Study on Correlation between IL-33 serum level, IL-33 Gene Single Nucleotide Polymorphism and Rheumatoid Arthritis Susceptibility

Hind Fouaad alhammami[1], Mahdi H. AL-Ammar[2]

[1]Department of Biology/ Faculty of Science / University of Kufa/Iraq

Abstract

Objective: To discuss the association between single nucleotide polymorphism (SNP) of rs1929992 in IL-33gene and IL-33 serum level in rheumatoid arthritis (RA) susceptibility among Iraq population. **Methods:** A total of 50 samples were collected from 35 RA patients from November 2018 untill end of January 2019 together with 15 healthy physical examines in the same period were chosen as the subjects. The serum IL-33 levels measured by commercial ELISA kits . Erythrocyte sedimentation rate ,white blood cell were measured by standard laboratory techniques The RFLP-PCR reaction technique was used to detect the genotype distributions for rs1929992 in IL-33gene was carried out by using restriction enzyme. The frequency of each allele and genotypes distribution was calculated so as to evaluate the association between genotype distribution and RA susceptibility. **Results:** Serum IL-33concentration was significantly higher in patients with RA than in control groups. The homozygous genotype AA recorded higher frequency in RA patients (42.9%) than controls (6.7%) with a significant difference (P-value 0.001). Homozygote genotype GG frequency (45.7%) was a significant in patients compared to controls subject (33.3%) with a significant difference (P-value 0.016), and the genotype heterozygous GA frequency (11.4%) were non-significant in patients compared to controls (60.6%). The allele frequency for allele G was (51.4%)in patients compared with controls (63.3%) with a significant difference (P-value 0.022)while for the allele A was (48.6%) in patients compared with controls(36.7%)with a significant difference (P- value 0.001). **Conclusions:** significant correlation between RA patients susceptibility and genotype AA and alleles at rs1929992in IL-33 gene is observed. From this study showed that the IL-33 levels were influenced by genetic variation at SNPs rs5743708and rs1929992, respectively.

Keywords: *rheumatoid factor, single nucleotide polymorphism, Rheumatoid arthritis.*

Introduction

Rheumatoid arthritis (RA) is one of the most common inflammatory autoimmune diseases. It is characterized by persistent synovitis, systemic inflammation and producetion of autoantibodies [1].The molecular mechanisms of RA pathogenesis are not fully understood it is believed that approximately half of the risk factors for RA are attributed to genetic factors such as the human leukocyte antigen (HLA) alleles while the other half of the risks are environmental factors including infection and smoking [2].The human IL-33 gene and protein ,structure of the human *IL-33* gene. The gene spans >42 kb, contains 8 exons, and is located on chromosome 9 at 9p24.1has also been described [3]. IL-33, a member of the IL-1 family, is a ligand for the ST-2R receptor , When binds to IL-33, it enhances inflammatory cytokines via the activation of nuclear factor-κB(NF-κB) and MAP kinases. Although it was initially thought that IL-33 was crucial for Th2cytokine-mediated immune responses, it is now known that, it can overcome to have a role in RA [4].

Methodology

A total of 50 samples were collected from 35 RA patients from November 2018 untill end of January 2019 together with 15 healthy physical examines in the same period were chosen as the subjects ,with age ranged between 30to 69 years old . the RA patients diagnosed by RFtest.. RFLP-PCR was used to detect the genotype distributions single nucleotide polymorphisms (SNP) in

the gene of IL-33 . About 6 ml of blood samples were drown from each patients and control, 4ml were collected in sterile test tube (plain tube) and allow to clot at room temperature for minutes to 1 hr., the sera were separated by centrifugation for 10 min. at 2500 r.p.m, (then serum would be divided into two Eppendorf tube, one tube for RF test, and other for IL-33 ELISA assay), the separated sera were labeled and stored at -30◦C until in-vitro tests were performed. other 2 ml of blood were collected in ethylinditetracitic acid (EDTA)tube and stored at 4◦C for ESR,WBC count and for DNA extraction for detection of IL-33 polymorphism gene by PCR-RFLP technique.

Peripheral blood DNA extraction

2 mL of fasting peripheral venous blood was taken from all subjects and 0.2% of EDTA-Na2 was used for anticoagulation. Modified salt fractionation was used for DNA extraction (Genomic DNA Extraction Kit (Human blood) ,Favorgen, USA) and the extraction was reserved in refrigerator at -20 ℃.

Primer design

Primer and probe were synthesized by Bio Labs, England Company with forward primer being F:5-GAAGTCATCATCAACTTGGAACC-3, and reverse primer being,R:5GGATTGGAATCCCATGGTC-3.

