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Original article

Serum hyaluronic acid and laminin as potential tumor markers for upper gastrointestinal cancers ☆

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ABSTRACT

Background: Early diagnosis of patients with upper gastrointestinal cancer is important because many cases are diagnosed in advanced stages and have poor prognosis. Several studies have reported increased serum levels of hyaluronic acid and laminin in various cancers and the correlation of the levels with poor prognosis. However, little data on the use of serum hyaluronic acid and laminin levels for early detection of esophageal and gastric cancers are available.

Methods: We assessed serum hyaluronic acid and laminin levels using enzyme-linked immunosorbent assay in 20 gastric cardia cancer, 23 gastric noncardia cancer and 20 esophageal squamous cell carcinoma incident cases and 25 controls in the Golestan Province, northern Iran, a high risk area for upper gastrointestinal cancers.

Results: Mean serum hyaluronic acid and laminin concentrations in cancer cases were higher than in controls in crude analyses. Significant correlations were observed between hyaluronic acid levels and gastric noncardia cancer (Beta-coefficient = 0.390; $P = 0.01$) and esophageal squamous cell carcinoma (Beta-coefficient = 0.332; $P = 0.05$) and between laminin levels and gastric cardia cancer (Beta-coefficient = 0.454; $P = 0.003$) in multivariate models. For esophageal squamous cell carcinoma, gastric cardia cancer, and gastric noncardia cancer, area under ROC curve (AUC) of hyaluronic acid was 0.708, 0.694, and 0.770, and of laminin was 0.706, 0.828, and 0.671.

Conclusions: Our study suggests that hyaluronic acid and laminin may be used to identify potentially high-risk groups of upper gastrointestinal cancers for further diagnostic work-ups, particularly in high incidence areas. Nevertheless, further studies with larger sample size and tumor staging information are warranted to clarify the clinical significance of hyaluronic acid and laminin in those cancers.

1. Introduction

Hyaluronic acid and laminin are among non-collagenous components of the extracellular matrix. Hyaluronic acid is distributed in the extracellular space and involved in several cell functions, including cell adhesion, migration, and proliferation [1]. Laminin is a major component of the basement membrane and involved in several activities, including cell adhesion, migration, differentiation and growth [2]. Several studies have reported an increase in hyaluronic acid levels in serum, saliva, urine, or tumor tissue of patients with a verity of malignant tumors, including multiple myeloma, mesothelioma, and cancers of the stomach, colon, lung, breast, head and neck, and genitourinary tract [3–13]. Elevated serum levels of laminin have also been reported in various cancers, including cancer of the stomach, colorectum, ovary, and hepatocellular carcinoma [2].

Gastric cancer is the second most common cause of mortality from cancer in the world [14]. A few tumor markers such as carcinoembryonic antigen and alpha-fetoprotein are reported useful to predict the prognosis of gastric cancer, but these serologic markers are most often elevated in patients with end-stage disease and are not useful for early detection [15]. Esophageal cancer is the sixth most common cause of

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death from cancer worldwide [14]. Although the incidence of esophageal adenocarcinoma is increasing in several industrialized countries, [16,17] the main histological subtype of the cancer worldwide is esophageal squamous cell carcinoma [14]. The majority of the esophageal squamous cell carcinoma patients is diagnosed with advanced metastatic cancer and have poor prognosis [18]. Early diagnosis may considerably increase the survival of the patients with gastric cancer or esophageal squamous cell carcinoma [19,20]. The standard method to diagnose these cancers is examination of tissue specimens obtained during upper gastrointestinal (UGI) endoscopy. Identification of reliable, less-invasive serum markers for detection of early-stage gastric cancer and esophageal squamous cell carcinoma may be helpful in improvement of the prognosis [19].

The majority of previous studies on the association of hyaluronic acid and laminin with cancer investigated the predictive value of those biomarkers among known cancer cases, which generally showed poorer prognosis with elevated biomarker levels. Very few studies have examined the efficiency of hyaluronic acid and laminin as diagnostic tumor markers, with both positive [6,8,13] and negative results [21]. So far, little data on the use of serum hyaluronic acid and laminin levels for early detection of esophageal and gastric cancers are available. To address this issue, we assessed serum concentrations of hyaluronic acid and laminin in patients with esophageal squamous cell carcinoma or gastric adenocarcinoma and in individuals without cancer in Golestan Province, a high incidence area for esophageal squamous cell carcinoma in northern Iran [22]. Gastric cancer, including cardia adenocarcinoma, is also a common malignancy in Golestan [23].

