

Research Article

Serum Cytokine Levels in Intestinal Helminth Infected Pre-School Aged Nigerian Children before and after Oral Rotavirus Vaccination.

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Abstract

Rotavirus is one of the leading causes of diarrheal episodes among children of low income countries of the world and its high prevalence among Nigerian children has been reported. Vaccination holds the best hope for reduction of rotavirus-associated mortality and morbidity but study on Rota virus immunogenicity in helminth infected subjects in Nigeria is unknown. This study therefore determined cytokine levels in helminth infected Nigerian Children following oral rotavirus vaccination. A total of 22 pre-school aged children (1-4 years old) comprising 12 (7 males, 5 females) helminth infected and 10 (5 males, 5 females) helminth-free children were vaccinated with Oral Rotavirus Vaccine (Rotarix, Glaxosmithkline). Blood samples were taken before and 4 weeks after vaccination and levels TNF- α , IL-10, IL-6 and IL-8 were determined in the serum using Enzyme Linked Immunosorbent Assay. Pre-vaccinated serum levels of IL-6 and IL-8 were significantly higher in helminth positive children compared with pre-vaccinated levels of IL-6 and IL-8 in helminth negative children. Post-vaccinated level of IL-8 was significantly raised in helminth positive children compared with post-vaccinated level of IL-8 in helminth negative children. Pre-vaccinated level of IL-8 was significantly higher in helminth positive children compared with post-vaccinated level of IL-8 in helminth positive children. Post vaccinated level of IL-10 was significantly raised in both helminth positive and helminth – negative children compared with its pre-vaccinated levels in both helminth positive and helminth – negative children. Post vaccinated level of IL-10 was also significantly raised in helminth positive children compared with pre-vaccinated level of IL-10 in helminth- negative children. There were no statistically significant differences in serum TNF- α levels pre- and post-vaccination in helminth negative and helminth positive children. Helminth infection significantly raised the serum levels of IL-6 and IL-8 in un-vaccinated children while Rota-virus vaccine significantly reduced the serum IL-8 level. Rotavirus vaccine reduces the inflammatory cytokine responses induced by helminths.

Keywords: Helminth, Cytokines, Vaccination, Children

INTRODUCTION

Rotavirus is the leading cause of diarrhoeal episodes in children and is responsible for about 45% of all diarrhoeal episodes in infants and children under 5 years of age worldwide (Parashar *et al.*, 2003; Glass *et al.*, 2005). It is estimated to cause more than 450,000 deaths per year globally (Tate *et al.*, 2012) with over 90% of these deaths occurring in low income countries of South Asia and Sub-Saharan Africa (Glass *et al.*, 2005). A review of studies in Africa shows that rotavirus is responsible for 25% of all diarrhoeal cases in Nigerian children over a period of 3 decades (Waggie *et al.*, 2010) and incidence rate of 13.8% rotavirus infection was reported among Nigerian children presenting with diarrhoea in a Teaching Hospital in a recent study (Junaid *et al.*, 2011). Vaccination against rotavirus infection using different strains of the rotavirus vaccine has demonstrated good responses in clinical trials conducted in developed countries (Europe and America) and has been introduced into routine immunization schedule of several countries in these regions (Ruiz-Palacios *et al.*, 2006). Despite being expensive, it appears to be less protective in developing countries (Hanlon *et al.*, 1987; Georges-Courbot *et al.*, 1991; Perez-Schael *et al.*, 1997).

Factors such as maternal trans-placental antibody titres, micronutrient malnutrition, breastfeeding practices, stomach acidity and interfering gut flora have been reported to be responsible for reduced response against rotavirus vaccination (Patel *et al.*, 2009).

Rotavirus stimulates Th1/Th2 lymphocyte responses in infected hosts (Azim *et al.*, 2003; Jiang *et al.*, 2003; Azevedo *et al.*, 2006). The initial expression of IL-6, TNF- α , and IL-12 signifies the immediate innate immune response and induction of specific response to the infection. Expression of IFN- γ and IL-10 which follows depict the Th1 and Th2 cytokine balance in the eventual response. IFN- γ and other Th1 cytokines play important role in intestinal immunoglobulin A responses and protection rates (Azevedo *et al.*, 2006).

