Original Article

Endoscopic Screening for Precancerous Lesions of the Esophagus in a High Risk Area in Northern Iran

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is a major health problem in many developing countries, including Iran. ESCC has a very poor prognosis, largely due to late diagnosis. As a first step in developing an early detection and treatment program, we conducted a population-based endoscopic screening for ESCC and its precursor lesion, esophageal squamous dysplasia (ESD), in asymptomatic adults from Golestan Province, northern Iran (a high-risk area for ESCC) to evaluate the feasibility of such a program and to document the prevalence and risk factor correlates of ESD.

Methods: This cross-sectional study was conducted among participants of the Golestan Cohort Study (GCS), a population-based cohort of 50,000 adults in eastern Golestan Province. Randomly selected GCS participants were invited by telephone. Those who accepted were referred to a central endoscopy clinic. Eligible subjects who consented were asked to complete a brief questionnaire. Detailed information about selected risk factors was obtained from the GCS main database. Endoscopic examination with was performed with Lugol's iodine staining; biopsies were taken from unstained lesions as well as the normally stained mucosa of the esophagus, and the biopsies were diagnosed by expert pathologists according to previously described criteria.

Results: In total, 1906 GCS subjects were invited, of whom only 302 (15.8%) were successfully enrolled. Esophagitis (29.5%) and ESD (6.0%) were the most common pathological diagnoses. Turkmen ethnicity (adjusted OR = 8.61; 95%CI: 2.48-29.83), being older than the median age (OR = 7.7; 95% CI: 1.99–29.87), and using deep frying cooking methods (OR = 4.65; 95%CI: 1.19-18.22) were the strongest predictors for ESD. There were significant relationships between esophagitis and smoking (p-value<0.001), drinking hot tea (*P* value = 0.02) and lack of education (*P* value = 0.004).

Conclusion: We observed a low rate of participation in endoscopic screening. The overall prevalence of ESD was 6.0%. Developing non-endoscopic primary screening methods and screening individuals with one or more risk factors may improve these rates.

Key words: Esophageal squamous cell carcinoma, endoscopic screening, northern Iran

Cite this article as: Roshandel G, Khoshnia M, Sotoudeh M, Merat S, Etemadi A, Nickmanesh A, Norouzi A, Pourshams A, Poustchi H, Semnani S, Ghasemi-Kebria F, Noorbakhsh R, Abnet C, Dawsey SM, Malekzadeh R. Endoscopic Screening for Precancerous Lesions of the Esophagus in a High Risk Area in Northern Iran. *Arch Iran Med*. 2014; **17(4):** 246 – 252.

Introduction

Esophageal cancer is the 8th most common cancer and the 6th most common cause of death from cancer worldwide.[1] High incidence rates of esophageal cancer have been reported across central Asia - from northern Iran to north-central China.[2–4] Esophageal squamous cell carcinoma (ESCC) is the most common histological type of esophageal cancer, especially in the developing world.[5,6] ESCC typically occurs by progression from a precursor lesion: esophageal squamous dysplasia (ESD).[7,8] Similar risk factors may affect the development of ESD and ESCC,[9] including genetic susceptibility,[10,11] environmental factors,[12,13] drinking alcohol,[14] smoking tobacco,[14,15] opium consumption,[15] drinking hot tea,[16] low socioeconomic status[17] and poor oral hygiene.[18]

One potentially effective method for controlling ESCC is to detect and eliminate ESD, preventing its progression into ESCC. This is the main aim of ESCC screening programs. Endoscopy with Lugol's iodine staining of the esophageal mucosa, called Lugol's chromoendoscopy, has been used in ESCC screening programs in different populations.[19–21] During chromoendoscopy, normal esophageal mucosa turns brown (iodine-positive) and dysplastic lesions remain unstained (iodine-negative), referred to as unstained lesions (USLs).

The Golestan Province, located in northeastern Iran, is a high-risk area for ESCC.[4,22] Most ESCC cases present at advanced stage, resulting in a poor prognosis with a 5-year survival of only 3.3%[23] Therefore, identification of earlier stage lesions by screening programs may potentially decrease the burden of this disease and improve health in this region. Endoscopic ESCC screening has been successfully conducted in several high-risk areas.[24] As a first step in developing an early detection and treatment program in Golestan, the aims of this study were to assess the feasibility of a population-based endoscopic screening program and to docu-

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Accepted for publication: 29 January 2014

ment the prevalence and risk factor correlates of ESD in asymptomatic adults from the Golestan Province.

