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Studying the polymorphism of TNF- and TNF- genes among people suffering from helicobacter pylori infection

Helicobacter pylori infection

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Abstract

Aim: One of the most important risk factors proposed for gastric cancer is Helicobacter pylori; however, the correlation between polymorphism of TNF-αα and TNFββ with Helicobacter infection has never been studied. The present research seeks to examine the correlation between polymorphism of these two genes in Helicobacter pylori infection and gastric cancer among those suffering from infection with this bacteria. Material and Method: This is a case-control research seeking to study the polymorphism of TNF-α and TNF-β genes among those suffering from Helicobacter pylori infection and compare it to healthy people. Polymorphism genotype of TNF-α-308 and TNF-α+254 genes was studied in 31 healthy cases, 50 cases with H. pylori, and 23 cases with Helicobacter pylori and gastric cancer using ARMS & PCR-RFLP polymerase chain reaction method. Results: According to the results obtained in this research, there is a significant correlation between TNF-308 A/A homozygote genotype and A allele in TNF-308 with H pylori. In other words, there is a correlation between TNF-308 A/A and the possibility of affliction with infection ($P < 0.05$). The correlation between TNF-308 G/A genotype and TNF-308 A/A genotype and TNF-308 An allele with affliction with cancer and H pylori was also significant ($P < 0.05$). Discussion: TNF-308 A/A homozygote genotype has a significant correlation with gastric cancer and H pylori. As a matter of fact, TNF-308 A/A has increased the possibility of affliction with H pylori and gastric cancer. A significant correlation was also observed between TNF-308 G/A genotype and TNF-308 A/A and TNF-308 An allele with affliction with gastric cancer and H pylori.

Keywords

Helicobacter Pylori; Gastric Cancer; Polymorphism

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Introduction

The genetic differences of people in each population are the major factor determining allergy towards various diseases, whether these diseases are infectious and contagious (like viral hepatitis) or non-contagious (like cancer) [1]. Furthermore, as cancer is caused by multiple factors, it is possible that genetic polymorphisms have a simultaneous interaction with environmental and other genetic factors affecting allergy on affliction with it [2, 3].

Helicobacter pylori is a growing microaerophilic gram-negative microorganism found in the stomach and duodenum, and it is associated with some diseases of stomach and duodenum. One of the most important risk factors playing a major role in causing gastric cancer is Helicobacter pylori bacteria. The role of this bacteria in this disease is so important it has come to be introduced as Class 1 carcinogenic factor by WHO [4]. By producing Cag A protein and entering it into epithelial cells of the stomach, the expression of various genes in these cells undergoes a major change thus affecting the hosting factors and making the individual prone to cancer [5]. Ever since a correlation has been established between Helicobacter pylori infection and gastric cancer, further researchers have sought to study the possibility of an infectious origin for other types of cancer. However, no case with such high level of influence like Helicobacter pylori has ever been introduced. The initial infection with Helicobacter pylori causes mild gastritis, and the resulting inflammation may end in an ulcer. If the pathogenic procedure continues and no measures are taken for appropriate treatment of ulcer, it will cause atrophic gastritis. People suffering from this inflammation are exposed to the danger of malignancy and cancer [6, 7].

Nowadays, various large-scale studies have been conducted on the correlation between genetic changes such as polymorphisms and the risk of affliction with various types of cancer [8]. Polymorphisms have their effect by increasing or decreasing the risk of a disease through several ways [9]. Polymorphisms may play a major role in making people prone to cancer [2]. Necrosis factor of an alpha tumor is an intracellular signaling protein that intervenes in the inflammatory system. The primary role of TNF is to adjust immunity cells. TNF may cause fever, an apoptotic death of the cell, and inflammation. It may also harness viral replication tumor. Failing to adjust TNF production (or lack of accurate correctness of its protein structure) may cause cancer, Alzheimer and bladder inflammation. Beta tumor necrosis factor is a multifunctional Cytokine protein mostly produced by T lymphocytes induced by mitogens or leukocytes. The range of TNF-α and TNF-β activities are identical, although the beta type has fewer capabilities. TNF-β induces interleukin-1 synthesis, collagenase and prostaglandin E2 in fibroblasts. TNF-α and TNF-β have cytotoxic and cytolytic effects on most tumor cells with the only difference being in their level of influence [10].

Dr. Hadi Ghafrani et al. (2003) conducted a research titled "Studying the role of Helicobacter pylori in Gastric Adenocarcinoma in terms of anatomic site" and found no significant difference in prevalence of Helicobacter pylori in cardiac and non-cardiac cancer (70% vs. 73.3%) [11].

