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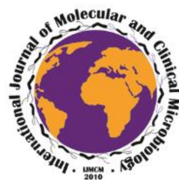

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# Evaluation of long non-coding RNA n34560, AF147447 and SNHG8 genes expression levels in gastric cancer patients and their association with *Helicobacter pylori* and Epstein-Barr virus infections

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ABSTRACT

The occurrence of gastric cancer is associated with numerous aspects, including the host's lifestyle and genetic history. Understanding gastric cancer molecular mechanisms can improve our insight into the early diagnosis, prognosis, and treatment. In this study, the RNA level of *SNHG8*, *AF147447*, and *n34560* genes in gastric tumor tissues was investigated and their association with *Helicobacter pylori* and Epstein-Barr virus infections was evaluated. Formalin-fixed paraffin-embedded (FFPE) tissues (100 samples), including 50 samples of gastric cancer tissues and 50 samples of healthy tissues were taken. The expression level of *SNHG8*, *AF147447*, and *n34560* genes in gastric cancer and control tissues were examined using the qRT-PCR technique. A significant association was observed between the expression level of *the SNHG8* gene in gastric tumor tissues compared to the healthy tissues ($P$=0.0003). Relative expression of *AF147447* and *n34560* genes did not show any significant difference among gastric tumor tissues compared to the normal tissues ($P$=0.2984, $P$=0.9158). In addition, pathological comparison of clinical data with the expression of *SNHG8, AF147447,* and *n34560* genes did not show any significant association in tumor and healthy tissues, but the expression level of *AF147447* gene in *Helicobacter pylori* infection ($P$=0.0458) and expression level of *n34560* gene in Epstein-Barr virus (EBV) infection ($P$=0.0362) showed significant association. In conclusion, we found a significant association between *SNHG8* gene expression levels and the possible cancer incidence. Also, a significant association was observed between the expression of *n34560* and *AF147447* genes relating to *H.pylori* and EBV infections in gastric cancer.

## 1. Introduction

Gastric cancer (GC) is recognized as the fourth common cancer and the third factor of death resulting from cancer worldwide (Siegel, Miller, & Jemal, 2016). Global distribution of this kind of cancer in geographical areas varies. The incidence of this cancer is higher in Asia than in American and European countries (Savabkar et al., 2013). The mean overall survival in GC patients remained smaller than one year (Camidge et al., 2012; Jemal et al., 2011; Locasale et al., 2011). Thus, study on the GC mechanisms such as reproduction, growth, migration, invasion, and apoptosis may greatly help to treat this disease (Deng, Wang, Guo, & Xia, 2016; Liz & Esteller, 2016).

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Long non-coding RNAs (lncRNAs) are a new group of non-coding RNA with a length of at least 200 nucleotides (Meller, Joshi, & Deshpande, 2015). Interestingly, growing research has found that lncRNAs play a role in tumorigenesis by acting as tumor suppressors or oncogenes. The biological purpose of lncRNAs, as well as their potential as diagnostic and prognostic biomarkers, have received increasing attention in recent years (Autuoro, Pirnie, & Carmichael, 2014; Maruyama & Suzuki, 2012). Although an increasing number of dysregulated lncRNAs have been discovered as variables that may affect the prognosis of cancer patients, the majority of lncRNAs remain unknown. Several studies have shown that dysregulated lncRNA expression has a role in GC. Small nucleolar RNA host gene 8 (*SNHG8*) is a member of the small nucleolar RNA host genes (SNHGs) family, with a length of 1062 nucleotides. *SNHG8* is overexpressed in malignancies and promotes cancer progression (H. Song, Song, Lu, & Li, 2019; P. Zhang, Li, Chen, Lu, & Zhang, 2020; Zhen et al., 2019). *SNHG8* was shown to have elevated expression in cancer cells in gastric cancer, and its knockdown inhibited cancer cell growth and invasion in vitro (P. Zhang et al., 2020). *SNHG8* expression was similarly increased in colorectal cancer tissues and cells, according to Zhen et al. (Zhen et al., 2019).

