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ORIGINAL ARTICLE

IL-18 Gene Expression in Patients with Peptic ulcers

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ABSTRACT
Peptic ulcer can be certain damage and injury in gastric mucosa (gastric ulcer) or small intestine, which can be caused by the irritating effect of acid and pepsin on the lumen. This is the most common type of ulcer in digestive tract, which is very painful. Helicobacter-pylori (H.pylori) can be one of the most common causes of the disease. The main objective of this study was analysis of Il-18 gene expression in patients with peptic ulcer. According to the investigations in field of same cytokines and gastric cancer and peptic ulcer and the investigations conducted on gastric tract ulcer and peptic cancer, the findings of the present study can be considerable. According to the results, analysis of the expression of the cytokine in different conditions of gastric tract such as normal, inflammatory, ulcer and cancer and more careful analysis of its correlation with H.pylori infection can be underlying.
Keywords*: Peptic ulcer, Helicobacter-pylori, Cure, Il-18 gene*

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INTRODUCTION

Peptic ulcer is one the main disease has been studied in previous studies to find its causes. The disease can be created or be intensified by medicines such as Aspirin or ibuprofen [1]. The main factor causing ulcer (up to 60% in peptic ulcer and up to 90% in duodenal ulcer) can be chronic inflammation caused by *H. pylori* concentrated in the mucosa [2]. Human immune system is not capable to diagnose and eliminate the infection.

While being infected to the bacterium and creation of colony, increased volume of stomach acid can help decline of mucosa and as a result, creation of peptic ulcer. Studies conducted on the disease in developing countries showing presence of numerous pillory colonies shows that diet plays key role in creation of the disease (3). Digestive bleeding is the most common complication for these patients and this happens when one of the arteries of digestive tract is destroyed or damaged as a result of the ulcer. In addition to *H. pylori* infections, using nonsteroidal anti-inflammatory drugs can be also another factor causing peptic ulcer. *H.pylori* and NSAIDs support each other to develop peptic ulcer disease (PUD) and it has been proved that eradication of *H. pylori* in patients using NSAIDs can decrease digestive ulcers [4].

IL-18 gene also called as interferon gamma inductor is one of the cytokines of IL-1 family and can be produced by wide range of immune cells such as monocytes and active macrophages [5, 6].

IL-18 is recognized as an important regulator in innate and acquired immune responses and can be expressed in chronic inflammatory diseases [7]. Many evidences have proved companionship of IL-18 and IL-12 for interferon gamma induction and Th1 response [8, 9]. IL-18 plays role in induction of Th2, IL-13 and Th2 response by itself (10, 11). In fact, imbalance of function of Th1 and Th2 can affect pathogenesis of allergic abnormalities [12, 13]. IL-18 can affect allergic inflammatory reactions by means of induction of B cells and induction of producing Th2 cytokines such as IL-4 and IL-3 [14]. Moreover, cytokine plays role in immune response by the mediation of Th1 and IgE synthesis inhibition [15].

IL-18 gene is placed on short chromosome-11arm(11q22) and contains several functional polymorphisms in its promoter area [16]. The cytokine can be made by both immune and non-immune cells like peripheral blood mononuclear cells and bronchial epithelial cells and plays key role in chronic inflammations, some infections and intensification of immune response by means of Th1 and Th2 [17].
Although the main role of this cytokine, as it was mentioned, as an inducing factor is interferon gamma induction [16], it has the induction potential of producing IL-14 and IL-13 in T cells, natural killer cells, mast cells and basophils [18, 19]. As *H.pylori* chronic infection is associated with interferon gamma production [20, 21] and the effect of IL-18 on production of interferon has been proved [17]; this study has analyzed the correlation between expression of the cytokine with or without *H.pylori* in addition to investigate the correlation between IL-18 gene expression and creation of peptic ulcer.