A research by Shahrokh Iravani (2013) titled "gastric cancer as a multifactorial disease" found that Helicobacter pylori infection, genetic background and environmental factors such as nutrition and sanity are considered risk factors that cause gastric cancer [12].

In a research by Saito et al. (2000), the effects of eradicating Helicobacter pylori on malignancy of Gastric adenoma was studied. It was finally concluded that eradication of Helicobacter pylori may prevent gastric adenoma from developing into gastric cancer [13].

As the correlation between polymorphisms of TNF-α and TNF-β genes and Helicobacter pylori infection has not been studied so far, the present research seeks to examine the correlation between the two above said factors to find proper molecular markers for predicting and preventing gastric cancer among those suffering from this bacteria's infection.

Material and Method

This is a control-case research studying the polymorphism of TNF-α and TNF-β genes among those suffering from Helicobacter pylori infection as the case and healthy people as control group. The statistical population included all the patients with Helicobacter pylori infection resorting to Omid, Ghaem, and Imam Reza hospitals of Mashhad and private clinic of Dr. Hadi Mohammad Doust and Imam Reza Hospital of Bojnourd. As many as 31 healthy cases, 50 cases with positive Helicobacter pylori, and 23 with both gastric cancer and positive Helicobacter pylori were selected for sampling through convenient sampling method. The informed consent of the individuals was obtained, and their demographic information (including age and gender) were asked. Using patients' files fro the hospital, their histopathological information was also written in the questionnaires.

In the next phase, blood samples were made in the above-said therapeutic centers with DNA PCR and Electrophoresis tests conducted on them. Next, polymorphism of TNF-α and TNF-β genes was determined according to RFLP-Restriction Fragment Length (polymorphism) and ARMS (Amplification refractory mutation system) methods in order to determine allele frequency of genotype in case and control groups. The H. pylori level was also determined and recorded in serum samples of patients. The collected information was then analyzed using SPSS v.20.

Results

There were 31 healthy participants in control group, while 50 people with H pylori infection and 23 with gastric cancer and H pylori infection were in case group. Table 1 shows distribution of age frequency. Table 2 represents the health status of the participants. Table 3 shows distribution of age groups in terms of health status.

Table 1. Distribution of the age frequency of participants

Average age	Frequency	Percentage
20 to 35	23	22.17
35 to 50	24	23.07
50 to 65	28	26.9
65 to 80	27	25.9
Older than 80	1	0.96
Total	104	100

Table 2. Health status of the participants

Health status	Frequency	Percent
Healthy	31	29.8
Positive H pylori	50	48.1
Positive cancer and H pylori	23	22.1
Total	104	100

Table 3. Distribution of age groups according to their health status

Health status	20 to 35	35 to 50	50 to 65	65 to 80	Older than 80	Total
Healthy	2	5	12	11	0	30
Positive H pylori	10	15	9	16	0	50
Cancer and positive H pylori	11	4	7	0	0	22
Total	23	24	28	27	0	102

While analyzing the research, two participants disappeared (one healthy person and one with both diseases)

Table 4 shows the frequency distribution of genotype and alleles of -305 G>A polymorphism in TNF gene promoter (TNF-α) and 252 A>G in the first intron of LTA (TNF-β) gene among patients with Helicobacter pylori infection and healthy control people in Khorasan province of Iran.

Table 4. Frequency distribution of genotypes and alleles of -308 G>A polymorphism in (TNF-α) TNF promoter and 252 A>G in the first intron of LTA (TNF-β) gene

	H-Pylori, n=50 (%)	Healthy Controls, n=30 (%)	P Value	OR (95% CI)
Genotypes				
TNF-308 G/G	30(60.00)	23(74.2)	-	-
TNF-308 G/A	4(8.00)	3(.679)	0.88	1.12 (0.77–1.34)
TNF-308 A/A	16(32.00)	5(16.13)	0.045	2.03 (0.65–2.12)
LTA 252 A/A	27(54.00)	17(54.8)	-	-
LTA 252 A/G	13(26.00)	9(29)	0.69	1.07 (0.63–1.84)
LTA 252 G/G	10(20.00)	8(25.8)	0.59	1.07 (0.63–1.84)
Alleles				
TNF-308 G	34(68.00)	26(83.87)	-	-
TNF-308 A	20(40.00)	8(25.8)	0.02	1.96 (0.80–1.91)
LTA 252 A	40(80.00)	26(83.87)	-	-
LTA 252 G	23(46.00)	17(54.83)	0.43	0.67 (0.54–1.65)

According to Table 5, TNF-308 A/A homozygote genotype and A allele have a significant correlation with H pylori in TNF-308. As a matter of fact, there is a correlation between TNF-308 A/A and the possibility of affliction with infection ($P < 0.05$).