2. Materials and methods

2.1. Study participants

Cases and controls were individuals with UGI symptoms who were referred to Atrak clinic, the only specialized clinic for UGI cancer in eastern Golestan, from February 2008 to September 2009 and agreed to participate in the study. All of the referred individuals underwent upper esophagogastroduodenoscopy at the clinic. Details of the diagnostic procedures are presented elsewhere [23]. Eligible case subjects were those who received diagnosis of an UGI cancer at the clinic in the specified time period. We selected the controls from those with normal endoscopy among the outpatients, who were referred to our clinic because of their benign UGI disorders. Eligible participants with a history of cancer, gastrointestinal bleeding, chronic liver disease, and any chronic inflammatory diseases (such as rheumatoid arthritis) were excluded. Finally, 20 patients with gastric cardia cancer, 23 with gastric noncardia cancer, 20 with esophageal squamous cell carcinoma, and 25 controls were enrolled in the study.

Data on demographic characteristics and habits, including tobacco and opium use were collected using structured questionnaires, which were administered by trained interviewers in face-to-face interviews. Ever opium use and ever cigarette smoking were defined as using opium at least once per week and smoking cigarette at least once per day for duration of at least 6 months.

One day before blood sampling, all cases and controls underwent upper esophagogastroduodenoscopy at the clinic. After that, fasting blood samples (5 ml) were obtained from all participants. Within 2 h following sample collection, the blood samples were centrifuged (at 2500 *g* for 5 min) and serum samples were stored in −20 °C freezers. Written, informed consent was obtained from all participants. The study was reviewed and approved by the Ethics Committee of Golestan University of Medical Sciences.

2.2. Hyaluronic acid assay

Hyaluronic acid levels were measured with enzyme-linked immunosorbent assay (ELISA), using hyaluronic acid-ELISA kits (hyaluronic acid-test, product number: K-1200, Echelon Bioscience Inc, USA) and an ELISA reader (Immunoscan, Lab System, Switzerland). The hyaluronic acid-ELISA is a competitive ELISA assay in which colorimetric signals are inversely proportional to the amount of hyaluronic acid present in the sample. Samples were first mixed with the detector, and then added to the hyaluronic acid ELISA plate for competitive binding. An enzyme-linked antibody and colorimetric detection was used to identify the hyaluronic acid detector bound to the plate. The concentration of hyaluronic acid in samples was determined by a standard curve using the reagent blank (0 ng hyaluronic acid/ml) and hyaluronic acid reference solutions (50, 100, 200, 400, 800 and 1600 ng hyaluronic acid/ml). Serum hyaluronic acid concentrations were determined in the same analytical batch in one working day. The coefficient of variation for intra-assay variability of the procedure was 5%.

2.3. Laminin assay

Serum laminin levels were measured with enzyme-linked immunosorbent assay, using laminin EIA kits (Takara Bio, code number: MK107) and an ELISA reader (Immunoscan, Lab System, Switzerland). The laminin-EIA kit is a solid phase EIA based on a sandwich method that utilizes two mouse monoclonal anti-laminin antibodies to detect laminin by a two-step procedure. One of the antibodies is bound to a microplate to create the solid phase. Non-specific binding is blocked by a blocking buffer. Samples and standards were incubated in microplate wells. After washing the plate, the second anti-laminin that was labeled with peroxidase (POD) was added to the wells and incubated. During these steps, laminin was captured onto the solid support on one side and tagged on the other by POD-anti-laminin. The reaction between POD and substrate (H2O2 and tetramethybenzidine) resulted in color development with intensities proportional to the amount of laminin present in the samples and standards. The amount of laminin was determined by measuring the absorbance's using an EIA plate reader. Accurate sample concentrations of laminin were determined by comparing their specific absorbance with those obtained for the standards plotted on a standard curve. A standard curve using 5, 10, 20, 40, 80, 160 and 320 ng/ml laminin was used to convert sample absorbance's into ng/ml of laminin. The coefficients of variation for intra-assay and inter-assay variability of the procedure were 4.0–5.7% and 0.3–5.0%. Again, serum laminin concentrations were determined in the same analytical batch in one working day.

2.4. Statistical analysis

Mean and standard deviation of age and frequency distribution of demographic characteristics, ever-cigarette smoking, ever-opium use, and history of cancer in family were reported for controls and cases with esophageal squamous cell carcinoma, gastric cardia cancer, and gastric noncardia cancer. The values for each cancer group were compared with of controls, using Student's *t*-test for continues and Fisher's exact test for categorical variables. The normality of the distribution of hyaluronic acid and laminin values for all participants and among controls was assessed by Q–Q plots and the Shapiro–Wilk W test. The distributions were found to be severely skewed; thus we used log-transformed values of hyaluronic acid and laminin. Distribution of laminin values was skewed even after log transformation. When 3 outliers for log-transformed laminin (1 from control and 2 from gastric cardia cancer groups) were not considered, normality was attained. Therefore, we excluded those 3 outlier values for geometric mean calculations and in regression models. We conducted linear regression models and reported Beta-coefficients and *P* values for relationship of hyaluronic acid and laminin, as dependent variables, with cancer status both in crude analyses and in multivariate models in which age, sex, ethnicity and ever-cigarette smoking were also included.