Furthermore, *SNHG8* knockdown had a clear inhibitory effect on colorectal cancer cell proliferation, migration, and invasion (Zhen et al., 2019). The functions of *SNHG8* in controlling NPC growth, however, are unknown. On the other hand, H. pylori infection affects a large percentage of GC patients. As a result, a thorough investigation of the mechanism and subsequent development of new targeted therapeutics for *H. pylori* infection-related GC is required (Osman, Bloom, & Tagoe, 2013; Zhu, Liu, Xu, Zhang, & Dai, 2015). Through lncRNA regulation, H. pylori infection may promote GC.

Zhou et al. 2016 discovered that *H. pylori* infection reduced the expression of lncRNA AF147447, which inhibits GC proliferation and invasion in vitro and in vivo and acts as a tumor suppressor in the formation of *H. pylori* driven GC (Zhou et al., 2016a). Hence, measuring the expression levels of these lncRNAs in combination with *AF147447* and *n34560* should help better predict the prognosis of *H. pylori* patients. Gastric cancer continues to be one of the leading causes of cancer death and health concerns around the world [20]. Traditional gastric cancer therapy options based on radical surgery, on the other hand, are not yet satisfactory. As a result, the discovery of the mechanisms underlying the occurrence and progression of stomach cancer is gaining traction in cancer research (Guo et al., 2009).

The purpose of this study was to look at the involvement of small nuclear RNA *SNHG8*, long non-coding RNA *AF147447*, and *n34560* in the pathogenesis of gastric cancer, and to see if there was any association between these genes and the infections caused by *H. pylori* and Epstein-Barr virus (EBV).

## 2. Materials and Methods

### *2.1. Study type and population*

In this case study, formalin-fixed paraffin-embedded (FFPE) tissues; 50 samples of individuals with GC, and 50 samples of healthy tissues were provided by Aramesh Lab, Tehran, Iran. The pathologist performed laboratory analysis for confirmation of GC tumor and non-tumor in all patients. The inclusion and exclusion criteria for the control group study were individuals were identified by observing the non-morphological changes under the microscope and without the family history of cancer. Demographic and clinicopathological information was provided through patient files and pathology reports. Patients and those under chemotherapy or radiation therapy were excluded. The Ethics Committee of the Islamic Azad University, Lahijan Branch (Ethic ID; IR.IAU.LIAU.REC.2021.072), approved this study.

### *2.2. Selection of the genes*

Previously identified lncRNAs molecular epidemiologic studies were carefully reviewed. The lncRNAs *SNHG8*, *AF147447*, and *n34560* were selected based on certain criteria which previously demonstrated association with cancer, especially in correlation with *H. pylori* and EBV infections.

### *2.3. H.pylori and EBV identifications*

The FFPE specimens were sliced into thin slices and placed in xylene at room temperature for 10 minutes until being cleaned twice in 99 percent ethanol. After drying the samples at room temperature, adding digestion buffer and Proteinase K, and centrifuged for 10 minutes at 14000 rpm, the supernatant was used for DNA extraction by the QIAamp DNA mini kit (QIAGEN-Hilden, Germany) according to the manufacturer's instructions. Procedures for EBV detection was adjusted according to prior reported technique (Hassani, Khan, & pathology, 2015). A 129 bp fragment of the EBNA-1 gene region was amplified to establish the occurrence of EBV in tumor tissue by the forward EBV, 5′-CCA GAC AGC AGC CAA TTG TC-3′ and reverse 5′-GGT AGA AGA CCC CCT CTT AC-3′ primers (Cui et al., 2011). In the PCR experiments for the 16S rRNA Region primers HP 1 .1 (5'-3' sequence CTG GAG ARA CTA AGY CCT CC, where) and HPX2 (5'-3' sequence GAG GAA TAC TCA TTG CGA AGG CGA,) were used with HP2.1 as a probe (5'-3' sequence ATT ACT GAC GCT GAT TGY GC.

Each individual's template DNA (1µL) was combined with 10µL Takara Mix and 1µL primers. An initial denaturation stage at 95 °C for 5 minutes was followed by 39 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds, and 72 °C for 30 seconds, with a final extension step at 72 °C for 3 minutes. To detect *H. pylori* strain in FFPE specimens from GC patients’ specific primers were used targeting 16S rRNA gene according to prior reported technique (Scholte, Van Doorn, Quint, & Lindeman, 1997).