Intestinal helminth infection ranks amongst the commonest causes of chronic infection in the world, with nearly one-third of the global population infected (Hotez *et al.*, 2008). The main species that infect people are the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and hookworms (*Necator americanus* and *Ancylostoma duodenale*) (WHO, 2016). Children are at greatest risk and

not less than 400 million school age children are chronically infected worldwide with about 90 million from Africa (Brooker *et al.*, 2010). Helminth infection classically induces Th2 immunity and has been linked to attenuation of some damaging Th1 – driven inflammatory responses (Wang *et al.*, 2008). It induces immuno-regulatory functions through proliferation of regulatory T-cells (Tregs) (Belkaid and Rouse, 2005) which play important role in regulating immune responses and maintaining homeostasis under various disease conditions including autoimmune disease, inflammation, cancer and microbial infections (Wang *et al.*, 2008).

The World Health Organisation (WHO) and GAVI Alliance (Global Alliance for Vaccines and Immunisation) has offered to assist low income countries of Africa and Asia through inclusion of rotavirus vaccination in the global program for childhood immunization in low income countries of Africa and Asia if efficacy of the vaccine can be demonstrated through clinical trials in these regions (WHO, 1997) as low immunogenicity to these vaccine has earlier been reported, when compared to reports from industrialized countries (Shin *et al.*, 2002; Armah *et al.*, 2012). Many factors have been considered to be responsible (Patel *et al.*, 2009) but no attention has been given to the effect of intestinal helminth infection on the vaccine efficacy in Nigerians. This study therefore determined the levels of serum cytokines in helminth infected Nigerian children vaccinated with oral rotavirus vaccine. The aim is to provide basis for compulsory deworming programme for children before oral vaccination.

MATERIALS AND METHODS

Study Participants

Children were recruited from schools and communities of Alabata and Laleye, two semi-urban suburbs of Ibadan, southwest Nigeria. The prevalence of helminth infection among the children recruited for this study has earlier been reported (Arinola *et al.*, 2015). Of the 349 children screened for helminth infection, 12 helminth - positive pre-school aged children between 1 and 4 years of age, were matched with 10 helminth - negative children. A structured questionnaire was administered to collect information about their previous history of vaccination, breastfeeding period, history of severe diarrhea or systemic infection before they were enrolled in the study.

Ethical Consideration

Ethical approval to carry out this study was obtained from the UI/UCH Research Ethics Committee of the University of Ibadan, Nigeria and the Research Ethics Committee of the Oyo State Ministry of Health, Nigeria. Participants were enrolled into the study after obtaining a written informed assent from their parents or guardian. Participants were also allowed to withdraw from the study at anytime during the study.

Collection and Analysis of Stool Samples

Fresh stool samples were collected into a labeled leak-proof stool container (polypots) by the children or their parent / caregiver who had been given containers to scoop a heap of child's stool. The stool specimens were examined microscopically within 12 hours of collection using the concentration technique. The magnifications of x10 and x40 were used to visualize and identify intestinal geohelminth ova

respectively. The number of helminth ova was counted using Kato-Katz method (Arinola *et al.*, 2015b).

Blood Samples Collection and Vaccination

Prior to vaccination, 3ml of venous blood was collected from all the subjects using a 23G vacutainer needle from the ante-cubital vein into a non anticoagulant vacutainer bottle. The participants were given oral rotavirus vaccine (Rotarix, Glaxosmithkine) and second blood sample was collected 4 weeks after the vaccination. The blood samples were allowed to clot. The clotted samples were retracted and centrifuged at 3000rpm for 10 minutes. The sera were then gently removed into cryovials and stored at -20°C until analysis.

Serum Cytokines Analysis

Serum levels of TNF- α , IL-10, IL-8, IL-6 were determined as previously described [24] using ELISA reagents from Leinco Technologies(USA), Invitrogen Corporation (USA), Life Technologies (USA) and AssayPro Inc.(USA).

Statistical analysis

The data obtained from this study were analysed using SPSS version 17 Statistical package (SPSS Inc., Chicago, Illinois, USA). All results are presented as mean \pm standard deviation (SD) and differences in means of the variables were assessed using student's t-test. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

The mean(\pm SD) level of each cytokine before and after oral vaccination is presented in Table I. 24 pre-school aged children between ages 1 and 4 that participated in this study were grouped into 12 helminth – positive and 10 helminth – negative subjects. Post vaccinated levels of IL-10 were significantly raised compared with its pre-vaccinated levels in both helminth positive and helminth – negative children. Also, post vaccinated levels of IL-10 were significantly raised in helminth – positive children compared with pre-vaccinated level of IL-10 in helminth- negative children. Pre-vaccinated levels of IL-8 were significantly higher in helminth positive children compared with pre-vaccinated levels of IL-8 in helminth negative children. Post-vaccinated level of IL-8 was significantly raised in helminth positive children compared with post-vaccinated level of IL-8 in helminth negative children. Pre-vaccinated levels of IL-8 were significantly higher in helminth positive children compared with post-vaccinated level of IL-8 in helminth positive children. Also pre-vaccinated levels of IL-6 were significantly higher in helminth positive children compared with pre-vaccinated levels of IL-6 in helminth negative children. The reduced levels of TNF- α following vaccination in both helminth positive and helminth negative children were not statistically significant.