The diagnostic accuracy of the serum hyaluronic acid and laminin was evaluated using receiver operating characteristic (ROC) curve

Table 1
Characteristics of controls and cancer cases [a].

Characteristics	Control n = 25	ESCC n = 20	ESCC n = 20	ESCC n = 23
Age				
Mean (SD), years	58.1 (10.2)	65.5 (13.0)	65.5 (11.2)	65.4 (10.0)
P value[b]	–	0.038	0.027	0.016
Sex				
Women/men	14 (56%)/11 (44%)	8 (40%)/12 (60%)	4 (20%)/16 (80%)	7 (30%)/16 (70%)
P value[c]	–	0.37	0.018	0.09
Place of residence				
Rural/urban	24 (96%)/1 (4%)	16 (80%)/4 (20%)	17 (85%)/3 (15%)	20 (87%)/3 (13%)
P value[c]	–	0.16	0.31	0.34
Ethnicity				
Turkmen/non-Turkmen	22 (88%)/3 (12%)	13 (72%)/5 (28%)	15 (75%)/5 (25%)	11 (48%)/12 (52%)
P value[c]	–	0.25	0.44	0.004
Formal education				
No/yes	17 (74%)/6 (26%)	16 (84%)/3 (16%)	17 (89%)/2 (11%)	22 (96%)/1 (4%)
P value[c]	–	0.48	0.26	0.10
Cigarette smoking				
Ever/never	24 (96%)/1 (4%)	12 (63%)/7 (37%)	18 (90%)/2 (10%)	19 (83%)/4 (17%)
P value[c]	–	0.014	0.58	0.18
Opium use				
Ever/never	23 (100%)/0 (0%)	11 (61%)/7 (39%)	15 (75%)/5 (25%)	16 (70%)/7 (30%)
P value[c]	–	0.001	0.016	0.009
Cancer in family				
No/yes	22 (92%)/2 (8%)	10 (56%)/8 (44%)	14 (70%)/6 (30%)	18 (78%)/5 (22%)
P value[c]	–	0.010	0.12	0.25

Abbreviations: ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia cancer; GNCA, gastric noncardia cancer.
[a] As a result of missing data, the sum of individuals in subgroups may be less than the total number of participants in those subgroups.
[b] *P* values for the difference between cancer and control groups were calculated using Student's *t*-tests.
[c] *P* values for the difference between cancer and control groups were calculated using Fisher's exact tests.

analysis, which correlates true- and false-positive rates (sensitivity and 1-specificity). As we used non-parametric ROC analyses, the original values of hyaluronic acid and laminin (rather than log-transformed values) were included in the analyses. The area under the ROC curves (AUC) and the corresponding 95% confidence intervals (CI) were also calculated. An AUC of 1.0 is characteristic of an ideal test, whereas 0.5 indicates a test of no diagnostic value [24]. Taking sensitivity and specificity into account, the optimal cutoff points were selected according to maximum values of sensitivity plus specificity. Accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using 2×2 tables. All statistical analyses were performed using STATA software, version 11.0. Throughout of this article, two-sided *P* values <0.05 are considered as statistically significant.

3. Results

Demographic characteristics of study participants are shown in Table 1. Cancer cases in average were older than controls; the approximate mean age was 58 years for controls and 65 years for cancer cases. While slightly more women than men were enrolled as controls, the number of men was higher in cancer groups. The majority

Table 2
Beta-coefficients (*P* values) for relationship of HA and LN with age, sex, ethnicity, cigarette and opium use, and history of cancer in family by cancer status [a].