*2.4. RNA extraction and cDNA synthesis*

Total RNA was extracted from the samples using the RNeasy DSP FFPE RNA extraction kit (Qiagen Co., Germany). The RNA concentration was quantified by a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies) and its quality was measured by the A260/A280 and A260/A230 ratios. The concentrations of the samples were normalized and 1µg of total RNAs were reverse transcribed to cDNA using the Revert Aid RT kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) according to the manufacturer's instructions.

*2.5. Quantitative real-time PCR*

qPCR was performed using a PCR cycler (Rotor-Gene Q MDx; Qiagen GmbH). The cDNA fragments were used as templates to amplify the *SNHG8*, *AF147447*, and *n34560* genes using SYBR® Premix Ex Taq™ (Takara Bio, Inc.), according to the manufacturer's protocol. The experimental protocol was performed as follows: an initial activation step for 30 sec at 94˚C; then, 35 cycles at 95˚C for 10 sec and 60˚C for 40 sec; and melting curve analysis. The primer sequences were designed using GeneRunner Software and then checked with Primer-BLAST (NCBI) for their specificity. The primer sequences are listed in Table 1. The *B2M* gene was used as a normalizer endogenous gene. The $2^{-\Delta\Delta Cq}$ method was used to determine the expression fold changes (patient vs. normal).

*2.6. Statistical analysis*

The SPSS 17.0 (SPSS Inc., Chicago, IL., USA) for windows was used to analyze data. The mean expression changes analysis and graph drawing were performed using Prism 8.0 software and data were evaluated using student’s t-test. The P-value<0.05 was considered as a significant difference.

**Table 1.** Primer sequences used for the Real-time PCR analysis

| No | Gene Name | Primer | Sequence(5'to3') | Primer size | GC% | Tm C˚ |
|---|---|---|---|---|---|---|
| 1 | *SNHG8* | Forward | AAGTTTACAAGCATGCGCGG | 20 | 50 | 60 |
| | | Revers | TCAAACTGACGGTTCTCGGG | 20 | 55 | 60 |
| 2 | *AF147447* | Forward | TCCTCTAATGCGTCTTGTCTCC | 22 | 50 | 59.57 |
| | | Revers | CCCATACCAAACTCTAACCACC | 22 | 50 | 58.38 |
| 3 | *n345630* | Forward | TCCGTTGAACCTTCCACAGT | 22 | 50 | 59.57 |
| | | Revers | ACTCTGCTCCGTTCCACATT | 22 | 50 | 58.38 |

## 3. Results

### *3.1. General characteristics*

In the present study, 50 samples from the patients with GC with the mean age of 60.18 ± 13.65 years old; 22.55 ± 4.9 body mass index and 50 samples from healthy individuals with the mean age of 36.72 ± 14.86 years old; 26.21 ±5.15 body mass index were studied. General information and demographic data of the studied patients include age, gender, smoking, stage, grade, tumor size, *H. pylori,* and EBV infections, which are shown in Table 2.

### *3.2. SNHG8, AF147447 and n34560 gene expression between tumor tissues and healthy tissues*

The *SNHG8* gene expression indicated a significant difference between tumor tissues and healthy tissues. Its expression in the tumor group increased significantly compared to the normal tissue (95% CI=1.357 to 4.428, *P*=0.0003) (Figure 1A). The *AF147447* and *n34560* genes expressions did not indicate any significant difference between tumor tissues and healthy tissues with 95% CI=-1.598 to 5.124, *P*=0.2984 and 95% CI=5.392 to -4.846, *P*= 0.9158, respectively (Figures 1B and 1C).

### *3.3. Relative expression of SNHG8, AF147447 and n34560 genes in sample tissues infected with H.pylori and HBV*

#### *3.3.1. SNHG8* gene

The results of comparing the *SNHG8* gene expression between grades I & II and between grades II & IV did not show any significant association (*P*=0.5622) (Figure 2A). Comparison of the expression of *SNHG8* gene in stages I & II with stage II (*P*=0.5363) and stage IV (*P*=0.8015), as well as stage III with stage IV (*P*=0.2749), did not indicate any significant association (Figure 2B). Tumors were divided into two groups of smaller than 5 cm and bigger than 5 cm. According to the results, no significant difference was observed between *SNHG8* gene expression level and tumor size (*P*=0.8641) (Figure 2C). According to the analysis, no significant association was observed between *SNHG8* gene expression and *H. pylori* and EBV infections (*P*=0.0988, *P*=0.3676). (Figures 2D and 2E).