Materials and Methods

This was a cross-sectional study conducted on a randomly selected sample of asymptomatic participants of the Golestan Cohort Study (GCS). The design and methods of the GCS have been described elsewhere.[25] Briefly, it is a prospective population-based cohort of 50,045 individuals, aged 40–75 years at baseline, in the eastern half of Golestan Province, Iran. It was primarily designed to investigate risk factors for upper gastrointestinal cancers.

For endoscopic screening, we aimed to randomly select about 300 GCS subjects, stratified on age group (< 50, 50–70, > 70 years) at GCS baseline (2004–2008), gender, and ethnicity (Turkmen or non-Turkmen). The first step of the screening project was telephone invitation. The initial telephone invitation lists included 2,000 randomly selected GCS participants. On telephone call, the aim of the study was explained briefly and the subjects were invited to participate. Subjects who accepted to participate in the study were picked up at their homes by drivers and taken to Atrak Clinic, a referral clinic for upper gastrointestinal diseases in eastern Golestan Province.

At Atrak Clinic, people with dysphagia, esophageal varices, cardiovascular disease, coagulopathies, severe pulmonary disease, cirrhosis of the liver, hypersensitivity to iodine solution, or a history of malignant disorders, myocardial infarction or stroke in the past 6 months were excluded. More detailed information about the project, including the procedures, risks, and benefits were provided to eligible subjects and informed consent was obtained. Subjects were then asked to complete a brief questionnaire including data on current age and gender. Detailed information about additional variables, including ethnicity (Turkmen, non-Turkmen), oral health, cooking methods, smoking, opium use, body mass index (BMI), tea temperature, and level of education, was obtained from the GCS main database. The methods of data collection in the GCS have been described previously.[16,18,25]

After completing the questionnaire, the subjects were transferred to the endoscopy room and administered Midazolam for sedation. Endoscopic examination of the esophagus, stomach and duodenum was performed, and 20cc of 1.4% Lugol's iodine was sprayed on the esophageal mucosa to identify abnormal lesions. Biopsies were taken from all USLs and from two normally-stained sites in the mid-esophagus. Endoscopic findings were recorded on endoscopic exam forms. Endoscopic biopsies were oriented on filter paper, placed in 70% ethanol and transferred to the pathology lab. The biopsies were processed to paraffin blocks, slides were prepared and stained with hematoxylin and eosin, and the biopsies were reviewed by expert pathologists. Histological diagnosis was made using previously described criteria[26] and the Vienna classification of gastrointestinal epithelial neoplasia.[27] Subjects with clinically important lesions, including moderate and severe dysplasia, were evaluated further, and appropriate therapeutic procedures were provided.

Adverse events during the endoscopic examination were recorded and reported using structured adverse events forms. The subjects' satisfaction with the endoscopic examination was assessed using a visual scale. Subjects were asked to select one of six scores, where 1 and 6 represented the best and worst satisfaction levels, respectively.

Based on their median current age, the participants were divided into younger (< 53 years) and older (≥ 53 years) age groups. They were also divided by the median of decayed, missing and filled teeth (DMFT) into those with good (DMFT < 17) and poor (DMFT ≥ 17) oral health. BMI was used to categorize the participants into normal (BMI < 25) and overweight/obese (BMI ≥ 25) groups. USLs were classified as small (less than 5 mm) or large (equal or more than 5 mm) lesions.

Multivariate logistic regression analysis was used to assess the relationship between different variables and the worst biopsy diagnosis of esophagitis or esophageal squamous dysplasia. Crude and adjusted odds ratios with 95% confidence intervals were calculated. *P* values of less than 0.05 were considered significant.

The study protocol was reviewed and approved by the Ethics Committees of the Digestive Disease Research Institute (DDRI) of Tehran University of Medical Sciences (TUMS) and Golestan University of Medical Sciences (GOUMS).

Results

In total, 1906 GCS subjects were successfully called by telephone, 346 (18.2%) agreed to come to Atrak Clinic for endoscopic screening, and after exclusions, 302 subjects (15.8%) were successfully enrolled in the screening program. The 302 subjects participating in the screening had a mean age (±SD) of 54.8 (±7.7) years and a median age (range) of 53 (42–80) years. 128 (42.4%) were male, and 108 (35.8%) were of Turkmen ethnicity.