Variables	Control		ESCC		GCA		GNCA		All participants	
	HA	LN	HA	LN	HA	LN	HA	LN	HA	LN
Age										
Crude	0.342 (0.09)	0.329 (0.12)	0.043 (0.86)	0.450 (0.05)	−0.003 (0.99)	0.311 (0.21)	0.376 (0.08)	−0.073 (0.74)	0.299 (0.005)	0.310 (0.004)
Adjusted	0.267 (0.23)	0.255 (0.24)	0.258 (0.43)	0.513 (0.12)	0.037 (0.89)	0.165 (0.61)	0.530 (0.04)	−0.047 (0.86)	0.239 (0.033)	0.292 (0.015)
Sex										
Crude	0.046 (0.83)	−0.253 (0.23)	0.237 (0.31)	0.018 (0.94)	0.455 (0.04)	0.068 (0.79)	0.252 (0.25)	0.074 (0.74)	0.271 (0.011)	0.066 (0.55)
Adjusted	−0.030 (0.89)	−0.323 (0.16)	0.163 (0.56)	0.139 (0.60)	0.363 (0.18)	0.094 (0.77)	0.363 (0.14)	0.200 (0.44)	0.213 (0.058)	0.034 (0.78)
Ethnicity										
Crude	−0.085 (0.67)	0.322 (0.13)	−0.544 (0.02)	0.213 (0.40)	0.277 (0.24)	−0.387 (0.11)	−0.033 (0.88)	0.156 (0.48)	0.061 (0.58)	0.154 (0.17)
Adjusted	−0.112 (0.60)	0.258 (0.24)	−0.604 (0.06)	0.222 (0.43)	0.288 (0.31)	−0.401 (0.25)	−0.325 (0.20)	0.062 (0.82)	0.031 (0.76)	0.111 (0.37)
Cigarette smoking										
Crude	0.319 (0.12)	0.132 (0.54)	0.126 (0.61)	0.357 (0.13)	0.191 (0.42)	0.173 (0.49)	0.033 (0.88)	−0.347 (0.11)	0.202 (0.06)	0.077 (0.49)
Adjusted	0.297 (0.19)	0.220 (0.33)	−0.167 (0.58)	0.052 (0.86)	0.012 (0.97)	0.292 (0.43)	−0.190 (0.46)	−0.364 (0.20)	0.046 (0.70)	−0.029 (0.82)
Opium use										
Crude	–	–	−0.018 (0.94)	−0.005 (0.98)	0.215 (0.36)	0.000 (1.00)	0.101 (0.65)	−0.132 (0.55)	0.205 (0.06)	0.053 (0.64)
Adjusted	–	–	0.119 (0.67)	0.150 (0.58)	0.308 (0.30)	−0.307 (0.39)	−0.077 (0.76)	−0.050 (0.86)	0.129 (0.27)	0.036 (0.77)
Cancer in family										
Crude	0.099 (0.65)	0.303 (0.16)	0.040 (0.87)	−0.635 (0.01)	−0.119 (0.62)	0.347 (0.16)	−0.159 (0.47)	−0.319 (0.14)	0.063 (0.57)	−0.030 (0.79)
Adjusted	−0.232 (0.46)	0.412 (0.17)	−0.445 (0.34)	−0.571 (0.19)	−0.009 (0.98)	0.128 (0.73)	−0.057 (0.83)	−0.317 (0.25)	0.079 (0.48)	0.018 (0.88)

Abbreviations: ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GCNA, gastric noncardia adenocarcinoma; HA, hyaluronic acid; LN, laminin.
[a] Variables were as follows: age (continuous variable in years), sex (men vs. women) ethnicity (non-Turkmen vs. Turkmen), cigarette smoking (ever vs. never), opium use (ever vs. never), and cancer in family (yes vs. no). Beta coefficients and *P* values were driven from linear regression models. Adjusted values were driven from multivariate models in which other variables in the table were included.

Table 3
Beta-coefficients for the association between serum HA and LN concentrations and cancer groups [a].

	Geometric mean (95% CI)	Crude B.coeff [b]	*P* value [b]	Adjusted B.coeff [c]	*P* value [c]
Hyaluronic acid					
Control	72.2 (49.7–104.8)	Reference	–	Reference	–
ESCC	135.6 (105.3–175.0)	0.387	0.009	0.332	0.049
GCA	136.0 (103.1–179.2)	0.381	0.010	0.249	0.12
GNCA	162.4 (125.2–210.8)	0.471	0.001	0.390	0.011
Laminin [d]					
Control	59.2 (54.6–64.3)	Reference	–	Reference	–
ESCC	68.2 (63.0–73.8)	0.363	0.015	0.151	0.34
GCA	73.8 (68.4–79.6)	0.530	<0.001	0.454	0.003
GNCA	66.5 (60.3–73.3)	0.270	0.066	0.208	0.24

Abbreviations: B.coeff, Beta-coefficient; CI, confidence interval; ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GCNA, gastric noncardia adenocarcinoma; HA, hyaluronic acid; LN, laminin.
[a] Levels of HA and LN are presented as ng/ml.
[b] Beta-coefficients and *P* values were calculated using linear regression models.
[c] Adjusted Beta-coefficients and *P* values are driven from multiple linear regression models in which age, sex, ethnicity, and ever-smoking were also included.
[d] All values calculated after exclusion of 2 outliers from GCA and 1 outlier from control groups.

of cases and controls resided in rural areas and did not have formal education. More esophageal squamous cell carcinoma cancer than controls had ever-smoked cigarette ($P=0.001$). Opium use was more common among cancer cases than controls; none of controls had ever used opium. History of cancer in family was also more common among esophageal squamous cell carcinoma cases than in controls ($P=0.01$).

