



Inflammation during *Schistosoma haematobium* infection and anti-allergy in pre-school-aged children living in a rural endemic area in Zimbabwe

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Abstract

BACKGROUND Allergies and autoimmune disorders are less prevalent in areas where parasitic infections are abundant. The relationship between schistosomiasis, Chitinase 3-Like 1 protein (YKL-40), an inflammatory marker, and antinuclear antibodies (ANA), an allergy marker, was investigated in pre-school-aged children (1–5 years old) living in an area endemic to *Schistosoma haematobium* infection.

METHODS Cross-sectional study including 145 participants, 66 females and 79 males. *S. haematobium* infection was diagnosed using the urine filtration technique. Levels of YKL-40 and antinuclear antibodies concentrations were determined using enzyme-linked immunosorbent assay. **RESULTS** The prevalence of *S. haematobium* infection was 21.4 % ($n = 31$) with 114 not infected, 18 with light and 13 with moderate infections. YKL-40 levels were higher in the *S. haematobium*-infected group than in the uninfected group ($P = 0.038$). However, *S. haematobium* infection intensity did not correlate with YKL-40 levels. ANA levels were significantly higher in uninfected children than in infected children ($P = 0.028$). There was a significant inverse relationship between ANA levels and schistosome infection intensity ($r = -0.225$, $P = 0.016$). The correlation between ANA levels and YKL-40 levels was not significant.

CONCLUSION Inflammatory marker in pre-school-aged children living in an area endemic for schistosomiasis indicate YKL-40 as a possible biomarker of *S. haematobium* infection in pre-school-aged children, warranting further investigations in a longitudinal study. The study gives an insight into allergy as ANA levels were higher in schistosome-uninfected than infected participants, further studies on allergies are needed.

keywords *Schistosoma haematobium* infection, intensity of infection, Zimbabwe, chitinase 3-Like 1 protein, antinuclear antibodies, pre-school-aged children

Introduction

Schistosomiasis is estimated to affect approximately a quarter of a million people worldwide, with the majority of them living in sub-Saharan Africa [1]. Studies were conducted in Mali, Niger, Sudan, Uganda and Zimbabwe have also shown prevalence rates of paediatric schistosomiasis infection ranging from 18% to 63% [2].

Children under 5 years of age are usually exposed to infected water very early in life when they accompany their caregivers to the schistosome-infested water bodies. Although initial infection may not be apparent, it generates inflammation that predisposes to organ fibrosis development, which can endure for many years [3]. Schistosomiasis has been called an indicator of poverty as it is often present among the poorest of the poor [4].

Urogenital schistosomiasis is caused by *Schistosoma haematobium*. In the later stages of its life cycle, *S. haematobium* female worms lay eggs equipped with terminal spines in the veins of the pelvis, which eventually rupture the endothelial linings of the vesical and urethral walls. This leads to granulomatous inflammation, ulceration, pseudopolyposis and haematuria [5]. The inflammation present during schistosomiasis is characterised by the presence of biomarkers such as C-reactive protein, Resistin and Chitinase 3-Like 1 protein (YKL-40).

YKL-40 is a biomarker of inflammation which has been associated with *Schistosoma japonicum* infection and with hepatic fibrosis due to schistosomiasis [6]. YKL-40 is a 40 kDa glycoprotein from the highly conserved 18 glycosyl hydrolase family [7]. The protein is structurally similar to chitinases and can bind chitin, but lacks

chitinase activity due to altered active site residues. Chitin in pathogenic organisms is detected by members of the 18 glycosyl family and sets off expression and activation of mammalian chitinase-like proteins. Mammalian chitinase-like proteins are also induced in response to chitin-lacking bacterial pathogens and even to non-infectious diseases such as cancer [8]. Neutrophils, fibroblasts, astrocytes and tumour cells secrete YKL-40 [9]. It is known that YKL-40 plays an important role in inflammation, proliferation and angiogenesis [10]. YKL-40 is a biomarker that acts independently from C-reactive protein [11]. YKL-40 has been found elevated in liver fibrosis [12,13], is involved in the activation of the innate immune system and is secreted by different cells [13]. Chitinase-like proteins are a family of mediators progressively more associated with infection, T-cell-mediated inflammation, wound healing inflammation and allergy [14]. YKL-40 has been linked to various allergic conditions such as asthma [15], respiratory syncytial virus-induced airway inflammation [16] and wheeze in pre-school children [17].

