Ahlam Kadhim Abed et al /J. Pharm. Sci. & Res. Vol. 11(2), 2019, 477-479

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Study genetic polymorphism of interlukin-4 and its relationship with peptic ulcer in Diyala governorate

Ahlam Kadhim Abed[*1], Maha Falh Nazzal[1], Mohmmed Abdul Daim Saleh[2], Sajid Ali Hussein[3]

1 *Biology Department, College of Education for Pure Sciences, University of Diyala, Iraq.*

2 *Biotechnology Department, College of Sciences, University of Diyala, Diyala, Iraq.*

3 *Gastroenterologist, Teaching hospital, Baquba, Diyala, Iraq*

Abstract

The aim of this study was to evaluate the frequency of polymorphism of interleukin-4 gene (IL-4) at position-590-rs2243250 in Peptic Ulcer Disease and in healthy controls subjects in 50 of Iraqi patients, (34 males & 16 females;12 -70 years) and 40 controls.(23 males & 17females ; 19-55 years) were enrolled in this study the polymorphism of IL4-590 were data waved by polymerase chain reaction-specific sequence primer (PCR-SSP) assay. Results revealed that comparing IL4-590 genotypes and alleles between PUD patients and controls frequencies of CC genotype and C allele (64 vs. 80%; P =0.01 respectively) were significantly high in patients in contrast to controls, (60 vs. 77.8%; P =0.01) and the related RR rates were 12.5 and 11.1, respectively, and the associated EF values were 1.24 and 1.16. In contrast TT genotype and T allele (4 vs. 20 %, P =0.01 respectively) frequencies were significantly decreased in patients, compared to controls (5.6 vs. 22.5%; P =0.01), and associated PF values were 0.0 and 3.1 , respectively., These findings suggest that IL4-590 SNP might have a role in the etiopathogenic mechanism of PUD and IL-4-590 polymorphism showed associations (positive and negative) with PUD in the samples of Iraqi patients. The results shown showed a clear increase in the interlukin-4 whitening level in the group of patients by (520.9±397.6) pg / ml compared with the control group, which recorded a significant decrease in the level by the ratio of (312.9±238.7) pg / ml.

Keywords: IL-4, peptic ulcer.

INTRODUCTION

Peptic ulcer disease (PUD) is a serious medical problem as it is a very common disease among people, caused by localized macular degeneration of the lining of the gastrointestinal tract (1).

The stomach ulcers are called gastric ulcers and sometimes occur in the duodenal Ulcers(DU) and rarely occur in the esophagus called Esophageal ulcers (EU) (2), previous studies have shown that PUD disease affects approximately 4 million people worldwide every year and that it affects the elderly more often than young people. More prevalent "in developing countries than in developed countries "Despite the large numbers of cases, the number of deaths is very low"(3).

A major cause of the gastrointestinal ulcer is the use of anti- inflammatory drugs, smoking and drug use, as well as the use of a high diet in acid and salt and the presence of a type of bacteria called Heliobactar pylori (H. tylori), which in most cases by 80% of cases of ulcers (4).

Other causes of these ulcers are the weakness of the immune response in people with inflammation. This occurs as a result of the attack of the H. pylori bacteria lining the gastrointestinal tract causing inflammation resulting in a reaction from the immune system by stimulating the production of cellular motions (Cytokines) In turn by an immunosuppressant to reduce inflammation o r prevent inflammation, it is called the other defense cells. Some cellular motions have genetic differences, which in turn affect the levels of cellular motility in the protective mucosa, making them Cytokines cellular motions (5)

Of the important cellular motility is the (IL-4), which is an important regulatory gene that promotes immune response t has been found that infection with gastrointestinal infections leads to an increase in localized response of **T**-cells producing the IL-4, (6).

The symptoms associated with the disease are not specific and the diagnosis according to these symptoms is inaccurate and unreliable, these severe recurrent symptoms in the abdomen are accompanied by nausea with flatulence and burning after eating with weight loss, in severe cases, pain is accompanied by anemia, (7)

MATERIALS AND METHODS

Subjects

The diagnosis and extent of disease was determined by conventional clinical 50 patients ; (34 males & 16 females) attended the hospital in Baquba for diagnosis and treatment during the period 1-10-2017 – 1-2-2018 in addition to 40 healthy controls (23males and 17females).(According to diagnosis, after an overnight fasting of 10–12 h in fasting state for all investigations). Blood samples were collected in EDTA .The samples were stored frozen at -20°C. PUD patients, and randomly selected healthy controls (HC). The patients age range was 12-70 years compared to healthy controls which was 19-55years, were enrolled in the study.

