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Studying the Association of Gene Expressions and Gene Polymorphisms of MAPK14 (rs28763973) and HTR2B (rs550352538) with Gastric Cancer in Isfahan Province

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A B S T R A C T

Objective: Gastric cancer (GC) is the fourth most common cancer worldwide, occurring in approximately two-thirds of developing countries, and is one of the leading causes of cancer deaths. The mitogen-activated protein kinase 14 (*MAPK14*) plays an important role in the conversion of extracellular stimuli to a wide range of cellular responses. The 5-hydroxytryptamine receptor 2B (*HTR2B*) increases the level of 5-HT; and as a result, platelet-derived 5-HT promotes tumor angiogenesis, tumor growth, and metastatic potential of cancer cells. Materials and Methods: Expression analysis was performed on 20 healthy cases and 20 cancerous cases for *MAPK14* and 20 healthy cases and 19 cancerous cases for *HTR2B* using real-time quantitative PCR (qRT-PCR) assay. Polymorphisms were evaluated in 38 healthy cases and 30 cancerous cases for rs550352538, and 35 healthy cases and 35 cancerous cases for rs28763973 via tetra-primer amplification refractory mutation system PCR (T-ARMS PCR) technique. Results: No significant difference was observed in *MAPK14* and *HTR2B* gene expression levels (P>0.05). In addition, there is no statistically significant association of different genotypes in rs28763973 from *MAPK14* gene and rs550352538 from *HTR2B* gene with gastric cancer (P>0.05). Conclusions: No significant difference was noted between patient and healthy individuals in expression levels and gene polymorphisms; nevertheless, results may vary by significantly changing the gene pool or population size.

Introduction

About 1 in 13 people who die from cancer worldwide suffer from gastric cancer (GC). This cancer often affects the male population. The most geographical areas affected by gastric cancer are East Asia, Eastern Europe, and South America, respectively; 5.7% of all new cases of all cancers were gastric cancer. Also, the mortality is 7.7% in both sexes. GC is extremely common within the Iranian male population and is considered the main cause of death through

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cancer, according to GLOBOCAN 2020 (Sung et al., 2021). Lauren's criteria subdivide the GC into intestinal and diffuse types. *Helicobacter pylori* (*H. pylori*) infection (Sitarz et al., 2018), lifestyle (e.g., obesity, poor diet, tobacco, and alcohol), stomach polyps, genetic susceptibility, geographical area, and gender are all important triggers for GC (Rawla & Barsouk, 2019; Yeoh & Tan, 2021).

Regardless of promising advancements in the knowledge of its molecular mechanisms, the median time to survive for sufferers with advanced GC is as low as approximately 12 months (Digklia & Wagner, 2016). Eighty percent of GC patients with *H. pylori* infection have no symptoms. Cytotoxin-associated gene A (CagA) - positive *H. pylori* is linked to the elevating risks of early-onset GC and has a poor prognosis. Also, the Epstein–Barr virus (EBV) is inserted into the stomach epithelial cells and can lead to genome instability in the host nucleus (Zhao et al., 2020).

To date, screening methods for accurate and early detection of gastric cancer have not been developed. Since patients have no clear symptoms, the early detection rate of gastric cancer is low, and over 70% of patients have advanced cancer (Tan, 2019).

GC's epigenetic alterations are diverse and exhibit multiple processes such as improper DNA methylation, histone alteration, micro RNAs (miRNAs), long non-coding RNAs (lncRNAs), and RNA editing. Epigenetics has undoubtedly appeared as a novel boundary in GC research; With the development of more powerful and inexpensive technologies (e.g., the use of whole-genome sequencing and profiling of gene expression) could help to make GC subtyping more comprehensive and impartial (Padmanabhan, Ushijima, & Tan, 2017).

The 5-hydroxytryptamine receptor 2B (*HTR2B*) gene, located at 2q37.1 (Safran et al., 2021) encodes one of the several different receptors for 5-hydroxytryptamine (serotonin) and is a member of G-protein coupled receptor 1 family. Serotonin is a biogenic hormone that has three different roles, a neurotransmitter, a hormone, and a mitogen. Many of the central and peripheral physiologic signaling pathways of serotonin are mediated by its receptors, for example, regulation of cardiovascular functions and also impulsive behavior. Notably, spliced transcript variants have also been found for *HTR2B* (Pruitt, Tatusova, & Maglott, 2005). Angiogenesis is one of the most important signs of cancer. Serotonin stimulates tumor angiogenesis through the signaling pathway of serotonin receptor 1B (5-HTR1B) and serotonin receptor 2B (5-HTR2B) activation. Serotonin can also directly proliferate tumor cells and plays a significant role in cancers such as breast, prostate, ovary, lung, and colon cancer (Peters et al., 2020) by promotion of proliferation through cell cycle progression, autophagy, and suppression of apoptosis (Karmakar & Lal, 2021).