DNA amplification and purification

PCR reaction system was 20 L, containing 100 ng of genomic DNA. The reaction conditions were as follows: predenaturing at 95 ℃ for 10 min, followed by 35 cycles of denaturing at 95 ℃ for 30 s, annealing at 61 ℃ for 30 ℃ s, and extension at 72 ℃ for 30 s, with a final extension at 72 ℃ for 7 min. Gel DNA Purification Kit was used for the purification of amplified production.

Genotyping

RFLP-PCR mix was prepared by using *SspI* restriction enzyme (New England Biolabs.UK); this mix has been done independently according to company , After PCR cycles was finished, (1 µl) unit of enzyme was added to(1µl) of IL-33 PCR produect with (5µl) of enzyme buffer, then incubated for 5-15 min in 37 ℃.After cooling down, they were put into mixed liquor of polyacry lamide and carbamide for electrophoresis for 3 h at 70 V.

Immunological investigations:

The serum IL-33 levels measured by commercial ELISA kits (USA, Elabscinece) according to the manufacturers guidelines. This study including RF test, ,CRP, in this method all the reagents preparation and assay procedures were carried out according to manufacturer's descriptions.

Statistical Methods

Sample collection of patients and genotype frequencies of IL-33gene were estimated by direct counts and expressed as percentage. The comparison between patients and control was analyzed by Unpairedχ^2cal. The statistical significance of the measured number was assessed by a special χ^2 formula. p-value (<0.05) was considered significant by using software packages SPSS the figures constructed was by SPSS program of Microsoft Office 2010. Significant association between RA susceptibility and the genotype distribution were assessed using Chi-squared test to estimate the Odd Ratio (OR) and 95% of confidence interval (CI) for genotype. IL-33 polymorphism was tested by using Chi-squared test. all significance were determined as being below the conventional level of *P= 0.05*.

Results

The clinical features of the35 RA patients and15 healthy controls were listed in Table1.Mean age of disease was 51.80±1.55. years, mean age of healthy control was 53.07±3.25. years. There were no statistical differences between the RA patients and controls regarding to age and , P> 0.05. The finding obtained from this study were illustred in table 1 that shows the gender distribution and reveal that the majority of patients with RA were females (88.6%)with significant difference at (p-value0.001)compared with control of females were(66.7%) while in males were(11.4%)in RA patients (females at more risk than males) compared with control of males were(33.3%) with non-significant at(p-value 0.73). As shown in table 1,the the mean of ESR ,WBC and IL-33 were significantly higher in RA group than control group.

Genetic polymorphism of IL-33 gene which was observed with three genotypes (GG,GA, and AA) as shown in table 2 which illustrated the distribution of genotypes of IL-33 in RA patients and healthy controls. The homozygous genotype AA recorded higher frequency in RA patients (42.9%) than controls (6.7%) with a significant difference (P-value 0.001). Homozygote genotype GG frequency (45.7%) was a significant in patients compared to controls subject

(33.3%) with a significant difference (P-value 0.016), and the genotype heterozygous GA frequency (11.4%) were non-significant in patients compared to controls (60.6%). The allele frequency for allele G was (51.4%) in patients compared with controls(63.3%) with a significant difference (P-value 0.022)while for the allele A was (48.6%) in patients compared with controls(36.7%)with a significant difference (P- value 0.001) as in table 3. The levels of IL-33 according to cytokine gene polymorphism in RA patients and the control groups have been demonstrated in Table 4.The mean serum level of IL-33 in patients with AA genotype (1.396)was higher than mean serum of GG(1.249),AG(1.362) genotype that in patients , but the differences were not statistically significant(p-value 0.687). Moreover, no significant differences were observed among RA patients with AA, GA or AA genotypes regarding the mean serum levels of IL-33. But the significant differences were observed between RA patients genotypes AA(p-value0.006) and GG(0.001) , AG(0.005) and healthy groups at rs1929992 with respect to the levels of IL-33 Table 4. The frequency of genotype AA recorded odd ratio (OR) 10.500 with a confidence intervals (CI) value between 1.240-88.920 under 95% it showed a significant difference (p-value=0.012) according to Pearson chi-square ,this genotype AA association with risk RA according to OR .The genotype AG recorded OR 0.086 with CI between 0.02-0.373 under 95% it showed a non- significant difference (p-value=0.001) according to Pearson chi-square. The frequency of genotype GG recorded OR 1.684 with CI between 0.476-5.954 under 95% it showed a non-significant difference (p-value=0.416) according to Pearson chi-square.

Table1: General Information and Clinical Features of RA Patients and Controls.