#### *3.3.2. AF147447 gene*

The relative expression of the *AF147447* gene demonstrated no significant difference between grades I & II vs. III & IV (*P*=0. 9269) (Figure 2F). Comparing the RNA expression of *AF147447* gene in groups stages I & II vs. stage III (*P*=0.0706) and stages I & II vs. stage IV (*P*=0.2839) as well as stage III vs. IV (*P*=0.27) did not show any significant association (Figure 2G). Also, no significant association was identified between the transcript level of *the AF147447* gene and tumor size (*P*=0.7755) (Figure 2H). Based on the analysis, a significant association was observed between *AF147447* gene expression level and *H. pylori* positive and negative infections (*P*=0.0458) (Figure 2I). While no significant association was observed between *AF147447* gene expression and EBV infection (*P*=0.2938) (Figure 2J).

#### 3.3.3. *n34560* gene

The results of comparing the expression of *the n34560* gene between grades I & II vs. grades III & IV did not indicate any significant association (*P*=0.5237) (Figure 2K). In addition, comparing the expression of *the n34560* gene in the stages I & II vs. stage III highlighted significant association (*P*=0.0385), but the stages I & II vs. IV (*P*=0.9528), as well as stage III vs. IV (*P*=0.0782), did not indicate any significant association (Figure 2L). Also, no significant difference was obtained between the expression level of the *n34560* gene and tumor size (*P*=0.7812) (Figure 2).

According to more analysis, a significant association was observed between *n34560* gene expression and EBV infection (*P*=0.0362). Nevertheless, no significant association was observed between *n34560* gene expression and *H. pylori* positive and negative infections (*P*=0.8792).

### *3.4. Evaluation of SNHG8 gene expression in tumor tissues as a biomarker*

The receiver operating curves (ROC) and area under the curve (AUC) were constructed for 50 tumors and 50 healthy samples to study *SNHG8* gene characteristics as the potential marker for GC. The ROC curve

analysis indicated AUC=0.7357 and 95% CI=0.6374 to 0.8339, $P$=0.001 for the *SNHG8* gene.

For the patients and normal tissue group, the degree of specificity was 71.43% and susceptibility was 67.92% (Figure 3).

**Table 2.** Demographic variables of the study population

| Characteristics | | Sex | | | Mean age at DX (years) | Risk factors | | | Tumor size | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | M/F ratio | | Smoking | *H. pylori* infection | EBV infection | ≤ 5 cm | >5 cm |
| Total controls | | 39 (78%) | 11 (22%) | 3.5 | 36.72 ± 14.86 | 12 (24%) | - | - | - | - |
| Total patients | | 41 (82%) | 9 (18%) | 4.5 | 60.18 ± 13.65 | 10 (20%) | 28 (56%) | 6 (12%) | 29 (58%) | 21(42%) |
| Tumor stage | I | 5 (12.2%) | 4 (44.4%) | 1 | 52.67 ±12.46 | 3 (33.3%) | 5 (62.5%) | 1 (11.1%) | 7 (77.8%) | 2 (22.2%) |
| | II | 2 (4.9%) | - | 2 | 69.50 ± 14.84 | - | 1(50%) | 1(50%) | 1 (50%) | 1 (50%) |
| | III | 17 (41.5%) | 4 (44.4%) | 4.25 | 59.81 ± 15.22 | 2 (9.5%) | 13 (61.9%) | 1 (4.8%) | 10 (47.6%) | 11(52.4%) |
| | IV | 17 (41.5%) | 1 (11.1%) | 17 | 63.33 ± 11.31 | 5 (27.8%) | 9 (50%) | 3 (16.7%) | 11 (61.1%) | 7 (38.9%) |
| Tumor grade | I | 3(7.3%) | 4 (44.4%) | 0.75 | 51.14 ± 12.70 | 2 (28.6%) | 4 (66.7%) | 1 (14.3%) | 6 (85.7%) | 1(14.3%) |
| | II | 16 (44.4%) | 2 (22.2%) | 8 | 59.89 ± 14.17 | 4 (22.2%) | 11 (61.6%) | 3 (16.7%) | 7 (38.9%) | 11 (61.1%) |
| | III | 22 (53.7%) | 3 (33.3%) | 7.3 | 62.92 ± 12.9 | 4 (16%) | 13 (52%) | 2 (8%) | 16 (64%) | 9 (36%) |