METHOD AND MATERIALS

A total number of 110 biopsy samples of people with gastric ulcer and healthy people were collected from Imam Reza Hospital in Chalus City with confirmation of the doctor after pathological analyses and were transferred to laboratory to extract the RNA.
RNA extraction: total RNA content of all biopsy samples was extracted using extraction kit made by Analytic Genna Company (Germany). Qualitative and quantitative analysis was done on all extracted samples. For qualitative analysis, 1% agarose gel and (RNA Loading Dye 2x) buffer was used. Quantitative analysis was also done using NanoDrop 2000 (Thermo Scientific) and the product was finally treated by 5μg DNase1 to remove DNA contamination.
Reverse transcription and PCR analysis: because of instability and short half lifetime of RNA, after taking qualitative and quantitative tests, cDNA was immediately extracted from the product. The reverse transcriptase enzyme used in this step was RevertAid H Minus First cDNA synthesis kit (Fermentas) and the OligodT was used also as the primer needed by this enzyme.
Using human Glyceraldehyde 3-phosphate primer, the accuracy of the process of converting RNA to cDNA was confirmed and the reaction was taken using PCR Premix kit made by BIONEER co (Korea) and under following thermal conditions:
5min at 95C;30 sec at 95C,1 min at 55^{0}C,1min at 72^{0}C(35 cycles)and 10 min at 72^{0}C
In next step, to analyze the IL-18 gene expression in samples, specific primers of the gene were used. This was done under following thermal conditions:
5min at 95^{0}C;30 sec at 95^{0}C,1 min at 52^{0}C,1min at 72^{0}C(35 cycles)and 10 min at 72^{0}C
In all steps, chain reaction of polymerase was done in volume of 20μl. To this end, 5μl cDNA was added to each tube and 1μl of each primer (10p mol) and 10μl of mastermix (2x) was added and reached to density of 20μl with distilled water. Moreover, the reaction was done in BIORAD thermocycler device.
After each step of PCR, the product was electrophoresed using 1.5% agarose gel and TBE buffer and the results were analyzed in trans laminator device (Uvi doc, England).
In general, this study used 2 pairs of primer as it is observed in table 1.

Table 1: list of primers used in this study

GAPDHF	Sense	**CCA TGG AGA AGG CTG GGG**
GAPDHR	anti-**sense**	**CAA AGT TGT CAT GGA TGA CC**
IL-18F	**Sense**	**GCT TGA ATC TAA ATT ATC AGT C**
IL-18R	Antisense	**CAA ATT GCA TCT TAT TAT CAT G**

RESULTS

Demographic information and patient profile: in general, 110 samples (50 normal and 60 patients with peptic ulcer) were collected from Imam Reza Hospital in Chalus City (51 male and 59 female) in age range of 18-75 years old. Also, the normal people and patients were matched in terms of gender and age. In this study, people were also analyzed in terms *of H.pylori* infections and it was found that 69 people were suffering from the bacteria, which shows the disease rate of 62.72%.
RNA extraction and qualitative and quantitative analysis: total RNA of all biopsy samples was extracted using extraction kit made by Analytic Genna Company (Germany). Qualitative and quantitative analysis was done on all extracted samples to confirm accuracy of extraction (using 1% agarose gel and NanoDrop 2000. Then, cDNA of all samples was taken using OligodT. Qualitative analysis is illustrated in figure 1.
Human Glyceraldehyde 3-phosphate dehydrogenase gene expression: to ensure of lack of any kind of inhibitor to take PCR and to confirm accuracy of prepared cDNAs, all samples were analyzed by PCR using specific primers of human Glyceraldehyde 3-phosphate dehydrogenase gene. Because of expression of

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Glyceraldehyde gene in all human tissues, this study has applied this gene as internal control. The final product of the reaction is a section with length of 377bp, qualitative analysis of which was done on 1.5% agarose gel.

Human IL-18 gene expression: finally, to analyze expression of this gene using specific primers, PCR reaction was taken and final product was a part with length of 335bp (figure 1).

Figure 1: IL-18 gene image; line 1, 3 and 4: IL-18 gene expression; line 2: no gene expression and M: molecular marker (100bp)

Statistical analysis

The correlation betweenIL-18 gene expression and age of participants using Chi-squared at the level of 95%

Table 2 has presented descriptive indices of age of participants. According to this table, mean age range of participants is 42.92±14.81 years old.

Table 2: descriptive indices of age of participants

Variable	Number of participants	Mean	SD
Age	110	45.92	14.81

As age is a continuous variable and IL-18 gene expression is a categorical variable, logistic regression analysis has been used to examine the correlation between the two variables. The results obtained from the analysis are presented in table 3. According to obtained results, age has no significant effect on probability of IL-18 gene expression (sig>0.05).

