The Role of the CDH1 Gene in the Incidence of Hereditary Gastric Cancer

Saleheh Jahani
Doctor of Medicine Diploma, **salehe.jahani@gmail.com**

Abstract

Introduction: Gastric cancer is a multifactorial disease, the fourth most common cancer globally and the second leading cause of cancer death. This study aimed to determine the expression of the CDH1 gene in tumor and peripheral tissue of healthy individuals with hereditary gastric cancer.
Methods: In this case-control study, 50 samples, including 25 samples from gastric tumor tissue and 25 samples from adjacent control tissue, were prepared from these patients. RNA was extracted from the target tissues. Then their cDNAs were made. Finally, the expression of CDH1 genes was measured using the Real-Time PCR technique. Paired t-test was used to analyze the amount of gene expression.
Results: In this study, in 87.5% of patients with gastric cancer, the expression of the CDH1 gene was reduced by 7.35 times compared to the healthy marginal tissue, which showed a significant difference between healthy and tumor tissue of these patients.
Conclusion: Due to the role of the CDH1 gene as a tumor suppressor gene, its expression is significantly reduced in the tumor tissue of individuals with hereditary gastric cancer.

Keywords: Gastric cancer, Multifactorial, CDH1, Tumor suppressant

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Introduction:

The genomes of all living organisms are permanently damaged by internal chemical processes in the body and by external factors such as chemicals and ionizing radiation. The present organism is protected by DNA repair from the mutagenic effects of these factors and the occurrence of cancer. Whenever a defect in the healing system is inherited, it causes cancer to develop and progress [1]. Cancer is the first and second leading cause of death in developed and developing countries. Gastric cancer is the fourth most common cancer globally and the second most common cause of cancer [2]. Gastric cancer is one of the most common malignancies worldwide. The prevalence of this cancer is due to creating a cancerous tissue in the stomach in several stages and is a part of multifactorial diseases [3]. In general, gastric cancer is a disease in older people, and the ratio of men to women is about 2 to 1 [4]. This cancer in blacks is twice as common as in whites and has the same sex ratio (male to female 2 to 1) [5]. According to 2005 statistics, the highest incidence of this cancer was observed in Japan, China and Russia, and the lowest incidence was related to the Western developed countries [6].

In the United States in the 1930s, gastric cancer was the second leading cause of cancer death among men and the third leading cause of cancer death among women, but today it is still the leading cause of death [4]. Gastric cancer is one of the most common cancers in Iran, which,

unlike in Western countries, is on the rise. The highest rate of gastric cancer in Iran has been reported in Ardabil province [7]. There is ample evidence that gastric cancer results from multiple genetic and epigenetic alterations in the genes that suppress tumorigenesis, Schneider and Schneider. There are many tumor suppressor genes in the stomach, CDH1 being one of the genes silenced by hypermethylation in gastric cancer; it has also been shown in various studies that specific epigenetic changes occur in some of the tumors of some tumor cell genes. These potential epigenetic changes may be good biomarkers for early detection and prevention and in the three areas of prognosis and treatment of cancer [8]. One to three percent of gastric carcinomas is caused by known inherited syndromes: HDGC, FAP, and Lynch. HDGC-induced gastric cancer is a predominant autosomal disease, affecting approximately 30% of individuals with an E-Cadherin-induced tumor in one of the tumor-inhibiting genes [9][10].

The E-Cadherin gene is located in region 1.22 on the upper arm of chromosome number 16 and in the distance between the base pair 68869444-68771127. This gene directs the production of a protein called epithelial cadre. Cadherin is a set of cell surface proteins that facilitate the attachment of adjacent cells to each other and prevent the formation of cellular tissue [11]. In addition to its role in cell binding, it can transmit chemical signals into the cell, control cell movement, and regulate active activity [12]. Some research has shown that the CDH1 gene acts as a tumor suppressor gene that keeps cells from growing and dividing uncontrollably. Since these proteins bind cells to help each other, it is possible to detach cancer cells from tumors, transmit them through the bloodstream and invade other tissues (metastasis) to prevent them [13]. Gastric cancer is usually diagnosed at an advanced stage and has a low chance of recovery. Our knowledge of the molecular factors of this cancer is less than that of other cancers. Although gastric cancer is still recognized as a deadly disease, the Identification of molecular, genetic, and epigenetic changes and new pharmacogenetic features improves the diagnosis and treatment of patients. And hopes that diagnostic methods and more effective treatment will be developed. Therefore, in this study, the genes in gastric cancer are effective in the Iranian population and investigated as potentially useful as a biomarker for early diagnosis of gastric cancer. Let's also answer the question of whether or not the expression of genes differs in different populations.

Method:

The present study was a case-control study based on 50 samples (25 samples from gastric cancer tissue and 25 samples from adjacent tissue), which were prepared from patients referring to Imam Khomeini Hospital in Tehran between 2009 and 2012. After preparation in liquid nitrogen and RNAase-free conditions, the samples were transferred to the laboratory and immediately stored in liquid nitrogen. The selection of patients undergoing radiotherapy, chemotherapy or immunotherapy was not used in this study. An official consent form has been obtained from all patients, and the Ethics Committee of the Cancer Institute has approved the study.

