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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 $21x10^6$ with more

than $2x10^5$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 lieshmaniasis (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 *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' -TAGGCTGAAAGGGGGAAAGC-3'and reverse primer5'-CTGTTCACCTCAACTGCTCC-3'; described by Mout *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

Genotype of VNTR	Cases N=200 (%)	Control N=200 (%)	P-value
3R/3R	63 (0.32)	79(0.39)	[a]*P* = 0.09 NS
3R/2R	112 (0.56)	92 (0.46)	[a]*P* = 0.045
2R/2R	24 (0.12)	29 (0.15)	[a]*P* = 0.46 NS
3R/1R	1 (0.005)	0	[b]*P*=0.5 NS
3R	119.5 (59.75%)	125 (62.5%)	NS [a]
2R	80 (40%)	75 (37.5%)	NS [a]
1R	1 (0.25%)	0 (0%)	NS [b]

[a]*P*-values were calculated by χ^2 tests.
[b]*P*-values were calculated by Fisher's exact test, It applied when observed frequency was <5.

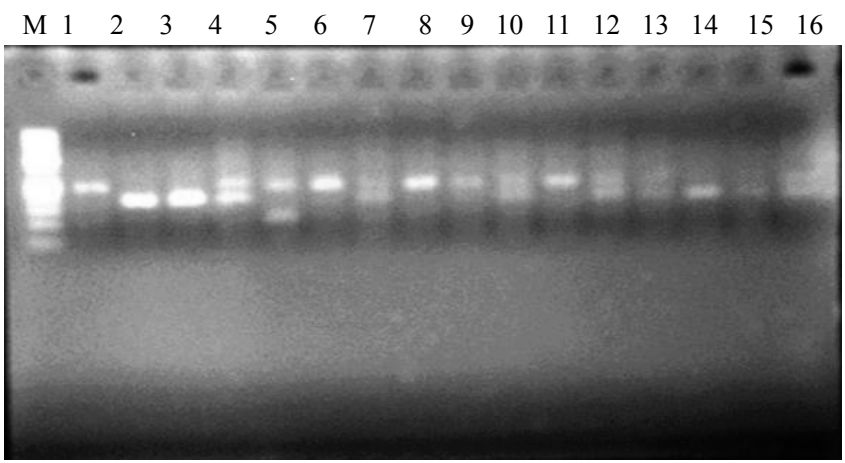

Fig. 1. Detection of the VNTR gene polymorphism of the IL-4 gene on 2% agarose gel electrophoresis. M = 50-bp marker. Lanes 1, 6, 8, 9, and 11 shows the 3R allele (253 bp). Lanes 4, 7, 10, 12, 13 and 16 shows the 3R2R (253 and 183bp) genotypes. Lanes 2, 3 and 14 shows the 2R2R genotype (183 bp). Lane 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 lieshmaniases (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 the2R3R 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.

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References

Ali, S., Vollaard, A.M., Widjaja, S., Surjadi, C., van de Vosse, E. & van Dissel J.T. (2006) PARK 2 /PACRG polymorphisms and susceptibility to typhoid and paratyphoid fever. Clinical and Experimental Immunology, **144**:425-431.

Ali, S., Vollaard, A.M., Kremer, D., de Visser, A.W., Martina, C.A., Widjaja, S., Surjadi, C., Slagboom, E., van de Vosse, E. & van Dissel, J.T. (2007) Polymorphisms in proinflammatory genes and susceptibility to typhoid fever and paratyphoid fever, **27**(4):271-279.

Cantagrel, A., Navaux, F., Loubet-Lescoulié, P., Nourhashemi, F., Enault, G., Abbal, M., Constantin, A., Laroche, M., Mazières, B. (1999) Interleukin-1beta, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: relationship to occurrence and severity of rheumatoid arthritis. Arthritis Rheumatology, **42**:1093-1100.

Crump, J.A., Luby, S.P. & Mintz, E.D. (2004) The global burden of typhoid fever. Bull World Health Organization, **82**:346-353.

