An association of VNTR polymorphism in intron3 of IL-4 gene with susceptibility to typhoid fever in Khartoum State, Sudan

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Abstract

Host genetic factors play a role in determining susceptibility to infectious diseases of humans. Polymorphisms in Interleukin 4 (IL-4), an anti-inflammatory cytokine that regulates the balance between T helper (Th1) and Th2 immune responses, has been reported to affect the risk of infectious and autoimmune diseases.

In this study we aimed to investigate the possible association of variable number of tandem repeat (VNTR) polymorphism in intron3 of IL-4 with susceptibility to typhoid fever in Sudanese patients. We analyzed the 70 bp intron-3 VNTR repeat polymorphism in 200 DNA samples obtained from patients with blood culture-confirmed typhoid fever and 200 samples from randomly selected healthy controls from the same city area. The intronic VNTR polymorphism was genotyped using PCR, followed by agarose gel electrophoresis. The result showed that the genotypes of the target VNTR polymorphism in both cases and controls were not deviated from Hardy-Weinberg equilibrium ($X^2, df=3=6.468, P>0.05$ and $X^2, df=1=0.07, P>0.5$ respectively). The observed difference of the R3R3 & R2R2 genotypes among cases and controls is not statistically significant ($P=0.09$ and $P=0.46$ respectively). However, a significant increase in the 2R3R genotype frequency among cases compared to the controls was observed ($P<0.05$), indicating that the heterozygote 2R3R of VNTR polymorphism of IL4 might affect individual susceptibility to typhoid fever in our population.

Keywords: Gene polymorphism; Khartoum; Sudan; typhoid fever; VNTR, IL-4 gene.

1. Introduction

Typhoid fever is a serious public health problem worldwide, especially in the developing countries. The disease is caused by Gram negative bacterium, Salmonella enterica serovar typhi (S. typhi). Globally, the number of cases exceeded $21 \times 10^6$ with more
than $2 \times 10^5$ deaths annually (Crump et al., 2004). In Sudan, the incidence of typhoid fever is 5 cases per 1000 population per year. Generally, contaminated water and food are important means for transmission of typhoid fever. In addition, poor housing, lack of latrines and personal hygiene are the risk factors of acquiring the disease. However, the outcome of the disease in our population is variable among subjects living in the same area, designated that our genetic makeup determines the different ways that we respond to the same infectious agents.

IL4, a pleotropic cytokine encoded by a gene located on the long arm of chromosome 5, is considered crucial for the development of Th2 responses, as it regulates the differentiation of precursor T helper cells into those of the Th2 subset that mediate humoral immunity and modulate antibody production (Romagnani, 1995). The expansion of IL4 producing Th2 cells was known to be correlated with susceptibility to parasitic infection and thereby plays a critical role in disease progression. More support for the role of IL4 in diseases’ outcome was demonstrated by anti-IL-4 antibody studies (Sadick et al., 1990; Uzonna & Bretscher, 2001) and from the susceptibility of IL-4 transgenic mice of resistant background (Leal et al., 1993). Functional promoter polymorphisms in this gene have been reported to favor a reduced protection against the majority of infectious agents such as Plasmodium sp. (Luzia et al., 2002; Verra et al., 2004), human immune deficiency virus (Nakayama et al., 2000), Mycobacterium tuberculosis (Vidyarani et al., 2006) and autoimmune disease (Cantagrel, et al., 1999) and asthma (Gervaziev et al., 2006). In Sudanese population, evidence for linkage of IL4 and IL9 polymorphisms and susceptibility to visceral leishmaniasis (VL) was reported (Mohamed et al., 2003).

Human case-control studies have documented that the genetic makeup may predispose individuals to acquisition of typhoid fever or development of severe disease (Dunstan et al., 2001; Ali et al., 2006, 2007). Therefore, we undertook the present study to determine, for the first time, whether or not the polymorphism in intron3 of IL4 contributes to susceptibility to typhoid fever in our population.

2. Materials and methods

2.1 Study area

The study has been conducted in Khartoum State, the national capital of Sudan. It was selected as the study area because the continued migration of villagers to this city making living conditions more and more crowded and food often prepared and distributed under unhygienic conditions. These conditions explain the possibility of typhoid fever infections. However, not all people exposed to such conditions get the disease, which indicates a possible genetic role in the response to typhoid disease.
2.2 Sample collection and DNA extraction

Diagnosis of typhoid was made on the basis of clinical symptoms and on positive blood culture.

Three ml of blood samples from patients (n=200) were collected with the collaboration of different Hospitals in Khartoum State and from randomly selected community controls (n=200). Case and control individuals were unrelated citizens and were matched for age, sex and for their residential location. Informed consent was obtained from all participants.

Genomic DNA was isolated from peripheral blood using Quick-gDNA™ MiniPrep DNA extraction kit. The quantity and quality of DNA was estimated by gel electrophoresis and spectroscopy.

