The frequency of interleukin-10 (IL-10) polymorphisms in patients with peptic ulcer and chronic gastritis

Meysam Rezaeishahmirzadi [1] Neda Motamedi Rad [1] Seyed Hamid Moosavy [2] Mohammad Shekari [3]

Deprtment of Medical Genetics [1], Hormozgan University of Medical Sciences, Bandar Abbas, Iran. Department of Internal Medicine [2] , Hormozgan University of Medical Sciences, Bandar Abbas, Iran. Deprtment of Genetics [3] , Molecular Medicine Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

(Received 9 Jun, 2014 Accepted 8 Mar, 2016)

Original Article

Abstract

Introduction: Peptic ulcer is a common problem in medicine with serious impacts on the quality of life of patients. It has been shown that Helicobacter pylori infection is related with inflammatory responses of gastric mucosa. However, some patients remain asymptomatic. Sustained colonization and chronic inflammation increase the risk of gastritis and peptic ulcer. The aim of this study is to investigate the genetic polymorphisms of inflammatory cytokine IL-10 and its relationship with peptic ulcer and gastric inflammation.

Methods: In this study, 107 people with symptoms of stomach pain and indigestion referred to a doctor with an indication for endoscopy were selected as the patient group. 107 healthy people without complications such as stomach pain and indigestion were selected as the control group. IL-10-592A/C polymorphism was examined by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Results: No significant difference was found between the patients with peptic ulcer and chronic inflammation and healthy people in the control group in terms of the C allele in the IL-10 gene (P=0.99; 0.48-1.80, CI 95%, OR 0.93, and P=0.26; 0.57-1.13, CI 95%, OR 0.81, respectively).

Conclusion: The results showed no significant correlation between the inflammatory cytokine IL-10 gene polymorphisms and peptic ulcer disease and chronic inflammation.

Correspondence:
Mohammad Shekari, MD, PhD.
Molecular Medicine Research Center, Hormozgan University of Medical Sciences.
Bandar Abbas, Iran
Tel:+98 76 33354939
Email: mshekari_ch@yahoo.com

Key words: Polymorphism, Interleukin-10, Peptic Ulcer, Chronic Gastritis

Citation: Rezaeishamirzadi M, Motamedi Rad N, Moosavy H, Shekari M. The frequency of interleukin-10 (IL-10) polymorphisms in patients with peptic ulcer and chronic gastritis. Hormozgan Medical Journal 2016;20(4):264-269.

Introduction:

Peptic ulcer include gastric and duodenal ulcers and has been a significant threat to the world's population with high mortality rates during the past two centuries (1). The major symptoms of peptic ulcer include epigastric pain along with other complications such as indigestion and bloating (2). Chronic ulcers may also be asymptomatic (3).

Peptic ulcer pathogenesis is complex and multifactorial caused by the imbalance of gastric attacking factors, acid and pepsin and mucosal barrier dysfunction. In addition, environmental and host factors participate in ulcer formation (4).

Environmental factors include smoking, alcohol consumption and non-steroidal anti-inflammatory drugs (NSAIDs) (5,6). Our understanding of the disease was changed with the discovery of Helicobacter pylori by Marshall and Warren in 1982 (7).

Many epidemiological evidence shows a relationship between Helicobacter pylori infection and peptic ulcer and chronic gastritis. But clinical effects of the disease are observed only in some people with Helicobacter pylori infection suggesting the role of other factors in the disease (8).

Neutrophils are activated in chronic inflammation of the stomach and mononuclear cells produce different types of cytokines which play a vital role in regulating inflammation. During inflammation, in addition to the inflammatory interleukin cytokines (IL-1B, IL-6, IL-8), anti-inflammatory cytokines such as IL-10 are also produced.

IL-10 limits the production of the inflammatory cytokines by restricting T helper lymphocytes and stimulating B lymphocytes and T helper 2 lymphocytes and therefore reduces inflammatory responses (9-11). Human IL-10 gene is located on the long arm of the chromosome 1, the band 31-32 and contains 5 exons and 4 introns (9-12) with three polymorphisms in the promoter region including -1082 Rs 1800896, -819 rs1800871 and -592 rs1800896. These polymorphisms affect the expression of IL-10 and are associated with increased risk of gastric cancer (13,14). The aim of this study is to investigate -592A/C polymorphism in the IL-10 promoter in patients with peptic ulcer and chronic inflammation.

