≤17.5mf/skin snip) micrfilaria density group (Table 2).

Table 2: Analysis of IgA antibodies and blood eosinophil percentage count by microfilaria load

Description	Sample size	OD values at 492nm >Cut-off point*	Eosinophil count Percentage
0 Mf load (n=33)	25 (75.8)	00.14±0.09	8.4±6.6§
0.5- 4.5 Mf (n=18)	12 (66.7)	0.17±0.15	14.9±7.9§
5.0 – 19.5Mf (n=22)	16 (72.7)	0.12±0.06	10.9±6.1
≥ 20 Mf (n=22)	15 (68.2)	0.15±0.12	10.6±6.1

*Cut-off point is mean plus 2 standard deviation of non-endemic control. § indicated significant difference between low microfilaria (Mf) level the zero and medium or high mf load using 3 way analysis of variant (ANOVA). OD= Optical density of enzyme linked immunosorbent assay reading at 492 nanometer (nm) wavelength.

The mean eosinophil level was significantly higher ($p<0.05$) in patients with optic nerve disease followed by those with no untoward clinical signs, as against the low level recorded for the chronic skin disease group. Onchocerciasis patients with optic nerve disease had 11.9±7.0% and 767±558 absolute value, while those presenting with chronic skin diseases and papular onchodermatitis had the least percentage, 8.9±7.4% and absolute count of 520±335, respectively. Individuals with no clinical manifestation of the disease had percentage count approaching the figures recorded for optic nerve disease as shown in Table 3.

Table 3: Effect of clinical manifestations on blood eosinophil levels

Description	Sample Size (%)	OD values 492nm	*Eosinophilia Percentage
*Clinical Sign negative (n=39)	24 (65.5)	0.14±0.1	11.08±6.9
POD (n=19)	12 (63.2)	0.14±0.08	9.5±5.8
CSD (n=16)	7 (43.8)	0.21±0.13§	8.9±7.4◊
OND (n=11)	8 (72.7)	0.15±0.11 §	11.7±7.4◊
OND+ (n=10)	6 (60.0)	0.12±0.95	12.2±7.1

*Eosinophilia count >7%, CSD= chronic skin disease, OND= optical nerve disease and OND+= those with other eye lesions, POD= papular onchodermatitis, n=sample size, SD = standard deviation, ◊ = significant difference ($p \leq 0.01$).

Among the skin snip and clinical signs negative, a suspected case of borderline leprosy with very high eosinophil counts of 32.5% and 200 cells per milliliter was recorded. Generally, very low level eosinophil degranulation and vacuolation were observed in patients with eosinophilia during the process of differential WBC count. Hence, these parameters did not attract further detail evaluation. Occasionally, *in situ* eosinophil lysis with resultant liberation of intact granules on thin films was noted.

There was no correlation between serum IgA and percentage and absolute eosinophil counts with regression coefficient, R^2 = 0.0008 and R^2 = 0.0009 as shown on Figures 1 & 2. Both serum IgA and blood eosinophil counts had strong association with decreased skin microfilaria load. Secondly, the higher IgA and eosinophil were associated with the development of chronic skin disease and optic nerve disease, respectively.

