Austin Journal of Tropical Medicine & Hygiene

Open Access

Austin Publishing Group

Research Article

Toll-Like Receptors 2 and 4 and their Association with the Pro/Anti Inflammatory Effects in Patients from Helminthiasis-Endemic Countries

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Received: December 25, 2014; **Accepted:** February 24, 2015; **Published:** February 26, 2015

Abstract

There is a growing interest in the role of Toll-Like Receptors (TLRs) and the innate immunity in the pathogenesis of various diseases. The role of TLRs may go beyond the recognition of microbial molecules to boosting immunity, modulating inflammation, or regulating autoimmune diseases. This work aimed to study the association between helminth infection and the expression of Toll-Like Receptors (TLR2 & TLR4) in infected individuals and possible association with asthma prevalence/severity in such individuals. Forty-two helminth-infected patients and 20 matched controls of uninfected persons were included. The CD14+ monocytes were analysed for TLR2 and TLR4 by flow-cytometry and TLR2 mRNA was measured by RT-PCR semi-quantitatively. The results showed an increased expression of TLR2 in the helminth-infected patients vs. uninfected persons. Mean Fluorescence Intensity (MFI) was 56.9 ± 13 vs. 31.2 ± 11, $p < 0.05$. The MFI in the patients with severe vs. mild intensity of infection was 73.03 ± 6 vs. 51.08 ± 7, $p > 0.05$). TLR2 mRNA was increased in helminth-infected vs. uninfected participants by RT-PCR ($p<0.05$). Asthma was found in 10/42 of the helminth-infected vs. 0/20 of the uninfected individuals (chi square 5.678, $p < 0.05$). No difference was found in TLR2-level in asthma vs. non-asthma patients (56.58±15 vs. 56.99±2.9, $p>0.05$), however there was a higher TLR2-expression with severe vs. less severe asthma ($p>0.05$). TLR4-MFI didn't show any significant difference in the studied groups. These results may underlie the role of TLR2 in the pathogenesis of both helminth infection and asthma and may introduce a novel approach to therapeutic developments.

Keywords: Toll-like receptors; TLR2; TLR4; Helminth infection; Asthma; Immune modulation; Immune stimulation; Inflammatory diseases

Introduction

The role of the innate immune mechanisms in microbial infections has gained growing international interest in the recent years since the discovery of the first human Toll-Like Receptor (TLR) in 1997 [1]. It was found that TLRs are crucial players in the innate immune response to microbial invaders; also they play a major role in the initiation of the protective immune responses and contribute to microbial elimination and tissue repair [2,3]. Moreover, TLRs are thought to be involved in the pathogenesis of the inflammatory responses, modulating inflammation or regulating autoimmune diseases [2, 4-8]. The cellular and molecular aspects of the innate immune response that can contribute to the initiation of the protective immunity following helminth infection were discussed [9]. Scientists suggested the activation and expression of TLRs along with other initiating events are implicated in the subsequent activation of the adaptive immune response against helminth parasites [9]. Investigating the link between helminth infection and TLRs activation has shown that TLRs maybe indirectly activated by the presence of helminth infection. However, direct stimulation was also seen with some helminthic derivatives such as the schistosomal lysophosphatidylserine (lyso-PS) which has been involved in the activation of TLR-2 [10]. Specific recognition of the helminth-derived excretory/secretory products by Antigen Presenting Cells (APC) is thought to be mediated by Pattern Recognition Receptors (PRR) including TLRs, C-type lectins, and intracellular Nod-Like Receptors (NLR) [7]. Signaling via PRR on the innate immune cells is critical for the recognition of parasites and other microbes and subsequent development of anti-pathogen immune responses and production of appropriate polarized T helper (Th) cell responses [6,11]. Recent evidence suggested that microbial stimulation through TLRs on the T cell, itself, can also directly influences T cell function and further immune reactions [12]. Among TLR family members, TLR2 and TLR4 have been identified as signaling receptors activated by helminthic components. Phospholipids from *Schistosoma mansoni* and *Ascaris lumbricoides* were found to have TLR2 activating capacity [11]. A parasitic derived substance, HSP60, in schistosomiasis was being able to activate TLR-2/4 [13]. Other Soluble Egg Antigen (SEA) preparations from *Schistosoma mansoni* contained a number of unique TLR ligands which stimulated the expression of TLR2 and TLR4 [14]. The filarial nematode excretory/secretory product (ES-62), which is a phosphorylcholine-rich glycoprotein was able to interact with TLR4 in a way distinct from that of the conventional TLR4 ligand [15]. An important association between TLR signaling and the pathological development of lymphangiogenesis in lymphatic filariasis due to TLR2-mediated enhancement of angiogenic growth factor were observed [16].

