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PREX2 gene's expression in gastric antral epithelial cells of patients with *H. pylori* infection

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ABSTRACT – **Background** – The Prex2 protein is a member of the Rac family proteins that belongs to small G proteins with a critical role in cell migration, cell proliferation, and apoptosis through its effects on PI3K cell signaling pathway and phosphatase activity of PTEN protein. The effect of *PREX2* gene expression has been shown in some cancer cells. A survey of *PREX2* gene expression in gastric antral epithelial cells of gastric cancer patients with *Helicobacter pylori* various genotypes infection can conduct to better understanding *H. pylori* infection's carcinogenesis. **Methods** – In a case-control study, *PREX2* gene expression was evaluated in gastric antral biopsy samples on four groups of patients referred to Sanandaj hospitals, including gastritis with (n=23) and without (n=27) *H. pylori* infection and gastric cancer with (n=21) and without (n=32) *H. pylori* infection. Each gastric biopsy sample's total RNA was extracted and cDNA synthesized by using Kits (Takara Company). The *PREX2* gene expression was measured using the relative quantitative real-time RT-PCR method and ΔΔCt formula. **Results** – The *PREX2* gene expression increased in gastric antral biopsy samples of gastritis and gastric cancer patients with *H. pylori* infection (case groups) than patients without *H. pylori* infection (control groups) 2.38 and 2.27 times, respectively. The patients with *H. pylori vacA s1m1* and *sabB* genotypes infection showed a significant increase of *PREX2* gene expression in gastric cancer antral epithelial cells. **Conclusion** – *H. pylori vacA s1m1* and *sabB* genotypes have the positive correlations with *PREX2* gene expression in gastric antral epithelial cells of gastritis and gastric cancer patients.

Keywords – Gastritis; gastric cancer; *PREX2*; *Helicobacter pylori*; virulence genes; real-time RT-PCR.

INTRODUCTION

Helicobacter pylori is the most common cause of human chronic bacterial infection with a high global prevalence[1]. On average, since past until now, *H. pylori* has infected over half of the world's population and causes various stomach diseases, including gastritis, peptic ulcers, and gastric cancer[1]. 15% of the people with *H. pylori's* chronic active infection progress to gastric cancer[2]. The prevalence of *H. pylori* infection and gastric cancer in countries wordwide has been reported differently[2]. The rate of *H. pylori* infection and gastric cancer prevalences are high in Iran, a country in the Middle East, and have been reported up to 69% and 1% of the total Iranian population, respectively[2]. Concerning *H. pylori* complicated interaction with the gastric antral epithelial cells, knowing the cellular and molecular mechanisms of the *H. pylori* long-term survival in the human stomach and causing cancer are still the most controversial issues[3,4].

H. pylori has a dozen virulence factors, including toxins, enzymes, and adhesins, that mediate bacterial pathogenesis[5]. The VacA (vacuolating cytotoxin A) and CagA proteins are the *H. pylori* main secretory virulence factor[5]. Numerous studies show that the *cagA* gene, located in the *H. pylori* cag pathogenicity islands, codes a pathogenicity critical determining factor, mainly involved in gastric epithelial cell damage, including tumorigenesis[6,7]. The VacA triggers apoptosis's internal pathway in gastric epithelial cells by releasing cytochrome C from the mitochondria[8,9]. The previous studies have shown antagonistic effects between VacA toxin and cagA protein effects through host cell proliferation changes[10]. *H. pylori* adhesin agents involve a wide range of bacterial surface proteins, including HopQ, HopS (BabA), HopC (AlpA), HopH (OipA), HopP (SabA), HopZ that bind the bacterium to the host cell[11,12]. An exciting aspect of the *H. pylori* infection is a persistent and long-term infection in interaction with the host mucosal immune system through regulating and altering its adhesin proteins' expression[11,13]. As a case in point, the interaction of SabA protein and its ligand increases *H. pylori* colonization[14]. SabB protein, another sab allele, is a homolog of SabA protein that its inactivation is associated with the duodenal ulcer but not the gastric ulcers[15].

H. pylori triggers a set of cell signalings, ultimately cause pathogenesis by changing the host cell skeleton and the expression of proinflammatory genes[9,16-18]. A complicated network of intermediate molecules has been identified as the gastric epithelial cells messengers that involve response to *H. pylori* infection[16]. Prex (phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor) proteins belong to the large family of GEFs (guanine nucleotide exchange factors) that regulate the Rho/Rac family of GTPs that control cell signaling using the phosphatidylinositol 3-kinase (PI3K) pathway[19]. The *PREX2* gene is located on chromosome 8 (8q13.2) in cancer and metastasis locus that its genes control cell proliferation and cell migration[19]. The previous studies show that Prex proteins are involved in several neoplasms' physiology[20-24].

