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Research Article

Role of *Toll-Like Receptor 2* (*TLR 2*) Genetic Polymorphisms in Modulating Susceptibility to Clinical Disease in Wuchereria Bancrofti

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Keywords

- *Toll-Like Receptor 2 (TLR 2)*
- Lymphatic filariasis
- Wuchereria bancrofti

Abstract

Background: Bancroftian filariasis a parasitic vector-borne disease among the communicable neglected tropical infectious disease. The disease has been found to compromise the well-being of large populations in endemic countries within the tropics and sub-tropics. Despite of the several studies attempted to document on the mechanism involved in the development of clinical disease, until now the pathogenesis of the disease is not yet clear to date, although there, several underlined aetiological factors being implicated. This study was conducted to determine the role of *TLR 2* –196 to –173 del and its association with asymptomatic bancroftian filariasis in endemic communities of Tanga region in north eastern Tanzania.

Methods: *TLR 2* -196 to -173 polymorphism in the 5' untranslated region using allele specific real time -polymerase chain reaction (RT-PCR) were tested in 79 individuals.

Results: *TLR 2* -196 to – 173 polymorphisms were tested positive in 36.7 % of the samples.

Conclusion: *TLR 2* -196 to -173 del polymorphisms occurrence among individuals infected with bancroftian filariasis disease highlights the potential for the susceptibility of bancroftian filariasis infection and importance of further genetic research for better understanding the mechanism of infection transmission and heterogeneity of the disease.

INTRODUCTION

Lymphatic filariasis is a mosquito-borne among the communicable neglected tropical infectious disease. The disease has been found to compromise the well being a large population in endemic countries within the tropics. During the early stage of the clinical progression, the disease presents with acute febrile conditions involving the inguinal lymph nodes where adults' filarial worms dwell and this can lead to adenolymphangitis (ADL) which progresses distally, a typical for Wuchereria bancrofti. These immune responses such as; pro-inflammatory cytokines, including IL-1Beta and TNF-alfa have been shown to upregulate the expression of VEGF-C, raising the possibility that pro-inflammatory cytokines affect the lymphatic vessels via VEGF-C [1]. Therefore, pro-inflammatory cytokines are able stimulate proliferation of lymphatic endothelia [2] and are elevated in lymph from parasitized lymphatics. If the ADL are secondarily affected by local skin bacteria or fungal infection the condition will be more broad hence adeno-dermatolymphangitis (ADDL). Individuals harbouring adult filarial worm in the lymphatics of the scrotal area will trigger inflammation in per-tunica vaginalis causing acute orchitis which will progess into chronic and eventually small hydrocele if not treated. This is now a clinical hydrocoele a condition that can be clinically diagnosed and surgically managed. The other clinical condition caused by the wuchereria bancrofti is the lymphoedema of lower limbs and upper. Although there two clinical condition emanating from one disease the pathogenesis of each one is different and unclear until to date, although there several underlined aetiological factors being implicated. For the condition to occur, some conditions have to be present, these includes; the mosquito- vectors, the infective larvae (L3), human host, the filarial nematode host of the wolbachia and the wolbachia endosymbionts bacteria. The disease has two clinical spectrum of presentation, one end with those amicrofilareamic and down-regulated through cell-mediated responses and having pathologies, whereas the other extreme are those microfilaraemics without pathologies

The immune responses differ between individuals who present pathologies and those who don't. One plausible explanation for the different responses is that, single-nucleotide polymorphisms (SNPs) alter the expression of or the activity of immune factors such as; MHC gene products, human host-wolbachia molecules (LPS-LBP-TLR 4)-mcD14-ScD14 and cytokines] leading to the

broad range of clinical-disease manifestations that are observed. On the other hand, the presence of adult filarial worms in the lymphatic vessels and lymph nodes is also a factor known to trigger the pathology [3]. Those worms in the vessels they alter the structural anatomy of the vessels leading to massive accumulation of lymph fluid resulting into lymphoedema and irreversible obstructive lesions within the vessels and their walls.