All 302 subjects completed the endoscopic screening exams successfully. Adverse events were reported in 8 subjects (2.6%), including agitation after Lugol's staining (1 case), chest pain after endoscopy (1 case), vomiting after endoscopy (4 cases), and cutaneous rashes (2 cases). The adverse events were not clinically significant, and all of them were resolved without treatment. The majority of subjects were satisfied with the endoscopic examination: 52.3%, 34.1%, 8.9%, 3.0%, 1.7% and 0% of participants selected satisfaction levels 1, 2, 3, 4, 5, and 6, respectively.

USLs were found on endoscopic examination of 108 subjects (35.8%). The size of lesions was recorded in 100 of these subjects with USLs, including 41 subjects (41%) with only small (<5 mm) USLs and 59 (59%) with at least one large (≥5mm) USL. In 66 (61.1%) of these 108 subjects, the USLs appeared as single lesions, and in 42 (38.9%), they were multiple. Most USLs (92.5%) were found in the middle and lower thirds of the esophagus.

The subjects' worst histologic diagnoses are shown in Table 1. Esophagitis (29.5%) and ESD (6.0%) were the most common diagnoses. The distribution of ESD by age and gender is shown in Table 2. The prevalence of ESD increased with age and was similar in men and women. Out of the 18 subjects with ESD, none had dysplasia in the upper third, 16 (88.9%) had dysplasia in the middle third, and 2 (11.1%) had dysplasia in the lower third of the esophagus. The worst histologic diagnosis in four subjects was high-grade (moderate or severe) dysplasia, of whom three were female and their age ranged between 53 and 66 years.

The correlation of the pathological diagnoses and the endoscopic appearances of the endoscopic biopsies sites is shown in Table 3. ESD was found in 15/76 (19.7%) of large USLs (≥5 mm), 0/55 (0.0%) of small USLs (< 5 mm), and 10/299 (3.3%) of biopsies of normal-staining mucosa. The mean size of USLs was also significantly larger in lesions with a diagnosis of ESD (19.1 ± 1.5 mm) than in those with a diagnosis of esophagitis (6.5 ± 0.6 mm)

Table 1. The worst biopsy diagnosis of the 302 participants in the endoscopic screening study in the Golestan Province, Iran

Histological diagnosis	Count	Percentage
Normal	181	59.9
Basal cell hyperplasia	5	1.7
Mild Esophagitis	66	21.9
Moderate Esophagitis	16	5.3
Severe Esophagitis	7	2.3
Atypical changes indefinite for dysplasia	9	3.0
Mild Squamous Dysplasia	14	4.6
Moderate Squamous Dysplasia	2	0.7
Severe Squamous Dysplasia	2	0.7
Total	**302**	**100.0**

Table 2. Number (N) and proportion (%) of participants with esophageal squamous dysplasia (ESD), by age and gender, in the endoscopic screening study in the Golestan Province, Iran

Age groups (years)	Men		Women		Total	
	All	ESD N (%)	All	ESD N (%)	All	ESD N (%)
< 50	31	1 (3.0)	54	2 (3.7)	85	3 (3.5)
50–59	55	3 (5.5)	84	4 (4.8)	139	7 (5.0)
60–69	33	3 (9.1)	29	3 (10.3)	62	6 (9.7)
≥ 70	9	0 (0.0)	7	2 (28.6)	16	2 (12.5)
Total	**128**	**7 (5.5)**	**174**	**11 (6.3)**	**302**	**18 (6.0)**

Table 3. Correlation of the biopsy diagnoses and the Lugol's staining patterns in 302 asymptomatic adults from the Golestan Province, Iran