Table 5 represents the frequency distribution of genotype and alleles of -308 G>A polymorphisms in TNF (TNF-α) gene promoter and 252 A>G in the first LTA intron of (TNF-β) gene among patients suffering from Helicobacter pylori infection and gastric cancer and healthy control participants in Khorasan province.

According to Table 5, the correlation between TNF-308 G/A genotype and TNF-308 A/A genotype and TNF-308 A allele with affliction with gastric cancer and H pylori is significant ($P < 0.05$).

Table 5. Frequency distribution of genotype and alleles of G>A -308 polymorphisms in TNF (TNF-α) gene promoter and 252 A>G in the first LTA intron of (TNF-β) gene

	H-Pylori, n=23 (%)	Healthy Controls, n=30 (%)	P Value	OR (95% CI)
Genotypes				
TNF-308 G/G	13(56.52)	23 (74.2)	-	-
TNF-308 G/A	5(21.7)	3 (9.67)	0.004	1.98 (0.76–2.02)
TNF-308 A/A	5 (21.7)	5 (16.13)	0.043	1.77 (0.83–1.90)
LTA 252 A/A	9(68.7)	17 (54.8)	-	-
LTA 252 A/G	8 (29.6)	9 (29.03)	0.98	1.05 (0.74–1.54)
LTA 252 G/G	6 (29.6)	8(25.8)	0.069	1.17 (0.45–1.32)
Alleles				
TNF-308 G	18 (78.26)	26 (83.87)	-	-
TNF-308 A	10(43.47)	8 (25.8)	0.005	2.85 (0.56–1.71)
LTA 252 A	17 (73.9)	26 (83.87)	-	-
LTA 252 G	14 (60.86)	17 (54.83)	0.064	1.27 (0.66–1.94)

Discussion

Helicobacter pylori and polymorphisms of the genes of some cytokines such as TNF increase the level of relevant proteins and cause surface inflammation of the stomach [10]. Atrophy of the gastric mucosa is another factor that contributes to cancer [10]. Keeping in mind the fact that the next step in causing cancer is chronic gastritis, it becomes clear that inflammation and inflammatory responses play a major role in various types of gastric cancer. Thus, the present research sought to study the polymorphism of various TNF and LTA genotypes in order to find the correlation between these inflammation mediators in Iranian race in Razavi Khorasan and Northern Khorasan provinces of Iran. As for the analysis of –TNFα polymorphism, the patients were divided into the positive and negative H pylori groups. The frequency of AA and A allele of TNF gene polymorphism among the patients who have gastric cancer with positive H pylori is significantly more common than healthy people. As a result, this polymorphism has had a greater influence on the possibility of affliction with cancer among people suffering from infection. But this state has not been observed for polymorphism studied by LTA. As a matter of fact, polymorphism of this gene has had no influence on the possibility of affliction with cancer whether with infection or without it.

Various researches have pointed to the fact that genetic changes in the area adjusting cytokine genes are associated with affliction with various diseases [5, 8]. For example, a change from G to A in -308 positios in α-TNF promoter increases the density of α-TNF and affliction of the individual with various diseases including gastric cancer. Although molecular mechanisms showing how genetic polymorphism influences cytokine gene is not mostly available, different researches show that -380 polymorphism can influence the connection between transcription factors and increase TNF-α gene expression [14]. Infection with Helicobacter pylori studied in this research indicates that effect of TNF-α in the presence of A308- allele in causing cancer is much more than G308-. This result highlights the importance of this region in adjusting α-TNF gene.

In 2001, a case-control study by Macron et al. on a Portuguese population of 252 cases of gastric cancer compared to 220 people in control group investigated polymorphisms of α-TNF

promoter in -238 and -308 positions in Helicobacter pylori. An analysis of +ureA versus –ureA showed that -308 polymorphism had no significant correlation with Helicobacter pylori infection [15]. This is in line with the results of the current research. However, other researchers have recently shown that a genotype change in -308 position from α-TNF position is more prominent among those patients with positive Helicobacter pylori than those with negative Helicobacter pylori [16, 17]. Therefore, the results may have been influenced by the difference between samples. If the correlation between these polymorphisms and Helicobacter pylori and gastric cancer is proved accurately, the polymorphism of the genes studied in this research is recommended to be taken into consideration as markers and used in clinical examinations.

Conclusion

On the strength of the present research, it can be concluded that the effect of α-TNF in the presence of –A308 allele on causing cancer is much more than that of –G308. A rise in the density of α-TNF as a result of –A308 polymorphism can change the defensive reaction of the body and prepare it for gastric infections such as Helicobacter pylori.

Human Rights Statement:

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Animal Rights Statement: Nonapplicable.

Conflict of Interest Statement:

The authors have no conflict of interest.

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