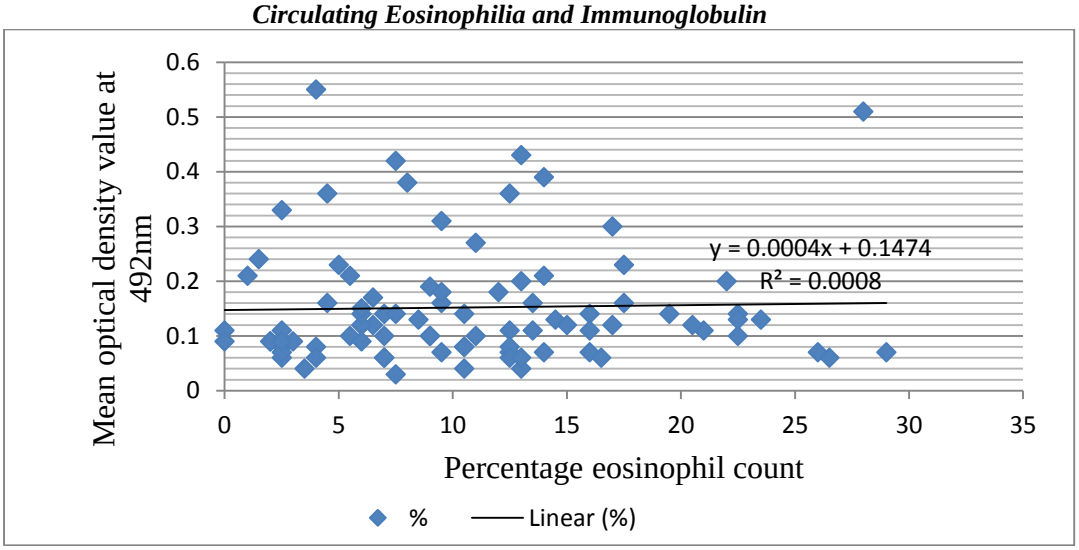

Figure 1: Correlation between mean optical density and percentage eosinophil count. The linear coefficient of regression analysis showed there is no correlation.

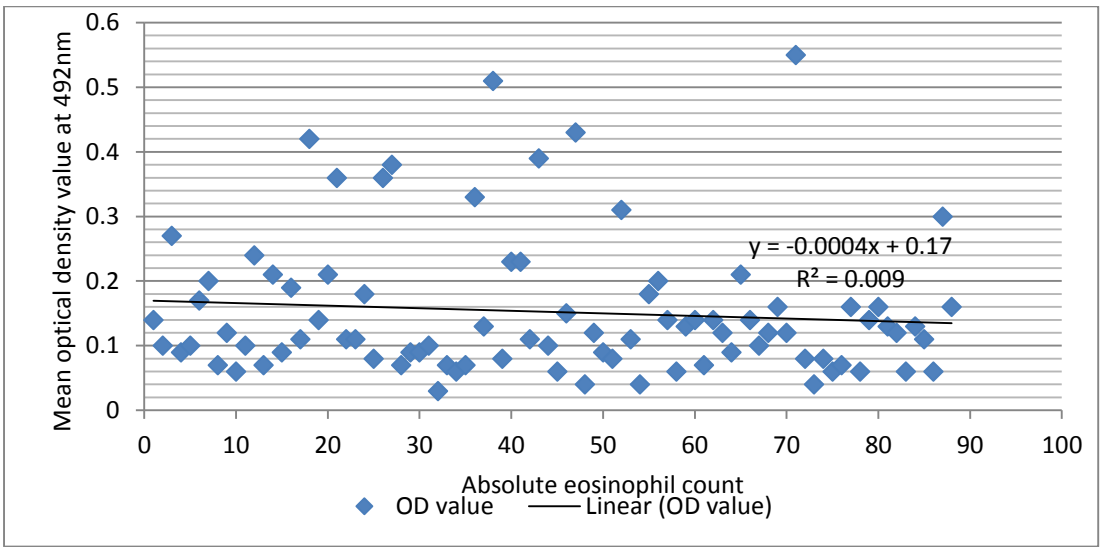

Figure 2: Correlation between mean optical density and absolute eosinophil count. The linear coefficient of regression analysis showed no correlation.

DISCUSSION

We examined the roles eosinophils and IgA antibody play in the cause and effect of modulating the mf and adult worm, association with dermal and ocular manifestation of onchocerciasis, working separately or jointly inducing combined effects. Our data have shown that moderate level of eosinophilia existed in the sample population drawn from onchocerciasis meso-endemic area which was expected. This lay credence to the proponent of using eosinophilia as first line of diagnosis since correlatively eosinophilia and increases in eosinophil protein levels are associated with ongoing helminth infections (Ehrens *et al*., 2022). In the same vain, there was no significant gender difference between male and female groups which is in agreement with Butt *et al*. (2017). An inverse relationship between the level of eosinophilia and the presence or in the absence of skin (mf+ and mf-) as shown on Table 1 directly aligned with the possible roles eosinophils played in killing mf. Also, it was reported that eosinophil degranulation was inversely correlated with neutrophil counts, and the recrudescence of *Wolbachia* can reconstitute the neutrophilia and 'rescue' the worms during the early stages of eosinophil infiltration (Brattig *et al*., 2001; Nfon *et al*., 2006).