Antinuclear antibodies (ANA) are an allergy marker and these were analysed in our study where the participants were exposed to *S. haematobium*. Allergies and autoimmune diseases are less common in countries with higher burdens of helminths and other parasitic organisms [18]. However, several countries that have eradicated those common parasitic infections are beginning to see the emergence of allergic and autoimmune diseases [19]. The hygiene hypothesis proposes that the stimulation of the immune system by microbes protects from the development of inflammatory diseases; therefore, a reduced exposure to infectious agents may explain the rise in allergic and autoimmune diseases in industrialised countries [20]. Helminths have many mechanisms which involve the generation of regulatory T cells and anti-inflammatory cytokines like interleukin-10 and transforming growth factor- β . Experimental studies in mice have shown that regulatory immune responses induced by helminth can repress Th2 and Th1/Th17 responses that mediate allergy and autoimmunity, respectively [21].

We sought to find out whether *Schistosoma* infection in pre-school-aged children (≤ 5 years) is associated with YKL-40 levels. We set out to assess whether schistosome-infected participants had higher levels of YKL-40 and vice-versa, and whether *S. haematobium*-uninfected children have higher levels of ANA than infected children. To determine the relationship between ANA and *S. haematobium* infection, ANA were measured and correlated to *Schistosoma* infection. Given the link between allergies and YKL-40, we hypothesised that chitinase levels could be associated with ANA levels.

Materials and methods

Study area and population

The children participating in this study were a subset of a cohort from the paediatric *S. haematobium* infection longitudinal study, which investigated immune responses of participants to the parasite on first infection. The study was conducted in 145 children aged 1–5 years old. To be included, participants met the following criteria: (i) informed consent from parents, (ii) age 1–5 years, (iii) no evidence of current or previous schistosome infection, (iv) lifelong residents of the area, (v) no previous anti-helminthic treatment and (vi) being at risk of schistosome infection. The participants were residents of Shamva district in Mashonaland Central province of Zimbabwe. The study area was chosen because the prevalence of *S. haematobium* in Zimbabwe there is $\geq 50\%$ in school-age children [22]. The residents rely on nearby rivers such as the Mupfure for their domestic needs and farming activities. Shamva has a humid subtropical climate with a wet season, which is humid and mostly cloudy, while the dry season is mostly clear and warm [23]. Sanitation in this area is poor; the majority of residents rely on open toilets for defecation.

Ethical approval and consent

Ethical and institutional approval was obtained from the Medical Research Council of Zimbabwe and the University of Zimbabwe (MRCZ/A/1173). The study aims and procedures were explained in the local language, Shona, prior to enrolment. Written informed consent was obtained from parents or guardians of the participants, and they were free to withdraw their children from the study without prejudice to any services they received from the health facility.

Parasitology

Urine and stool specimens were collected from each participant between 10:00h and 14:00h, and processed within 2 h of collection. The urine filtration technique was used to diagnose *S. haematobium* infection as described by Mott *et al.*, (1982) [24]. In brief; the urine was filtered through a nitrile filter membrane. The filter membrane was stained with Lugol's iodine and examined with a $\times 10$ light microscope objective lenses. *S. haematobium* infection intensity was expressed as the number of eggs detected per 10 ml urine. The same procedure was repeated for three consecutive days to prevent misdiagnosis due to day-to-day variation of egg excretion [25].

The Kato Katz technique was used to determine *S. mansoni* and soil-transmitted helminths (STH) and those positive were excluded from the study [26]. Briefly, stool samples were sieved and at least two slides per sample prepared using standard 41.7 mg templates and stained with 50 % glycerol-malachite green to easily identify the helminths. Slides were examined microscopically and egg counts per gram of stool determined for the diagnosis of *S. mansoni* and STH.

Blood collection

Up to 5 ml blood was collected into plain tubes (BD vacutainer, Fisher Scientific) from each participant with the aid of a trained nurse. Blood samples were left to clot at room temperature (25° C) for 1 h. The clot was removed by centrifugation at 2555 g, and the serum was then collected into 1.5 ml Eppendorf tubes. The serum was stored at -20°C until used for detection of YKL-40 and ANA.

Determination of YKL-40

YKL-40 concentrations were determined by ELISA according to the manufacturer's instructions (Duoset ELISA, DY2599). Optimisation was done to ensure the best conditions to carry out the assay. 100 µl of each serum sample was diluted in 13 mM phosphate-buffered saline, pH 7.2 containing 1% bovine serum albumin. The samples as well as standards were added to polystyrene microtiter plate wells (Corning, New York) coated with capture antibody (mouse anti-human YKL-40 capture antibody) and incubated for 2 h at room temperature. After washing the plates four times with 0.05 % Tween in PBS (pH 7.2), 100 µl of the detection antibody (Biotinylated Mouse Anti-human YKL-40 detection antibody) was added, followed by incubation for 2 h at room temperature. The washing was repeated as before, streptavidin horse radish peroxidase (HRP) was added and plates were incubated for 20 min at room temperature in the dark. The plates were washed once again, and 100 µl of the substrate solution o-phenylenediamine dihydrochloride (OPD) was added followed by incubation for 20 min at room temperature. Absorbance was measured using a microplate reader (Biotek, USA) at 450 nm.