Considering the high prevalence of gastric cancer and *H. pylori*

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infection in Kurdistan province, located in the West of Iran, and the known role of the *PREX2* gene in regulating cell proliferation, the survey *PREX2* gene expression in gastric antral epithelial cells of gastric cancer patients with *H. pylori* infection can shed more light on the *H. pylori* carcinogenesis.

METHODS

A case-control study was designed using Cochran's sampling formula and gastric carcinoma patients' prevalence in Sanandaj city[25,26]. 53 gastric carcinoma patients and 50 gastritis patients were introduced to the study, including 21 and 23 gastritis and gastric carcinoma patients with *H. pylori* infection as the case groups. The patients without *H. pylori* infection were considered as the control groups. After diagnosing diseases by a gastroenterologist, the patient selection was made by a sequential and available method. Patients treated with antibiotics, alcohol, and tobacco consumption was excluded from the study. After receiving patients' consents and observing medical ethics, demographic characteristics, including age, sex, and geographic locations, were registered in the questionnaires. Diagnosis of *H. pylori* active infection was performed using the urease breath test. A gastric antral epithelial cell specimen was collected from each patient referred to Tohid and Shahid Ghazi hospitals of Sanandaj city for 18 months from September 2018 to March 2019. The gastric antral epithelial cells biopsy specimen of each patient was dropped in 1cc RNALater solutions vials (Roche Co.). The biopsy specimens were transferred to the Department of Microbiology research laboratory, Kurdistan University of Medical Sciences.

The *H. pylori* virulence and *PREX2* gene primers were designed by primer 3 online software (TABLE 1 and 2). For each gastric antral biopsy sample, total RNA was extracted. *H. pylori* virulence and *PREX2* genes cDNAs were synthesized using the Takara kits. The PCR method was used with *vacA s2* genes' specific primers to confirm *H. pylori* cDNAs. After sequencing, the *vacA s2* PCR product was registered in GenBank with accession number MK642592.1. The *H. pylori* virulence genes cDNAs profile was detected using specific primers and the Gradient Thermocycler PCR method (Biorad company, Germany). PCR master mix for each sample was included buffer 10X (2.5 microlitre), DNA Taq polymerase 5 U/microliter (0.25 microlitre), dNTPs 10 mM (0.5 microlitre), $MgCl^2$ 50 mM (1 microlitre), cDNA (2 microlitre), forward and reverse specific primers 10 picoliter (each one 0.5 microlitre) and RNase-free water (17.75 microlitre) in final volume 25 microlitre. The thermal cycling PCR steps were involved an initial denaturation at 94°C for 5 minutes, a denaturation at 94°C for 30 seconds, a primer annealing for 45 seconds (primers temperatures have been shown in TABLE 1), an extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes.

The *PREX2* and GAPDH (reference gene) genes' expression in gastric antral biopsy samples of gastritis and gastric carcinoma patients with and without *H. pylori* infection were evaluated by the Corbett real-time PCR machine (rotor gene 6000 software) and Takara kit (One Step PrimeScriptTM RT-PCR) based on manufactures. Real-time PCR master mix was included buffer 10X (2.5 microlitre), DNA Taq polymerase 5 U/microliter (0.25 microlitre), dNTPs 10 mM (0.5 microlitre), $MgCl^2$ 50 mM (1 microlitre), cDNA (2 microlitre), forward and reverse specific primers 10 picolitre (each one 0.5 microlitre), RNase-free water (11.75 microlitre) and SYBR Green 1 microlitre in final volume 20 microlitre. The PCR Thermocycler machine program was involved an initial denaturation at 94°C for 5 minutes, a denaturation at 94°C for 30 seconds, a primer annealing for 45 seconds (the temperatures have been shown in TABLE 2), an extension at 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. The denaturation through extension step was repeated for 30-35 cycles. Fold changes of *PREX2* gene's expression in case and control groups were determined using the comparative real-time RT-PCR method and ΔΔCt formula[27-29].

TABLE 1. The forward and reverse specific primers of *H. pylori* virulence genes with significant effects on *PREX2* gene expression in patients' gastric antral epithelial cells.