Recently, it has been established that eosinophil granules can be released by crosslinking of Fc receptors such as FcγRI, FcγRIII, FcγRII, FcαRI and FcϵRI by IgG1, IgG3, IgG2, IgA and IgE respectively. Antibody-dependent cellular cytotoxicity (ADCC) occurs, leading to cell degranulation, activation and/or phagocytosis. This may be responsible for the lower numbers observed in mf- groups on Table 2. It could either occur as a consequence of secondary filarial infections through re-infection and as observed after vaccination, eosinophil-mediated ADCC plays an important role in the killing of the filariae (Martin *et al*., 2000, LeGoff, *et al*., 2000, Huang *et al*., 2015b). Similarly, the involvement of *O. volvulus* parasite-specific IgA antibody responses coincidentally followed the same trend like that of the eosinophil levels. The combined action of eosinophils and IgA antibodies appeared to be more potent way of mf clearance. Involvement of neutrophil in DNA extracellular trap cell death has been reported for human onchocerciasis by Tamarozzi *et al*., (2016) *in vivo* around onchocercomata, subcutaneous nodule containing adult filariae worms. Similar observation has been reported for eosinophil on mf by (Ueki *et al*., 2013, 2016) where various mechanisms of degranulation and release of granule proteins are known to occur. However, it is unclear whether the eosinophils are involved in parasite killing or if they are attracted secondarily to dying worms (Hansen *et al*., 2011). Some biomolecules like the major basic protein (MBP), eosinophil derived neurotoxin (EDN), and eosinophil peroxidase (EPO) have been implicated in host defense mechanism (Acharya and Ackerman, 2014) including galectin protein. A study by Euki *et al*. (2018) had reported the deposition of Charcot-Leyden crystal (CLC) proteins (lyposophospholipase) leading to DNA extracellular trap cell death contributing to eosinophil ETosis of mf.

Whereas there appeared to be direct relationship between peripheral blood eosinophil levels higher in optic nerve disease and those with other ocular lesions (Table 3). The observed significant difference appeared to be equivocal in so far there was no significant difference between OND /OND+ groups and the clinical signs negative group. On the contrary, those with chronic skin disease (onchodermatitis), had higher IgA antibody and vice versa for antibody responses in ocular groups. The apparent negative association existing between mild eosinophilia is in accordance with Boyer, (2016) and parasitological status; adult worms in nodules and skin microfilaria density, or clinical outcome of disease depicted the GEO form at pre-treatment. Eosinophilia due to infection has been reviewed by O'Connell and Nutman, (2015) include parasitic and mycotic infections. Evidence of considerable changes in blood eosinophils with initial decrease and then increase in number following treatment with DEC/ivermectin have been well documented (Ehrens *et al*., 2022).

The hypothesis that the primary function of eosinophils is to defend hosts against infection by relatively large organisms such as parasitic helminths is based on the accumulation of observations that: (1) eosinophils degranulate onto the worm and can kill helminths *in vitro* in the presence of antibody and/or complement; (2) they move from the blood and aggregate in the locality of helminths *in vivo*; (3) large numbers of eosinophils are often seen in close association with both intact and damaged helminths *in vivo*; and (4) they clearly degranulate in the vicinity of or on to the surfaces of, helminths *in vivo* (Meeusen and Balic, 2000). Eosinophils are also responsible for considerable pathology in mammals because they are inevitably present in large numbers in inflammatory lesions associated with helminth infections or allergic conditions (Behm and Ovington 2000).

Also, it was reported that eosinophil degranulation was inversely correlated with neutrophil counts, and the recrudescence of *Wolbachia* can reconstitute the neutrophilia and 'rescue' the worms during the early stages of eosinophil infiltration (Brattig *et al*., 2001; Nfon *et al*., 2006). The specific role played by eosinophils has continued to attract attention. In a study where neutrophils were reported to be attracted to *Wolbachia*, but following antibiotic chemotherapy they were replaced by eosinophils that degranulate on the worm cuticle.