Determination of ANA

The levels of ANA were determined in serum using an ANA screen ELISA kit (Catalog number KA0939,

Abnova) following the manufacturer's instruction, where the ANA antibodies were measured by ELISA against nuclear antigens including DsDNA, SSDNA, RNP, Sm, SSA and SSB. The intensity of the colour generated at the end is proportional to the amount of IgG specific antibody in the sample.

Statistical methods

The data described for this study were continuous. Graph pad prism was used to analyse the results. The Mann-Whitney U-test was used to analyse if the difference in YKL-40 levels between infected and uninfected individuals at a significance level of $P < 0.05$. Correlation analyses were conducted using the Spearman's rank method to assess the relationship between YKL-40, with schistosome infection intensity with P values < 0.05 being significant. Mann-Whitney U-test was also used to determine whether the levels of ANA were higher for those positive or negative for schistosomiasis with results being significant for $P < 0.05$. The Kruskal-Wallis test was used to analyse the difference in ANA levels according to infection intensity for $P < 0.05$.

Results

Schistosoma haematobium infection, YKL-40 and ANA levels

Schistosoma haematobium infection was diagnosed by urine filtration technique. The prevalence of urogenital schistosomiasis among the 145 participants was 21.4 % ($n = 31$). There were 66 females (45.5 %) and 79 males (54.5 %).

YKL-40 is increasingly associated with infection, T-cell-mediated inflammation, wound healing, allergy and asthma. The levels of YKL-40 were analysed by ELISA; no correlation between infection intensity and YKL-40 levels (Figure 1) was detected. YKL-40 levels were significantly higher in schistosome-infected than in uninfected participants ($P = 0.038$; Figure 2).

ANA levels were measured by ELISA in schistosome-infected and uninfected participants and were significantly higher in uninfected than infected individuals ($P = 0.028$; Figure 3), and inversely proportional to infection intensity ($r = -0.225$; $P = 0.016$; Figure 4). This trend is further highlighted in Figure 5, which reveals a gradual decrease in ANA levels for non-infected to moderately infected individuals. Although there was a positive relationship between YKL-40 and ANA levels, this was not significant ($r = 0.162$, $P = 0.156$).

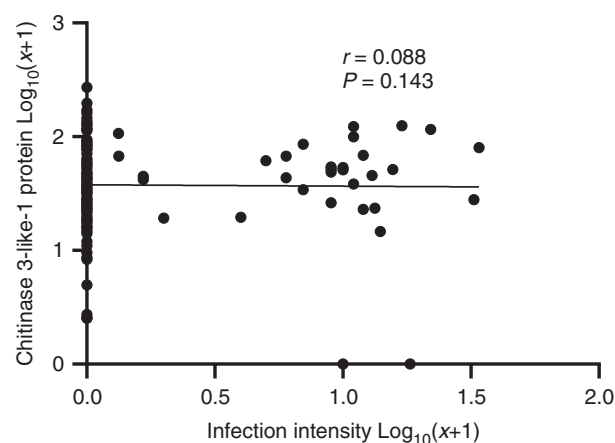


Figure 1 Chitinase 3-Like-1 Protein relationship with infection intensity. Spearman's rank method was used to analyse the relationship between Chitinase-3-Like 1 Protein with and infection intensity.



Figure 3 ANA levels by Infection status. The black and open bars represent ANA levels for those positive and negative for *Schistosoma haematobium* infection, respectively. Mean ANA levels are shown with error bars representing standard error of the mean.

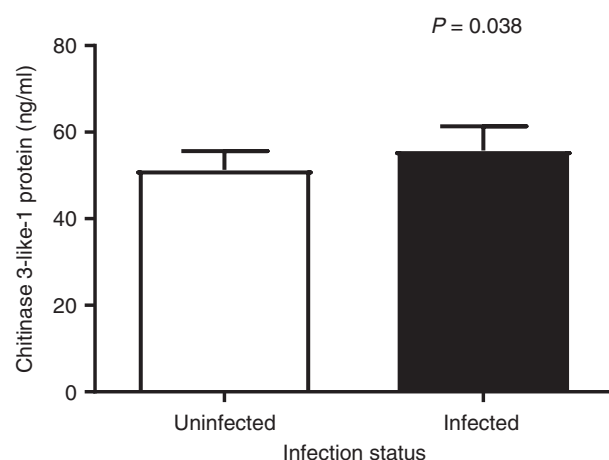


Figure 2 Chitinase-3-Like 1 Protein levels according to Infection status. The black and open bars represent YKL-40 levels for those positive and negative for *Schistosoma haematobium* infection, respectively. Mean YKL-40 levels are shown with error bars representing standard error of the mean.

