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IL1β exon5 3954 C/T Polymorphism: A potential Risk factor for the Susceptibility to Rheumatoid Arthritis in Sudanese Patients

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease, usually causes progressive destruction of the joints. The genetic information is important for understanding the mechanisms of the disease. Cytokines are considered to play an important role in the pathogenesis of RA, as the imbalance between pro- and anti-inflammatory cytokines was known to promote the induction of autoimmunity, inflammation and joint destruction. This study aimed to demonstrate "for the first time" a possible association of the 3954 C/T polymorphism (rs1143634) in exon 5 of IL-1β and the RA predisposition in Sudanese patients. A cross sectional association study was conducted, involving 84 unrelated RA patients and 93 healthy controls. The IL-1β (rs1143634) polymorphism was detected by PCR followed by RFLP technique using *Taq*I restriction enzyme. The result showed that 83.3% of the examined RA patients are females with high significant difference between cases and controls and five folds increased risk of female than males (P=0.001, OR=5.33, CI=2.64-10.77). 48.8% of the cases are at age less than 35 years. Family history of RA was reported in 26% of the cases, with no significant difference between cases and controls (p=0.07). None of the participants was smoker, 100% of the cases live sedentary live. The genotype analysis of IL1β exon5 3954 C/T (rs1143634) polymorphism showed a significant association with RA (P=0.001). In addition, the analysis considering the mutant genotypes CT+TT versus the wild genotype CC also suggests a high significant association of IL1β exon5 polymorphism with the risk of RA susceptibility in our patients and it increase peoples' risk 2.6 folds to RA predisposition (P=0.001, OR=2.64, CI= 1.38-5.03). Carriage of the rare mutant IL-1 β (+3954) allele T was higher in arthritis patients as compared to the controls, however, the difference was insignificant (P=0.75). 68% of the RA patients showed articular manifestation, of which 70.6% have the mutant T allele. The result of this study, for the first time in Sudan, suggest that the 3954 C/T IL1 β (rs1143634) polymorphism is associated with the risk of RA, and the carriage of the mutant allele might be a predictive factor for the onset of clinical manifestation of rheumatoid arthritis in Sudanese patients.

Keyword: Rheumatoid arthritis, risk factors, Gene polymorphisms, Interleukin (IL)-1 β.

Introduction

Rheumatoid arthritis (RA) is a chronic, complex and heterogeneous autoimmune disease, characterized by persistent chronic inflammation of the synovial joints which may lead to progressive joint destruction and disability. RA is estimated to affect approximately 1% of the world's population (Gibofsky, 2012) and the associated mortality rates are higher among RA patients than in the general population (Rojas-Villarraga et al., 2008). Although the etiology of rheumatoid arthritis (RA) is uncertain, there is a convincing evidence that genetics play an important role in understanding the pathogenesis of the disease, however the results were controversial (Cox et al., 1999, Cantagrel et al., 1999, Cvetkovic et al., 2002, Camargo et al., 2004 , Rego-Perez et al., 2008, Paradowska-Gorycka et al., 2010, Kurko et al., 2013, Felson and Klareskog 2015, Ismail et al., 2016). Cytokines are known to play an important role in the pathogenesis of RA, as the imbalance between pro- and anti-inflammatory cytokines promote the induction of autoimmunity. (Arend, 2001, Zwerina et al., 2005, Moudgil and Choubey ,2011)

IL-1α and IL-1 β both are pro inflammatory cytokines that initiate immune and inflammatory responses. In rheumatoid arthritis; IL-1β production lead to erosive damage in RA, as it stimulates synovial fibroblasts and chondrocytes in the articular cartilage to secrete cytokines and enzymes that degrade proteoglycans and collagen (Jacques et al., 2006).

Previous studies demonstrate the association of IL1 polymorphisms with the RA predisposition in different ethnic groups (Crilly *et al*., 2000, Huang et al., 2001, Cvetkovic et al., 2002, Chaudhary et al., 2008, Jung et al., 2010, Magyari et al., 2014).

Moreover, IL-1β polymorphisms were known to decrease the repair process by inducing many inflammatory genes (Abramson and Amin, 2002, Lange *et al*., 2010).

Based on the above, this study aimed to investigate a possible association of the 3954C/T IL-1β polymorphism (rs1143634) with RA susceptibility in our patients.

Patients and methods

A cross sectional study was carried-out included 84 of the clinically confirmed RA patients and 93 age matched controls. Samples were collected from Police Hospitals and Modern Medical Center in Khartoum State. The demographic data of all subjects were obtained using a well-designed questionnaire. Ethical approval was obtained from the ethical Committee of the Faculty of Medical Laboratory-AlNeelain University. Verbal consent was obtained from all patients and controls.

In this study we conducted a (PCR-RFLP) analysis of IL-1β exon 5 polymorphism (rs1143634).