Table 3: results of logistic regression analysis of the effect of age on IL-18 gene expression

Variable	Regression coefficient	Wald	Sig
Age	-0.006	0.207	0.649ns
Constant	0.492	0.609	0.435ns

*refers to significance at the level of 5%; ns: not significant

Correlation between IL-18 gene expression and gender of participants using chi-squared at the level of 95%

In order to test correlation between gene expression and gender of participants, chi-squared test is used in the adaptive table. Table 4 is the adaptive table between the two variables.

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Table 4: adaptive data of correlation between two variables of gender and IL-18 gene expression

gender / IL-18 gene expression	Male	Female
Negative	20	29
Positive	31	30

Figure 2 has illustrated frequency of IL-18 gene expression separated for female and male genders. According to the figure, in female, there is no significant difference between expression and lack of expression of IL-18 gene; although in males, the gene expression was observed in majority of cases. However, there was no significant difference between male and female genders in terms of gene expression (figure 2).

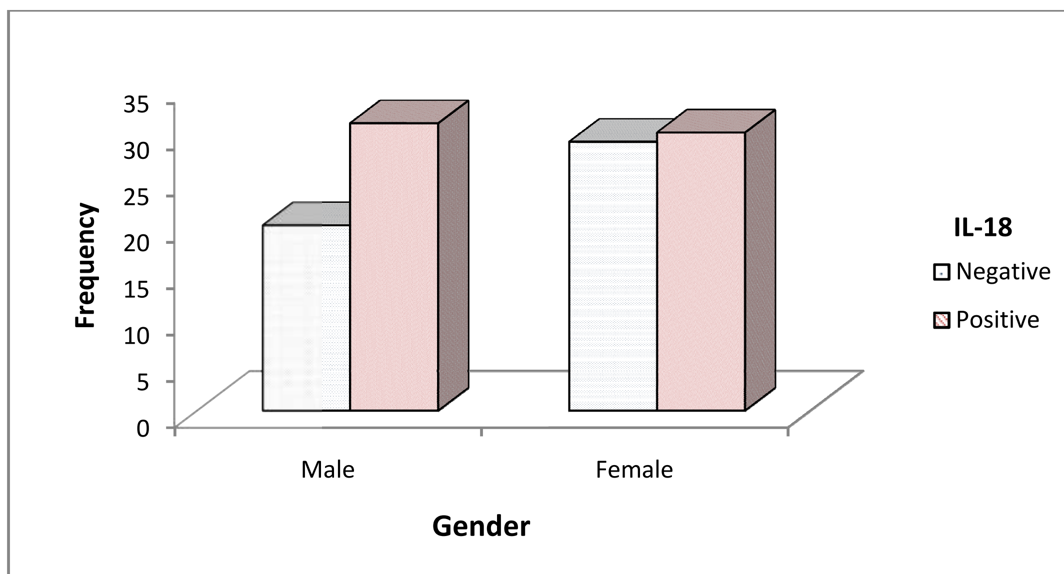

Figure 2: comparing mean effect of gender on IL-18 gene expression

In order to examine the correlation between gender and IL-18 gene expression, chi-squared (x2) test was used. Moreover, as adaptive table is a 2*2 table, the fisher test results has been also reported (for accuracy of the results). The results of two mentioned tests are presented in table 5. According to obtained results from x2 and fisher test, there was no significant correlation between gender and IL-18 gene expression.

Table 5: results of x2 and fisher test in terms of the effect of gender on IL-18 gene expression

Test	Stat	Sig
Chi-squared (x2)	1.093	0.296ns
Fisher	-	0.339ns

*:significant at the level of 5% and ns: no significant

Correlation between IL-18 gene expression and bacterial contamination in participants using chi-squared test at the level of 95%

In order to test the correlation between IL-18 gene expression and bacterial contamination, chi-squared test was used. The adaptive table between two variables is as follows (table 5):

Table 5: adaptive data for correlation between bacterial contamination and IL-18 gene expression

IL-18 gene expression / H.pilory	Negative	Positive
Negative	33	16
Positive	8	53

Figure 3 has presented the results of comparing mean effect of presence of absence of bacteria on IL-18 gene expression. According to the diagram, Il-18 gene expression in presence of bacteria is higher than gene expression in absence of bacteria.