M, male; F, Female; M/F, Male to Female

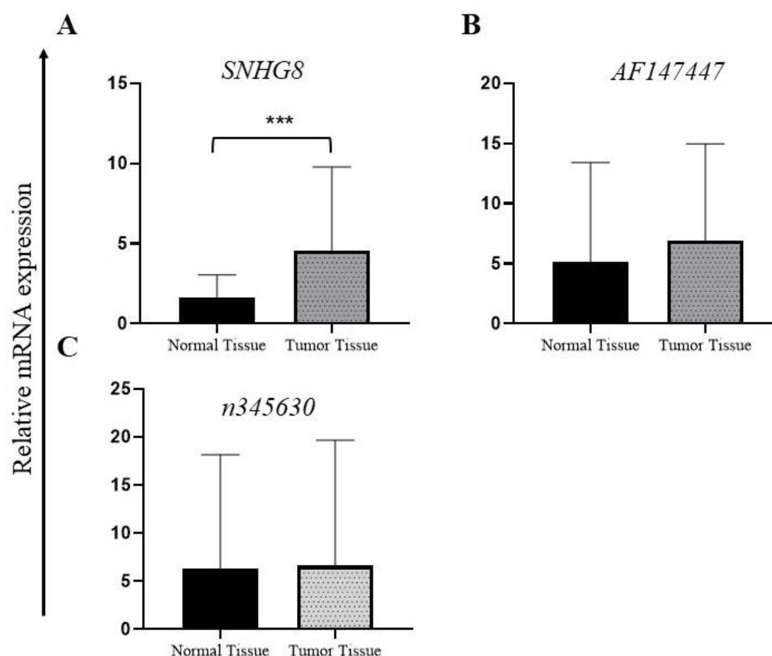


**Figure 1.** A comparison of the gene-relative expressions between the tumor tissue and the normal tissues. (A) *SNHG8* -relative expression, (B) *AF147447*-relative expression, (C) *n345630*-relative expression. *$P$<0.05, **$P$<0.01, ***$P$<0.001