RNA extraction:

ACCORDING TO COMPANY PROTOCOL, all RNA was extracted from 100 mg tissue using Invitrogen Trizol USA Carlsbad solution.RNA purity and concentration were measured using a nanodrop spectrophotometer (Bio-Tek-USA), and its integrity was investigated with 1% agarose electrophoresis gel. RNAs were stored at 80 ° C until cDNA synthesis.

cDNA synthesis:

Synthesis of cDNA from the whole mRNA using the fermentate company kit based on the company protocol at temperatures of (25 ° C for 5 min and 42 ° C for 5 min). Concentration and purity were measured by a nano-drop device based on the above descriptions. All cDNAs were stored at 20 ° C.

Quantitative Real-time PCR :

Expression levels of this gene were measured by PCR-qRT using a USA RAD-BIO thermocycler. The reaction of CDH1 gene by method and using Takara kit (Japan, TAKARA) in 10 µL volume (5µL sybrgreen), (2µL nuclease-free H2O), (1µL cDNA), primer (1L-F), primer (1L-F) The basis of the company protocol was done. To determine the efficiency, the real-time PCR reaction with dilutions (1-1 / 0-01 / 0-001) of the primers was performed, and a standard curve slope indicating the efficiency of the primer was also used. Primer design was performed by alleleID-7 software. The properties of the primers are shown in (Table 1).

Primer name	Primer sequence	Primer length	Connection temperature
CDH1-Forward primer	AGAACGCATTGCCACAT	21	50.8
CDH1-Reverse primer	GAGGATGGTGTAAGC GATGG	20	50.8

Table 1. Primer sequence

DNA sequencing:

To confirm the identity of the PCR fragments, the region containing each band was cut from the gel, and each band was sequenced by Bioneer South Korea and compared to the Gene bank 100% similar to CDH1 for observation and confirmation of the result.

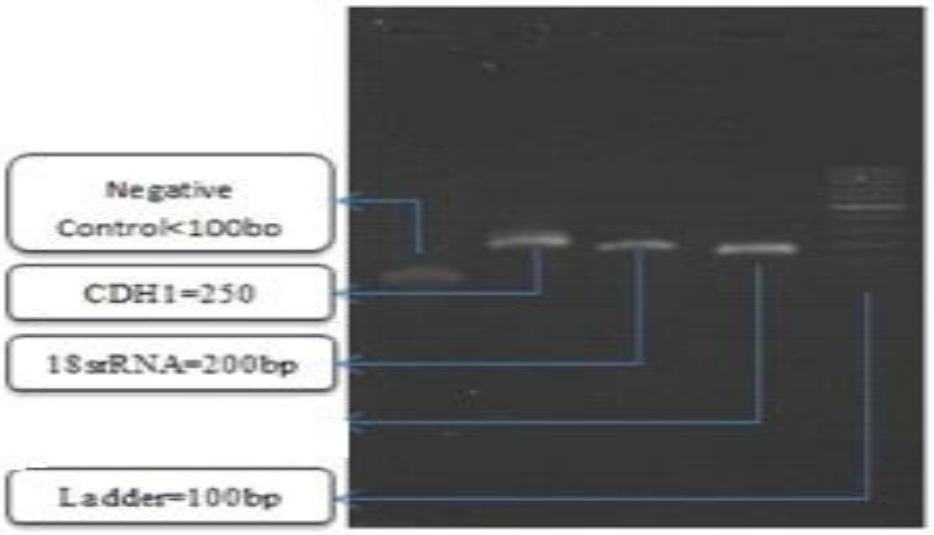

Figure 1. CDH1 gene electrophoresis

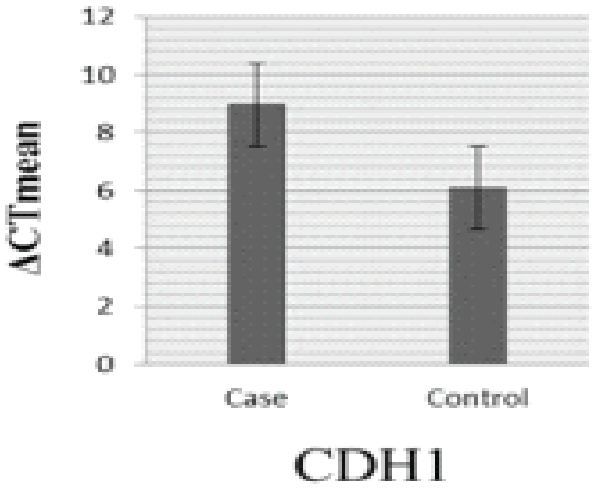

Figure 2. Frequency distribution of CDH1 gene

Gender	Age	Tumor stage	Tumor size	Vascular invasion
Man	>59	Stage I	>5	Yes
Female	<59	Stage II	<5	No

Table 2. Characteristics of the studied samples

The mean expression of CDH1 was 6.08 in healthy tissue and 8.95 in cancerous tissue. The average folding change was 0.136, which indicates a decrease in CDH1 expression in cancerous tissue compared to the adjacent healthy tissue (Figure 2). Paired t-test analysis also showed that the difference in CDH1 expression level in both healthy and cancerous textures was significantly different (P = 0.000)), in this study, in 87.5% of patients with gastric cancer in the tumor tissue, compared to the healthy marginal tissue, the expression of CDH1 gene decreased by 7.35 times and reduced significantly. ROC curve analysis was performed to evaluate the diagnostic value of CDH1 in differentiating tumor tissue samples from healthy peripheral tissue (Figure 3).