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Citation: El-Moamly A, El-Sweify M and Rashad R. Toll-Like Receptors 2 and 4 and their Association with the Pro/Anti Inflammatory Effects in Patients from Helminthiasis-Endemic Countries. Austin J Trop Med & Hyg. 2015;1(2): 1007.

Although, TLRs are believed to play a major role in the initiation of the protective immune responses to various microbes, the extensive release of TLR-triggered pro-inflammatory mediators is thought to harm the host [17]. TLRs exaggerated mediators were possibly involved in the pathogenesis of sepsis and various chronic inflammatory diseases [18]. In contrary, some believe the helminth-mediated exacerbation of TLR signaling underlies the ability of helminths to modulate the exaggerated immune responses observed in the inflammatory and autoimmune conditions [19-22]. These studies showed that although the Dendrite Cells (DC) recognizes the microbes by TLR ligation typically results in their phenotypic maturation and is characterized by increased expression of co-stimulatory molecules and the production of pro-inflammatory cytokines; in contrast, helminth-stimulated DCs are notable for their relatively immature status. They often express low levels of co-stimulatory molecules and pro-inflammatory cytokines and could be rendered refractory to subsequent stimulation through TLR activation with subsequent down regulation of the inflammatory response [19-22]. Also, antigens of helminths such as *Schistosoma* were able to differentially condition DC to promote either Th2 polarization or specifically induce IL-10 producing regulatory T cells (Treg) [10]. In this respect, scientists suggest that helminths products could represent an evolutionary pathway to limit inflammation while selectively allowing expression of Th2 cytokines needed for immune protection against parasites [9]. Scientists have been divided about the role of helminth-mediated TLRs on the allergic and inflammatory responses [23-29]. What is certain, in the view of many experts, is that we are at an early stage of our understanding of the roles of TLRs in disease. The relationship between helminth infection and TLRs activation, and the impact of this relationship on the pathogenesis of allergic diseases are not well-understood. Yet, we have to determine whether we wish to antagonize TLR signals to ameliorate severe inflammatory disease [30], use TLR agonists to boost protective immunity or modulate local inflammatory and immune response [31], or utilize helminth extract to alleviate allergic conditions [9].

This, *in vivo*, study aimed to determine the association between TLR2 / TLR4 expression and the presence of intestinal helminth infection and explore possible impact on the course of bronchial asthma in infected / uninfected patients.

