Annals of Global Health
© 2015 The Authors. Published by Elsevier Inc.
on behalf of Icahn School of Medicine at Mount Sinai

VOL. 81, NO. 5, 2015
ISSN 2214-9996
http://dx.doi.org/10.1016/j.aogh.2015.12.008

ORIGINAL RESEARCH

Serum Levels of Cytokines and IgE in Helminth-Infected Nigerian Pregnant Women and Children

Ganiyu Olatunbosun Arinola, PhD, Olajumoke Abimbola Morenikeji, PhD, Kazeem Sanjo Akinwande, MSc, Ayodele Olasoji Alade, MSc, Oluwakemi Olateru-Olagbegi, MSc, Ponmile Emmanuel Alabi, MSc, Sheu Kadiri Rahamon, PhD

Ibadan, Nigeria

Abstract

BACKGROUND Helminth infection is an important health challenge. Because of modulation of the immune response toward T-helper 2 (Th2) cells, the immunologic interplay that manifest during the coexistence of helminth infection with other conditions is still poorly understood.

OBJECTIVE This study determined the pattern of alteration in selected cytokines and immunoglobulin E (IgE) in pregnant women, preschool aged children, and school-aged children with helminth infection compared with uninfected groups.

METHODS Seventeen pregnant women, 42 preschool-aged children, and 60 school-aged children with helminth infection (HI) were recruited into this study. They were matched with 21 pregnant women, 42 preschool-aged children, and 50 school-aged children without helminth infection (HN) who served as controls. Venous blood samples were collected from each participant and analyzed for serum levels of tumor necrosis factor α (TNF-α), interleukin-10 (IL-10), interleukin-8 (IL-8), interleukin-6 (IL-6), and IgE. Statistical analysis was done using the Student *t* test, and $P < .05$ was considered as statistically significant.

FINDINGS Only serum level of IgE was significantly elevated in HI pregnant women compared with HN pregnant women. In HI preschool- and school-aged children, serum levels of IL-8, IL-6, and IgE were significantly elevated compared with HN children. However, preschool- and school-aged children with HI had similar levels of serum TNF-α and IL-10 compared with their corresponding HN groups.

CONCLUSIONS It could be concluded that altered cytokines expression in children and pregnant women with helminth infection might have some implications on need for deworming programs to improve pregnancy outcomes and vaccine responses.

KEY WORDS children, cytokines, helminth infection, IgE, pregnant women

INTRODUCTION

Helminth infections are one of the most common chronic infections in countries with poor hygienic conditions.[1] Roundworm, whipworm, and hookworms are the main species that infect people of all ages, including pregnant women.[2]

About 2 billion people are infected with helminthes worldwide. People living in endemic areas often suffer from single to multiple infections of

The authors declare no conflicts of interest. All authors read and approved the contents of this manuscript.
From the Department of Chemical Pathology, College of Medicine (GOA, KSA, OO-O, SKR); Department of Zoology, University of Ibadan, Ibadan, Nigeria (OAM, AOA, PEA). Address correspondence to G.O.A. (drarinolaog64@yahoo.com).

Annals of Global Health, VOL. 81, NO. 5, 2015
September–October 2015: 689–693

several helminth species, thereby causing marked morbidity and disability.[3]

Infection with helminthes have been reported to have profound immunomodulatory effects on the host as it can inhibit, alter and/or modify other ongoing immune responses.[3-5] During chronic helminth infection, there is increased production of cytokines such as interleukin-4 (IL-4) and interleukin-10 (IL-10) as well as increased activation and expansion of eosinophils, mast cells, basophils, and the antibody isotypes immunoglobulins G1 (IgG1), G4 (IgG4), and E (IgE).[6,7] This form of response clearly shows that there is a shift toward T-helper 2 (Th2) cells and anti-inflammatory immune response.