Methods:

This is a case-control study. Considering P=0.77, z=0.08 and d=0.08, 107 people were selected for the patient group and 107 people were considered for the healthy group. Accordingly, 107 people with symptoms of stomach pain and indigestion referred to a doctor with necessary indication for endoscopy were considered as the patient group. The gastric or duodenal ulcers in patients were demonstrated by performing endoscopic examination. Gastritis in this group of patients was detected by pathological tests. During sampling, people who were taking non-steroidal anti-inflammatory drugs (NSAIDs) or those with autoimmune and alcoholic gastritis were excluded. 5 ml blood was taken from healthy people without digestive problems who had not consumed any gastric medication such as antacids and proton pump inhibitor (PPI) drugs. Helicobacter pylori IgG serum test was performed to examine a previous encounter with Helicobacter in patients. After the test, IgG positive people were considered as the control group. The study was approved by the ethics committee and a written consent was obtained from both cases and controls to participate in the research project.

The genomic DNA from patients and controls was respectively extracted from biopsy specimens and peripheral blood samples by DNA mini kit Qiagene. -592A / C polymorphism in the IL-10 gene was studied by PCR-RFLP method. The primers used are as follows:

Table 1. Primer Sequences of IL10

Primers	Sequence
IL10: F	[5'GGTGAGCACTACCTGACTAGC3']
IL10: R	[5'CCTAGGTCACAGTGACGTGG3']

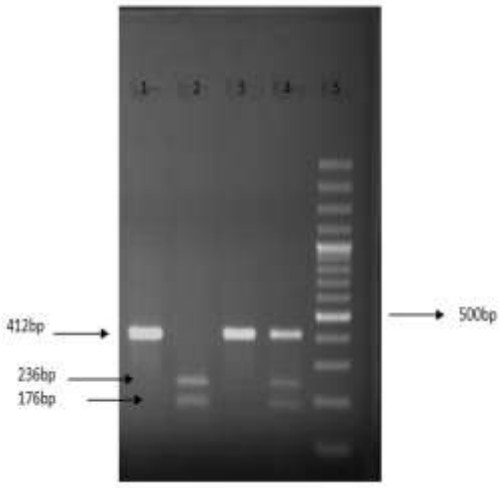
The polymerase chain reaction (PCR) was performed in a total volume of 25μL containing the buffer 10X KCL, 1.5 mM $MgCL_2$, 0.2 mM of each dNTP and 25 pmol of both primers, polymerase U1 and 100ng of extracted DNA.

Polymerase chain reaction was performed by PCR-RFLP method under the following conditions:

A primary denaturation stage at 95°C for 5 minutes, 35 cycles at 95°C for 40 seconds, annealing at 63°C for 1 minute and at extension at 72°C for 40 seconds and a final amplification stage at 72°C for 10 minutes. The resulting piece with a length of 412bp was digested with RsaI limiting enzyme. The enzyme product was electrophorized on the agarose gel 2% containing ethidium bromide and then was examined. The allele A created a 412bp band and the allele C created the basic bands of 236 and 176 while both AC alleles created three basic bands of 412, 236 and 176.

The frequency of alleles and genotypes in both cases and controls were studied. Chi-square tests were used to evaluate and compare the genotype and allele frequencies and the Hardy-Weinberg

equilibrium in patients and controls. Statistical surveys and χ^2 analysis were performed with the help of SPSS 16. Differences in genotype and allele frequencies in cases and controls were evaluated by Chi-square test and Odds Ratio (OR) to examine the -592A / C polymorphism in the IL-10 gene.



Results:

The allele and genotypic frequencies of the -592A/C polymorphism in the IL-10 gene are shown in Table 1. The frequencies of alleles A and C in patients with peptic ulcer were 73.2% and 26.8%, respectively. The corresponding values in patients with gastritis were 75.3% and 24.7%, respectively. The frequencies of A and C alleles in the control group were 73.4% and 26.6%, respectively. The Hardy-Weinberg equilibrium test was conducted for the SNP and the null hypothesis was not rejected. No significant difference was found between genotypic and allelic frequencies of the -592A/C polymorphism in the IL-10 gene in patients with peptic ulcer and chronic gastritis and in the controls. No significant difference was observed between the patients with peptic ulcer and controls in terms of CC and AC genotypes in the IL-10 gene (P=0.4; 0.54-10.01, CI 95%; OR 2.32; and P=0.89; 0.45-1.74, CI 95%, OR 0.89, respectively).