Detection of IL4 Polymorphism

Genomic DNA was extracted from EDTA blood using Wizard Genomic DNA Purification Kit (Promega, USA). The polymorphism was detected at –IL4-590 positions of the promoter region 590 by polymerase chain reaction-

specific sequence primer (PCR-SSP) assay, followed by electrophoresis on 2% agarose-gel, by using Go Taq Green Master Mix Kit (Promega, USA). The thermocycling conditions were: initial denaturation at 94˚C for 2 minutes, followed by denaturation at 94˚C for 15 seconds, and then 10 cycles of annealing and extension at 65˚C for 60 seconds. This was followed by denaturation at 94˚C for 15 seconds, and then 20 cycles of annealing at 61˚C for 50 seconds and extension at 72˚C for 30 seconds.

Statistical Analysis

Genotypes of IL4-590 were presented as percentage frequencies, and significant differences between their distributions in PUD patients and controls were assessed by two-tailed Fisher's exact probability (P). addition, relative risk (RR), etiological fraction (EF) and preventive fraction (PF) were also estimated to define the association between a genotype with the disease. These estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available for free at http://www.brixtonhealth.com.

RESULTS

Genetic polymorphism of IL4 gene was determined in the promoter region at position -590 (IL4-590SNP), which was presented with three genotypes (TT, TC and CC) that corresponded to two alleles (T and C). Among PUD patients, no significant difference was observed between the observed and expected frequencies of the three genotypes (a good agreement with Hardy-Weinberg equilibrium; HWE), while in controls, a departure from HWE was observed (i.e. a significant difference between the observed and expected genotype frequencies they were significantly deviated in controls ($P \leq 0.01$).); however, comparing patients to controls results in some significant differences (Table -1). The frequencies of CC genotype and C allele were significantly increased in patients (64 and 80%, respectively) compared to controls (60.06 and 77.5%, respectively). The relative risks (RRs) of such positive associations were 12.5% and, 11.1% respectively and (PFs) 1.24and 1.16 . In contrast, TT genotype and *T* allele frequencies were significantly decreased in patients (4 and 20%, respectively) compared to controls (5.06 and 22.5%, respectively) the (RR) 0.0 and 3.1. The preventive fractions (PFs) of such negative associations were 0.80 and 0.86 respectively (Table -2).

The results shown in Table (3) showed a clear increase in the intrelukin-4 whitening level in the group of patients by(520.9± 397.6) pg / ml compared with the control group, which recorded a significant decrease in the level by the ratio of (312.9±238.707)pg/ml.

Table 1: Observed numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of(IL-4-590 genotypes and alleles) in PUD patients and controls.

Groups	***IL4* Genotype or Allele**							HWE
			CC	**CT**	**TT**	**C**	**T**	***X2* P≤**
Disease (No. = 20 Peptic ulcer	Observed	No.	13	6	1	32	8	N.S.
		%	64	32	4	80	20	
	Expected	No.	12.8	6.4	1			
		%	2.6	1.2	0.2			
Controls (No. = 20	Observed	No.	12	7	1	31	9	o.o1
		%	60.06	34.88	5.06	77.5	22.5	
	Expected	No.	12.01	6.98	1.06			
		%	2.9	1.4	0.2			

Table 2-: Statistical analysis of associations between IL-4-590 genotypes or alleles in PUD patients and controls.

Type of Comparison	**Statistical Evaluation**			**Fisher's Exact Probabilit**	**95 %Confidence Intervals**
	IL-4-590 Genotype or Allele	**Relative Risk %**	**Preventive or Fraction Etiological**		
PUD Versus Controls	CC	12.5	1.24	0.01	0.45-4.32
	TC	7.1	1	0.01	0.22-2.90
	TT	0.0	0.80	0.01	0.06-15.99
	C	11.1	1.16	0.01	0.40-3.35
	T	3.1	0.86	0.01	0.30-2.48

Table 3: Statistical analysis of associations between IL-4-590 in PUD patients and controls.

Pg/ml		Study group	
		patients	control
SerumIL-4	Mean	520.9	312.9
	SD	397.6	238.7
pvalue			0.01