PCR amplification was performed using (iNtRON Biotechnology, Inc., pre mix) mixture containing: 1 μL of 10 pmol/μL of each primer (forward primer 5'GTTGTCATC-AGACTTTGACC 3' and reverse primer 5'TTCAGTTCATATGGA-CCAGA3), was added to 3-5μL of 50ng/ μL of template DNA, the mixture completed to 12.5ul. The PCR program was as follows: 95˚C for 5 min for initial denaturing, 30 cycles of denaturing at 95˚C for 30 s, annealing at 55˚C for 1 min, followed by extension at 72 °C for 30 s. Final extension at 72 °C for 10 min.

2ul of the PCR product of 249bp was incubated for three hours with 0.5U of the Taq1 restriction enzyme (New England Biolab),1 μL Tango buffer and the mixture was completed to 10 μL.

The homozygous wildtype genotype (CC) of the 3954C/T polymorphism (rs1143634) in exon 5 of IL-1β was cleaved into fragment 136 and 113bp. The homozygous mutant genotype was un cleaved giving a band of 249 bp. The heterozygous genotype was indicated by the presence of three fragments, 249 bp, 136 bp and 113 bp.

Statistical Analysis

SPSS version 21 statistical package was used to count the genotype, chi square, P value, odds ratio (OR) and 95% confidence interval (CI). Statistical significance was determined at $p<0.05$. ORs with 95% CI were used to assess the strength of the association of the tested variables with the risk of rheumatoid arthritis in our population.

Results

This is a cross sectional study; included177 sample 84 cases of rheumatoid arthritis (RA) and 93 healthy controls. The highest frequency of RA (83.3%) was observed among females and approximately 48.8% of RA patients were aged less than 35years with high significant differences between cases and controls with regard to gender and age (P=0.001, P=0.005 respectively) (Table 1). 26% of the cases have family history of RA disease with no significance for RA susceptibility (P= 0.071). The genotype analysis suggests that IL1 β exon5 3954 C/T (rs1143634) polymorphism is associated with the risk of RA susceptibility in our patients (P=0.001). Moreover, the genotype analysis of the IL1β C > T (rs1143634) polymorphism considering the mutant genotype versus the wild genotype (CT + TT vs. CC), showed a significantly higher frequency in RA patients compared to the controls, and was associated with about three folds increased risk of RA (P = 0.001, OR = 2.63, CI = 1.38 to 5.03). Carriage of the rare mutant IL-1 β (+3954) allele T was higher in arthritis patients as compared to the controls, however, the difference was insignificant (Table 1).

68% of the RA patients showed articular manifestation, of which 70.6% have the mutant T allele. The mutant CT and TT genotypes were observed with 43.8% and 18.8% frequency respectively among those who have family history.

None of the participants was smoker, 100% of the cases live sedentary live. 70% of the RA patients live in the Capital.

Table 1: **Comparison of risk factors between Rheumatoid Arthritis patients (RA cases) and Healthy controls**

	Patients (N=84)(%)	Controls(N=93)(%)	P, OR, CI 95%
% Males	14(16.7%)	48(51.6%)	P=0.001,
% Females	70(83.3%)	45(48.4%)	
			OR=5.33, CI=2.64-10.77
Age:	41(48.8%	27(29.0%)	
	43(51.2%)	66(71.0%)	P=.005, OR=0.43,
≤35			
		43(46.2%)	CI=0.23-0.80
≥ 35		29(31.2%)	
IL1β C > T	CC 20 (23.8%)	21(22.6%)	
Homozygous wildtype			
	CT 49 (58.3%)		P=0.001
Heterozygous		0.62	
Homozygous mutant type	TT 15 (17.9%)	0.38	OR=2.64, CI= 1.38-5.03
		0.0%	
		0.0%	

Wild allele C	0.53		
Mutant allele T	0.47	0.0% 100%	P=0.75
Other diseases:			
Diabetes	16.3%	100%	
Hypertension	18.8%	100%	
Physical activities			
Yes	0.0%		
No	100%		
Smoking			
Yes	0.0%		
No	100%		
Residence			
in Khartoum the Capital	70%		
Out Khartoum	30%		

(1A) (1B)