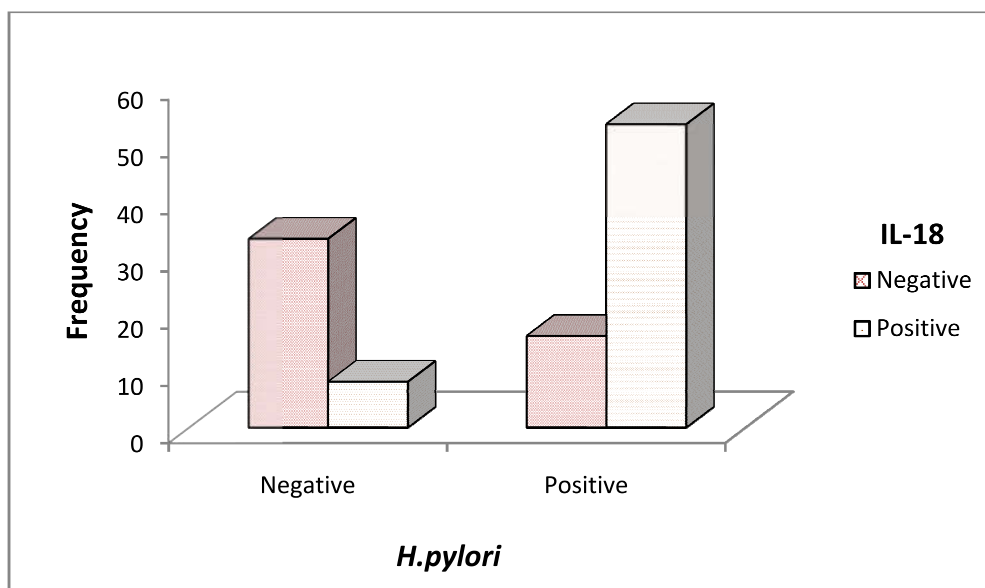

Figure 3: comparing mean effect of presence of absence of bacteria on IL-18 gene expression

In order to examine the correlation between IL-18 gene expression and bacteria contamination, in addition to Ch-squared test, fisher test has been also used for more reliability of the results. The results obtained from both tests are presented in table 6. According to obtained results from both tests, there was significant correlation at the level of 95% between bacteria contamination and IL-18 gene expression.

Table 6: results of chi-squared and fisher tests in terms of effect of bacterium contamination on IL-18 gene expression

Test	Stat	Sig
Chi-squared	34.182	0.00*
Fisher	-	0.00*

*: significant at the level of 5% and ns: not significant

Correlation between IL-18 gene expression and normal or ulcer participants using chi-squared test at the level of 95%

Table 7 is adaptive table to test correlation between IL-18 gene expression and normal or ulcer participants. The table can be used to perform chi-squared test.

Table 7: adaptive data to determine correlation between normal and ulcer participants and I-18 gene expression

IL-18 gene expression / Condition	Normal	Ulcer
Negative	37	12
Positive	13	48

The diagram in figure 4 has illustrated frequency of Il-18 gene expression separated for two conditions of normal or ulcer. As it is observed, IL-18 gene expression was mainly observed in people with ulcer and significant difference was observed between normal and ulcer participants in terms of IL-18 gene expression (figure 4).