Toll-like receptors (TLRs) are pattern-recognition receptors (PRRs), that recognize evolutionarily conserved structures on the invading pathogens [4]. *TLRs* trigger a number of pro inflammatory and anti-microbial responses, through ligands binding for defence against the pathogens and promoting adaptive immunity responses. *TLR 4* is the signal transducing element of the lipopolysaccharide (LPS) receptor complex1 and is also involved in the signalling response to other exogenous stimuli [4]. *TLR 5* binds flagellin, a bacterial protein involved in motility and *TLR 9* recognizes CpG, a repetitive sequence of unmethylated nucleic acids (guanine and cytosine) [4]. Growing amounts of data suggest that single nucleotide polymorphisms (SNPs) on the various human *TLR* proteins are associated with altered susceptibility to infection. *TLR-2*, as a heterodimer with *TLR-1* or *TLR-6*, two synonymous SNPs on *TLR 2* gene have been associated with asymptomatic bancroftian filariasis [5]. A-196 to -173 deletions polymorphisms in the 5' untranslated region of *TLR2* gene and two synonymous SNPs, 597C>T (rs3804099, Asn199ASN) and 1350C>T (rs3804100, Ser450Ser) in exon 3 are associated with asymptomatic bancroftian filariasis [5]. However other studies have indicated that *TLR 2* polymorphisms do not correlate with lymphatic filariasis infection status or disease phenotype of lymphatic filariasis [6]. Since there is limited data regarding *TLR 2* mutations, additional genotyping studies on *TLR 2* and other candidate genes involved in susceptibility to lymphatic filariasis will be required to determine their contribution to the heterogeneous pattern of infection and filarial disease. Therefore, the aim of this objective is to determine the role of deletion polymorphisms in *TLR 2* gene and their potential association to filarial infection. Exploring the role of the *TLR 2* polymorphisms is important to the understanding of the pathogenesis and protection against filarial infection.

MATERIALS AND METHODS

Study site

Tanga region lies between longitude of 35 E and latitude of 2.75. It consists of different ethnic groups and large minority being Wazigua. The livestock species kept are primarily cattle, goats, sheep and donkey. The area has two rainy seasons: the short rainy season and the long rain season with annual precipitation between 500 and 1,000mm. The study was carried out in five villages of Tanga District located in North eastern Tanzania an area known to be endemic for bancroftian filariasis. The villages included Tanga city, Pangani, Muheza and Mkinga.

Study design

The study involved the samples collected from previously cross sectional survey conducted in September 2016 in the study villages (Figure 1). Samples were collected from lymphatic filariasis (lymphoedema, hydrocele, CFA positives, 18-74 years old) patients resides in endemic communities of Tanga region.

Figure 1 Map of Tanga showing the location of study villages.

Blood collection

A total of 10 mls of EDTA monovette of blood were collected from each volunteer; plasma was separated and stored at -80°C. The blood pellets were mixed 1:1 with 8M Urea and transport to laboratory.

DNA extraction

Genomic DNA was extracted from peripheral blood preserved in an equal volume of 8M urea from the patients using the QIAmp® kit from Qiagen (Hilden, Germany) as instructed by the manufacturer.

Plasmids preparation.

E. coli bacteria were grown on 15 mls of Lysogen Broth medium with appropriate temperature of 37°C overnight. Lysogen Broth medium allows bacteria that have been successful transformed to grow uninhibited. Overnight culture was then transferred to 1.5 mls Eppendorf tube and spin down the cell culture at high speed for 1 minute, 250 mls of lysis buffer solution were added and mix gently by inverting the tube 6-8 times followed by 250 mls of neutralizing buffer solution. Bacteria precipitates were observed around the tube. Centrifugation were done at 13000 rpm for 10 minutes then the supernatant was transferred to 1.5 mls Eppendorf tube and plasmid DNA pellets were resuspended by TE buffer.

DNA concentration measurements

The concentration of DNA was measured using the spectrophotometer by measuring the absorbance at 260 nm where DNA absorbs light mostly strong and the number generated allowed to estimate the concentration of the solution. The copy number of the standard were calculated using the following mathematical correlation formulas as a guideline

For average molecular weight of	Calculation
ds DNA	(number of base pairs) x (660 daltons/base pairs)
ss DNA	(number of base pairs) x (330 daltons/base pairs)
ss RNA	(number of base pairs) x (340 daltons/base pairs)

MW=Molecular weight (g/mol)

1mol=6x10^23 molecules (=copies)

6x10^23 (copies/mol) x concentration (g/mol) = amount (copies/µl)

MW(g/mol)

Real time Polymerase chain reaction

The molecular detection of the of the TLR 2 -196 to -173 del polymorphism was performed using the RT-Polymerase chain reaction system (Rotor gene 6000 Real Time PCR system). The primers used were, TLR – 2 Forward: (5'- cgg agg cag cga gaa a-3') and TLR – 2 Reverse: (5'- ctg ggc cgt gca aag aag-3') and the TaqMan deletion probe (5'-agc cag gtg act gc- 3') and wild type probe (5'- acg ccg agc agc cg-3') targeting 23 base pair fragment of the TLR 2 In/del. The results of the assay were assessed by the Ct value.