DISCUSSION

Diarrhoeal episodes due to rotavirus infection still remain a burden in Sub-Saharan Africa where 90% of deaths due to rotavirus gastroenteritis occur (Glass *et al.*, 2005). Unfortunately, vaccination against rotavirus has shown low immunogenicity in African children (Patel *et al.*, 2009) apart from being expensive and not affordable by average Nigerian.

Moreover, heminthiasis is a serious burden in Nigerian children (Arinola *et al.*, 2012). This study determined the effect of helminth infection on rotavirus vaccination by analysing serum cytokine levels before and after oral rotavirus vaccination.

Results from this study showed that post vaccinated serum levels of IL-10 were significantly increased in helminth-positive children and helminth-negative children compared with pre-vaccinated levels. Also, post vaccinated levels of IL-10 were significantly increased in helminth-positive children compared with pre-vaccinated level of IL-10 in helminth-negative children. IL-10 plays important role in regulating immune responses to parasitic infections and attenuation of disease pathogenesis (Sher and Coffman, 1992). It is a downmodulatory factor in allergic disease and is responsible for asymptomatic phenotypes observed in many parasitic diseases (Mahanty, 1996). IL-10 is secreted by a set of helminth – induced suppressive regulatory T-cells (T-regs) which constitutes about 5% to 10% of peripheral CD4+ T cells in humans (Takahashi *et al.*, 2000). High levels of IL-10 have been observed in individuals heavily infected with chronic intestinal helminth infections (Figueiredo, 2010). Our earlier study has reported increased level of IL-10 in helminth infected children when compared with helminth-free children (Arinola *et al.*, 2015). The result from this study is also in consonance with findings of Azim *et al.*, (1999) and Azevedo *et al.*, (2006) who demonstrated increased level of IL-10 after rotavirus vaccination in children and pigs respectively. This result suggests that the increased post vaccinated levels of IL-10 in helminth-positive children compared with pre-vaccinated levels may indicate up-regulation of T-regs after vaccination of helminth – infected children, while the increased post vaccinated levels in helminth-positive children compared with pre-vaccinated level in helminth-negative children (control) may indicate the up-regulation of T-regs both in helminth infection and vaccination. However, the post vaccinated increased levels of IL-10 in helminth-negative

children compared with its pre-vaccinated levels may confirm involvement of T-reg cells in vaccination. This may occur in order to regulate the possible excessive effect of vaccines.

This study also showed that pre-vaccinated levels of IL-8 were significantly higher in helminth positive children compared with pre-vaccinated levels of IL-8 in helminth negative children. Post-vaccinated level of IL-8 was significantly raised in helminth positive children compared with post-vaccinated level of IL-8 in helminth negative children. Pre-vaccinated levels of IL-8 were significantly higher in helminth positive children compared with post-vaccinated level of IL-8 in helminth positive children. This implies that IL-8 was up-regulated due to helminth infection which is the counteracted by the effect of the vaccine. IL-8 is a pro-inflammatory chemokine, produced by a wide variety of cells including monocytes, T-lymphocytes, neutrophils, vascular endothelial cells, dermal fibroblasts, keratinocytes and hepatocytes (Strieter *et al.*, 1989; Thornton *et al.*, 1990), whose major function is neutrophil activation and recruitment (Harada *et al.*, 1994). Eosinophils are known to produce and secrete IL-8 (Hansel and Walker, 1993) which can be stimulated by Th2 cell-derived cytokines (Nakajima *et al.*, 1996).

Increased eosinophils are observed in acute helminth infection through the helminth-induced IL-5 secretion which induces eosinophil proliferation and differentiation (Maizels and Yazdanbakhsh, 2003). Earlier and this present study have shown statistically significant differences in serum levels of IL-8 in helminth positive and helminth negative subjects which might be due to the effect of eosinophil. However, increased IL-8 secretion occurs in active and inactivated rotavirus infection through NF-κB mediated epithelial expression (Sheth *et al.*, 1996; Clemente *et al.*, 2015). This may also occur after rotavirus vaccination. The findings of this study therefore suggest that helminth infection may down-regulate NF-κB mediated expression of IL-8, while it up-regulate its secretion through induction of eosinophils.