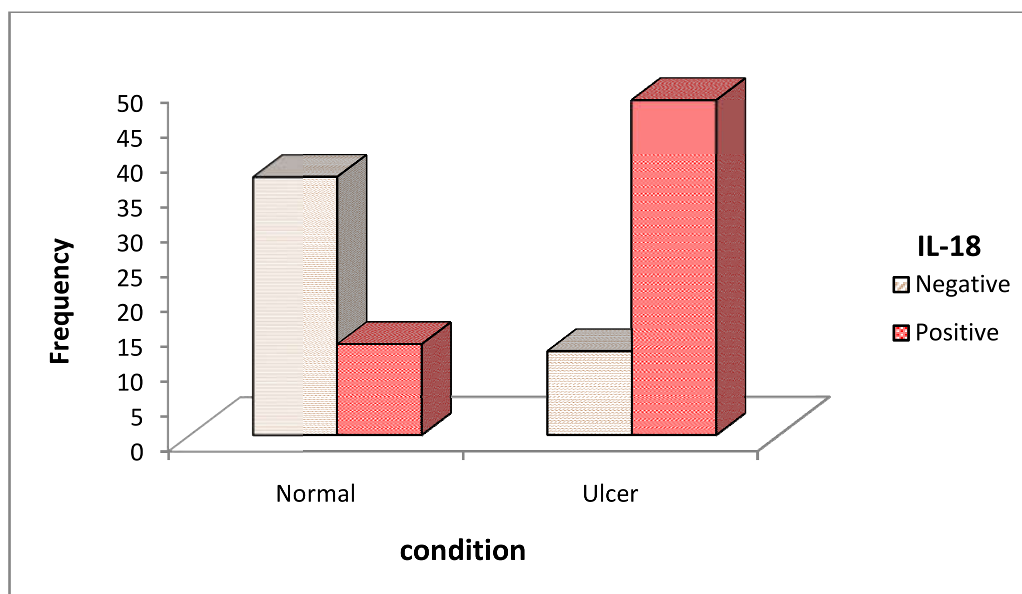

Figure 4: comparing mean effect of normal or ulcer condition of participants on Il-18 gene expression

In order to test the correlation between Il-18 gene expression and normal or ulcer condition, chi-squared test was used. As the adaptive table is a 2*2 table, the results of fisher test have been also reported to test the accuracy of results. The results of the two tests are presented in table 8. According to obtained results, significant correlation was observed between IL-18 gene expression and normal or ulcer condition of participants in both tests (sig>0.05).

Table 8: results of chi-squared and fisher tests in terms of the effect of conditions of participants on IL-18 gene expression

Test	Stat	Sig
Chi-squared	32.194	0.00*
Fisher	-	0.00*

*: significant at the level of 5%; ns: not significant

DISCUSSION

IL-18 is a cytokine with different functions increased under different inflammatory conditions of human body [22]. Increase in expression and transcription of this gene under inflammatory conditions in liver and pancreatic [23] and in colonic mucosa has been proved [24]. An underlying note about IL-18 gene is its vital role in immune and inflammatory responses such as NK Cells irritation and T Cells and increased response of Th1 [25]. On the other hand, in addition to inflammation, increased expression and secretion of IL-18 gene was observed in different cancer cells [26].

In this study,IL-18 gene expression was studied in patients with gastric ulcer and patients with H.pylori infection.

After the statistical analysis, it was fund that *H.pylori* infection can cause expression of IL-18 compared to normal people, so that majority of patients with this infection showed expression of IL-18 gene (76%). The results are in consistence with findings of Masaaki et al in this field and it was found that *H.pylori* infection can cause upregulation of the cytokine in mucosa tissue [27].

An important note about IL-18 is the synergetic effect of the cytokine with IL-12 in anti-tumor activities [28] and angiogenesis process inhibition mechanism with secretion of INF-y [29]. In regard with patients with gastric cancer, studies show that 70% of these patients show IL-18 gene expression [30]. Although similar studies on other types of cancer have also confirmed the evidences on high expression of IL-18 gene [31], expression of IL-18 in patients with gastric cancer at 26%, which was not in consistence with findings of previous studies [32].

In general, it was found that in majority of patients, expression of this gene was happened in majority of patients (80%). Also, statistical analysis showed that IL-18 gene expression and gastric ulcer are significantly correlated. In other words, in patients with the disease, the probability of expression of IL-18 gene is higher than others. In this study, both factors including peptic ulcer and *H.pylori* infection were assessed independently. In fact, even patients without infection or those with *H.pylori*+ infection without

symptoms of peptic ulcer were also assessed. However, it was found that both factors can also cause the cytokine independently.
In similar study, Kyoka *et al* studied expression level of 3 cytokines such as IL-18 in patients with *H.pylori* infection and showed that this infection can make human gastric mucosa with *H.pylori* show increased upregulated expression [33].

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