Subjects and Methods

This descriptive case-control study was conducted at a Ministry of Health hospital, Riyadh, Saudi Arabia. The study targeted the foreign expatriates who came for work in Saudi Arabia from different helminthiasis-endemic countries. Stool and blood specimens were collected from the children aged 2-12 years who attended the hospital for routine investigations during the study period. The participants who denied any current clinical illness, had normal body temperature, and had no history of prior anthelmintic drugs or systemic corticosteroids usage for the preceding 4 weeks and whose guardians gave consent for participation were included. Examination of 3 stool samples on ten days/person for helminth ova/ parasites was done by formalin ethyl acetate concentration technique [32]. All stool samples were stained by trichrome and kinyoun acid fast staining methods [32] to exclude other intestinal protozoa and coccidia. To exclude bacterial infections, specimens were also tested using conventional methods. Quantitative estimation of the intensity of helminth infection in stool samples was performed on Kato-Katz slides [32]. The egg counts were classified as light, moderate, and heavy infection as per WHO recommendations [33]. For *A. lumbricoides*: light (1-4,999 egg per gram of stool; epg), moderate (5,000-49,999 epg), and heavy (≥50,000 epg); for *T. trichiura*: light (1—999 epg), moderate (1,000-9,999 epg), and heavy (≥10,000 epg); for hookworm: light (1–999 epg), moderate (2,000-3,999 epg), and heavy (≥ 4,000 epg); and for *Schistosoma*: light (1–99 epg), moderate (100–399 epg) and heavy (≥ 400 epg). A randomly selected control group of parasite-free individuals matched for age, sex, residence, socioeconomic status of the same study population were included. Diagnosis of asthma was made according to the asthma guidelines of the British Thoracic Society [34]. The presence of more than one of the followings support the diagnosis: 1) Wheeze, cough, and dyspnea particularly if frequent and recurrent. 2) Symptoms worsen at night and in the early morning. 3) Symptoms occur in response to or are worse after exercise or other triggers such as exposure to pets, cold or damp air, or with emotion or laughter, or occur apart from cold. 4) Personal history of atopic disorder. 5) Family history of atopic disorder and/or asthma. 6) Widespread wheeze on auscultation. 7) History of improvement in history or lung functions in response to adequate therapy. Asthma severity was defined as follow. Mild intermittent asthma: Symptoms < 2 days/week and < 2 nights/month. Mild persistent asthma: Symptoms > 2 days/week but < 1 time/day and nighttime symptoms > 2 nights/month. Moderate persistent asthma: Symptoms daily and nighttime symptoms >1 night/week. Severe persistent asthma: Continuous daily symptoms and frequent nighttime symptoms. Institutional approval to conduct the study was obtained from the Institutional Review Board.

Laboratory procedures

Flow Cytometry to estimate the expression of TLR2 and TLR4 on monocytes [35]: Heparinized blood samples were collected from helminth-infected participants and control group. Specimens were centrifuged over Ficoll Hypaque density gradients (Ficoll paqueTM plus, StemCell Technologies, Vancouver, BC, Canada) to prepare peripheral blood mononuclear cells (PBMCs). PBMC (1×10^5) were incubated on ice for 2 min with 10 µg of human IgG, then a 30-min incubation with 5 µl of anti-CD14 FITC antibody or monoclonal anti-TLR-2 antibody, anti-TLR-4 antibody (clone TL2·1 and HTA125, respectively, bioscience, San Diego, CA, USA), or mouse IgG1 control. The primary antibodies were washed off in PBS/ 0·5% BSA, 0·1% azide and the cells incubated for a further 30 min on ice with 2·5 µl of rabbit anti-mouse IgG1-pe. The secondary antibody was washed off as previous and labelled cells were acquired on the Vantage flow cytometer (FACS, Becton Dickinson Immunocytometry Systems, and Mountain View, CA, USA). Monocytes were gated on the basis of forward and side scatter and were >95% positive for CD14. Expression of phycoerythrin-labelled TLR-2 and TLR-4 on the gated population (1×10^4cells) was analysed using Expo 32 software (Beckman-Coulter). Results were expressed as mean fluorescence intensity (MFI) obtained for the isotype control antibody.

Reverse Transcriptase PCR (RT-PCR) [36]: Extraction of the RNA was performed from ($1x10^6$) CD14+ monocytes using the RNeasy Mini kit from Qiagen (Valencia, CA, USA) according to the manufacturer's instructions. Individual samples of RNA (1µg) in a

Table 1: Distribution of helminth infection and asthma in 62 study participants.

	Helminth infection	No Helminth infection	Total
Asthma	10	0	10
No asthma	32	20	52
Total	42	20	62