Polymerase chain reaction parameters

The polymerase chain reaction (PCR) was carried as follows. The 20 µl reaction mixture contained 2 µl of 50 ng of template DNA, 10 µl of Quantinova master mix, 0.6 µl of forward and reverse primer respectively, 0.2 µl of wild type probe and deletion probe respectively, 6.6 µl of deionized water. The amplification was carried out using the Rotor gene 6000 Real Time PCR system. The amplification profile consisted of: 95°C for 2 minutes; followed by 45 cycles of 95 °C for 5 seconds and 58°C for 30 seconds. The amplification of all DNA samples was repeated three times in order to see variability, if any in the amplification pattern.

Statistical analysis

FamHap software was used to carry the statistical analysis. The goodness of fit to Hardy–Weinberg Equilibrium (HWE) was performed using a chi-square ($\chi 2$) test. Determination of allele and genotype were determined by direct counting and then divided by the number of chromosomes to produce an allele frequency or by the number of subjects to produce the genotype frequency. Allele or genotype frequencies were compared between bancroftian filariasis patients, lymphedema, hydrocele and normal controls to ascertain the association. *P*-values were considered significant at ≤ 0.05.

RESULTS

The molecular detection by real time PCR indicated that 36.7 % of the samples were positive for TLR 2 -196 to – 173 del genotype Figure 2.

polymorphisms	Lymphoedema (n=26)	Hydrocele (n=28)	Genotypic model	OR	P-Armitage value
–196 to –173 del					
wt/wt	17	16	0.561	0.818	0.622
wt/del	0	1			
del/del	9	11			

DISCUSSION

This study has demonstrated the evidence of TLR 2 -196 to -173 polymorphism in the 5' untranslated region in individuals reside in the endemic communities of Tanga region, North Eastern Tanzania. The study was carried 14 years after the implementation of the mass drug administration using the drug combination of ivermectin and albendazole with the aim of interrupting the bancroftian filariasis infection. During the course of the study, the cross sectional survey findings demonstrated a considerable reduction in filarial infection [8].

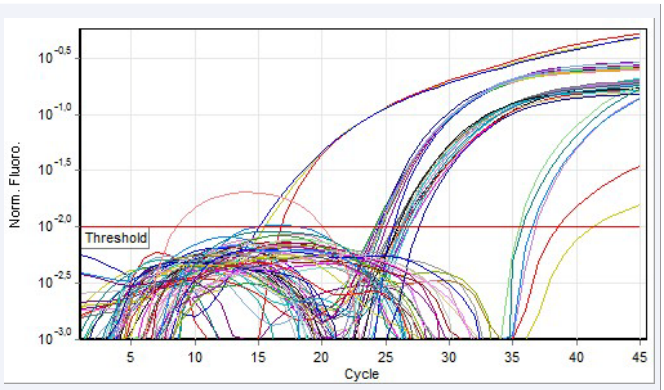

Figure 2 Quantitation data showing RT-PCR products of TLR 2 -196 to -173 del.

The TLR 2 haplotype -196 to -173 del/ +597C/+1350C have been reported to strongly associated with an increased risk of asymptomatic bancroftian filariasis in Thailand [9]. No previous reports of association have been reported in the area. A case control study conduted in Ghana revealed the strong association of TLR 2 -196 to -173 del with asymptomatic bancroftian filariasis. (jubin et al., 2017 unpublished data). However, the same investigation could not detect the association in individual tested in Tanga region. Due to limited resources only a subset of positive samples was subjected to RT-PCR analysis and this is considered to be limitation of this study. Other studies have demonstrated the role of polymorphisms in various host genes and susceptibility and clinical manifestation of lymphatic filariasis such as Vascular Endothelial Growth Factors and Mannose-binding lectin [9]. However little is known regarding the pathogenesis of the disease and this calls for more research in the area for better understanding of the disease, considering the fact that there are potential mosquito vectors in the area, the risk of disease emergence is still high.