Distribution of hyaluronic acid and laminin levels in serum by disease status is shown in the Supplementary material. Beta-coefficients and *P* values for relationship of hyaluronic acid and laminin with age, sex, ethnicity, cigarette and opium use, and history of cancer in family by cancer status are shown in Table 2. Among controls, both age and cigarette smoking were associated with hyaluronic acid and laminin levels, while sex (men vs. women) was inversely associated with laminin levels. However, none of the association was statistically significant. When all participants were combined, age was significantly associated with hyaluronic acid (Beta-coefficient for one year increase in age = 0.239; $P=0.03$) and laminin (Beta-coefficient = 0.292; $P=0.02$). The associations with cigarette smoking were disappeared after adjustment for other factors, including age. There were sporadic associations among cases that were not observed among controls, such as the association between hyaluronic acid and ethnicity among esophageal squamous cell carcinoma cases and between hyaluronic acid and sex among cancer cases.

Table 3 shows geometric mean (95% CI) of hyaluronic acid and laminin by disease status, as well as Beta-coefficients and *P* values for the relationship of hyaluronic acid and laminin with cancer status in univariate and multivariate linear regression models. In multivariate models, hyaluronic acid was associated with gastric noncardia cancer (Beta-coefficient = 0.390; $P=0.01$) and esophageal squamous cell carcinoma (Beta-coefficient = 0.332; $P=0.05$) and laminin was associated with gastric cardia cancer (Beta-coefficient = 0.454; $P=0.003$).

Results of ROC curve analyses are shown in Table 4 and Figs. 1 and 2. AUC (95% CI) for hyaluronic acid was as following: 0.708 (0.549–0.869) with esophageal squamous cell carcinoma, 0.694 (0.537–0.851) with gastric cardia cancer, and 0.770 (0.635–0.904) with gastric noncardia cancer. Sensitivity of hyaluronic acid for diagnosis of esophageal squamous cell carcinoma was 80% for cut-off point of 101 ng/ml and 75% for cut-point of 109 ng/ml; the specificity was not very different (68% vs. 72%). Sensitivity of hyaluronic acid for diagnosis of gastric cardia cancer was 75% for cut-off point of 82 ng/ml and 65% for cut-point of 125 ng/ml; the corresponding specificity values were 60% and 72%. For diagnosis of gastric noncardia cancer using hyaluronic acid levels, sensitivity of cut-off points of 81 and 105 ng/ml was 91% and 74%, the respective specificity were 56% and 72%. When we assigned a single cut-off point (101 ng/ml) to hyaluronic acid for discrimination of all cancer cases combined from controls, sensitivity and specificity were 73% and 68%. AUC (95% CI) for laminin with regard to gastric cardia cancer was 0.828 (0.711–0.945). Sensitivity was 90% for cut-off point of 62 ng/ml and 80% for cut-off point of 67 ng/ml; the respective specificity was 60% and 68%. Sensitivity and specificity of laminin for discrimination of esophageal squamous cell carcinoma and gastric noncardia cancer were lower, particularly for gastric noncardia cancer. Sensitivity and specificity of laminin using a single cut-off point (62.1 ng/ml) for discrimination of all cancer cases combined were 73% and 60%.

4. Discussion

This study found higher serum hyaluronic acid and laminin levels in UGI cancer cases than in control group. Multivariate analyses adjusted for several factors, including age, sex, ethnicity, and cigarette smoking, confirmed significant relation of hyaluronic acid with esophageal squamous cell carcinoma and gastric noncardia cancer and of laminin with gastric cardia cancer. Therefore, it is unlikely that

Table 4
ROC analysis of HA and LN levels for differentiating cancer cases from controls[a]. Sensitivity, specificity, positive and negative predictive values, and accuracy for each marker are presented for two selected cut-off points.