Pearson Chi square: 5.678, $p < 0.05$

13µl volume were transformed to cDNA by using dNTP and OligodT primer (Invitrogen, Carlsbad, CA, USA). cDNA was then amplified in a 20µl final volume using the amplification kit, Superscript™ III (Invitrogen, Carlsbad, CA, USA). The reaction mixture (Bioneer, Daejeon, Korea) was used for the RT-PCR analysis; it contains 250 µM dNTP, 1U Taq polymerase, 10mM Tris–HCl (PH 9.0), 1.5mM $MgCl_2$, 40mM KCl, and 10 pmol of primers for TLR2. For the co-amplification primer, glyceraldehydes-3-phosphate (GAPDH) was used. The reaction was performed in a DNA Thermal Cycler (RTC-200, MJ Research, MA, and USA). The reverse transcription RT was performed at 47°C for 30 min followed by 94°C for 2 min to inactivate the reverse transcriptase enzyme. For TLR-2 and TLR-4 amplification, PCR was performed with 30 cycles of 94°C for 20 s, 54°C for 45 s and 72°C for1 min. For the GAPDH housekeeping gene, PCR was performed with 20 cycles of 94°C for 30s, 60°C for 30 s and 72°C for 45s. The sense and anti-sense primers sequences used for amplification were: TLR2, 5′ GGCCAGCAAATTACCTGTGTG-3′ and 5′-AGGC GGACATCCTGAACCT-3′; GAPDH, 5′ AGTCAACGGATTTGGTCGTA-3′ and 5′-GGAA CATGTAAACCATGTAG-3′. The intensities of the PCR bands were measured to quantify the transcripts semi-quantitatively by densitometry using the Image-Pro plus Version 4.5 (Media Cyber tics Co, MD, USA), and the intensities were expressed relative to GAPDH as a percentage of GAPDH expression.

Statistical analysis

The frequencies of asthma, expression of TLR2 and TLR4 on individuals- monocytes, and the intensity of TLR2-mRNA were compared between the test and control groups. Independent-sample *t* tests were used to compare means for continuous variables, and the chi-squared test was used to compare nominal variables. Pearson's correlation analysis was performed to determine the correlation between the TLRs level and the intensity of both helminth infection and asthma. For all tests, a two-tailed p <0.05 were considered significant. All analyses were done with SPSS version 10 software (SPSS, Chicago, IL).

Results

Helminth infection was detected in 42 out of 425 participants from endemic countries (India, Pakistan, and Bangladesh). Thirty four were from rural and 8 from urban settings. A control group of 20 matched persons with no parasitic infection were included. The detected helminthes and intensity of infections were as follow: Total helminth infections 42 (3 heavy, 15 moderate, and 24 mild infections); 17 *Ascaris lumbricoides* (1 heavy, 8 moderate, and 8 mild); 10 *Trichuris trichiura* (2 heavy, 4 moderate, and 4 mild); 7 *Hymenolepis nana* (2 moderate, 5 mild); 5 Hookworm (1 moderate and 4 mild); 2 *Enterobius vermicularis* (2 milds); 1 *Schistosoma mansoni* (1 mild); while mixed infections were excluded. Mean age of the infected patients was 9.5±2.3 yrs (range 5-15 yrs), male to female was 26:16, and past history of gastrointestinal symptoms in the form of diarrhea, nausea, vomiting, abdominal pain, or dyspepsia were found in all patients. Past history of parasitic infections was reported in 15/42 infected patients and in 2/20 control-individuals. Of the 42 patients with the helminth infections, asthma diagnosis was made in10 patients, while no asthma was detected in the 20 control participants (Table 1). Five patients (4 males, 1 female) were having moderate to severe asthma. Of those, moderate persistent asthma was found in 3 males and 1 female, and severe persistent asthma was found in 1 male. Mild asthma was detected in 5 patients (3 males, 2 females). Of those, mild intermittent asthma was detected in 2 males and 2 females, and mild persistent asthma was detected in 1 male. Mean disease duration of asthma was 3.5±1.5 years. Five out of 10 asthma-patients (4 with moderate persistent and1 with severe persistent asthma) were having long-term inhaled corticosteroid plus long acting inhaled beta 2-agonist. While 3 patients (with mild asthma) were having either inhaled beta 2-agonist or theophylline as long term treatment, and another 2 patients (with mild asthma) were having the same drugs intermittently. Additionally, all included asthma-patients had occasionally taken quick-relief medications during the acute attacks of asthma in the form of short-acting inhaled beta2-agonists bronchodilator with/without oxygen or parasympatholytic agent as a single nebulizer treatment, or short course of systemic corticosteroids.