Parameters Groups	**Patients n=35**	**Control n=15**	**Sig.**
Age (year)	51.80±1.55	53.07±3.25	0.692
Gender Female n(%) Male n(%)	31(88.6%) * 4(11.4%)	10(66.7%) 5(33.3%)	0.001 0.73
RF n(%) N P	0(0.0%) 35(100%)	15(100%) 0(0.0%)	–
ESR ((mm/hr)	46.80±1.90 *	13.07±0.92	<0.0001
WBC (X3L)	15.66±0.37 *	7.6±0.50	<0.0001
IL-33 (pg/ml)	1.32±0.08 *	0.24±0.043	<0.0001

Abbreviations: CRP, C-reactive protein; SD,standarddeviation;WBC,whitebloodcount. a Values are expressed as No. ormean±SD.

Table 2: Distribution of IL-33 –rs1929992genotype between patients and controls.

Genes	genotypes Groups	Patients n=35	Control n=15	Sig.	Chi-Square	OR CI (95%) SIG.
IL-33	GG	16 45.7%	5 33.3%	0.016	5.762	1.684 0.476-5.954 0.416
	AG	4 11.4%	9 60.0%	0.166	1.923	0.086 0.02-0.373 0.001
	AA	15 42.9%	1 6.7%	0.001	12.250	10.500 1.240-88.920 0.012
	Sig.	0.022	0.041			

Table 3: Distribution of IL-33 allele between patients and controls.

Gene				study groups: patients	study groups: control	Total	SIG.
IL-33	freqauncy allels	A	Count	34	11	45	0.001
			% within study groups	48.6%	36.7%	45.0%	
		G	Count	36	19	55	0.022
			% within study groups	51.4%	63.3%	55.0%	
			SIG.	0.811	0.144		

Table 4. Association between SNP IL-*33* rs1929992 genotypes and allele, and total IL-33 serum level.

	SNP	Study groups				
		RA patients n=35		Control n=15		
		Mean	Std. Error	Mean	Std. Error	Sig.
IL-33 serum level	GG	1.249	0.142415	0.15260	0.022126	0.001*
	AG	1.362	0.199045	0.31800	0.054142	0.005*
	AA	1.396	0.090706	0.22100	0.00.	0.006*
	Sig.	0.687		0.066		

*Significant Differences at p value <0.05

Discussion

Rheumatoid arthritis (RA) is a long-lasting autoimmune disorder that primarily affects joints. It typically results in warm, swollen, and painful joints. Pain and stiffness often worsen following rest. Most commonly, the wrist and hands are involved with the same joints typically involved on both sides of the body. The disease may also affect other parts of the body. This may result in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Fever and low energy may also be present. Often, symptoms come on gradually over weeks to months [5]. This study showed this disease occur in all ages but the highest frequency of patient age infected with RA was in 50 -59 years from other group . this study agree with[6],they found the disease affects all ages, but the rate of infection increases with age and the severity of the disease with age between 60 to 40 years. This results which showed the female more than male in study groups with infected in *RA* were agreement with results of Gabriel [et al.,7] the cohort of RA patients were predominantly female, which is similar to findings from other parts of the world, including the USA , The dysregulation of the oestrogen level might explain why women are much more likely to develop RA than men, whereas androgens may play a suppressive role in the development of the disease [8]. In this study show the level of IL-33 in serum of RA patients was significantly higher than that in the control group this agreement with [9]also reported that in patients with RA, the serum level of IL-33 andST2 was significantly higher than that of healthy controls. this study demonstrated that the patients withRA had detectable levels of IL-33 in serum, supporting the idea that IL-33 is implicated in the pathogenesis of RA,this result similar with study of [10] which showed that IL-33 has been implicated in joint inflammation and destruction in animal models. This study show Genetic factors contribute to the development of RAthat IL-33 gene plays an important role in the pathogenesis of RA and indicate that the IL33 genetic variants associated with RA ,this result agree with [11] they found The gene encoding IL-33 may serve as a genetic factor and be associated with the risk of RA. Human IL-33 gene is mapped on chromosome 9p and several SNPs have been reported in the cytokine gene,[12]. The association of the SNP rs1929992 with several non-malignant diseases such as ankylosing spondylitis [13].and Behcet's disease [14]. SNPs within IL33 seem to be an asthma-susceptibility gene, IL33 encodes a cytokine belonging to the IL1 superfamily, and is the natural ligand for the IL1RL1 receptor [12] which has been previously implicated in asthma, inflammation, and a number of immune disorders [15, 16]. Another study show that the implication of 6 genetic IL33-IL1RL1 variants inthe susceptibility to several inflammatory diseases[16]. Regarding genetic studies, polymorphisms located bothin IL33 andIL1RL1 havebeen associated with autoimmunity, cytokine pathwaygenes, whichhave critical modulatory effects on innate and adaptive immunity, have been shownto represent akey component ofthe genetic network associated with immune-mediatedprocesses [16]. In conclusion, this study to discuss the association between IL-33 gene polymorphisms and the risk of RA. The present research suggested that the IL-33 gene rs1929992 was related to RA susceptibility in Najaf population. These data showed that the IL-33 levels were influenced by genetic variation at SNPs rs5743708and rs1929992, respectively.