Specific primers	Sequence	Anealing (°C)	product length (bp)	Ref
vacA s1/s2 F *vacA* s1/s2 R	ATGGAAATACAACAAACACAC CTGCTTGAATGCGCCAAAC	55	259/286	Atherton et al.[9]
vacA m1/m2 F *vacA* m1/m2 R	TGGATAGTGCGACTGGGTTT TCCATGCGGTTGTTGTTGTT	54	205/220	this study
sabA F *sabA* R	TCTCTCGCTTGCGGTATCAT AGCTCAATGTTGTTGGCGTT	56	204	this study
sabB F *sabB* R	GCATTCAAACGGCGAACAAC TCCTGTGCAGTTCCCATCTT	56	248	this study

TABLE 2. Forward and reverse specific primers *PREX2*.

Specific primers	Sequence (5'->3')	Anealing (°C)	product length (bp)	Ref
PREX2	F: CACCCCGAACCTAATGCTCA R: TCTGTGTTCTTCCGTCCTCC	59	191	this study
GAPDH	F: GAAGGTGAAGGTCGGAGTCAAC F: CAGAGTTAAAAGCAGCCCTGGT	59	71	this study

Statistics

First, the relationship between patients' qualitative demographic variables was examined by chi-square tests, including Fisher's exact[26]. To evaluate statistical relationships between *H. pylori* virulence genes cDNA and the *PREX2* gene expression, the Pearson and Spearman tests were used to measure normal and abnormal distributions of the *PREX2* gene ΔΔCt, respectively[26]. The statistical relationships between *H. pylori* infection and patients groups were evaluated using Mann-Whitney and Kruskal-Wallis tests. Similarly, the statistical relationship between *PREX2* gene expression and *H. pylori* virulence genes subgroups were investigated[26].

RESULTS

This study's results included statistically significant correlations related to demographics and gene expression in patients' cells. Concerning patients' demographics, gender and aging were associated with the incidence of gastric cancer. TABLE 3 shows a significant relationship between gastric cancer incidence among men in the Kurdistan province (P=0.024). As expected, the incidence of gastric cancer increased with age, so that the highest incidence of gastric cancer was in the age group of 76 to 85 years and the lowest incidence was in the age group of 18 to 30 years (P=0.000). In our study, no statistically significant relationship was observed between clinical outcomes and *H. pylori* infections (P=0.513). Our results showed no significant correlations between *H. pylori* infection and patients' demographic data (TABLE 4).

TABLE 5 shows the correlations between *PREX2* gene expression and demographic data of patients. *PREX2* gene expression showed a statistically significant difference between men and women (P=0.036). Also, the expression of the *PREX2* gene in gastric antral epithelial cells of patients with *H. pylori* infection was twice as high as cells without *H.pylori* infection (P=0.016). The Kruskal-Wallis statistical test results in TABLE 6 show no statistical relationship between *H. pylori* virulence genes cDNA subtypes with *PREX2* gene expression in gastric antral epithelial cells (P>0.05). The results of *H. pylori* genotypes showed an increase in *PREX2* gene expression in gastric antral epithelial cells of individuals infected with *vacA s1m1* and *sabB H. pylori* genotypes infection (TABLE 7).

TABLE 3. Demographic characteristics of patients and correlation of demographic variables.

	Gastritis	Gastric cancer	Total	*P*-value
H. pylori infection (positive/negative)	23/27	21/32	44/59	0.513
Sex (male/female)	24/26	37/16	50/53	0.024
Age group (18–30/31–45/46–60/61–75/76–80)	10/18/17/5/0	0/1/14/29/9	10/19/31/34/9	0.000

TABLE 4. Demographic characteristics of patients with *H. pylori* infection.

H. pylori infection (n: 103)	Sex (n)		Age group (n)				
	Male	Female	18–30	31–45	46–60	61–75	76–85
H. pylori infection (positive)	28	16	2	11	14	16	1
H. pylori infection (negative)	33	26	8	8	17	18	8
Total	61	42	10	19	31	44	9

TABLE 5. Correlation of *PREX2* gene expression in gastric antral epithelial cells of gastritis and gastric cancer patients with patients' demographic characteristics.