The mitogen-activated protein kinase 14 (*MAPK14*) gene, located on 6p21.31 (Safran et al., 2021), encodes a member of the protein kinases family containing a threonine/tyrosine residue with a 40–46 kDa molecular weight (Sudo, Yagasaki, Hama, Watanabe, & Osada, 2002) and are involved in cascades of cellular responses such as cell proliferation, differentiation, transcriptional regulation, and development. Proinflammatory cytokines and environmental stresses via phosphorylation by mitogen-activated protein kinase kinases (MKKs) and also it is notable that alternative splicing results in different variants of protein (Consortium, 2019; Pruitt et al., 2005). MAPK family members are four, and they are named *MAPK11*, *MAPK12*, *MAPK13*, and *MAPK14*. *MAPK14* and *MAPK11* are the most important and famous variants (Browne et al., 2016; Li et al., 2019).

This study aimed to investigate the relation of the expression pattern of the *5HTR2B* gene and *MAPK14* gene in patients with gastric cancer in the Iranian population from Isfahan; also, the second aim was to find the relation of rs28763973 from the *MAPK14* gene and rs550352538 from the *5HTR2B* gene with gastric cancer in the Iranian population from Isfahan.

2. Materials and Methods

2.1. Sampling

Some samples of stomach formalin-fixed paraffin-embedded (FFPE) tissues were collected from Al-Zahra Hospital of Isfahan, from 2016 to 2018; then, pathological confirming tests were applied in the cases and controls. A number of the total sample size were selected for each gene, and

their demographic characteristics are provided in Table 1.

Table 1- Demographic characteristics used for each gene.

Gene	Groups	N	Sex	N
MAPK14	Cases	30	Male	19 (63.33%)
			Female	11 (36.67%)
	Controls	38	Male	29 (76.31%)
			Female	9 (23.69%)
HTR2B	Cases	35	Male	23 (65.71%)
			Female	12 (34.29%)
	Controls	38	Male	25 (65.79%)
			Female	10 (34.21%)

N = Number

A series of the samples were randomly selected from both groups to analyze gene expression (20 cases and 20 controls for *MAPK14* and 19 cases and 20 controls for *HTR2B*).

2.2 RNA extraction and gene expression

The RNA was extracted from stomach FFPE tissues using the RNA Extraction kit (Pars Tous, Mashhad, Iran), which was strictly followed by the supplier's instructions. In the next step, RNA concentration was measured using nanodrop, and its integrity was determined by 1% gel electrophoresis. Finally, 10 µg of total RNA was utilized to synthesis the first cDNA strand using the cDNA Synthesize Kit (Pars Tous, Mashhad, Iran). The StepOne™ Real-time PCR System device (Applied Biosystems, USA) was employed for quantifying the amounts of cDNA using the SYBR Green-based qRT-PCR assay. Following are the steps used to perform the qRT-PCR reactions: first denaturation at 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 30 s (*18S rRNA*: 60 °C, *MAPK14*: 63.2 °C, *HTR2B*: 63.2 °C), and 72 °C for 40 s, followed by 1 cycle of final elongation at 72 °C for 10 min; in the end, the melting curve was obtained over the range 60–95 °C. *18S rRNA* gene (Ebrahimi, Kordi-Tamandani, & Arish, 2016) was used as an internal control for normalizing *MAPK14* and *HTR2B* genes; Finally, the gene expression level was acquired using the $2^{-\Delta\Delta ct}$ formula. *MAPK14* and *HTR2B* gene expression primers were adopted from the PrimerBank (PrimerBank ID: 194578904c1 and 222080048c1, respectively) (Spandidos, Wang, Wang, & Seed, 2010). The primer sequences for gene expression are listed in Table 2.

Table 2 - Primer Sequences for qRT-PCR.

Genes	Sequences (5´→3´)	Product size (bp)	Annealing temp (^{0}C)
MAPK14	F: CCCGAGCGTTACCAGAACC R: TCGCATGAATGATGGACTGAAAT	136	63.2
HTR2B	F: TGATTTGCTGCTTGGATTGTTTG R: ATGGATGCGGTTGAAAAGAGAA	173	63.2
18S rRNA	F: GTAACCCGTTGAACCCCATT R: CCATCCAATCGGTAGTAGCG	112	60

F = Forward, R = Reverse, bp = base pair

2.3. DNA extraction and gene polymorphism

Genomic DNA was isolated from stomach FFPE tissues using DNA Extraction Kit (Ziaviz, Tabriz, Iran), which has magnetics nanoparticles to capture DNA, according to the manufacturer's protocol.