DISCUSSION

According to the presented results, IL4-590 SNP can be highlighted as an important genetic marker in the pathogenesis of PUD presented with three genotypes (TT, TC and CC) that corresponded to two alleles (*T* and *C*). These genotypes were in a good agreement with Hardy-Weinberg equilibrium (HWE) in patients, but they were significantly deviated in controls ($P \leq 0.01$).The present study illustrated that IL-4-**590** is important genetic marker in the pathogenesis of PUD especially if we consider RR values as 12.5 and 11.1for it was showed that the frequency of CC genotype and that of *C allele* (64*vs*. 80% ; P =0.01 respectively) were significantly increased in patients contrast to controls, (60 *vs*. 77.5% ; P =0.01), and the associated EF values were 1.24 and 1.16 , respectively. g. In contrast, TT genotype and *T allele* (4 *vs*20% , P = 0.01 respectively) frequencies were significantly decreased in patients, compared to controls (5.06 *vs*. 22.5% ; P =0.01), and the associated PF values were 0.0 and 3.1 respectively. According to these findings which agree with previous results, it can concluded that IL-4-590 SNP might have a role in the etiopathogenic mechanism of PUD, However, other studies investigated other polymorphisms in intron and promoter regions of IL4 gene and the results were almost conflicting due to ethnic variations, but they agreed that IL-4 is an important cytokine involved in immunity and its polymorphisms play a critical role in PUD development. [11,12.13]. The above results show a clear increase in the ovarian group 4 in patients with gastrointestinal ulcers, which indicates an increase in the inflammatory process, prompting the immune system to respond by stimulating the local response of **T** cells that are predominant in the **T** cells that produce IL-4 whitening(8).

As shown above, the IL-4 whitening has an important role in the formation of gastric ulcers by increasing the inflammatory process(9,10).

REFERENCES

1- Graham, D.Y. History *of Helicobacter Pylori*, duodenal ulcer, gastric ulcer and gastric cancer. World .J.Gastroenterol. 2014, 14;20(18):5191-5204.doi:10.3748/ wjg.v20.i18.5191.PMCID:PMC4017034.

2- Mohammed Ali;Zahraa,A.G;AL-Mulla Hummadi,Y.M. and Kamal,N.A. Triple and Quadruple Eradication Therapy for *H.Pylory* in Iraq pations with peptic ulcer disease a comparativ stude .British Journal of Medicine. 2015, 7(3).231-240.

3- Zelickson,M.S.;Johnson,B.L;Camunas,J.A;Smith,D.E;Taylor,JM. Helicobacter pylori is not the predominant etiology for peptic ulcer requiring operation Amsurg . 2011, 77:1054-1060.PMID:219445230.

4- Lau,J.Y.M. Systematic review of the epidemiology of complicated peptic ulcer disease incidence ,visls factors and motality . 2011, 84:102-113.pmib:21494041.

5- Gastroenterol, J. Influence of interleukin polymorphisms on deveiopment of gastric cancer and peptic ulcer . March 14 ; 2010, 16(10): 1188-1200.

6- Lohoff, M.; Rollinghoff, M.and Sommer, F. *Helicobacter pylori* gastritis: Ath1 mediated disease.J.Biotechnol., 2000, 83:33-36

7- Malfertheiner, p.; Megraud, F.; Morain, C.A. European Helicobacter study Group. Management of Helicobacter pylori infection-the Maastricht IV/Florence Consensus Report .Gut 2012, 61(5):646-664.

8- Orsini,B.;Ottanelli,B.;Amedei,A.;Surrenti,E.;Capanni,M.;Delprete,G .;Amorosi,A.;Milani,S,;Delios,M.and Surrenti,C. Helicobacter pylori cag pathogenicity island is associated with reduced expression of interleukin-4 (IL-4) Mrna and modulation of the IL-4&2 mRNAisoform in human gastric mucosa J Infection and Immunity , 2003, 71(11):6664-6667.

9- Zauros, y. ;Rathinavelu, S.; Kao, J.Y.; Todisco, A.; Delvalle, J.; Low, M.J.; Merchant, J.L. Treament of Helicobacter gastritis with IL-4 requires somatostation proc Natl Acad Sci USA. 2003, 100:12944-12949.

10- Liu, X.; Chu, K.M. Gastric cancer con sequence, and application Biomed Rest Int, 2014, p.637308.

11- Sugimoto, M.; Yamaoka, Y.; Furut, Q.T. Infuence of interleukin polymorphisms on development of gastric cancer and peptic ulcer .Mar : 2010, 14.16(10):1188-200 .

12- W12-Wu, M. C.; Wu, C.Y.; Chen, C.J.; Lin, M.T.;; Shun, C.T.; Lin, J.T. Interleukin -10 genotypes associate with the risk of gastric carcioma in taiwanes chinese . Int J Cancer. 2003, 104 pp.617-623 .

13- Omar, E. M..; Rabkin, C.S.; Gammon, M. D.; Vaughan, T. L.; Risch, H.A.; Schoenberg, J.B.; Stanford, J.L. Increased risk of noncardia gastric cancer associated with proin flammatory cytokine gene polymorphisms. 2003, 123:1193-1201.