	Sex	Age group	Disease	*H. pylori* infection	Gastric cancer area	Tumor grade
PREX2 correlation	0.207	-0.049	-.0.104	-0.239	-0.002	0.202
P-value	0.036	0.620	0.269	0.016	0.991	0.146

TABLE 6. Correlation between *PREX2* gene expression in gastric antral epithelial cells of gastritis and gastric cancer patients with *H. pylori* virulence genes using Spearman test.

	vacA s1m1/ s1m2	cagA	cagA-EPEAYC	cagT	cagY	cagE	sabA/B	babA2/B	hopQI/II	alpA/B	oipA	iceA1/2
PREX2 correlation	0.378	-0.096	-0.152	-0.154	-0.199	-0.153	-0.477	-0.147	0.065	0.133	0.043	0.027
P-value	0.013	0.533	0.336	0.318	0.196	0.321	0.015	0.341	0.675	0.390	0.784	0.862

TABLE 7. Analysis of the relationship between sex, *H. pylori* infection, expression of *vacA* and *sab* genes of *H. pylori* variables with the expression of *PREX2* gene in gastric antral epithelial cells of gastritis and gastric cancer patients with and without *H. pylori* infection using Mann-Wittny test. Gene expression changes in different subtypes were calculated using the ΔΔCt formula.

		N	Mean rank	Z	*P*-value	Mean ΔCt *prex2*	Fold change
Sex	Male	61	46.89	-2.094	0.036	8.7078	1.39
	Female	42	59.43			9.1843	
H. pylori infection	Positive	44	43.76	-2.417	0.016	8.3275	2.00
	Negative	59	58.14			9.3307	
vacA	s1m1	19	17.00	-2.477	0.013	7.5536	2.57
	s2m2	25	26.68			8.9157	
SabA/B	Negative	12	15.17	-2.339	0.000	9.1438	5.52
	sabB	11	8.55	–	–	6.6775	–
	sabA	17	17.53	–	–	7.2796	–
	sabB	11	9.82	-2.423	0.007	6.6775	3.64

DISCUSSION

The results of the present study show a significant relationship between gastric cancer and gender and age group. In previous studies, the prevalence of this kind of cancer was higher in men[2,30]. The results of a review study by Forman et al. showed that the cases of gastric cancer in the age group of 60–80 years, and men were twice as high as women[1]. Although *H. pylori* is the main cause of gastric cancer and gastritis, the present study results showed no significant relationship between gastric cancer and gastritis with active *H. pylori* infection.

Based on previous studies' results, increased expression of the *PREX2* gene has an essential role in cancer and can be a biomarker in diagnosis and prognosis trends[20-24]. Although our results showed that the expression of the *PREX2* gene is increased in patients with grade 2 gastric cancer, there was no statistically significant relationship between tumor grade and expression of the *PREX2* gene (data has not shown). Prex2 plays a vital role in regulating Wnt/β-catenin signaling in GC cells so that inhibition of Prex2 inhibits Wnt/β-catenin signaling in gastric cancer[21,22]. In the current study, the relationship between *PREX2* gene expression and age and sex of patients was investigated, but no significant relationship was observed. However, the expression of the *PREX2* gene in people with gastritis and *H. pylori* infection is 2.38 times higher than in people with gastritis without *H. pylori* infection, that is supporting result to relevant previous studies on bacterium's role in cell cycle interference, apoptosis, and gastric epithelial cell proliferation. On the other hand, increasing of the an increasing of PREX2 gene expression by 2.27 times in cancer samples with *H. pylori* infection compared to cancer samples without *H. pylori* infection can support the role of *H. pylori* infection in increasing of the *PREX2* gene expression.

H. pylori infection increases the expression of PRDX2 by increasing NF-κB activity[31]. The study by Wang et al. shows that PRDX2 protects DNA damage and cell death against *H. pylori* oxidative stress and promotes gastric cancer[31]. A study by He et al. in 2016 showed that the *PREX2* gene upregulation leads to the proliferation and migration of hepatocellular carcinoma cells via PTEN-AKT signaling and the *PREX2* gene had increased in transcriptional and protein levels compared to expected[22]. A study by Li et al. in 2017 showed that the function of the *PREX2* gene in hepatocellular carcinoma is high in 54.9% of carcinoma specimens, and Its mRNA level was high in tumor tissues and adjacent tumors[23]. A study by Yang et al. in 2016 on examining the *PREX2* gene's characterization showed the proliferation, invasion, and cell migration in pancreatic cancer controlled by the PI3K signal pathway[20]. Their study showed that repeated expression levels of *PREX2* in cancer patients showed a sharp increase compared to normal tissues[20]. Berger et al. also reported recurrent mutagens of the *PREX2* gene in a 2012 study of squamous cell carcinoma genome sequencing[19]. They showed that the expression of various mutagens derived from the *PREX2* gene facilitated cancerous tumors' formation and involved the progression and severity of cancer and the proliferation of associated cells[19].