In patients with chronic gastritis, no significant difference was found between both study groups in terms of CC and AC genotypes (P=0.1; 1.17-2.70, CI 95%; OR 1.78 and P=0.12; 0.51-1.06, CI 95%; OR 0.74, respectively).

Table 2.Or and 95% Cl for IL-10 genotypes among gastritis case and control

Polymorphism	Geontype	Gastritis (79)	Control (107)	OR (95% Cl)	P-value
IL-10	AA	14 (50)	51 (47.7)	-	-
	AC	13 (46.4)	55 (51.4)	0.74 (0.51-1.06)	0.12
	CC	1 (3.6)	1 (0.9)	1.78 (1.17-2.70)	0.10
	AC+CC	14 (50)	56 (52.3)	0.81 (0.57-1.13)	0.26

Table 3.OR and 95% Cl for IL-10 genotypes among peptic ulcers case and control

Polymorphism	Geontype	Peptic Ulcer (28)	Control (107)	OR (95% Cl)	P-value
IL-10	AA	14 (50)	51 (47.7)	-	-
	AC	13 (46.4)	55 (51.4)	0.89 (0.45-1.74)	0.89
	CC	1 (3.6)	1 (0.9)	2.32 (0.54-10.01)	0040
	AC+CC	14 (50)	56 (52.3)	0.93 (0.48-1.80)	0.99

Conclusion:

Specifically, the host immune system responses including the activity of cytokines play a vital role in the gastrointestinal tract inflammation caused by infections including Helicobacter pylori infection. It ultimately plays a role in disease progression and the creation of more advanced clinical effects including gastric ulcers, especially gastric and duodenal ulcers as well as gastrointestinal tract

cancer. Many genetic studies suggest the relationship between cytokines and immune-related illnesses. However, it should be noted that despite many studies on cytokines and the role of genetics in cytokines and different genotypes as well as their polymorphisms, the relationship between different polymorphisms of host cytokines and the disease severity or nature is not entirely clear (15).

In particular, experiments were performed on the anti-inflammatory cytokine IL-10. In fact, the anti-inflammatory cytokine IL-10 regulates the development and differentiation of B-cell and inhibits immunological activity and B-cell activity. According to literature, IL-10 is one of the key cytokines against anti-inflammatory cytokines TNF-α (14,16). Experiments on these two groups showed no significant correlation between the carriers of each allele or a combination of alleles and the risk of inflammation and peptic ulcer disease.

In agreement with the results obtained in this study, Rad *et al.* (2004) found no significant correlation between the low frequency allele carriers or the so-called risks alleles in IL-10-592 in atrophic gastritis and intestinal metaplasia (17). In another study in China, no significant correlation was observed between intestinal metaplasia and IL-10 polymorphisms [18] conforming our results on IL-10-592 polymorphisms. Hunt Pj and Yao-Yam found a significant correlation between IL-10-592 polymorphism and the IL-4 gene intron 3 variable number tandem repeat (VNTR) with other diseases such as rheumatism, arthritis, asthma, rhinitis, atopic dermatitis, gravis disease, multiple sclerosis and bladder cancer (19,20). This can be attributed to the role of immune system and immune responses in causing this disease. Shekari *et al.* conducted many statistical studies on IL-4 and IL-10 in patients with cervical cancer and in normal people. They found no significant difference between non-smokers with the CC and AC genotypes in IL-10 and normal non-smokers. Passive smokers with at least one allele A are more at risk (1.7 fold), but no significant correlation was found between people with the genotypes CC and passive smokers (21).

According to Shekari *et al.,* being passive smoker increases the risk of polymorphism and a certain genotype for cervical cancer.

According to the results, it can be concluded that the presence of this polymorphism in the cytokines does not lead to increased or decreased risk of gastritis and peptic ulcer disease following Helicobacter pylori infection regardless of other intervening and environmental factors only with regard to age and sex. However, as previously discussed, since the role of these cytokines in inflammatory diseases is not entirely clear, these results cannot be emphasized. The other reason for uncertainty is due to the role of haplotypes in these cytokines. According to literature, different haplotypes affect the secretion of cytokines; a point that was not examined in this study.