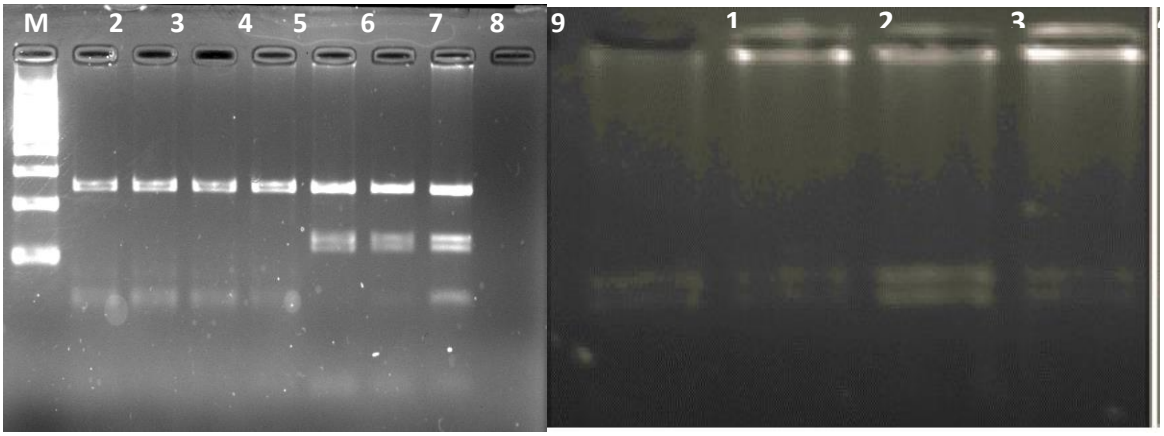

Fig. The above figure shows the genotypes of IL-1 β exon5 C/T gene polymorphism (1A): M represents molecular 100bp DNA ladder, lane 2,3,4,5 shows CC wildtype (249bp), lane 6,7,8 shows TC heterozygote (249 bp, 136 bp and 113 bp), lane 9 shows the -ve control. **(1B):** Lane 1,2,3,4 shows TT wild type (136 bp and 113 bp)

Discussion

Chronic inflammation and joint problems, make rheumatoid arthritis (RA) a serious problem that affect the activity and productivity of the affected individuals. RA is a multifactorial disease; where both

environmental and genetic factors contribute to the disease, the later contribute 50% to 60% of the risk of developing RA (Lebba et al., 2011, Kurko et al., 2013). Of the genetic contributors are the cytokines that have been implicated as important mediators of inflammation and joint destruction in RA (Symons et al., 1989, Cox et al.,1999, Arend et al., 2001, Zwerina et al., 2005, Dayer et al., 2017).

In this study we succeed to demonstrate the association of the IL-1β +3954 C/T gene polymorphism with the susceptibility to rheumatoid arthritis (RA) in Sudanese patients; as the frequency of the mutant CT and TT of the+3954 IL-1β are significantly differ in RA cases compared to controls (P=0.001) and was also found to expose patients to an increased risk of the disease, with an odds ratio of (OR=2.64, CI= 1.38-5.03). Our finding agreed with studies from different populations that confirm a strong association of IL-1β +3954 C- T with RA (Pociot et al., 1992, Buchs et al., 2001, Arman et al., 2006, Lagha et al., 2015). The frequency of the mutant allele T was 47% and 38% in RA patients and controls respectively moreover, 43.8% and 18.8% of those who have family history carry the CT and TT genotypes respectively. These indicate the presence of a considerable frequency of the mutant allele in the population and might possibly make the controls "in presence of other risk factors" at risk of developing RA. In addition, this study revealed that 68% of the RA patients showed articular manifestation, of which 70.6% have the mutant T. So if we linked this finding with the previous studies that proved not only the association of the IL-1β +3954 C- T polymorphism with RA susceptibility; but also its role in the severity of the disease (Jouvenne et al., 1999, Vuolteenaho et al, 2003), we suggested that the IL-1β +3954 C- T polymorphism might play a significant role in the destructive process of the joints in our patients and could be a valuable predictive measure for early detection of RA manifestation. Other demographic data revealed that the frequency of RA is significantly higher among female (83.3% of the RA patients are females) which is consistent with previous study (You et al., 2007). This could be related to the hormonal changes during females` live or during or after pregnancy or menopause. 48.8% of the RA patients were aged less than 35years, which is a reproductive age, indicating the adverse effects of RA on the quality of life of patients and their families. Un expectedly none of the participants was smoker; this could be explained by traditions and cultural constrains. 100% of the cases live sedentary live which is luckily is one of the modifiable RA risk, that could be avoided to decrease or inhibit the tragic complications and irreversible changes associated with destructive arthritis thereby enables prolonged healthy productive life.

Conclusion and Recommendation:

In this study, although the unrelated participants; both RA patients and healthy controls, are not belonging to homogeneous ethnic lineage, we succeed to demonstrate the association of the *IL-1β exon 5* Polymorphism (rs1143634) with rheumatoid arthritis (RA) predisposition in Sudanese patients. Moreover, a considerable frequency of the mutant allele among both RA cases and controls (47% and 38% respectively), indicating a non-negligible presence of the mutant allele in the population and this allele could be a candidate predictive factor of RA in our population.

Rheumatoid arthritis is a disabling disease which have adverse and great economic burden to both families and healthcare providers. Understanding the genetic contributor to RA susceptibility, may help in

the preventive measures by identifying those who are at risk of developing the disease and also enables a provision of targeted therapy that inhibit the tragic complications associated with the disease, thereby enables prolonged healthy productive life

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Competing interest:
The authors declare that they have no competing interests.

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