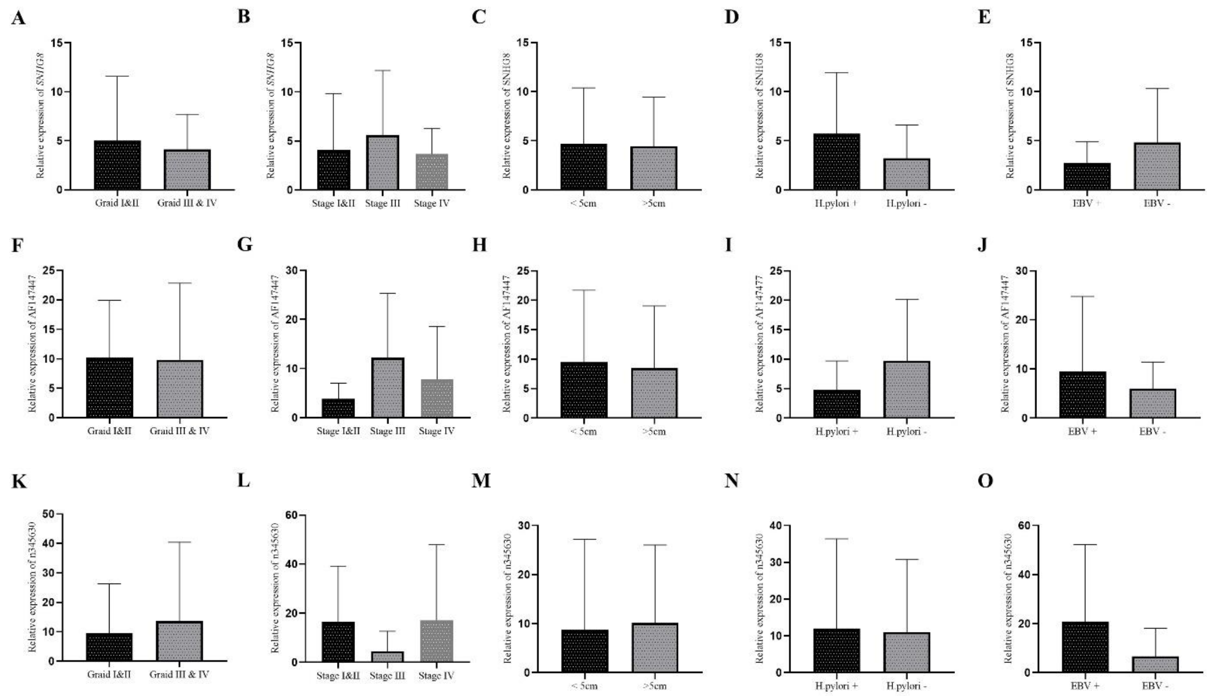


**Figure 2.** Relative RNA expression between the *SNHG8*, *AF147447* and *n345630* genes with clinicopathological feature, *H.pylori* and HBV infections.

(A) Relative expression of *SNHG8* between the different grade of gastric cancer tissues, (B) Relative expression of *SNHG8* between the stage of the tumor tissue, (C) Relative expression of *SNHG8* between the two-tumor size group ≤ 5, > 5 cm, (D) Relative expression of *SNHG8* between the negative and -positive H. pylori patients, (E) Relative expression of *SNHG8* between the negative and -positive EBV infection, (F) Relative expression of *AF147447* between the different grade of gastric cancer tissues, (G) Relative expression of *AF147447* between the stage of the tumor tissue, (H) Relative expression of *AF147447* between the two-tumor size group ≤ 5, > 5 cm, (I) Relative expression of *AF147447* between the negative and -positive H. pylori patients, (J) Relative expression of *AF147447* between the negative and -positive EBV infection, (K) Relative expression of *n345630* between the different grade of gastric cancer tissues, (L) Relative expression of *n345630* between the stage of the tumor tissue, (M) Relative expression of *n345630* between the two-tumor size group ≤ 5, > 5 cm, (N) Relative expression of *n345630* between the negative and -positive *H. pylori* patients, (O) Relative expression of *n345630* between the negative and -positive EBV infection.

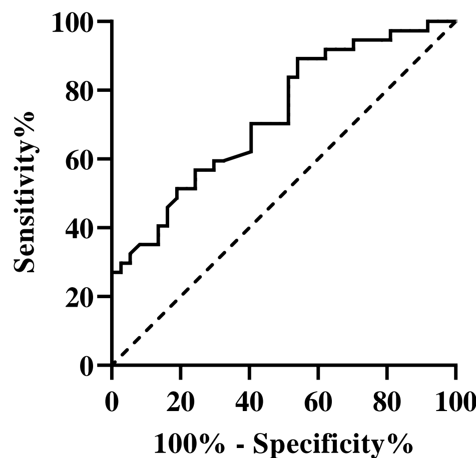


**Figure 3.** The receiver operating curves (ROC), area under the curve (AUC) in 50 tumor tissues and 50 healthy samples for *SNHG8* gene

## 4. Discussion

Our results indicated that *SNHG8* gene expression in gastric tumor tissue increased significantly compared to this gene expression in normal tissue. Similar results were achieved by Dong et al., (2018) who found that *SNHG8* gene expression significantly increased in cancer tissues and had a positive association with tumor recurrence, but it had no relation with other paraclinical characteristics (Dong et al., 2018).

As a tumor suppressor gene, LncRNA *SHNG8* is down regulated in the colon and gastric cancer. (Huang et al., 2016a; Siprashvili et al., 2016; Zhao et al., 2016). Following that, Kaplan-Meier data revealed that *SHNG8* expression was inversely associated with overall survival in pancreatic cancer patients. As a result, *SHNG8* was predicted to represent a unique prognostic marker for pancreatic cancer (Y. Song et al., 2018).