Pregnant women are among the groups vulnerable to helminth infection.[8] During pregnancy, there is production of pregnancy-related hormones that modulate immunologic responses in different stages of pregnancy.[9] This interplay was hypothesized to avoid damage to the fetus and to prevent spontaneous abortions.[10,11] Cytokine expression during chronic helminth infection is similar to cytokine responses in the third trimester of pregnancy. There is an increased activity of Th2 cells and increased production of anti-inflammatory cytokines during pregnancy.[12] In contrast, the activities of Th1 cells, inflammatory macrophages, and natural killer cells and production of inflammatory cytokines are reduced especially during the third trimester of pregnancy.[10] Therefore, helminth infection during third trimester of pregnancy further favors a shift toward Th2 immune responses, thus increasing the susceptibility of pregnant women to infections that require Th1 immune response to control. Therefore, severity and susceptibility to intracellular pathogens are increased.[13] For example, a report from Uganda [14] found that there was a significant association between increased HIV viral load and infections with hookworm and *Trichuris* in HIV-infected pregnant women. The viral load was found to reduce in these women after treating with albendazole.

Pregnant women and children are groups of people mostly vaccinated because of the high burden of different infections in these groups of people from developing countries. It is therefore necessary to determine serum levels of representatives of Th1 and Th2 arms of immune response and IgE in these vulnerable groups. The outcome of this study will be implicated in the prediction of vaccine responses in pregnant women and children. This study was therefore carried out to determine the pattern of alteration in selected cytokines and IgE in pregnant women and children with helminth infection.

MATERIALS AND METHODS

Study Participants. The study center, participant selection, collection of stool specimens and examination for helminthes, and blood sample collection and storage have earlier been reported.[15] Briefly, 245 pregnant women in their third trimester and 349 children were screened for helminth infection. Seventeen pregnant women, 42 preschool-aged children, and 60 school-aged children had helminth infection (HI) and they were matched with 21 pregnant women, 42 preschool-aged children, and 50 school-aged children without helminth infection (HN) who served as controls.

Informed Consent and Ethical Approval. Ethical approvals were obtained from the University of Ibadan/University College Hospital Joint Ethics Committee and the Oyo State Ministry of Health before the commencement of the study. Also, written informed consent or assent was obtained from each study participant or their parents.

Laboratory Analysis. Serum levels of TNF-α, IL-10, IL-8, IL-6, and IgE were determined as previously carried out[4,16] using ELISA from Leinco Technologies (Fenton, MO), Invitrogen Corporation, (Waltham, MA), Life Technologies, (Carlsbad, CA), and Assaypro (St. Charles, MO).

Statistical Analysis. The independent Student *t* test was used to assess the differences in means of the variables at $P < .05$. All results are presented as mean ± standard deviation.

RESULTS

The prevalence and types of helminth infection among the study participants have earlier been reported.[15]

As shown in Table 1, only serum level of IgE was significantly elevated in HI pregnant women compared with HN pregnant women. Serum levels of TNF-α, IL-8, and IL-6 were not significantly different in HI pregnant women compared with HN pregnant women. In HI preschool- and school-aged children, serum levels of IL-8, IL-6, and IgE were significantly elevated compared with their corresponding HN groups. However, preschool- and school-aged children with HI had

Table 1. Serum Levels of Selected Cytokines and IgE in Pregnant Women, Preschool-Aged Children and School Aged Children With Helminth Infection (HI) and Without Helminth Infection (HN)

	TNF-α (pg/mL)	IL-10 (ng/mL)	IL-8 (pg/mL)	IL-6 (pg/mL)	IgE (ng/mL)
Pregnant women					
HN (n = 21)	100.00 ± 50.00	ND	22.00 ± 7.10	52.80 ± 39.60	581.52 ± 232.32
HI (n = 17)	100.00 ± 60.00	ND	24.30 ± 3.50	57.80 ± 32.80	799.92 ± 231.84
P	1.000	NA	.198	.670	.006*
Preschool-aged children					
HN (n = 10)	49.32 ± 12.42	0.10 ± 0.05	493.30 ± 266.40	8.66 ± 4.01	ND
HI (n = 12)	45.64 ± 13.17	0.13 ± 0.08	966.90 ± 552.80	22.29 ± 17.03	ND
P	.510	.222	.022*	.018*	NA
School-aged children					
HN (n = 10)	44.05 ± 11.32	0.23 ± 0.14	562.90 ± 298.40	4.89 ± 2.36	1806.40 ± 222.40
HI (n = 12)	49.70 ± 11.45	0.26 ± 0.20	995.20 ± 334.20	16.56 ± 12.20	3156.20 ± 388.70
P	.225	.745	.002*	.006*	.000*