H. pylori active infection causes inflammation, which is a early stage the tumor begins[32,5,7]. *H. pylori* activate multiple cellular pathways in epithelial cells including Gsk3B, PI3K/AKT, NF_kB, MAPK, and Wnt/B_Catenin, which produce inflammatory cytokines, alter cell proliferation, differentiation, and cell apoptosis, which eventually leads to cellular apoptosis. Become cancerous[7].

According to our study results, the cDNA of *Helicobacter pylori vacA* and *sabB* genes concludes the expression of the *PREX2* gene in gastric antral epithelial cells. The previous studies concluded that the intensity of *vacA* and *cagA* genes' expression in *H. pylori* was consistent with gastritis severity in patients[6,10].

Various studies have shown that sabB gene concludes more expression in inflamed tissues than in healthy tissues and will cause pathogenesis[13,15]. On the other hand, the *sabB* gene's off-status is associated with duodenal ulcers but is not associated with gastric ulcers, and SabB is less common in cancers and lymphomas[13]. During *H. pylori* infection, the amount of Sab glycoprotein is strongly associated with the extent of tissue damage, which is consistent with our study's results[13]. Jonge and et al. in 2004 have reported a significant relationship between gastritis and duodenal ulcer with SabB protein expression; an odds ratio of less than one with the significant predictive value for duodenal ulcer[15]. In contrast, SabA status is associated with intestinal metaplasia and gastric cancer but is negatively associated with duodenal ulcers[14]. According to previous studies, it can be concluded that the expression of the sabB gene in the stomach of patients with gastritis and gastric

cancer infected with *H. pylori* infection increases the expression of the *PREX2* gene of gastric epithelial cells, which is consistent with the results of our studies[13,15].

In conclusion, *H. pylori* increases tumorigenesis in gastric epithelial cells through increasing *PREX2* gene expression.

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Authors' contribution

Hedayati MA: chief and executor of research projects; corresponding author; conception and design; analysis and interpretation of the data; drafting of the article. Ahmadi S: collecting samples and the data. Servatyari K: drafting of the article. Sheikhesmaeili F: clinical consultant and preparation of samples.

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RESUMO – **Contexto** – A proteína Prex2 é membro das proteínas da família Rac que pertencem a pequenas proteínas G com um papel crítico na migração celular, na proliferação celular e na apoptose através de seus efeitos na via de sinalização celular PI3K e atividade fosfatase da proteína PTEN. O efeito da expressão genética *PREX2* tem sido mostrada em algumas células cancerosas. Um levantamento da expressão genética *PREX2* em células epiteliais antrais gástricas de pacientes infectados com vários genótipos de *Helicobacter pylori* pode conduzir a um melhor entendimento da carcinogênese da infecção por *H. pylori*. **Métodos** – Em estudo de caso-controle, a expressão genética *PREX2* foi avaliada em amostras de biópsia antral gástrica em quatro grupos de pacientes encaminhados aos hospitais de Sanandaj, incluindo gastrite com (n=23) e sem (n=27) infecção por *H. pylori* e de câncer gástrico com (n=21) e sem (n=32) infecção por *H. pylori.* O RNA total de cada amostra de biópsia gástrica foi extraído e cDNA sintetizado por meio de kits (Takara Company). A expressão genética *PREX2* foi medida utilizando-se o método RT-PCR em tempo real quantitativo relativo e a fórmula **ΔΔ**Ct. **Resultados** – A expressão genética *PREX2* aumentou em amostras de biópsia antral gástrica de pacientes com gastrite e câncer gástrico com infecção por *H. pylori* (grupos de casos) em relação aos sem infecção por *H. pylori* (grupos de controle) 2,38 e 2,27 vezes, respectivamente. Os pacientes com infecção por genótipos *H. pylori vacA s1m1* e *sabB* apresentaram um aumento significativo da expressão genética *PREX2* em células epiteliais antrais de câncer gástrico. **Conclusão** – Os genótipos *H. pylori vacA s1m1* e *sabB* têm correlações positivas com a expressão genética *PREX2* em células epiteliais antrais gástricas de pacientes com câncer gástrico e gastrites.

Palavras-chave – Gastrite; câncer gástrico; *PREX2*; *Helicobacter pylori;* genes de virulência; RT-PCR em tempo real.

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