The risk of gastric cancer (GC) from birth to 74 years old is 1.87% in men and 0.79% in women worldwide. GC is the 4th most common cancer in men and 7th most common in women, with mortality rates varying depending on diet, *H. pylori* infection, Epstein–Barr virus (EBV) infection, and habitat (He et al., 2016; Rawla & Barsouk, 2019). The most common reasons for GC occurrence are *H. pylori* infection and the major predisposing factors include excessive salt consumption, smoking, and family genetic background. Early prevention such as *H. pylori* eradication is recommended (Choi et al., 2018).

lncRNA plays a role in various stages of tumor formation and could be used as a new diagnostic and therapeutic target (Chandra Gupta & Nandan Tripathi, 2017). Some studies have discovered that lncRNA plays a pathogenic function in gastric cancer. Fattahi et al. (2020) investigated novel biomarkers for diagnosing and managing GC treatment, they proposed that lncRNAs could be used as therapeutic targets for GC. The lncRNAs such as *PVT1, UCA1, HOTAIR, H19*, and *LINC00152* can provide potential diagnosis and prognosis in patients with GC (Fattahi et al., 2020). Therefore, recognizing new lncRNAs related to the tumor growth and invasion can greatly help to personal treatment of GC. T. Yang et al., (2016) studied the role of lncRNAs in GC. They observed that lncRNAs applied changes in GC and played an important role in this cancer. They also observed that lncRNAs decrease or increase in *H. pylori*-positive patients serum and their overexpression in the presence of *H. pylori* leads to an increase in the risk of GC prevalence (Q.-Q. Yang, Deng, & pathology, 2014). In another study, it was indicated that lncRNAs *H19* and *C00152* can be used as biomarkers for the diagnosis and prognosis of GC mostly for the cases with *H. pylori* infection (Q.-Q. Yang et al., 2014). Thus, a study on various lncRNAs roles in GC will help profoundly to diagnose this kind of cancer. In this regard, identifying new lncRNAs related to the growth and invasion of tumors can greatly help to diagnosis, prognosis, and treatment of individuals with GC.

Most importantly, previous studies were performed on the lncRNAs role in GC patients infected with EBV. Huang and colleagues studied the lncRNA pattern in GC relative to EBV. They found that the expression of *SNHG8, RP11-359D14.3, H19 RNU12*, and *Mir-143* in GC infected with EBV had an increasingly significant association compared to the GC non-infected with EBV (Huang et al., 2016b). In vitro and in vivo studies indicated that knockdown of *SNHG8* lncRNA by hairpin RNA leads to the inhibition of cell proliferation and colony formation in GC related to EBV (Liu et al., 2018). According to Tao Huang et al. study, *SNHG8* interacts with the EBV proteins LF3, BHLF1, BHRF1, and BNLF2a, and modulates the expression of TRIM28, EIF4A2, NAP1L1, PLD3, RPL18A, and TRPM7, TRIM28, EIF4A2, NAP1L1, PLD3, RPL18A, and TRPM7 were found to have direct roles in gastric cancer after functional analysis. This contributes to a better understanding of carcinogenesis by revealing the regulatory roles of lncRNAs and viruses in gastric carcinoma (Huang et al., 2016a). But in our study, there was no significant association between *SNHG8* gene expression and *H. pylori* and EBV infections.

*H. pylori* is a Gram-negative motile-curved bacterium that causes stomach cancer, peptic ulcers, gastritis, and mucosa-associated lymphoid tissue lymphoma (MALT) [38, 39]. Despite the strong relationship between *H. pylori* infection and the growth of gastric cancers, the mechanisms underlying this process

are not completely understood (Yousefi et al., 2015; Y. Zhang et al., 2019).