Table 1: Serum Cytokines in Helminth Positive and Helminth Negative Pre – School aged Children before and after Rotavirus Vaccination. Values are expressed as Mean (± SD)

		TNF-α (pg/ml)	IL-10 (ng/ml)	IL-8 (pg/ml)	IL-6 (pg/ml)
Pre-Vaccination	Helminth Positive (n=12)	45.64 ± 13.17	0.13 ± 0.08	996.85±552.76	22.89 ± 17.03
	Helminth Negative (n=10)	49.32 ± 12.42	0.10 ± 0.05	493.32±266.41	8.66 ± 4.01
Post-Vaccination	Helminth Positive (n=12)	40.78 ± 12.66	0.25 ± 0.15	545.26±273.29	14.35 ± 10.70
	Helminth Negative (n=10)	42.50 ± 10.26	0.18 ± 0.09	312.84±170.33	11.95 ± 5.80
	t-test ^a	0.670	1.028	2.630	2.574
	p-value ^a	0.511	0.316	0.016*	0.018*
	t-test ^b	0.345	1.292	2.333	0.634
	p-value ^b	0.734	0.211	0.030*	0.533
	t-test ^c	0.922	2.445	2.537	1.471
	p-value ^c	0.367	0.023*	0.019*	0.156
	t-test ^d	1.589	3.015	0.471	1.586
	p-value ^d	0.128	0.007*	0.642	0.128
	t-test ^e	1.464	2.571	2.035	1.476
	p-value ^e	0.177	0.030*	0.072	0.157

- a- Helminth Positive pre-vaccination vs Helminth Negative pre-vaccination
 - b- Helminth Positive post-vaccination vs Helminth Negative post-vaccination
 - c- Helminth Positive pre-vaccination vs Helminth Positive post-vaccination
 - d- Helminth Negative pre-vaccination vs Helminth Positive post-vaccination
 - e- Helminth Negative pre-vaccination vs Helminth Negative post-vaccination
- * Statistically significant (p<0.05)

This study also showed that pre-vaccinated levels of IL-6 were significantly higher in helminth positive children compared with pre-vaccinated levels of IL-6 in helminth negative children. IL-6 is a multifunctional pleiotropic cytokine produced by different cell types, with pro- and anti-inflammatory properties depending on the prevailing circumstance. It is involved in the regulation of immune responses, inflammation, activation and differentiation of immune cells such as macrophages and lymphocytes (Smith and Maizels, 2014). The result of this study 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. Although not statistically significant, this study also showed a raised post-vaccinated level of IL-6 in helminth positive children compared with post-vaccinated level of IL-6 in helminth negative children and raised post-vaccinated levels of IL-6 in helminth negative children compared with pre-vaccinated levels of IL-6 in helminth negative children. This indicates that IL-6 is raised following vaccination which may not be affected by helminths. Raised levels of IL-6 are observed in active rotavirus infection (Jiang *et al.*, 2003; Shan-Ming *et al.*, 2014; Clemente *et al.*, 2015) and after attenuated rotavirus injection in animals (Azevedo *et al.*, 2006), the action of which may occur after vaccination which is in consonance with this study.

Pre- and post-vaccinated levels of TNF- α were higher in helminth negative children (non-significantly) compared with helminth positive children. Pre-vaccinated levels of TNF- α were higher (non-significantly) in helminth- negative children compared with post-vaccinated levels in helminth negative children. This indicates that TNF- α , a pro-inflammatory cytokine, is down-regulated in helminth infection. This supports earlier reports which showed reduced TNF- α levels in helminth infected asthmatic patients compared with controls (Arinola *et al.*, 2014) and further demonstrates the antagonistic effects of Th1 and Th2 cytokines to each other (Araujo *et al.*, 2000). However, the reduced TNF- α levels in helminth-negative children following vaccination is not in agreement with earlier finding (Azevedo *et al.*, 2006) in animal models.

Conclusively, helminth infection significantly raised levels of IL-6 and IL-8 in un-vaccinated children while Rotavirus vaccine significantly reduced IL-8 level in helminth-positive children. This may also contribute to low immunogenicity of oral vaccines among children of developing countries with high burden of intestinal helminth infection

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