Marker	Cancer	AUC (95%CI)	Cut off point ng/ml	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
HA	ESCC	0.708	101.0	80.0	68.0	66.7	81.0	73.3
		(0.549–0.869)	109.0	75.0	72.0	68.2	78.3	73.3
	GCA	0.694	82.0	75.0	60.0	60.0	75.0	66.7
		(0.537–0.851)	125.0	65.0	72.0	65.0	72.0	68.9
	GNCA	0.770	81.0	91.3	56.0	65.6	87.5	72.9
		(0.635–0.904)	107.0	73.9	72.0	70.8	75.0	72.9
	All	0.726	101.0	73.0	68.0	85.2	50.0	71.6
		(0.692–0.860)						
LN	ESCC	0.706	63.1	70.0	60.0	58.3	71.4	64.4
		(0.555–0.857)	67.3	60.0	68.0	60.0	68.0	64.4
	GCA	0.828	62.1	90.0	60.0	64.3	88.2	73.3
		(0.711–0.945)	67.3	80.0	68.0	65.2	77.3	73.3
	GNCA	0.671	57.0	78.3	48.0	58.0	70.6	62.5
		(0.516–0.827)	70.6	48.8	84.0	71.4	61.8	66.7
	All	0.732	62.1	73.0	60.0	82.0	46.9	69.3
		(0.618–0.746)						

Abbreviations: AUC, area under the ROC curves; ESCC, esophageal squamous cell carcinoma; GCA, gastric cardia adenocarcinoma; GNCA, gastric noncardia adenocarcinoma; HA, hyaluronic acid; LN, laminin; PPV, Positive predictive value; NPV, negative predictive value; ROC, receiver operating characteristic; 95% CI, 95% confidence interval.
[a] Sensitivity, specificity, positive and negative predictive values, and accuracy for each marker are presented for at least two selected cut-off points.

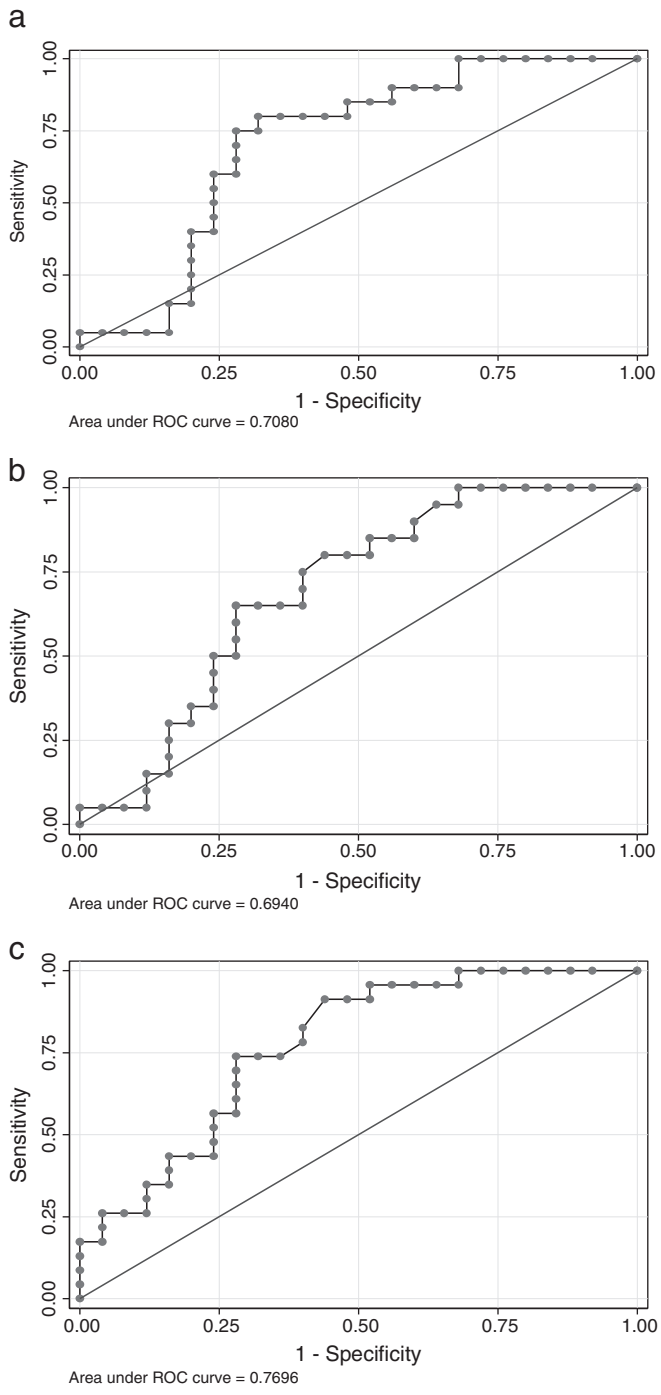

Fig. 1. ROC curves of hyaluronic acid for discrimination of esophageal squamous cell carcinoma, gastric cardia adenocarcinoma, and gastric noncardia adenocarcinoma cases from control. a. ROC of hyaluronic acid for diagnosis of esophageal squamous cell carcinoma. b. ROC of hyaluronic acid for discrimination of gastric cardia adenocarcinoma. c. ROC of hyaluronic acid for discrimination of gastric noncardia adenocarcinoma.