A recent independent study by Zhou et al. determined how *H. pylori* infection led to the decreased expression of the *AF147447* gene and increased malignancy of GC. Therefore, a significant association was observed between the expression of the *AF147447* gene and *H. pylori* infection. These findings were similar to the ones we found in our research. The impact of *H. pylori* on the lncRNA *AF147447* gene plays a key role in the progression of GC. Some studies imply that *H. pylori* infection can alter the expression of lncRNAs. For example, Zhou et al indicated that the *AF147447* gene can be related to the E2F1 transcription factor and its expression may be affected by E2F1. The *E2F1* is a transcription factor that is expressed during *H. pylori* infection and plays a key role in the suppression of the infection (Zhou et al., 2016b).

Furthermore, according to the research by Zhu et al., (2015), lncRNAs of *XLOC-004122* and *XLOC-014388* in *H. pylori*-positive patients had decreased expression, and following the deletion of *H. pylori* infection these lncRNAs showed increments (H. Zhu et al., 2015). It was discovered by Zhu et al., that the expression of *n345630*, *XLOC-004787*, *LINC00473*, and *n378726* genes decreased in the GC patients with positive *H. pylori* infection It was discovered by Zhu et al., that the expression of *n345630*, *XLOC-004787*, *LINC00473*, and *n378726* genes decreased in the GC patients with positive *H. pylori* infection (Dastmalchi, Khojasteh, Nargesi, Safaralizadeh, & disease, 2019). According to a previous functional study by Yang et al., long non-coding RNAs (lncRNAs) showed different expressions in GC. They found that *H19* and lncRNAs were reregulated in *H. pylori*-positive patients' serum and that their overexpression in the presence of *H. pylori* was linked to an elevated risk of GC(T. Yang et al., 2016). They concluded that *H19* and *LINC00152* genes can be used as biomarkers for the diagnosis and prognosis of GC, especially in the cases where *H. pylori* infection is present (Ghafouri-Fard, Esmaeili, Taheri, & Pharmacotherapy, 2020).

AF147447 expression was evaluated after *H. pylori* co-culture with GC cells, expression was reduced in three different gastric epithelial cells (Zhou et al., 2016a). Also, the expression of *AF147447* in *H. pylori*-positive gastric tissues was studied in mice. *AF147447* expression was low during infection, and the lncRNA *AF147447* inhibited cell growth and migration both in vitro and in vivo (Zhou et al., 2016a). Also, our results showed that *AF147447* gene expression in the patients' tissue was significant compared to the *H. pylori* infection. Studies indicated that *H. pylori* infection leads to increased malignancy of GC following the decrease in lncRNAs *AF147447* gene expression (Zhou et al., 2016b).

In addition, the results of our study showed that *n34560* gene expression had a significant association between the stages I & II vs. III and a significant association between expression of *n34560* gene and EBV infection in GC, studies indicated that lncRNAs *n34560*, *XLOC-004787*, *n378726*, and *LINC00473* in *H. pylori*-positive stomach tissues exhibited decreased expression(H. Zhu et al., 2015). Also, in the epithelial cells (GES-1) infected with *H .pylori*, 21 and 23 lncRNAs showed decreased and increased expression, respectively (Hsieh et al., 2015).

Zhao et al. 2016 found that *n345630* lncRNA had lower expression in *H. pylori* -positive stomach tissues. Unfortunately, the mechanism of action of this gene is still indistinct. Therefore, due to significant changes, this gene can be maybe able used as a suitable biomarker for improving GC diagnosis strategies.

We acknowledge that our study has several limitations. Firstly, we suggest that cellular mechanisms and other sub-units of *SNHG8*, *AF147447*, and *n34560* be investigated. Secondly, *AF147447* and *n34560* have only been studied in a few research, which limits our discussion because we cannot fully compare our findings to those of other ethnic groups. Furthermore, there are restrictions on specific information about our healthy controls and patients' lifestyles, which limit our results. Thirdly, due to the small sample size in our study, more research is needed to confirm the clinical importance of *SNG8*, *AF147447*, and *n34560* in genes in gastric cancer patients.

## Conclusion

The outcome of this study indicated significant changes in the cancer tissues by the

increased *SNHG8* gene expression compared to the non-tumor tissues. In addition, our results on *the AF147447* gene implied a significant association of *AF147447* gene expression level with *H.pylori*. Additionally, a significant association was obtained between the expression level of *n345630* gene expression and EBV infection and the disease stage.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**Refereces**

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