These data signify that the IL-33 levels were influenced by genetic variation at SNP rs1929992,a significant differences in the frequencies of genotypes AA at SNP rs1929992 in IL-33 gene between RApatients and controls, the genotypes AA at SNP rs1929992 association to patients RA this result agree with [16] they showed that rs 1929992 IL-33 gene polymorphisms may be associated with susceptibility to RA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

FundinG: Self-funding

References

1- Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet.; (2010). 376:1094–1108.

2- McInnes IB and Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med.; (2011). 365:2205– 2219.

3- Liew FY, Girard JP, Turnquist HR. Interleukin-33 in health and disease. Nat Rev Immunol. 2016;16:676-689.

4- Xiangyang, Z.; Lutian, Y.; Lin, Z.; Liping, X.; HuiSh, and Jing Lu (2012): Increased levels of interleukin-33 associated with bone erosion and interstitial lung diseases in patients with rheumatoid arthritis. Cytokine (2012) 58 6–9.

5- Geuskens GA, Burdorf A, Hazes JM. Consequences

of rheumatoid arthritis for performance of social roles--a literature review. *J Rheumatol*; (2007). 34:1248-60.

6- Jawaheer ,D.; Lum, R..; Gregersen, P.K. and Criswell, L.A. Influence of male sex on disease phenotype in familial rheumatoid arthritis. *Arthritis Rheum*.; (2006). **54(10)**:3087–3094.

7- Hong, Y. S. ; Moon, S. J. and Joo, Y. B. "Measurement of interleukin-33 (IL-33) and IL-33 receptors (sST2 and ST2L) in patients with rheumatoid arthritis," *Journal of Korean Medical Science*, (2011). 26:9, 1132–1139,.

8- Palmer, G. ; Talabot-Ayer, D. ; Lamacchia, C.; Toy, D.; Seemayer, C.A. Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis. *Arthritis Rheum*; (2009). 60:738-49.

9- Chun Li, Rong Mu, Jianping Guo1, Xinyu Wu, Xin Tu, Xu Liu, Fanlei Hu Shiwei Guo, Jiaxin Zhu, Huji Xu3 and Zhanguo Li1.(2014).Genetic variant in IL33 is associated with susceptibility to rheumatoid arthritis

10- Yu, J.T. ; Song, J.H. ; Wang, N.D. ; Wu, Z.C. ; Zhang, Q. and Zhang, N. Implication of IL-33 gene polymorphism in Chinese patients with Alzheimer's disease. *Neurobiol Aging* ; (2012). 33:e11–14.

11- Fan, D. ; Ding, N. ; Yang,T. ; Wu, S. ; Liu, S. and Liu, L. Single nucleotide polymorphisms of the interleukin-33 (IL-33) gene are associated with ankylosing spondylitis in Chinese individuals:a case-control pilot study. *Scand J Rheumatol*. ; (2014). 43:374–379.

12- Koca, S.S. ; Kara, M. ; Deniz, F. ; Ozgen, M. ; Demir, C.F. and Ilhan, N. Serum IL-33 level and IL-33 gene polymorphisms in Behcet's disease. *Rheumatol Int.* ; (2015). 35:471–477.

13- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA: IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptorrelated protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005, 23:479–490.

14- Liew FY, Pitman NI, McInnes IB: Disease-associated functions of IL-33: the new kid in the IL-1 family. Nat Rev Immunol 2010, 10:103–110

15- Latiano A. ; Palmieri O. Pastorelli L. ; Vecchi, M. ; Pizarro, TT. ; Bossa F. Associations between genetic poly morphisms in IL-33,IL1R1and risk for inflammatory bowel disease. Plo Sone (2013). 8:e62144. doi: 10.1371/*journal. pone*. 0062144PMID: 23634226

16- López-Mejías, R. ; Genre, F. ; Remuzgo, MartínezS,Robustillo-VillarinoM,García-Bermúdez M, Llorca, J. Protective Role of the Interleukin 33rs 3939286 Gene Polymorph is min the Development of Subclinical A the rosclerosisin Rheumatoid Arthritis Patients. (2015). PLoSONE10(11):e0143153.doi:10.1371/*journal. pone*.014315