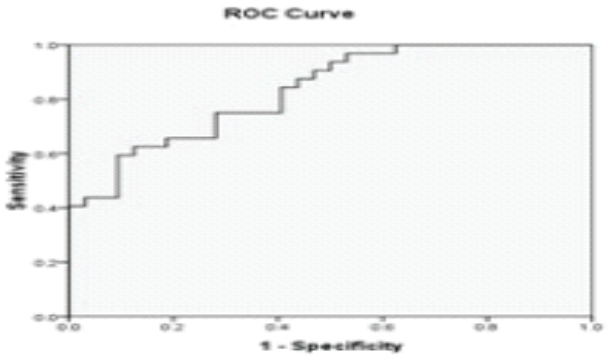

Figure 3. ROC curve in the CDH1 gene

Discussion:

Given that gastric cancer is still recognized as a deadly disease, To identify molecular markers, genetic and epigenetic changes, and characteristics of pharmacogenetics new, the state of the diagnosis and treatment of patients improves and hopes for the development of diagnostic

methods, and more effective treatment creates and increased awareness about the creation and the development of gastric cancer it becomes. In recent years, the link between RNAs and their target genes in gastric cancer has come to the fore[14]. And since research on RNA-type markers has expanded with advances in real-time-PCR techniques, especially RT-PCR, Early diagnostic methods can be sought to increase patients' chances of recovery and reduce their costs of diagnosis and treatment [3]. This study showed that in 87.5% of patients with gastric cancer, the tumor tissue was reduced to 7.35 times relative to the tissue. And tumors have been shown in these patients. Therefore, this study identified the CDH1 gene as a possible diagnostic biomarker in gastric cancer. These findings confirm most previous reports. One of the studies conducted by Shino et al. in 1995 examined the expression of E-cadherin in gastric cancer. Low expression of E-cadherin was not significantly differentiated from adenocarcinoma, and decreased E-cadherin expression was associated with increased tumor growth, distant metastasis and low survival[15]. Also, based on the study results in gastric cancer, E-cadherin expression decreased between 17% to 92% of cases[16][17]. In the present study, 87-5% of patients had reduced expression of the E-cadherin gene. In general, in a study conducted by Alzar et al. in 2008 in Romania[18]. Also, other studies [19] have reached the following conclusions: 1. The abnormal expression of E-cadherin is significantly more significant in the type of cancer spread. 2- E-cadherin's abnormal expression is associated with distant metastasis - less patient survival and more tumor spread because E-cadherin is involved in cellular binding and cellular movement control [12].

The results obtained above can be justified. These findings indicate that this biomarker can be used as a factor in disease progression and diagnosis. Despite the reports mentioned above, a study conducted by Zheng et al. In 2004 in the country concluded that cadherin-E alone has no role in gastric cancer, but its effect with MUC2 could play a synergistic role in gastric cancer [20]. Also, in another study, there was no correlation between the reduced expression of E-cadherin and sex, age, tumor site, pT, pN, pTNM, and vascular-lymphatic invasion[21]. A study conducted by Almondehri et al. In 2010 on 105 environmental blood samples was performed on methylation of CDH1, P16, P, and RUNX3 genes by pyrosequencing technique. This study showed that an increase in methylation in these genes was seen in elderly patients. In particular, an increase in the specific methylation of the promoter of these genes is seen. Finally, they concluded that general and specific DNA methylation analysis could be used as an important prognostic factor [22]. Several studies have also studied the correlation of polymorphisms of genes associated with cell proliferation and gastric cancer. In a meta-analysis of 55 research papers, it has been suggested that these polymorphisms can be used as biomarkers for low-risk

prognosis [23]. Decreased expression of CDH1 gene in the present study due to their role as a tumor suppressor that prevents cells from growing and dividing uncontrollably. On the other hand, the high sensitivity of the gene studied in the Rock curve could indicate the diagnostic value of this gene as a possible biomarker in the diagnosis of gastric cancer.

Conclusion:

The results of this study showed that the expression of the CDH1 gene in 87.5% of samples decreased 7.35 times in malignant tissues of patients with gastric cancer compared to healthy tissue; therefore, due to the role of the CDH1 gene as a tumor suppressor gene that protects cells from uncontrolled growth and proliferation, it can be used against cancer And can be considered as a diagnostic biomarker in the prognosis, diagnosis and treatment of hereditary gastric cancers. It is hoped that the results of this study will shed light on the treatment of this disease.

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