T-ARMS PCR was employed for the assessment of the genotypic analysis. The conditions that performed for the technique are included: I) initial denaturation at 95 °C for 10 min; II) 35 cycles of 95 °C for 30 s, 60 °C (rs550352538) or 61 °C (rs28763973) for 40 s, and 72 °C for 5 min; and III) 1 cycle of final elongation at 72 °C for 10 min. The PCR products were validated using 2 percent agarose gel electrophoresis. Primer3 software was used to design rs550352538 and rs28763973 polymorphism primers (Sherry, Ward, & Sirotkin, 1999; Untergasser et al., 2012), which are listed in Table 3.

Table 3 - Primer sequences used in T-ARMS PCR.

SNPs		Primer sequences (5´→3´)	Product size (bp)	Tm (°C)
rs55035253 8	F inner	ACTAACCATGCTGACCACCTGT	425	60
	R inner (mutant)	ACTAACCATGCTGACCACTGC		
	F outer	TTCAAACACGACTCTACAACTAC	135	
	R inner (wild)	GATAACGTGAACAAAGGTGCTC		
rs28763973	F outer	TCGACTTGCTGGAGAAGATGCT	1011	61
	R outer	AACACAACAGAAACCAGGTGCT		
	F inner (wild)	TGCCTACTTTGCTCAGTACCAC	941	
	F inner(mutant)	TGCCTACTTTGCTCAGTACCAA		

F = Forward, R = Reverse, Tm = melting temperature, bp = base pair, SNP = Single nucleotide polymorphism

2.4. Statistical analysis

Data were analyzed using the SPSS version 25.0 (SPSS, Chicago, IL); Mann–Whitney test was performed for comparing gene expression data between patient and healthy groups. Also, the classified data of polymorphisms were obtained by Pearson's χ^2. All statistical significance levels were fixed at $P<0.05$.

3. Results

3.1. Expression Results

Our findings suggest there is no statistical difference exist in gene expression between cases and control groups in *HTR2B* and *MAPK14* genes ($p>0.05$); therefore, no notable association was obtained between the changes in mRNA level and gastric cancer; Table 4 shows statistical analysis of the gene expression results.

Table 4 - Comparison of relative gene expression for *MAPK14* and *HTR2B* between cases and healthy controls.

Gene	Groups	N	Mean ± S.D.	P-value *
MAPK14	Cases	20	9.24 ± 17.21	0.30
	Controls	20	12.06 ± 22.14	
HTR2B	Cases	19	3.26 ± 5.72	0.24
	Controls	20	2.58 ± 5.00	

N = Number, S.D. = Standard Deviation, * = obtained from Mann–Whitney U test

3.2. Polymorphism results

As Table 5 shows, according to the P-value, there is no statistically significant association of different genotypes in rs550352538 from the *HTR2B* gene with gastric cancer. In the frequency of AA, AG, and GG genotypes, there was no statistically significant difference between healthy and patient groups in rs550352538 polymorphism of the *HTR2B* gene. The frequency of A and G alleles at the rs550352538 in the *HTR2B* gene showed no significant difference between patients and controls.

Table 5 - Statistical result about the association of rs550352538 from *HTR2B* gene with gastric cancer.

	Cases (N=35)	Controls (N=35)	OR	CI (95%)	P-value *
AA	32 (91.42 %)	29 (82.85 %)	Reference	Reference	Reference
AG	2 (5.71%)	4 (11.42 %)	0.453	0.077-2.660	0.380
GG	1 (2.85%)	2 (5.71%)	0.453	0.039-5.264	0.527
AG+GG	3 (8.57%)	6 (17.14 %)	0.453	0.103-1.979	0.292
A	66 (94.28 %)	62 (88.57 %)	Reference	Reference	Reference
G	4 (5.71%)	8 (11.42 %)	0.469	0.134-1.638	0.235

N = Number, OR = Odd ratio, CI = Confidence interval; * = obtained from Pearson's $\chi 2$ test

As Table 6 shows, according to the P-value, there is no statistically significant association of different genotypes in rs28763973 from *MAPK14* gene with gastric cancer. In the frequency of AA, AG, and GG genotypes, there was no statistically significant difference between healthy and patient groups in rs28763973 polymorphism of *MAPK14* gene. The frequency of A and G alleles at the rs28763973 in the *MAPK14* gene showed no significant difference between patients and controls.

Table 6 - Statistical result about the association of rs28763973 from *MAPK14* gene with gastric cancer.