Discussion

YKL-40 has been found elevated in several conditions such as psoriasis, a common inflammatory skin disorder [10], and schistosomiasis [27]. In this study, we investigated YKL-40 and its association with schistosomiasis in pre-school-aged children. ANA levels were also determined, and the relationship with schistosome infection observed in the same population. We found higher levels of the YKL-40 in schistosome-infected than uninfected

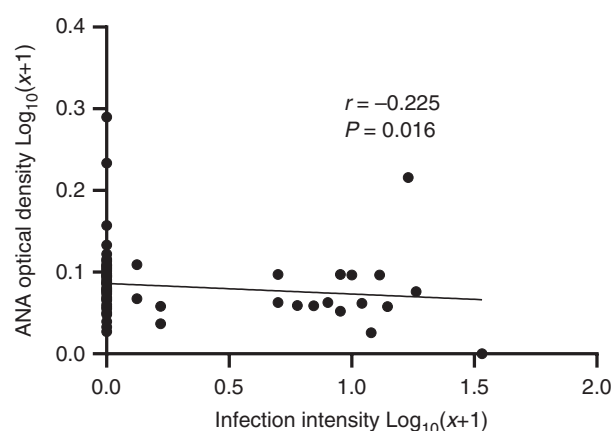


Figure 4 Relationship between ANA and infection intensity. The relationship between ANA levels with infection intensity was analysed using Spearman's rank method.

participants. The results are of public importance as they give an insight into inflammation in schistosomiasis and point towards a biomarker, especially if a longitudinal study to confirm this was carried out. Of note are higher levels of allergy marker ANA in schistosome-uninfected than infected participants. As the demand for pre-school-aged children to be treated for schistosomiasis increases, it is also important to consider that by treating the children the effects of treatment may increase allergies. This occurred in Indonesia, where mass drug administration (MDA) with albendazole to treat STH increased allergic sensitisation after a year [28].

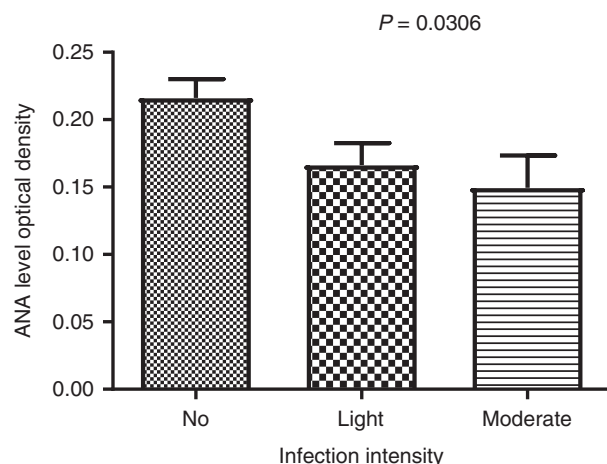


Figure 5 ANA level by infection levels. A bar graph of ANA by infection levels as described by WHO, no infection – 0 eggs, light infection – 1–9 eggs, medium infection – 10–49 eggs was plotted.

YKL-40 levels were higher in schistosome-infected than uninfected participants. YKL-40 levels have also been reported to be higher in the schistosome-infected than uninfected participants for the youngest age group of 2–10 years [27]. In our study, we focussed on pre-school-aged children only and not on schoolchildren, as they have been neglected in schistosome-control programs. We found significant results for this age group. Elevated levels of this marker are associated with inflammatory conditions, and this could explain why the levels were higher in infected individuals [29]. However, infection intensity did not correlate with YKL-40 levels.

Higher levels of ANA were observed in schistosome-uninfected than infected participants. The levels of ANA were also inversely proportional to infection intensity, which was similar to findings of a study conducted in Mashonaland East Province of Zimbabwe by Mutapi *et al.* (2011) [30]. Our results support the hygiene hypothesis, which proposes that the decreasing incidence of infections in western countries and lately in developing countries is the basis of the rising incidence of both autoimmune and allergic diseases [19]. ANA may indicate autoimmune disorders that are Th1-mediated diseases, but also due to the Th17 cell whose main product is IL-17, a major pro-inflammatory cytokine, which promotes autoimmunity [31]. ANA levels were not significantly correlated with YKL-40 levels. This finding contrasts with other studies, which showed a correlation with asthma [32], and that allergen exposure induced YKL-40 secretion [33]. More studies on these relationships after treatment with praziquantel would improve the knowledge gained from the current study, which was

conducted in children in their early years of development and during the period of receiving major vaccine antigens.

Conclusion

Our results give an insight into inflammation and point to YKL-40 as a possible biomarker of *S. haematobium* infection in pre-school-aged children. Further investigations in a longitudinal study are needed. ANA levels were higher in schistosome-uninfected than infected participants; hence, further studies on allergies may have to be carried out.

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Data availability statement

The statistical data on the parasitology and ELISA used to support the findings of this study are available from the corresponding author upon request.

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