Expression of TLR2 and TLR4 in helminth infected patients

Flow-cytometry testing showed an increased expression of TLR2 on the monocytes of the helminth-infected patients compared to the uninfected ones (p <0.001) (Table 2). The TLR2-MFI was increased in severe compared to moderate infections (p = 0.321) and in moderate compared to mild infections (p = 0.003) (Table 3). The expression of TLR2 was positively correlated with the intensity of helminth infections (r = 0.547, p <0.001). On the other hand, TLR4-MFI didn't show significant difference in infected vs. uninfected subjects (21.2 vs. 20.8), p = 0.37 or in severe compared to moderate or mild infections (20.2, 21.3, and 21.218 respectively), p = 0.8. By using RT-PCR, the TLR2 mRNA transcripts were expressed in all included specimens of

Table 2: TLR2-MFI in participants with/without helminth infection.

	Number of cases	Minimum TLR2-MFI	Maximum TLR2-MFI	Mean ± Std. Deviation
Helminth infection	42*	34.0	82.0	56.895 ± 13.211
No helminth infection	20**	17	53.7	31.195 ± 10.898

$p < 0.001$

P value was calculated with the independent-sample *t* test

*10 of them had asthma ** none of them had asthma

TLR2: Toll-like receptor 2; MFI: Mean fluorescence intensity

Table 3: TLR2-MFI level with severity of helminth infection.

Helminth Infection	Number of cases	Minimum TLR2-MFI	Maximum TLR2-MFI	Mean ± Std. Deviation	*P* value
Severe	3	68.2	79.4 66.8	73.033 ± 5.755	0.321*
Moderate	15	34.0	82.0	62.980 ± 16.440	0.003
Mild	24	41.8	66.8	51.075 ± 7.089	

P value was calculated with the independent-sample *t* test

TLR2: Toll-like receptor 2; MFI: Mean fluorescence intensity

* Although the difference between severe compared to moderate group was not significant, comparing results of severe to mild group was significant (p <0.05)

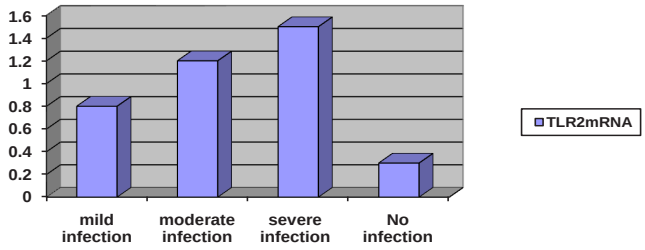

Figure 1: Increased median values of mRNA expression of TLR2 in helminth-infected patients compared to non-infected controls. Levels of TLR2 mRNA were increased with severity of infection ($p < 0.05$). *P* value was calculated with the ANOVA test.
Y axis represents TLR2 mRNA % of GAPDH (control).

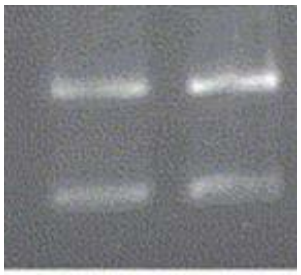

Figure 2: RT-PCR: The lower bands represent the TLR2 mRNA in relation to GAPDH upper control bands on the gel (non- original photo). The semi-quantitative assay was done in our study by comparing the relative density of TLR2 mRNA band of each specimen to the band size of the control (GAPDH) using the Image-Pro Plus Version 4.5 (Media Cybertics Co, MD, USA). Intensities were expressed as a percentage of GAPDH expression.

the test and control groups. On semi-quantifying these expressions, the median value of TLR2 mRNA was increased in the monocytes of helminth-infected vs. uninfected participants and in severe compared to moderate and mild infections, $p < 0.05$ (Figures 1 & 2).

Expression of TLR2 and TLR4 in asthma patients

In the overall participants, patients with asthma have shown higher TLR2-MFI versus non-asthma patients (56.580±15.033 vs 47.071±17.472, $p = 0.113$) (Table 4). However, in the helminth-infected patients, there was no detected difference in the expression of TLR2 in asthma-patients comparing to those without asthma (MFI: 56.58 ±15 vs. 56.99±12.9), $p = 0.932$ (Table 5). The expression of TLR2 was higher with severe asthma (TLR2-MFI = 79.40) than with moderate asthma (TLR2-MFI= 66.10), $p = 0.212$ and in moderate than mild asthma (TLR2-MFI = 44.40), $p = 0.005$ (Table 6). The TLR2-MFI levels in different study groups were shown in (Table 7). In regard to TLR4, the mean fluorescent intensities didn't show significant difference in asthma versus non-asthma patients (21.6±2.5 vs. 20.95±1.4, $p = 0.24$).