Dharmana, E., Joosten, I., Tijssen, H.J., Gasem, M.H., Indarwidayati, R., Keuter, M., Dolmans, W.M., Van Der Meer, J.W. (2002) HLA-DRB1*12 is associated with protection against complicated typhoid fever, independent of tumour necrosis factor alpha. European Journal of Immunogenetics, **29**:297-300.

Dunstan, S.J., Stephens, H.A., Blackwell, J.M., Duc, C.M., Lanh, M.N., Dudbridge, F., Phuong, C.X., Luxemburger, C., Wain, J., Ho, V.A., Hien, T.T., Farrar, J. & Dougan, G. (2001) Genes of the class II and class III major histocompatibility complex are associated with typhoid fever in Vietnam. Journal of Infectious Diseases, **183**:261-268.

Dunstan, S.J., Nguyen, T.H., Rockett, K., Forton, J., Morris, A.P., Diakite, M., Mai, N.L., Le, T.P., House, D., Parry, C.M., Ha, V., Nguyen, T.H., Dougan, G., Tran, T.H., Kwiatowski, D. & Farrar, J.J. (2007) A TNF region haplotype offers protection from typhoid fever in Vietnamese patients. Human Genetics, **122**(1):51-61.

Fadl, M.A., Miller, N., Mohamed, H., Elhassan, E., Ibrahim, M.E. & Blackwell, J.M. (2011) 5q34-35: One of the visceral leishmaniasis susceptibility loci demonstrated by a Genome wide search in Sudanese population. Sudan Medical Monitor, **6**(3):253-257.

Gervaziev, Y.V., Kaznacheev, V.A. & Gervazieva, V.B. (2006) Allelic polymorphisms in the interleukin-4 promoter regions and their association with bronchial asthma among the Russian population, **141**(3):257-264.

Hue, N.T., Lanh, M.N., Phuong, L.T., Ha Vinh, Chinh, N.T., Hien, T.T., Hieu, N.T., Farrar, J.J., Dunstan, S.J. (2009) Toll-Like Receptor 4 (TLR4) and Typhoid Fever in Vietnam. Plos One, **4**(3):e4800.

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 Lafaille & 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.

Miller, N.E., Fadl, M.A., Mohamed, H.S., Elzein, A., Jamieson, S., Cordell, H.J., Peacock, C.S., Fakiola, M., Raju, M., Khalil, E.A., Elhassan, A., Musa, A.M., Ibrahim, M.E. & Blackwell, J. M. (2007) Y chromosome lineage- and village-specific genes on chromosomes 1p22 and 6q27 that control visceral leishmaniasis in The Sudan. PLoS Genetics, **3**(5):679-688.

Mohamed, H.S., Ibrahim, M.E., Miller, E.N., Peacock, C.S., Khalil, E.A., Cordell, H.J., Howson, J.M., El Hassan, A.M., Bereir, R.E. & Blackwell, J.M. (2003) Genetic susceptibility to visceral

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

Mout, R., Willemze, R. & Landegent, J.E. (1991) Repeat polymorphisms in the interleukin-4 gene (IL4). Nucleic Acids Research, **19**(13):3763.

Nakayama, E.E., Hoshino, Y., Xin, X., Liu, H., Goto, M. & Watanabe, N. (2000) Polymorphism in the interleukin-4 promoter affects acquisition of human immunodeficiency virus type 1 syncytium-inducing phenotype. Journal of Virology, **74**(12):5452-5459.

Romagnani, S. (1995) Biology of human TH1 and TH2 cells. Journal of Clinical Immunology, **15**(3):121-129.

Roy, S., McGuire, W., Mascie-Taylor, C.G., Saha, B., Hazra, S.K., Hill, A.V. & Kwiatkowski, D. (1997) Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy. The Journal of Infectious Diseases, **176**(2):530-532.

Sadick, M.D., Heinzel, F.P., Holaday, B.J., Pu, R.T., Dawkins, R.S. & Locksley, R.M. (1990) Cure of murine leishmaniasis with anti-interleukin 4 monoclonal antibody. Evidence for a T cell-dependent, interferon gamma-independent mechanism. Journal of Experimental Medicine, **171**:115-127.