CONCLUSION

TLR 2 -196 to -173 del occurrence among individuals infected with bancroftian filariasis disease highlights the importance of further research for better understanding the mechanism of infection transmission and heterogeneity of the disease.

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Table 1: Genotype frequencies of TLR2 -196 to – 173 del in Lymphedema and Hydrocele.

Polymorphisms	bancroftian filariasis patients (n=6)	Lymphoedema (n=26)	Genotypic model	OR	P-Armitage value
–196 to –173 del					
wt/wt	4	17	0.998	0.944	0.952
wt/del	0	0			
del/del	2	9			

Table 2: Genotype frequencies of TLR2 -196 to – 173 del in bancroftian filariasis patients and Lymphedema.

Polymorphisms	Bancroftian filariasis patients	Normal controls	Genotypic model	OR	P-Armitage value
–196 to –173 del					
wt/wt	4	9	0.907	0.643	0.658
wt/del	0	0			
del/del	2	7			

Table 3: Genotype frequencies of TLR 2 -196 to – 173 del in bancroftian filariasis patients and endemic normal.

Polymorphisms	Bancroftian filariasis patients	Hydrocele	Genotypic model	OR	P-Armitage value
–196 to –173 del					
wt/wt	4	16	0.845	0.773	0.721
wt/del	0	1			
del/del	2	11			

Table 4: Genotype frequencies of TLR2 -196 to – 173 del in bancroftian filariasis patients andhydrocele.

Polymorphisms	Lymphoedema (n=26)	Hydrocele (n=28)	Genotypic model	OR	P-Armitage value
–196 to –173 del					
wt/wt	17	16	0.561	1.202	0.622
wt/del	0	1			
del/del	9	11			

Table 5: Genotype frequencies of TLR2 -196 to – 173 del in Lymphoedema andhydrocele.

Polymorphisms	Endemic normal (n=16)	Lymphoedema (n=26)	Genotypic model	OR (95%CI)	P-Armitage value
–196 to –173 del					
wt/wt	9	17	0.839	1.469	0.554
wt/del	0	0			
del/del	7	9			

REFERENCES

1. Ristimäki A, Narko K, Enholm B, Joukov V, Alitalo K. "Proinflammatory cytokines regulate expression of the lymphatic endothelial mitogen vascular endothelial growth factor-C. J Biol Chem. 1998; 273: 8413–8418.

2. Rao UR, Zometa CS, Vickery AC, Kwa BH, Nayar JK, Sutton ET. "Effect of Brugia malayi on the growth and proliferation of endothelial cells in vitro. J Parasitol. 1996; 82: 550–556.

3. Pfarr KM, Debrah AY, Specht S, Hoerauf A. "Filariasis and lymphoedema. Parasite Immunology. 2009; 31: 664–672.

4. Hise A, Hazlett F, Bockarie M, Zimmerman P, Tisch D, Kazura J. "Polymorphisms of innate immunity genes and susceptibility to lymphatic filariasis. Genes Immun. 2003.

5. Skevaki C, Pararas M, Kostelidou K, Tsakris A, Routsias JG. "Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious diseases. Clin Exp Immunol. 2015.

6. Trejo-De La OA, Hernández-Sancén P, Maldonado-Bernal C. "Relevance of single-nucleotide polymorphisms in human TLR genes to infectious and inflammatory diseases and cancer. Genes Immun. 2014; 15: 199-209.

7. Batsa L. "Anti-Wolbachia Treatment of Lymphatic Filariasis and

Genetic Analysis of the Pathology of Lymphedema as a Clinical Manifestation of the Disease. 2012.

8. Shana HJ, Baraka V, Misinzo G, Makunde WH. "Current Epidemiological Assessment of Bancroftian Filariasis in Tanga Region , Northeastern Tanzania. J Trop Med. 2016; 1–6.

9. Nuchprayoon S, Junpee A, Poovorawan Y. "Random Amplified Polymorphic DNA (RAPD) for differentiation between Thai and Myanmar strains of Wuchereria bancrofti. Filaria J. 2007; 6: 6.

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