There is no absolute eosinophil level in the peripheral blood at which treatment is deemed necessary in completely asymptomatic patients (Gotlib 2014). With only few degranulation observed *in situ* on stained slides, on the contrary, this allays the fear whereof high count degranulated eosinophils would potend high risk of cardiac damage, which has been found to correlate with a degranulated eosinophil count of 1 × 109/l or more (Wright *et al*., 2016). This is the reason why onchocerciasis induced eosinophilia are associated with absence of identified organ damage such as TPE characterized by ashma-like syndrom and endomyocardial diseases that may both require initiating treatment for eosinophilic-associated inflammatory conditions (Lombardi *et al*., 2022). The role of IgA in modulating or counter-balancing the allergic role of its counterpart, IgE remained to be thoroughly investigated. Of the four basic proteins in the eosinophil granules, the MBP, ECP and EPO are deposited on dead or dying mf in the skin especially after DEC treatment and to lesser extent after ivermectin treatment, they are sequestered in lymph nodes (Ehrens *et al*., 2022). The eosinophil and IgA pre-treatment levels could influence the severity of post-treatment reactions particularly that of Mazzotti after DEC and adverse event attributed to ivermectin in deterministic manner. This is an aspect requiring further research interrogation not just for onchocerciasis but for other drug induced adverse effects.

It can be inferred that the observed increased blood eosinophilia has a causal relationship with the development of optic nerve disease manifestation among the sample population. Similarly, it has been amply demonstrated that combined higher serum IgA and blood eosinophils have strong association with decreased skin microfilaria load. Secondly, the higher IgA and corresponding higher peripheral eosinophil were associated with the development of chronic skin disease and optic nerve disease, respectively. According to Topic and Dodig, (2011), eosinophils function through several factors; eotaxin chemotatic protein (ECP) for example, is primarily excreted by degranulating eosinophils and its secretion can be elicited in an either antibody-dependent or antibody-independent (complement) manner. Although its anti-helmintic properties remain unclear, but it is assumed that ECP works on microfilaria rather than adult worms.

Clinical dichotomy in pathology of filariasis depended on the filarial stage of *W. bancrofti*, *Brugia malaye*, *Loa loa*) whose adult worms are subjected to inflammatory immune reaction leading to lymphedema, hydrocele and elephantiasis, respectively. On the other hand, dermatitis and ocular lesions in onchocerciasis patients and TPE in LF is caused by the responses to dead MF (Ehrens *et al*., 2022). Although blood eosinophilia in travelers returning from developing countries had limited predictive value for the presence of travel-related infections, the likelihood of presence of helminth infections increases considerably with the extent of eosinophilia (Schulte *et al*., 2002). It is important knowing the life cycle of parasites in determining the true positivity or negativity of the findings. The exact timings of possible exposures are important in both infectious and non-infectious eosinophilia (Checkley *et al*., 2010).

Eosinophilia may be transient in association with migration phase of infection, which occurs during pre-patent period, when parasite eggs and larva are not detected. Among the common causes of asymptomatic eosinophilia are due to intestinal helminthes (Whetham *et al*., 2003). This may best explain the observed high eosinophilia among the CS- group who may be harbouring other intestinal parasites.

CONCLUSION

This study has amply demonstrated the possible direct influence of IgA antibodies played on the pathology of skin clinical manifestations (chronic onchodermatitis) compared to the likely role of eosinphils in associated with the development of optic nerve disease.

We therefore support advocates of population screening in tropical disease endemic countries for eosinophilia and increases in eosinophil protein (ECP and EDN in serum) levels as first diagnostic indicator to identify those with ongoing helminthic infections, eosinophilic-associated allergies, and hyper-eosinophilic syndrome of unknown origin. It will be highly cost effective, simple to perform, doesn't require sophisticated laboratory equipment and specialized skills. It can be integrated into the primary health care programme and other national intervention strategies against neglected endemic parasitic diseases. Also, both differential and total eosinophil counts can be performed as adjunct to routine procedure in haemoparasites diagnosis based on stained blood smear and full blood count for anaemia can be performed on same sample at no extra cost. This paper has demonstrated the neen to step further research on interaction between eosinophil and IgA as protecting the parasite and or causing pathology.

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Conflict of Interest: We state categorically that none of the authors has derived any personal financial benefit from this study carried out as part of institutional and academic research activities devoid of any interest whatsoever.

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