References:

1. Sonnenberg A. Causes underlying the birth-cohort phenomenon of peptic ulcer: analysis of mortality data 1911–2000, England and Wales. International Journal of Epidemiology. 2006;35(4):1090-1097.
2. Malfertheiner P, Dent J, Zeijlon L, Sipponen P, Veldhuyzen Van Zanten SJ, Burman CF, et al. Impact of Helicobacter pylori eradication on heartburn in patients with gastric or duodenal ulcer disease—results from a randomized trial programme. Alimentary Pharmacology & Therapeutics. 2002;16(8):1431-1442.
3. Dew MJ. Asymptomatic peptic ulcer disease. British Medical Journal (Clinical research ed). 1987;295(6595):401.
4. Flemström G, Turnberg L. Gastroduodenal defence mechanisms. Clinics in gastroenterology. 1984;13(2):327-354.
5. Kato I, Nomura AM, Stemmermann GN, Chyou PH. A prospective study of gastric and duodenal ulcer and its relation to smoking, alcohol, and diet. American Journal of Epidemiology. 1992;135(5):521-530.
6. Rosenstock S, Jørgensen T, Bonnevie O, Andersen L. Risk factors for peptic ulcer disease: a population based prospective cohort study comprising 2416 Danish adults. Gut. 2003;52(2):186-193.
7. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. The Lancet. 2009;374(9699):1449-1461.

8. El-Omar EM, Carrington M, Chow W-H, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404(6776):398-402.
9. Mocellin S, Marincola FM, Young HA. Interleukin-10 and the immune response against cancer: a counterpoint. Journal of Leukocyte Biology. 2005;78(5):1043-1051.
10. Avradopoulos K, Mehta S, Blackinton D, Wanebo HJ. Interleukin-10 as a possible mediator of immunosuppressive effect in patients with squamous cell carcinoma of the head and neck. Annals of Surgical Oncology. 1997;4(2):184-190.
11. Perrin GQ, Johnson HM, Subramaniam PS. Mechanism of interleukin-10 inhibition of T-helper cell activation by superantigen at the level of the cell cycle. Blood. 1999;93(1):208-216.
12. Turner D, Williams D, Sankaran D, Lazarus M, Sinnott P, Hutchinson I. An investigation of polymorphism in the interleukin-10 gene promoter. European Journal of Immunogenetics. 1997;24(1):1-8.
13. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. Gastroenterology. 2003;124(5):1193-1201.
14. Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5′ flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis & Rheumatism. 1999;42(6):1101-1118.
15. Murphy G, Thornton J, McManus R, Swan N, Ryan B, O'Morain C, et al. Association of gastric disease with polymorphisms in the inflammatory related genes IL-1B, IL-1RN, IL-10, TNF and TLR4. European Journal of Gastroenterology & Hepatology. 2009;21(6):630-635.
16. Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. The Journal of Immunology. 2001;166(6):3915-3922.
17. Rad R, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, et al. Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during Helicobacter pylori infection. Gut. 2004;53(8):1082-1089.
18. Leung WK, Chan MC, To K-F, Man EP, Ng EK, Chu ES, et al. H. pylori genotypes and cytokine gene polymorphisms influence the development of gastric intestinal metaplasia in a Chinese population. The American Journal of Gastroenterology. 2006;101(4):714-720.
19. Hunt P, Marshall S, Weetman A, Bell J, Wass J, Welsh K. Cytokine gene polymorphisms in autoimmune thyroid disease . Journal of Clinical Endocrinology & Metabolism. 2000;85(5):1984-1988.
20. Zhu S, Chan-Yang M, Becker AB, Dimich-Ward H, Ferguson AC, Manfreda J, et al. Polymorphisms of the IL-4, TNF-α, and Fc α RI β Genes and the Risk of Allergic Disorders in At-risk Infants. American Journal of Respiratory and Critical Care Medicine. 2000;161(5):1655-1659.
21. Shekari M, Kordi-Tamandani DM, Malekzadeh K, Sobti RC, Karimi S, Suri V. Effect of anti-inflammatory (IL-4, IL-10) cytokine genes in relation to risk of cervical carcinoma. American Journal of Clinical Oncology. 2012;35(6):514-519.