Histological diagnosis	Lugol's staining pattern						Total	
	Normal Staining		Unstained Lesions (USLs)					
			Small* USLs		Large* USLs			
	N	%	N	%	N	%	N	%
Normal	216	72.2	34	61.8	32	42.1	282	65.6
Basal cell hyperplasia	2	0.7	2	3.6	3	3.9	7	1.6
Mild Esophagitis	56	18.7	11	20.0	15	19.7	82	19.1
Moderate Esophagitis	9	3.0	4	7.3	6	7.9	19	4.4
Severe Esophagitis	1	0.3	2	3.6	3	3.9	6	1.4
Atypical changes indefinite for dysplasia	5	1.7	2	3.6	2	2.6	9	2.1
Mild Squamous Dysplasia	7	2.3	0	0.0	10	13.2	17	4.0
Moderate Squamous Dysplasia	3	1.0	0	0.0	2	2.6	5	1.2
Severe Squamous Dysplasia	0	0.0	0	0.0	3	3.9	3	0.7
Total	299	100.0	55	100.0	76	100.0	430	100.0

* Small USLs: < 5mm; Large USLs: ≥ 5mm

or normal mucosa (6.8 ± 1.0 mm) (*P* value < 0.001). In the multivariate analysis, Turkmen ethnicity (adjusted OR = 8.61; 95% CI: 2.48–29.83), being older than the median age (adjusted OR = 7.7; 95% CI: 1.99–29.87) and using deep frying methods for cooking (adjusted OR = 4.65; 95% CI: 1.19–18.22) were significantly associated with ESD (Table 4).

Most (82/107; 76.6%) of the esophagitis biopsies were graded as mild, and most (96/107; 89.7%) came from the middle third of the esophagus. About half (49.4%) of subjects with a worst pathologic diagnosis of esophagitis were male, and this diagnosis occurred most commonly in the 50–59 (48.3%) and 60–69 year (24.7%) age groups. There were also significant relationships between a worst diagnosis of esophagitis and smoking (adjusted OR = 5.81; 95% CI: 2.43–13.88), drinking hot tea (adjusted OR = 2.27; 95% CI: 1.15 – 4.47), and lack of education (secondary school education vs. none, adjusted OR = 0.23; 95% CI: 0.09–0.56) (Table 5).

The endoscopic examinations showed hiatal hernia in 39 subjects (12.9%). Gastroesophageal reflux disease (GERD) was reported in 28.2% and 4.9% of subjects with and without hiatal hernia, respectively (*P* value < 0.001). The prevalence of hiatal hernia was significantly higher in subjects of Turkmen ethnicity (18.5%) than in non-Turkmens (9.8%; *P* value = 0.03). The prevalence of GERD was also higher in Turkmen (12.0% vs. 6.7%; *P* value = 0.08). No significant relationship was found between the presence of hiatal hernia or GERD and other variables including age, gender, ethnicity, BMI or smoking.

Discussion

Esophageal squamous cell carcinoma (ESCC) is a common fatal cancer across central Asia, including the Golestan Province, Iran. The high fatality rate of this cancer is largely due to the late development of symptoms, which leads to late diagnosis, after the tumor has spread outside the esophagus. One potential way to reduce mortality from such a cancer is earlier diagnosis of the tumor or its precursor lesions, when the disease is still curable. For ESCC, in which nearly all early lesions are asymptomatic, this will require screening asymptomatic high-risk people. The only screening technique to date that has shown a high sensitivity for identifying esophageal squamous dysplasia (ESD), the precursor lesion of ESCC, in asymptomatic people is Lugol's chromoendoscopy. The aims of this study were to evaluate the feasibility of this technique and to document the prevalence and risk factor correlates of ESD among asymptomatic adults in the Golestan

Table 4. Relationship between different variables and esophageal squamous dysplasia in asymptomatic adults from the Golestan Province, Iran