	Cases (N=30)	Controls (N=38)	OR	CI (95%)	P-value *
AA	26 (86.66 %)	33(86.84 %)	Reference	Reference	Reference
AG	4 (13.33 %)	5 (13.15%)	1.015	0.247-4.166	0.983
GG	-	-	-	-	-
AG+GG	4 (13.33 %)	5 (13.15%)	1.015	0.247-4.166	0.983
A	56 (93.33 %)	71 (93.42%)	Reference	Reference	Reference
G	4 (6.66%)	5 (6.57%)	1.014	0.260-3.954	0.983

N = Number, OR = Odd ratio, CI = Confidence interval, * = obtained from Pearson's $\chi 2$ test

4. Discussion

Our result indicated there are no statistical associations between *MAPK14* and *HTR2B* with the progression of gastric cancer. Also, based on the results, there is no statistically significant association of different genotypes in rs28763973 from *MAPK14* gene and rs550352538 from *HTR2B* gene, with gastric cancer.

Gastric cancer is a severe clinical problem with more and more new cases each year worldwide (Tan, 2019; Thrift & El-Serag, 2020). Until now, it has been one of the most occurring cancers in men and women. Scientists in different research centers try to find strategies to prevent gastric cancer development and treat it and increase the life quality of gastric cancer patients. Gastric cancer has far more acute effects in developing countries, but, improvement in hygiene and eradication of H. Pylori has notably decreased the statistics of gastric cancer in those populations (Sexton, Al Hallak, Diab, & Azmi, 2020).

There are very few therapies that provide an important benefit to surviving patients and developing their life qualities (Eusebi, Telese, Marasco, Bazzoli, & Zagari, 2020; Johnston & Beckman, 2019). Many various emerging therapies, methods, and targets are being evaluated and are at the clinical trial level. These days, as research progresses, hopefully, there will be more achievements in finding more impressive therapeutic ways to increase the survival statistics rate of this deadly cancer worldwide (Sexton et al., 2020). To develop therapies, it is good to know that there are differences in the epidemiological characteristics, clinicopathological features, tumor biological characteristics, treatment patterns, and drug designs through gastric cancer patients from the Eastern and Western countries, so there should be different methods all over the world (Wang et al., 2021).

Serotonin promotes cancer development by affecting cancer cells through different mechanisms like proliferating cells via cell cycle progression, autophagy, and apoptosis suppression. This could guide us to explore the potential of other molecules that regulate the serotonergic system in current meliorating methods or creating new therapeutic ways for cancer. This allows us to study new treatment methods in rare tumors, for example, brain tumors and sarcomas, and other tumors that there are not fine treatment methods available now (Karmakar & Lal, 2021).

MAPK14 products are signaling molecules that play an important function in the conversion of extracellular motivators to a wide variety of cellular responses. This finding will guide us to the development of inhibitors of these signaling ways and finding new cancer treatments. Also, *MAPK14* can be used as a potential target molecule to find new treatment methods for hematological malignancies, breast cancer cells, and glioblastoma (Browne et al., 2016).

It will be truly hard to find the right molecular pathology of future GCs. Until now, it is found that pathogenic germline variants may be significant risk factors in society about GC, even for those who don't have *H. pylori* infection, and it is because of their lifestyle. For example, it is proved that chronic gastritis is a result of salty and smoky foods, smoking, oxidative stresses, and chemical agents that modulate host immunity, as well as other yet unidentified factors. In these years, to all studies about the somatic and germline genetic causes of GCs, in addition to patients' lifestyles, it came to light those molecular mechanisms of both the current and also novel types of GC in the upcoming *H. pylori*-negative era are personalized (Katoh & Ishikawa, 2021).

One of the latest methods in cancer treatment is gene therapy. As we know, cancer is a disease with a lot of genetic changes in its regulation; therefore, by modifying particular genes such as specific tumor suppressor genes, we could find new treatment approaches for gastric cancer. In addition, it should be known that immunotherapy is another important cancer treatment approach in these years. It is so obvious that in the near future, the development of new drugs and gene therapy methods will improve Treatment approaches in diseases such as cancer in a profitable way (García-Hernández et al., 2022).

In this study, we examined the role of the genes of interest from two aspects (SNP and gene expression); in its kind, the information reveals a relatively vivid outline of the genetic and epigenetic status of gastric cancer in our population study. The results showed that there was no significant difference in the expression patterns of the *MAPK14* and *HTR2B* genes between patients and healthy subjects. Based on the results, there is no statistically significant association of different genotypes in rs28763973 from *MAPK14* gene and rs550352538 from *HTR2B* gene with gastric cancer; nevertheless, results may vary by significantly changing the gene pool or population size. The phenotypic effect of gene polymorphisms is always affected by genetic and environmental factors, and also gene-gene interactions.

Conflict of interests

The authors declared no conflict of interests.

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Ethical consideration

Informed consent was obtained from all human adult participants and the parents or legal guardians of minors in Al-Zahra Hospital in Isfahan, Iran.

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