Table 4: TLR2-MFI in 62 asthma and non-asthma persons with/without helminth infections.

	Number of cases	Minimum TLR2-MFI	Maximum TLR2-MFI	Mean± Std. Deviation
Asthma	10*	34.0	79.4	56.580±15.033
No Asthma	52**	17	82.0	47.071±17.472

$p = 0.113$
*All had helminths infection. ** 32 had helminths and 20 had no helminths.
P value was calculated with the independent-sample *t* test
TLR2: Toll-like receptor 2; MFI: Mean fluorescence intensity.

Table 5: TLR2-MFI in asthma/non-asthma patients with helminth infections.

	Number of cases	Mean TLR2-MFI	Std. Deviation
Asthma/helminth infection	10	56.580	15.003
Non-asthma/helminth infection	32	56.994	12.852

$p = 0.932$
*All had helminths infection. ** 32 had helminths and 20 had no helminths.
TLR2: Toll-like receptor 2; MFI: Mean fluorescence intensity

Table 6: TLR2-MFI with severity of asthma.

Asthma severity	Number of cases	Mean TLR2-MFI	St. Deviation	*P* value
Severe	1	79.40	--	0.212*
Moderate	4	66.10	7.52	0.005
Mild	5	44.40	7.73	

P value was calculated with the independent-sample *t* test
TLR2: Toll-like receptor 2; MFI: Mean fluorescence intensity
* Although the difference between severe compared to moderate group was not significant, comparing results of severe to mild group was significant ($p < 0.05$)

Table 7: Overall distribution of TLR2- MFI in different study groups.

Helminths infection	Asthma	Number of cases	Mean TLR2-MFI	Std. Deviation
Mild	No asthma	24	51.075	7.089
Moderate	No asthma	8	74.750	9.270
	Mild asthma	5	44.400	7.733
	Moderate asthma	2	62.350	10.394
Severe	Moderate asthma	2	69.850	2.333
	Severe asthma	1	79.400	--
No helminth	No asthma	20	31.195	10.898

TLR2: Toll-like receptor 2; MFI: Mean fluorescence intensity

Discussion

The present study showed a higher expression of TLR2 in patients with helminth infections comparing to helminth-free individuals, and in patients with higher versus lower intensity of infections (Tables 2 and 3). Also, the prevalence of asthma in helminth-infected patients was higher comparing to the control group: 10/42 vs. 0/20, respectively, $p = 0.017$, (Table 1). The current results also showed that, in helminth-infected patients, there was no significant difference between the TLR2 mean level in patients with asthma compared to those without asthma, $p = 0.9$ (Table 5). Although there was a higher expression of TLR2 with severe asthma compared to less severe asthma, this result was statistically non-significant (Table 6). These findings may propose that helminth infection is associated with higher expression of TLR2, and also with higher prevalence of bronchial asthma in the infected patients. One may suggest that TLR2, which is activated as a response to helminth infection, can be involved in the higher susceptibility of the infected persons to the effects of other environmental allergens. However, the causative role of the expressed TLR2 in helminth-infected patients as a provoking factor for the occurrence or the severity of asthma can't be approved by the available results. The current cross-sectional study can't conclusively prove a cause and effect relationship between the three studied variables (helminth infection, TLR2, and asthma). Looking at other research works, one can see several studies that have assessed the association of helminth infection with allergic diseases but have shown discrepant