IgE, immunoglobulin E; IL, interleukin; ND, not determined; NA, not applicable; TNF-α, tumor necrosis factor α.
* Significant at $P < .05$.

similar levels of serum TNF-α and IL-10 when compared with their corresponding HN groups.

DISCUSSION

Because of poor sanitation and poor personal hygiene, helminth infections continue to be a major health challenge in the developing countries, affecting especially schoolchildren and pregnant women. Reports have found that infection with helminthes has marked immunomodulatory effects that could be fatal in vulnerable groups such as children and pregnant women.[3]

IL-6 is a multifunctional pleitropic cytokine produced by different cell types. It has both pro- and anti-inflammatory properties depending on the prevailing immune context; thus, it is regarded as a prominent target for clinical intervention.[17] IL-6 is involved in the regulation of immune responses and inflammation and in the activation and differentiation of immune cells such as macrophages and lymphocytes.[18,19] An experimental study revealed that IL-6 limits the Th2 response, modifies the Treg-cell phenotype, and promotes host susceptibility to helminth infection.[19] Our observed elevated IL-6 levels in preschool- and school-aged children support the result of Nagy et al.,[20] who reported elevated IL-6 level in children with *Toxocara canis* infection. Our observation might be a reflection of the function of IL-6 as an enhancer of the differentiation of Th2 cells, which are predominantly involved in the control of helminth infection. However, the insignificantly elevated level of IL-6 in the pregnant women with HI contradicts our observation in children with HI. This observation might indicate that there is lower burden of helminth antigen in pregnant women that requires a stronger Th2 cell differentiation compared with the children. This is in line with our report,[15] which found that the pregnant women were infected with only *Ascaris lumbricoides,* whereas the children were infected with *Ascaris lumbricoides*, hookworm, *Fasciola hepatica,* and *Trichuris trichiura,* with some of the children even having coinfection with different species of helminth. Nagy et al.[20] reported that the higher the helminth antigen, the greater the production of IL-6. Also, it is possible that reproductive hormones during pregnancy might be responsible for the nonsignificant difference in serum levels of cytokines observed in HI pregnant women compared with HN pregnant women.

IL-8 is a potent proinflammatory cytokine that has chemotactic activity for neutrophils, basophils, eosinophils, and lymphocytes.[21] It is released from various cell types in response to an inflammatory stimulus. It is an important cytokine in phagocytosis because it is involved in neutrophil activation.[22] The observed elevated IL-8 levels in preschool- and school-aged children might be as a result of increased production of IL-8 from eosinophils. Cytokines produced by Th2 cells during helminth infection stimulate eosinophils, which are known to produce and secrete IL-8.[23] However, pregnant women with HI had insignificantly elevated level of IL-8 compared with HN pregnant women. This observation might support our earlier

Annals of Global Health, VOL. 81, NO. 5, 2015
September–October 2015: 689–93

suggestion that the pregnant women with HI might have lower helminth antigen, thereby culminating in nonsignificant production of IL-8 and IL-6.