2.3 Genotyping of \( IL4 \) intron3 variable number of tandem repeats (VNTR) polymorphism

Polymorphic site located in intron3 region contains a 70-bp length of VNTRs of IL-4 gene. The allelic form consists of three 70 base-pair (bp) repeats (3R=253bp) (Mout \textit{et al.} (1991)); allele with two repeats (2R=183bp) and much rarer allele with four repeats (4R) or one repeat (R1=113). Forward primer 5’-TAGGCTGAAGGGGGGAAGC-3’ and reverse primer5’-CTGTTCACCTCAACTGCTCC-3’; described by Mout \textit{et al.} (1991); was used for PCR amplification. 25-μL PCR reaction mix containing 20 pmol forward and reverse primers, 0.4 mM each dNTP, 2 mM MgCl\(_2\), 1X Taq buffer, 1 U NEB Taq DNA polymerase (New England Biolabs, Beverly, MA, USA) and 1 μL of 10 ng/μL of DNA template. The initial denaturation was set up for 5 min at 94°C followed by 38 cycles of denaturation for 1 min at 94°C, annealing for 30 s at 60°C, and extension for 1 min at 72°C and final extension for 5 min at 72°C. The amplified PCR product was run in 2% agarose gel stained with ethidium bromide, visualized and photographed by gel documentation system.

2.4 Statistical analysis

The chi-square test was used to determine whether the obtained genotypes of both cases and controls are in Hardy–Weinberg equilibrium. SPSS statistical program was used to test if there is a significant difference in the allele frequency of \( IL4 \) VNTR variants in cases compared to controls. Fisher exact test was used, where expected frequency was less than 5.

3. Result

Our result showed that, in addition to the two alleles 3R and 2R; a much rarer allele 1R has been observed in one of the typhoid patient (Figure 1, Table 1). All the \( IL-4 \) genotype frequencies in both typhoid cases and controls were in accordance
with Hardy–Weinberg equilibrium ($X^2$, $df=3=6.468$, $P>0.05$ and $X^2$, $df=1=0.07$, $P>0.5$ respectively. Among the four genotypes of VNTR in intron3 of $IL-4$; the 3R2R heterozygous was the most common genotype (Table 1) and is significantly higher in patients than controls ($P<0.05$), while 3R1R genotype was found to be the rarest genotype, as it was observed in one case subject only in proportion of 0.5%. The other homozygote genotypes: R3R3 & R2R2 (Figure1) are not much different in the frequencies between the two groups. In addition the frequencies of all allele:3R, 2R and 1R did not differ in patients compared to controls (Table 1).

Table 1. The genotypic and allelic frequencies of VNTR polymorphism in intron3 of $IL-4$ in cases of typhoid fever and randomly selected community controls

<table>
<thead>
<tr>
<th>Genotype of VNTR</th>
<th>Cases N=200 (%)</th>
<th>Control N=200 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R/3R</td>
<td>63 (0.32)</td>
<td>79 (0.39)</td>
<td>$^aP = 0.09$ NS</td>
</tr>
<tr>
<td>3R/2R</td>
<td>112 (0.56)</td>
<td>92 (0.46)</td>
<td>$^aP = 0.045$</td>
</tr>
<tr>
<td>2R/2R</td>
<td>24 (0.12)</td>
<td>29 (0.15)</td>
<td>$^aP = 0.46$ NS</td>
</tr>
<tr>
<td>3R/1R</td>
<td>1 (0.005)</td>
<td>0</td>
<td>$^bP=0.5$ NS</td>
</tr>
<tr>
<td>3R</td>
<td>119.5 (59.75%)</td>
<td>125 (62.5%)</td>
<td>NS$^a$</td>
</tr>
<tr>
<td>2R</td>
<td>80 (40%)</td>
<td>75 (37.5%)</td>
<td>NS$^a$</td>
</tr>
<tr>
<td>1R</td>
<td>1 (0.25%)</td>
<td>0 (0%)</td>
<td>NS$^b$</td>
</tr>
</tbody>
</table>

$^a$P-values were calculated by $\chi^2$ tests.

$^b$P-values were calculated by Fisher’s exact test, It applied when observed frequency was <5.

Fig. 1. Detection of the VNTR gene polymorphism of the IL-4 gene on 2% agarose gel electrophoresis. M = 50-bp marker. Lanes 1, 6, 8, 9, and 11 shows the 3R allele (253 bp). Lanes 4, 7, 10, 12, 13 and 16 shows the 3R2R (253 and 183bp) genotypes. Lanes 2, 3 and 14 shows the 2R2R genotype (183 bp). Lane 5 shows the 3R1R (253 and 113bp) genotypes.
4. Discussion

To date, several case-control studies in different ethnic groups have been carried out to investigate the association between genes` polymorphisms and the risk of several infectious diseases (Roy et al., 1997; Wattavidanage et al., 1999; Miller et al., 2007; Fadl et al., 2011) including S. typhi infection (Dunstan et al., 2001; Dunstan et al., 2007; Dharmana et al., 2002, Ali et al., 2006; Hue et al., 2009). However, results from different articles remain controversial. Genetic association has been identified between IL-4 polymorphisms and diseases caused by the intra-macrophage pathogens, such as pulmonary tuberculosis (Vidyarani et al., 2006) and visceral leishmaniasis (Mohamed et al., 2003). In this study, we tested the hypothesis that the intronic VNTR polymorphism of IL4 would participate in predisposing individuals to acquire typhoid disease in our population.