Variables	Total number of subjects*	Number (%) of subjects with dysplasia	Univariate analysis			Multivariate analysis		
			Crude OR	95% CI	*P*-value	Adjusted OR	95% CI	*P*-value
Gender								
Male	128	7 (5.5)	—	—	0.76	—	—	0.16
Female	174	11 (6.3)	1.17	0.44–3.10		2.89	0.65–12.87	
Age group								
<53 years	145	4 (2.8)	—	—	0.03	—	—	0.003
≥53 years	157	14 (8.9)	3.45	1.28–12.75		7.70	1.99–29.87	
Ethnicity								
Non-Turkmen	194	5 (2.6)	—	—	0.002	—	—	0.001
Turkmen	108	13 (12)	5.66	1.11–10.74		8.61	2.48–29.83	
Oral health								
Good	139	8 (5.8)	—	—	0.78	—	—	0.46
Poor	153	10 (6.5)	1.14	0.44–2.99		0.65	0.21–2.03	
Using deep frying method								
No	159	5 (3.1)	—	—	0.03	—	—	0.03
Yes	133	13 (9.8)	3.34	1.16–9.62		4.65	1.19–18.22	
Smoking								
No	239	15 (6.3)	—	—	0.87	—	—	0.95
Yes	53	3 (5.7)	0.9	0.25–3.21		0.94	0.16–5.54	
Opium use								
No	260	16 (6.2)	—	—	0.98	—	—	0.66
Yes	32	2 (6.2)	1.02	0.22–4.64		1.52	0.23–10.02	
Body mass index								
Normal	78	5 (6.4)	—	—	0.92	—	—	0.78
Overweight/obese	214	13 (6.1)	0.94	0.32–2.74		0.84	0.25–2.82	
Tea temperature								
Cold	211	12 (5.7)	—	—	0.59	—	—	0.71
Hot	81	6 (7.4)	1.33	0.48–3.66		1.25	0.39–4.04	
Education								
None	100	7 (7.0)	—	—	0.79	-	—	0.14
Primary school	72	5 (6.9)	0.99	0.30–3.26		4.58	0.88–23.77	
Secondary school or higher	120	6 (5.0)	0.70	0.23–2.15		5.31	0.85–33.23	

* Subjects with missing data were excluded

Province.

Only 18.2% of this representative sample of the general population of eastern Golestan Province agreed to be screened by endoscopy, and after ineligible subjects were excluded, only 15.8% of the originally invited subjects underwent endoscopy. Endoscopic examination is an invasive procedure; therefore, this low compliance rate is not too surprising in a general population, especially in response to a single telephone invitation. This procedure may be more acceptable to subgroups of individuals who are persuaded that they have an exceptionally high risk for developing ESCC, such as patients with a previous head and neck cancer, [20] alcoholic subjects, [28] or others shown to be at especially high risk by other risk-stratification models. [9, 29] Concentrating the risk in such ways should also increase the proportion of screened subjects with ESD lesions. Of note in our study, once subjects were enrolled in the screening program, they all completed endoscopy, there were no significant adverse events, and most were quite satisfied with their experience.

The prevalence of ESD in our study was only 6.0%, including 4.6% of participants with low-grade (mild) dysplasia and 1.4% with high-grade (moderate or severe) dysplasia. Three previous papers have reported much higher rates of total ESD (21.5% – 31.6%) and high-grade ESD (12.9% - 17.5%) from high-risk areas of China. [24, 30, 31] These differences probably reflect differences in ESCC rates among the screened populations.

Our results showed a positive correlation between the prevalence of ESD and the participants' age, and a similar prevalence in men and women. This data will inform the design of future screening efforts in this population, which may include only older adults (e.g. subjects older than 50 years).

The correlation of biopsy diagnoses and Lugol's staining pattern was consistent with the mechanism of Lugol's staining and the results of previous studies. Iodine reversibly binds to glycogen, which is abundant in normal superficial squamous epithelial cells but is scant in rapidly-dividing cells such as those in high-grade esophagitis or dysplasia. Thus, most biopsies that were histologically normal or showed mild esophagitis came from mucosa that appeared brown after application of Lugol (normally stained),

Table 5. Relationship between different variables and esophagitis in asymptomatic adults from the Golestan Province, Iran