IL-10 is an anti-inflammatory cytokine that regulates the inflammatory process. It is produced by many different myeloid and lymphoid cells. During infection, more than 1 population of IL-10–producing cells are induced, resulting in inhibition of the activities of Th1 cells, natural killer cells, and macrophages.[24] In allergic diseases, IL-10 is a down-modulatory factor that functions to induce modified Th2-cell phenotype.[3,25] The observed elevated levels of IL-10 in preschool- and school-aged children are in line with the reports of Goddey et al.[26] and Sanchez et al.[27] This observed elevated IL-10 might be necessary to dampen excessive inflammation with a view to protecting host tissues. Experimental studies found that IL-10–deficient mice with helminth infection had high morbidity and mortality.[28,29] This probably shows that IL-10 is important in controlling pathology associated with helminth infection.[5] This IL-10 action, however, could facilitate host tolerance to helminth infection and cause immune hyporesponsiveness against chronic helminth infection.[24,30] Thus, this allows the helminth to live for a long time (as long as 20 y as in the case of *Schistosoma* spp) if not treated.

Infection with helminthes has been found to be the most effective and reliable inducer of IgE responses. During helminth infection, total serum IgE levels may rise up to 100-fold.[31,32] Our observed elevated IgE in both children and pregnant women with helminth infection are not surprising. Gebreegziabiher et al.[33] and Arinola et al.[16] reported elevated IgE levels in pregnant women and children with helminth infection. IgE performs an important role in the control of helminth infection. Because helminths are large parasites that cannot be engulfed by phagocytes, the prevailing Th2 cytokines milieu will induce the B cells to switch the immunoglobulin class. The IgE produced coats the helminth to enable tissue mast cells and circulating eosinophils to bind via their IgE receptors leading to cascade of immunologic events resulting in the extracellular killing of parasite.[34]

CONCLUSIONS

The results from this study indicate that inflammatory cytokines (IL-8 and IL-6) were significantly raised in helminth-infected children but not in pregnant women with helminth infection. Therefore, infectious agents that require Th1 immune responses to control may thrive more in children infected with helminth compared with pregnant women with helminth infection. Also, vaccines that stimulate Th1 immune responses may not be effective in helminth-infected individuals, especially children. Insignificant differences in inflammatory cytokines (IL-6, IL-8, and TNF-α) in helminth-infected pregnant women compared with pregnant women without helminth infection might result in normal pregnancy outcomes in helminth-infected pregnant women. Therefore, altered cytokines and IgE production in helminth-infected individuals call for regular deworming program, especially for pregnant women and children.

REFERENCES

1. Lechner CJ, Komander K, Hegewald J, et al. Cytokine and chemokine responses to helminth and protozoan parasites and to fungus and mite allergens in neonates, children, adults, and the elderly. Immun Ageing 2013;10:29.
2. World Health Organization. Fact Sheet (No. 366). Soil-transmitted helminth infections. Available at: http://www.who.int/mediacentre/factsheets/fs366/en/. Accessed August 24, 2015.
3. Maizels RM, Yazdanbakhsh M. Immune regulation by helminth parasites: cellular and molecular mechanisms. Nat Rev Immunol 2003;3:733–44.
4. Arinola G, Oluwole O, Oladokun R, Adedokun B, Olopade O, Olopade C. Intestinal helminthic infection increases serum levels of IL-2 and decreases serum TGF-beta levels in Nigerian asthmatic patients. Open J Immunol 2014;4:1–8.
5. Helmby H. Human helminth therapy to treat inflammatory disorders—where do we stand? BMC Immunol 2015;16:12.
6. Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infection. Nat Rev Immunol 2007;7:975–87.
7. Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. Nat Rev Immunol 2011;11:375–88.
8. Katona P, Katona-Apte J. The interaction between nutrition and infection. Clin Infect Dis 2008;46:1582–8.
9. Arinola OG, Louis JS, Tacchini-Cottier F, Aseffa A, Salimonu LS. Pregnancy impairs resistance of C57BL/6 mice to Leishmania major infection. Afr J Med Med Sci 2005;34:65–70.

10. Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune responses and disease pathogenesis. Horm Behav 2012;62: 263–71.
11. Abdoli A, Pirestani M. Are pregnant women with chronic helminth infections more susceptible to congenital infections? Front Immunol 2014;5:53.
12. Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. Immunol Today 1997;18: 478–82.
13. Salgame P, Yap GS, Gause WC. Effect of helminth-induced immunity on infections with microbial pathogens. Nat Immunol 2013;14: 1118–26.
14. Webb EL, Kyosiimire-Lugemwa J, Kizito D, et al. The effect of anthelmintic treatment during pregnancy on HIV plasma viral load: results from a randomized, double-blind, placebo-controlled trial in Uganda. J Acquir Immune Defic Syndr 2012;60:307–13.
15. Arinola OG, Morenikeji OA, Akinwande KS, et al. Serum micronutrients in helminth-infected pregnant women and children: suggestions for differential supplementation during anti-helminthic treatment. Ann Global Health, in press.
16. Arinola OG, Yaqub SA, Rahamon SK. Reduced serum IgE level in Nigerian children with helminthiasis compared with protozoan infection: implication on hygiene hypothesis. Ann Biol Res 2012;3:5754–7.
17. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. Nat Immunol 2015;16:448–57.
18. Akdis M, Burgler S, Crameri R, et al. Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. J Allergy Clin. Immunol 2011;127:701–21. e1–70.
19. Smith KA, Maizels RM. IL-6 controls susceptibility to helminth infection by impeding Th2 responsiveness and altering the Treg phenotype in vivo. Eur J Immunol 2014;44: 150–61.
20. Nagy D, Bede O, Danka J, Szénázi Z, Sipka S. Analysis of serum cytokine levels in children with chronic cough associated with Toxocara canis infection. Parasite Immunol 2012;34: 581–8.
21. Erger RA, Casale TB. Interleukin-8 is a potent mediator of eosinophil chemotaxis through endothelium and epithelium. Am J Physiol Lung Cell Mol Physiol 1995;268:L117–22.
22. van Damme J, Rampart M, Coning R, et al. The neutrophil-activating proteins interleukin 8 and beta-thromboglobulin: in vitro and in vivo comparison of NH2-terminally processed forms. Eur J Immunol 1990;20:2113–8.
23. Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. J Immunol 1996;156:4859.
24. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. J Immunol 2008;180:5771–7.
25. Jeannin P, Lecoanet S, Delneste Y, Gauchat JF, Bonnefoy JY. IgE versus IgG4 production can be differentially regulated by IL-10. J Immunol 1998;160:3555–61.
26. Goddey NOP, Osagie ID, Maliki A. Serum cytokines profiles in Nigerian children with *Ascaris lumbricoides* infection. Asian Pac J Trop Med 2010;3:288–91.
27. Sanchez AL, Mahoney DL, Gabrie JA. Interleukin-10 and soil-transmitted helminth infections in Honduran children. BMC Res Notes 2015;8:55.
28. Wynn TA, Cheever AW, Williams ME, et al. IL-10 regulates liver pathology in acute murine Schistosomiasis mansoni but is not required for immune down-modulation of chronic disease. J Immunol 1998;160: 4473–80.
29. Schopf LR, Hoffmann KF, Cheever AW, Urban JF Jr, Wynn TA. IL-10 is critical for host resistance and survival during gastrointestinal helminth infection. J Immunol 2002;168:2383–92.
30. Figueiredo CA, Barreto ML, Rodrigues LC, et al. Chronic intestinal helminth infections are associated with immune hyporesponsiveness and induction of a regulatory network. Infect Immun 2010;78:3160–7.
31. Jarrett E, Bazin H. Elevation of total serum IgE in rats following helminth parasite infection. Nature 1974;251: 613–4.
32. Bell RG. IgE, allergies and helminth parasites: a new perspective on an old conundrum. Immunol Cell Biol 1996;74:337–45.
33. Gebreegziabiher D, Desta K, Desalegn G, Howe R, Abebe M. The effect of maternal helminth infection on maternal and neonatal immune function and immunity to tuberculosis. PLoS One 2014;9:e93429.
34. Amarasekera M. Immunoglobulin E in health and disease. Asia Pac Allergy 2011;1:12–5.