Despite that the sample size is a limitation in this study, our finding revealed that the distribution of VNTR genotypes in both cases and controls was consistent with the Hardy-Weinberg equilibrium and of the three genotypes observed among cases and controls, only the 2R3R genotype revealed an association with typhoid susceptibility (P= 0.045). This result suggests that 2R3R heterozygote for the intronic VNTR of IL4 might be a possible genetic risk factor for typhoid disease predisposition and this is consistent with the previous studies that demonstrated the genetic role in typhoid susceptibility in different population from the East (Dunstan et al., 2007; Dharmana et al., 2002; Ali et al., 2006; Hue et al., 2009).

5. Conclusion

In this study we demonstrated the association of IL4 VNTR polymorphism with typhoid fever predisposition. Due to the limitations of the sample size in this study, and as the typhoid disease might be an oligogenic disease, further investigations with IL4 VNTR polymorphism and other cytokine genes` polymorphisms are needed to find a more conclusive association between genes` polymorphisms and typhoid risk in our population.

6. Acknowledgment

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References


Leal, L.M., Moss, D.W., Kuhn, R., Muller, W. & Liew, F.Y. (1993) Interleukin-4 transgenic mice of resistant background are susceptible to Leishmania major infection. European Journal of Immunology, 23:566-569.

Luzia, H., Carvalho, Sano, G., Julius, C.R., Hafalla, Morrot, A., Maria A., Curotto de La FAILLE & Zavala, F. (2002) IL-4-secreting CD4+ T cells are crucial to the development of CD8+ T-cell responses against malaria liver stages. Nature Medicine, 8:166-70.


leishmaniasis in the Sudan: linkage and association with IL4 and IFNGR1. Genes and Immunity, 4:351-355.


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في VNTR من الجينات جمعية لتعداد ترابط الأشكال المتعددة من IL-4 مع قابلية التعرض للحمى التيفوئيد في ولاية الخرطوم، السودان من جين VNTR من قبل فضل، 1، مودة عيدروس، 2، كانغوان ماو، 3، أفشنان باسمين.

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خلاصة

تلعب العوامل الوراثية للمضيف دوراً في تحديد قابلية الإصابة بالأمراض المعدية للبشر. إن تراكيم عامل خلوي مضاد للالتهابات وينظم التوازن بين TH1 و TH2 (انترولوكين 1) عامل خلوي مضاد للالتهابات وتوزع وينظم التوازن بين TH1 و TH2 (انترولوكين 4) (IL-4) لتأثیر على احتمال الإصابة بالأمراض المعدية والمناعة الذاتية.

كما نهدف في هذه الدراسة للتحقيق من علاقة محتملة بين العدد المتغير من تكرار VNTR وعدد أشكالها الموصولة في VNTR من جين IL-4 من قبل قابلية التعرض للحمى التيفوئيد في المنطقة المعدية للبشر. ومن خلال تحليل 70 ازواج تكرار من تكرار VNTR في إنترولوكين 3 في VNTR من 200 مع قابلية التعرض للحمى التيفوئيد في المنطقة المعدية للبشر.

تم تأكيدها من خلال استزاع الدم ومن 200 عينة أخرى من لاصحاء تم اختيارهم عشوائياً من VNTR لنتائج الدراسة المستقلة في الدراسة المستقلة.

ANOVA (intrinsic) باستخدام لأسلاك الدنا المعدلة PCR ولفصل الكهربائي بالاغراز. وأظهرت النتيجة أن الأناضات الجينية للسلسل المستهدف في VNTR في الأصبع والصابان لم تترحف عن توزن Hاردي وليبرغ (0.5، 0.07، P > 0.05). على التوالي. على التوالي. على التوالي. على التوالي. على التوالي.

الفرق الملحوظ بين الأناضات الجينية R2R2 و R3R2 بين الحالات المرضية وبين المجموعة الضابطة ليست ذات دلالة إحصائية (P = 0.46) و P = 0.09. ومع ذلك، لوحظ وجود زيادة كبيرة في وثيقة التركيب الوراثي بين الحالات المرضية والمت濯ة بالكثير من الضابطة (P < 0.05)، قد يؤثیر على قابلية الفرد للإصابة بحمى التيفوئيد في VNTR من IL-4.

كلمات البحث: النماذج الجينية المتعددة، الخرطوم; السودان. حمي التيفوئيد. IL-4.