Uzonna, J.E. & Bretscher, P.A. (2001) Anti-IL-4 antibody therapy causes regression of chronic lesions caused by medium-dose Leishmania major infection in BALB/c mice. European Journal of Immunology, **31**:3175-184.

Verra, F., Luoni, G., Calissano, C., Troye-Blomberg, M., Perlmann, P., Perlmann, H., Arca, B., Sirima, B.S., Konate, A., Coluzzi, M., Kwiatkowski, D. & Modiano, D. (2004) *IL4*-589C/T polymorphism and IgE levels in severe malaria. Acta Tropica, **90**(2):205-209.

Vidyarani, M., Selvaraj, P., Prabhu, S., Jawahar, M.S., Adhilakshmi, A.R. & Narayanan, P.R. (2006) Interferon gamma (IFNγ) & interleukin-4 (IL-4) gene variants & cytokine levels in pulmonary tuberculosis. Indian Journal of Medical Researches, **124**:403-410.

Wattavidanage, J., Carter, R., Perera, K.L., Munasingha, A., Bandara, S., McGuinness, D., Wickramasinghe, A.R., Alles, H.K., Mendis, K.N. & Premawansa, S. (1999) TNFalpha*2 marks high risk of severe disease during Plasmodium falciparum malaria and other infections in Sri Lankans. Clinical and Experimental Immunology, **115**(2):350-355.

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من الجينات جمعية لتعدد ترابط الأشكال المتعددة من VNTR في intron3 من جين IL-4 مع قابلية التعرض للحمى التيفوئيد في ولاية الخرطوم، السودان

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خلاصة

تلعب العوامل الوراثية للمضيف دورا في تحديد قابليته للإصابة بالأمراض المعدية للبشر. انترلوكين عامل خلوى مضاد للالتهابات وينظم التوازن بين T المساعد (TH1) و TH2 خلال الاستجابات المناعية. وقد سبق الإبلاغ عن أشكال من انترلوكين 4 (IL-4) لها تأثيرعلى احتمال الإصابة بالأمراض المعدية والمناعة الذاتية.

كنا نهدف في هذه الدراسة للتحقيق من علاقة محتملة بين العدد المتغير من تكرار (VNTR) وتعدد أشكالها المرصودة في intron3 من IL-4 مع قابلية التعرض لحمى التيفوئيد في المرضى السودانيين. قمنا بتحليل 70 ازواج قاعدة من تكرار (VNTR) في إنترون 3- VNTR في 200 عينة من الحمض النووي التي تم الحصول عليها من المرضى الذين يعانون من حمى التيفوئيد التي تم تأكيدها من خلال استزراع الدم ومن 200 عينة اخرى من لاصحاء تم اختيارهم عشوائيا من منطقة المدينة نفسها. تم تحديد الأشكال النطية الجينية الانترونية (intronic) لتتابعات ال VNTR باستخدام تقنية بلمرة الدنا المسلسل PCR والفصل الكهربائي بالاغاروز. وأظهرت النتيجة أن الانماط الجينية للتسلسل المستهدف في VNTR في الاصحاء والمصابين لم تنحرف عن توازن هاردي واينبرغ، ($X^2_{,\ DF=3} = 6.468, P > 0.05$ and $X^2_{,\ DF=1} = 0.07, P > 0.5$)على التوالي.

الفرق الملحوظ بين الأنماط الجينية R3R3 و R2R2 بين الحالات المرضية وبين المجموعة الضابطة ليست ذات دلالة إحصائية ($P = 0.09$ و $P = 0.46$ على التوالي). ومع ذلك، لوحظ وجود زيادة كبيرة في وتيرة التركيب الوراثي 2R3R بين الحالات المرضية بالمقارنة مع الضابطة ($P<0.05$)، مشيرا إلى احتمال أن شكل R3R2 من VNTR في IL4 قد يؤثر على قابلية الفرد للاصابة بحمى التيفوئيد بين سكان السودان.

كلمات البحث: النماط الجينية النتعددة، الخرطوم؛ السودان. حمى التيفوئيد. VNTR، جين IL-4 .