results. Some related the recent dramatic increase in the prevalence of allergic disorders in the developed vs. underdeveloped countries, although largely not yet explained, either to the protective effect of the intestinal parasitic infections in the rural regions or to the exposure to environmental pollutants in the industrialized countries [37]. A meta-analysis of many studies investigating the association between the presence of geohelminth eggs in stool samples and asthma concluded that parasite infections do not in general protect against asthma, but infection with hookworm may reduce the risk of this disease [38]. Three studies have demonstrated a clear inverse relation between intestinal parasite infection and markers of atopy or allergic diseases in tropical societies [39-41], while other researchers have detected a positive association between *Ascaris* and asthma among children from asthmatic families [42]. On the other hand, the role of TLRs in the pathogenesis of allergic and inflammatory disorders was also studied in some research works. Epidemiological studies supported by laboratory research have suggested that the innate immune responses are involved in the adverse effects of ambient air pollution on the prevalence of asthma [43]. Variant alleles of TLR2 and TLR4 genes were found to influence the susceptibility to the adverse effects of traffic-related air pollution on childhood asthma. However, the exact mechanisms of this influence are not yet elucidated [43]. Recently, scientists claimed that TLRs may be involved in activities other than recognition of microbial surface structures and nucleotides to enable vertebrates to detect the Pathogen-Associated Molecular Patterns (PAMPs) and subsequent activation of the adaptive immune response and contributing in microbial elimination [44,45]. Moreover, scientists proposed that TLRs may be involved in the pathogenesis mechanisms of some inflammatory diseases [2]. It was found that TLRs may induce anti-inflammatory effects in some contexts and pro-inflammatory effects in others and this may depend on the involved cells, the ligand, and the general milieu of the local and systemic immune responses [4]. In parasitic diseases, the *In-vitro* studies suggested that the expression of HSP60, as a parasitic derived products in *Schistosoma*, stimulates PBMCs of patients with inflammatory diseases such as Behcet's disease, resulting in excess production of Th1 cytokine with subsequent potent inflammatory response via activation of TLR2 and TLR4 [13,46]. On the other hand, Harn et al.[46], found that helminth extracts (mainly glycans) can drive CD4+ T-helper cell responses towards Th2 type and induce Alternatively-Activated Antigen-Presenting Cells (APCs) with the induction of immune suppression or energy. Though the mechanism of APC activation has not been fully elucidated, it was suggested that this may involve C-type lectin ligation on the surface of APCs, with subsequent antagonism of TLR signaling [47]. *In vivo,* however, it is not clear yet, what kinds of stimuli and mechanisms in helminth infection are responsible for the up/down regulation of TLRs and what the expected subsequent effects on the inflammatory disorders are. The current *in vivo* study is adding another confirmatory piece of knowledge to this controversial topic. Results of the current study showed that TLR2 is expressed at a higher level in children with helminth infection than uninfected children, while TLR4 levels were unaffected. Neither TLR2 nor TLR4 showed any difference in patients with bronchial asthma compared to asthma-free individuals. However, more exploratory studies are required to verify the causative relationship between helminth infection, TLRs, and asthma variables. Additional studies are needed to find out whether treating intestinal helminths is useful to reduce TLRs levels and ameliorate the severity of asthma and inflammatory symptoms or the reverse is true. Further defining the nature of the helminth-derived products, the cell types and signaling pathways which involve TLRs activation (or other effectors such as IL10 or IgE) and its possible role in modulating or provoking the inflammatory responses are also needed.

Conclusion

There is very little known about the relationship between helminth infection, TLR expression, and the inflammatory diseases. This descriptive case control study demonstrates that TLR2 was expressed at a higher level in children with helminth infection than uninfected children, while TLR4 levels were unaffected. Neither TLR2 nor TLR4 expressions showed any difference in patients with bronchial asthma compared to asthma-free individuals. Much works remains to be done to determine whether these finding can be generalized to other patients in different localities. Investigating different populations of helminth-free individuals with asthma and larger groups of participants to assess the individual helminth effect would improve the accuracy of future comparisons. Not only studying the involved mechanisms in the relationship between TLRs, helminth infection, and the inflammatory effects in various diseases are required, but also translational research studies are needed to get use of these findings from bench to bed-side and to provide new insights for the development of therapeutic options.

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Austin J Trop Med & Hyg - Volume 1 Issue 2 - 2015
ISSN: 2472-3681 | www.austinpublishinggroup.com
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Citation: El-Moamly A, El-Sweify M and Rashad R. Toll-Like Receptors 2 and 4 and their Association with the Pro/Anti Inflammatory Effects in Patients from Helminthiasis-Endemic Countries. Austin J Trop Med & Hyg. 2015;1(2): 1007.