بررسی فراوانی پلی‌مورفیسم ژن اینترلوکین ۱۰ در بیماران مبتلا به زخم پپتیک و گاستریت مزمن

میثم رضایی شهمیرزادی [۱] ندا معتمدی‌راد [۱] سید حمید موسوی [۲] محمد شکاری [۳]

[۱] گروه ژنتیک انسانی، دانشگاه علوم پزشکی هرمزگان، بندرعباس، ایران. [۲] گروه داخلی، دانشگاه علوم پزشکی هرمزگان، بندرعباس، ایران. [۳] گروه ژنتیک، دانشگاه علوم پزشکی هرمزگان، بندرعباس، ایران.

مجله پزشکی هرمزگان سال بیستم شماره چهارم ۹۵ صفحات ۲۶۹-۲۶۴

چکیده

مقدمه: *زخم پپتیک مشکل معمول در پزشکی می‌باشد که اثرات جدی در کیفیت زندگی بیماران دارد. مشخص شده که عفونت هلیکوباکتر پیلوری با پاسخ‌های التهابی موکوس معده ارتباط دارد اما تعدادی از افراد بدون علامت باقی می‌مانند. کلونیزاسیون پایدار و التهاب مزمن، خطر التهاب معده و زخم پپتیک را افزایش می‌دهد. شواهد قوی وجود دارد که نشان می‌دهد پاسخ‌های التهابی، بخش اساسی بیماری زایی در زخم پپتیک می‌باشد. همچنین ازآنجایی که بعد از تولید سایتوکین‌های التهاب آور میزان زیادی سایتوکین ضدالتهاب در موکوس معده تولید می‌شود، تولید بیش از اندازه این سایتوکین‌ها می‌تواند یکی از فاکتورهای اساسی التهاب همراه با هلیکوباکتر پیلوری باشد. در این مطالعه، هدف ما بررسی پلی مورفیسم ژنتیکی در سایتوکین ضدالتهابی IL-10 و بررسی وجود یا عدم وجود ارتباط آن با بیماری زخم پپتیک و التهاب معده می‌باشد.*

روش کار: *در این مطالعه، ۱۰۷ نفر با علائم درد معده و سوء هاضمه که به پزشک مراجعه کرده‌اند و اندیکاسیون لازم برای اندوسکوپی را دارا می‌باشند، به عنوان گروه بیمار و ۱۰۷ نفر افراد کاملاً سالم که عوارضی مانند درد معده و سوءهاضمه ندارند را به عنوان گروه کنترل قرار می‌دهیم. پلی‌مورفیسم IL-10-592A/C به روش PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) مورد بررسی قرار می‌گیرد.*

نتایج: *حضور آلل C در ژن IL-10 تفاوت معنی‌داری را در بین بیماران مبتلا به زخم پپتیک و التهاب مزمن در مقایسه با گروه کنترل نشان نداد (P=۰/۹۹; OR, ۰/۹۳; ٪۹۵ CI, ۰/۴۸-۱/۸۰ و P=۰/۲۶; OR, ۰/۸۱; ٪۹۵ CI, ۰/۵۷-۱/۱۳) به ترتیب.*

نتیجه‌گیری: نتایج ما نشان می‌دهد که بین این پلی مورفیسم در ژن سایتوکاین ضدالتهابی IL-10 و بیماری زخم پپتیک و التهاب مزمن همراهی وجود ندارد.

کلیدواژه‌ها: *پلی مورفیسم، اینترلوکین ۱۰، زخم پپتیک، گاستریت مزمن*

نویسنده مسئول:
دکتر محمد شکاری
مرکز تحقیقات پزشکی مولکولی، دانشگاه علوم پزشکی هرمزگان
بندرعباس - ایران
تلفن: ۳۳۲۵۴۹۲۹ ۷۶ ۹۸+
پست الکترونیکی:
mshekari_ch@yahoo.com

نوع مقاله: پژوهشی

دریافت مقاله: ۹۳/۳/۱۹ اصلاح نهایی: ۹۴/۹/۹ پذیرش مقاله: ۹۴/۱۲/۱۸

ارجاع: رضایی شهمیرزادی میثم، معتمدی راد ندا، موسوی سید حمید، شکاری محمد. بررسی فراوانی پلی‌مورفیسم ژن اینترلوکین ۱۰ در بیماران مبتلا به زخم پپتیک و گاستریت مزمن. مجله پزشکی هرمزگان ۱۳۹۵؛۲۰(۴):۲۶۹-۲۶۴.