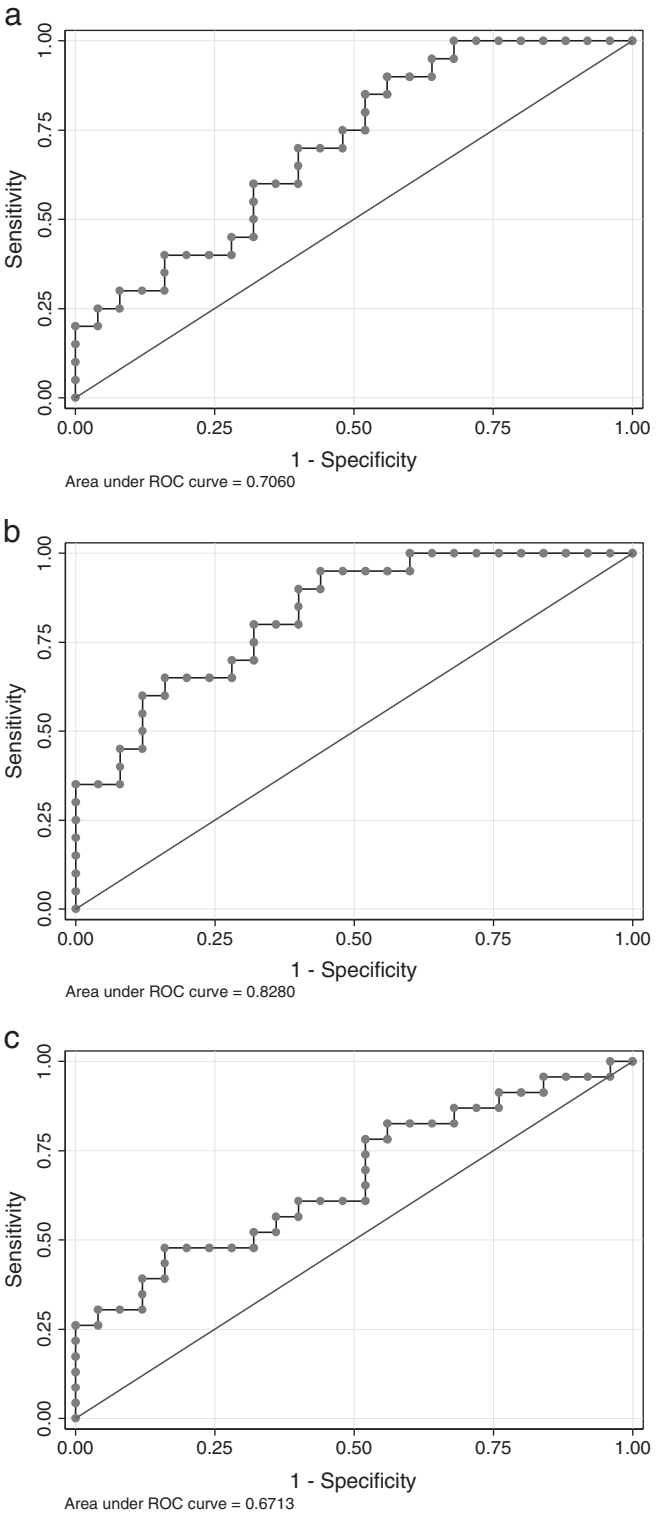

Fig. 2. ROC curves of laminin for discrimination of esophageal squamous cell carcinoma, gastric cardia adenocarcinoma, and gastric noncardia adenocarcinoma cases from control. a. ROC of laminin for discrimination of esophageal squamous cell carcinoma, b. ROC of laminin for discrimination of gastric cardia adenocarcinoma. c. ROC of laminin for discrimination of gastric noncardia adenocarcinoma.

the above findings are spurious associations related to the influence of other factors. Hyaluronic acid showed fairly good sensitivity and specificity for discrimination of UGI cancer cases, particularly esophageal squamous cell carcinoma and gastric noncardia cancer cases, from controls. While similar findings were found with regard to laminin and gastric cardia cancer, the efficacy of laminin for discrimination of esophageal squamous cell carcinoma and gastric noncardia cancer was less promising.

Our results suggest that hyaluronic acid and laminin may be used as tumor markers for the above UGI cancers. Nevertheless, increase in serum hyaluronic acid and laminin is not specific to those cancers. In addition to several other cancers, increased serum hyaluronic acid has been reported with liver disease and various inflammatory conditions, including rheumatoid arthritis, psoriasis, scleroderma, and osteoarthritis [25,26]. Elevated serum laminin is also reported not only with other

cancers but also with liver disease, including chronic hepatitis, cirrhosis, and liver fibrosis [27–29]. Although these biomarkers are not specific to UGI cancers and diagnosis of those cancers should be confirmed by standard methods, serum hyaluronic acid and laminin levels may be helpful to identify potentially high-risk groups for further diagnostic work-ups or closer follow-ups; this can be an important issue in high incidence areas of UGI cancers, such as Golestan.