Variables	Total number of subjects*	Number (%) of subjects with esophagitis	Univariate analysis			Multivariate analysis		
			Crude OR	95% CI	*P*-value	Adjusted OR	95% CI	*P*-value
Gender								
Male	119	44 (37.0)	—	—	0.21	—	—	0.94
Female	151	45 (29.8)	0.72	0.43–1.20		1.03	0.50–2.12	
Age group								
<53 years	134	37 (27.6)	—	—	0.06	—	—	0.57
≥53 years	136	52 (38.2)	1.62	0.97–2.70		1.19	0.65–2.20	
Ethnicity								
Non-Turkmen	179	55 (30.7)	—	—	0.27	—	—	0.43
Turkmen	91	34 (37.4)	1.34	0.79–2.28		1.28	0.69–2.37	
Oral health								
Good	128	32 (25.0)	—	—	0.007	—	—	0.20
Poor	132	54 (40.9)	2.08	1.22–3.53		1.49	0.81–2.75	
Using deep frying method								
No	144	46 (31.9)	—	—	0.66	—	—	0.17
Yes	116	40 (34.5)	1.12	0.67–1.88		0.64	0.34–1.21	
Smoking								
No	211	58 (27.5)	—	—	<0.001.00	—	—	<0.001
Yes	49	28 (57.1)	3.52	1.85–6.68		5.81	2.43–13.88	
Opium Use								
No	232	74 (31.9)	—	—	0.25	—	—	0.51
Yes	28	12 (42.9)	1.60	0.72–3.56		0.73	0.28–1.89	
Body mass index								
Normal	69	22 (31.9)	—	—	0.81	—	—	0.09
Overweight/obese	191	64 (33.5)	1.08	0.60–1.94		1.84	0.91–3.72	
Tea temperature								
Cold	191	59 (30.9)	—	—	0.21	—	—	0.02
Hot	69	27 (39.1)	1.44	0.81–2.55		2.27	1.15–4.47	
Education								
None	89	39 (43.8)	—	—	0.03			0.004
Primary school	63	18 (28.6)	0.51	0.26–1.02		0.37	0.16–0.85	
Secondary school or higher	108	29 (26.9)	0.47	0.26–0.86		0.23	0.09–0.56	

* Subjects with missing data were excluded

while most biopsies of higher-grade esophagitis or dysplasia came from USLs. These findings were similar to those in previous reports. [32]

We also found a significant relationship between the size of USLs and the histologic diagnosis of their biopsies. All biopsies from USLs that showed dysplasia came from USLs ≥5mm in greatest dimension. This result confirms the rationale for the practice of previous authors who only biopsied USLs ≥ 5mm in size. [28, 33]

The prevalence of ESD was significantly higher in older subjects, Turkmens, and those who used deep frying methods for cooking. Genetic susceptibility has been suggested as a risk factor for ESD and ESCC in previous studies, [10, 11, 34] and Akbari et al. reported a strong familial component to ESCC among Turkmens.[35] In addition, Hakami et al., reported an increased risk of ESCC among subjects from this region who use deep frying to cook their food. [36] Thus, these results from our study are in line with previous reports suggesting that risk factors for ESD are similar to those for ESCC. [9] However, we found no significant relationship between ESD and other variables that have been reported as risk factors for ESCC in northern Iran, including smoking tobacco, [15] opium consumption, [15] drinking hot tea, [16] poor oral health, [18] and low socioeconomic status. [17] This may be due to the small number of ESD lesions found in our study, and the resulting low power of our study to detect such associations.

Esophagitis was the most common abnormal pathology in our subjects, and was the worst histologic diagnosis in 29.5%. Over

75% of the esophagitis biopsies were graded as mild, similar to previous esophageal screening studies. [24, 30, 31] We found a significant relationship between smoking tobacco, drinking hot tea and lack of education and the risk of esophagitis. These variables have also been suggested as risk factors for ESCC in our region. [15-17]

In our study, the prevalence of hiatal hernia and GERD were approximately twice higher in Turkmen than non-Turkmen subjects. Recently, an increasing rate of esophageal adenocarcinoma was reported in the Golestan Province. [37] In addition, Ehsani et al., have reported a high frequency of GERD in another Iranian population. [38] Additional studies of trends in GERD, Barrett's esophagus and esophageal adenocarcinoma, both in Golestan and throughout Iran, are warranted.

In conclusion, we found a low compliance rate for endoscopic screening and a low prevalence of esophageal squamous dysplasia among asymptomatic adults in the Golestan Province, suggesting the need for developing less invasive non-endoscopic methods for primary ESCC screening which can accurately triage the highest-risk individuals to endoscopy. Risk-stratification by genetic or environmental risk factors may also improve the yield of such a screening program.

Acknowledgments

The authors would like to thank Dr. Sharifi, Ms. Kor, Dr. Khalilipour, Dr. Abaie, Mr. Yolmeh, Mr. Ataie, Mrs. Nasi and Mrs. Mohammadi for their valuable work in data collection and sample processing. This study was supported by the Digestive Disease Research Institute (DDRI) affiliated with Tehran University of Medical Sciences (TUMS); the Golestan Research Center of Gastroenterology and Hepatology (GRCGH) affiliated with Golestan University of Medical Sciences (GOUMS); and in part by the Intramural Research Program of the National Cancer Institute, NIH.

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