Hyaluronic acid may be produced by tumor cells or as a result of interactions between tumor and the surrounding connective tissue [13,30]. Surgical removal of tumor has been associated with a reduction in serum hyaluronic acid levels in a few studies that investigated such an association [31]. Two different patterns have been suggested for expression of hyaluronic acid in tumors arising from simple and stratified epithelia [12]. With the first group, such as cancers of the breast, colon, and stomach, the expression correlates with tumor grade and invasiveness. With poorly differentiated tumors and local or distant invasion, more cancer cells will be hyaluronan-positive or will have more intense hyaluronan staining. On the other hand, early stage cancer arising from stratified epithelia may have increased hyaluronan expression compared to normal epithelium, but high-grade, aggressive squamous cancers are associated with decrease in hyaluronan expression. It is not clear if any pattern also exists with serum hyaluronic acid levels. The upper limit of hyaluronic acid levels in controls of our study (95% CI: 50–105 ng/ml) was comparable with reported levels among healthy individuals in other countries (range: 0–100 ng/ml), [3,21] but higher than the levels in other parts of Iran (95% CI: 0–68 ng/ml) [28,31]. Higher age of our study controls may partly explain the higher levels in our study. Our controls were referred to our clinic because of their benign UGI disorders. It is not clear whether benign inflammatory processes in UGI are also associated with increased levels of hyaluronic acid in serum.

The exact source of increased serum laminin in patients with malignant tumors is not clear. Tumor cells may release the enzymes that can dissolve extracellular matrix, which may lead to release of laminin into serum [32]. Furthermore, tumor cells may produce laminin and other element of the basement membrane [33]. There are reports showing a decrease in serum laminin following surgical excision of tumors [34]. Serum laminin levels among controls in our study (95% CI: 55–64 ng/ml) were comparable to reported values from healthy individuals in another area of Iran (95% CI: 26–66 ng/ml) [28].

One of the strengths of this study is recruitment of both non-cancer individuals and incident, newly diagnosed cancer cases. To our knowledge, this is the first report on the pattern of serum hyaluronic acid and laminin levels in esophageal squamous cell carcinoma and gastric cancer by subtype (cardia and non-cardia) compared to non-cancer individuals. Furthermore, many of previous studies on the association of hyaluronic acid and laminin with cancers did not consider potential confounding factors, such as age, that might have influenced their results. We used multivariate analyses with adjustments for several other factors to examine the relationship of hyaluronic acid and laminin with cancers of interest.

Limitations of our study include the fairly small sample size and lack of staging information for cancer cases. As hyaluronic acid and laminin are also associated with aggressiveness of tumors, increased levels of the biomarkers in our study, or a part of it, may be related to presence of local or distant invasion by tumor rather than early-stage cancer. We did not have information on the stage of tumors. Although it is unlikely that the majority of cases had very advanced disease, because they were newly diagnosed cases and generally without overt signs of distant metastasis, the cancer cases might have referred with tumors in different stages. Further studies with staging information are warranted to study hyaluronic acid and laminin levels in different stages of UGI cancers.

5. Conclusions

In summary, our study found elevated levels of serum hyaluronic acid and laminin in UGI cancers, suggesting that those biomarkers may be used for early diagnosis purposes. Increased levels of hyaluronic acid and laminin may also be seen in certain other cancers and inflammatory disease. Nevertheless, those biomarkers may be helpful to identify potentially high-risk groups for further diagnostic work-ups or closer follow-ups, particularly in high incidence areas of UGI cancers, and as it is shown in other studies to predict the prognosis of those cancers. Further studies with larger sample size and tumor staging information are required to clarify the clinical significance of serum concentration of hyaluronic acid and laminin as tumor markers for esophageal squamous cell carcinoma, gastric cardia cancer, and gastric noncardia cancer.

Learning points

- Serum hyaluronic acid and laminin levels measurement in cases with UGI cancers may be helpful to identify potentially high-risk groups for further diagnostic work-ups.

Abbreviations

ESCC	esophageal squamous cell carcinoma
GCA	gastric cardia adenocarcinoma
GCNA	gastric noncardia adenocarcinoma
HA	hyaluronic acid
LN	laminin

Conflict of interest

There is no conflict-of-interest or financial disclosure in the manuscript.

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Appendix A

Supplementary data to this article can be found online at doi:10.1016/j.ejim.2011.07.018.

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