RESEARCH ARTICLE

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Association between Serum Progastrin Biomarker Level and Gastric Cancer

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Abstract

Background and Objective: gastric cancer is the fifth most prevalent cancer and the fourth cause of death because of cancer. In Iran, northern and northwestern regions are considered gastric cancer hot spots. Identifying serum biomarkers could be helpful in early diagnosis of patients with gastric adenocarcinoma (GAC). Increase in progastrin level has been reported in different cancers. Given the diagnostic value of this biomarker, this study aimed to determine the diagnostic role of progastrin serum biomarker in patients with gastric cancer. **Methodology:** In this case-control study, forty patients with gastric cancer who were diagnosed by endoscopy and pathologic findings and visited Mazandaran Comprehensive Cancer Center. The participants had received no treatment yet and entered this study. The participants in case group were compared with the control group including forty-two individuals with no history of gastrointestinal cancer in their first-degree relatives and visiting the lab for routine tests. Progastrin serum level was assessed using ELISA kit. The Kruskal-Wallis test and Mann Whitney test, both non-parametric) were used for statistical analysis and the relation between the variables was examined using Pearson's correlation coefficient at 95% confidence level in SPSS 16. **Findings:** In this study, progastrin serum level was significantly higher in patients with gastric cancer compared with normal participants (P = 0.035). Progastrin serum level had no significant relation with tumor clinicopathologic parameters (p-value > 0.05). **Conclusion:** Increase in progastrin may be utilized as a predictive factor for gastric cancer.

Keywords: Progastrin- gastric cancer- biomarker- ELISA

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Introduction

Gastric adenocarcinoma is the fifth most prevalent cancer and cause of death by cancer (769,000 mortality rate in 2020) after lung, colorectal, and liver cancers (Sung et al., 2021). It has been known as the most common cancer in men and it is the main cause of death by cancer in several western Asian countries including Iran, Turkmenistan, and Kyrgyzstan (Arnold et al., 2020; Bray et al., 2018). In northern provinces of Iran such as Mazandaran, Gilan, and Golestan, the number of reported cases is more than other regions and given the high rate of mortality caused by gastric cancer, it is necessary to actively examine control and prevention programs(Atoof et al., 2010). Although there has been substantial progress in increasing cancer patients' survival, gastric cancer is mostly diagnosed at advanced stages. Moreover, given the high rate of recurrence, gastric adenocarcinoma has undesirable prognosis and its five-year survival is less than 30%, mostly in patients who have underwent surgery. However, patients' five-year survival can be increased to more than 90% and even lead to full recovery with early treatment (Sotoudeh and Sedghi, 2002). If discovered and treated in early stages, gastric cancer is recoverable (Kasper et al., 2015). Accordingly, studying methods of early diagnosis and management of gastric cancer, particularly pathological biomarkers, can be valuable as diagnosis of presence of biomarkers may be helpful in diagnosing cancer in early stages. Recently, researchers have focused on a new marker, assessing its role in different cancers. Progastrin is a marker encoded by the GAST gene; it is a 90-amino acid protein and the precursor of gastrin, a gastrointestinal hormone. Progastrin is synthesized by the G cells in the gastric antrum and then, it is processed into gastrin hormone through several enzymatic stages (Benoit et al., 2020). Gastrin stimulates secretion of hydrochloric acid (HCL) by gastric mucosa and production of digestive enzymes in pancreas. In addition, it increases blood flow and secretion of water in the stomach and intestine by contraction of smooth muscles (Prosapio, 2021). It was

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initially believed that progastrin is bioinactive, but later it was revealed that it affects formation of tumors by destructing cell junctions, cell proliferation, inhibiting apoptosis, regulating cancer stem cells (CSCs), and angiogenesis(Benoit et al., 2020). In colorectal cancer (CRC), progastrin is believed to harbor tumor promoting functions with impacts on cancer stem cell self-renewal (Giraud et al., 2016). Targeting human progastrin by exclusive antibody leads to increased apoptosis, reduced proliferation, and migration and invasion of colorectal cancer cells(Prieur et al., 2017). GAST gene is directly activated by the oncogenic pathway Wnt/β-catenin/Tcf4 (Koh et al., 2000). As this pathway has a role in many human cancers and survival of CSCs, progastrin is probably expressed in different cancers (Benoit et al., 2018). Also, some controversy exists in the literature regarding its tumorigenesis functions. There are evidences that treatment with gastrin17 inhibits proliferation and induces apoptosis in some CRC cell lines (Müerköster et al., 2005). G17 has a proapoptotic effect and increases sensitivity to chemotherapeutic agent (5-FU) (Sebens Müerköster et al., 2008). Given the high prevalence of gastric cancer in Iran, particularly Mazandaran Province, and the role of progastrin in stomach and its expression in different cancers, this study aimed to examine progastrin serum level in patients with gastric cancer in comparison with healthy individuals. The results of this study could help early diagnosis and examination of response to treatment in patients with gastric cancer.

Materials and Methods

This case-control study aimed to examine progastrin serum level in patients with gastric cancer and its relation with clinical-pathological findings in comparison with the control group. The case group included forty patients with gastric cancer and the control group included 42 healthy individuals. The case group participants were selected from patients with gastric cancer (clinical suspicion confirmed by the pathologist) and the control group participants were selected from individuals without any inflammatory problems and with no history of gastrointestinal cancer in their first-degree relatives visiting Imam Khomeini Hospital in Sari, Iran for routine tests. The demographical information of patients (age, sex, etc.), stage of tumor (TNM), and the type of tumor were extracted from patients' profile. The two groups were standardized in terms of age and sex. Personal consent was obtained from patients and the study was registered at the university ethics committee by registration no. IR.MAZUMS.IMAMHOSPITAL.REC.1397.3534.

Determining progastrin concentration by ELISA

The participants' blood samples were centrifuged for 15 minutes at 10,000 × g to determine the progastrin concentration in serum samples. The supernatant was separated and kept at the freezer until performing ELISA. Human ProGRP (Pro-Gastrin Releasing Peptide) ELISA Kit 96 by Elabscience (USA) was used to perform the test according to the kit instructions. In Sandwich ELISA, first, the serum sample with undetermined concentration of the protein was added to the standard sample with determined concentration. Next, biotinylated detection Ab for Human ProGRP was added. After incubation and washing, the complex was completed with the addition of avidin horseradish peroxidase (HRP) (avidin-biotin connection increases reaction sensitivity). Then, with the addition of substrate, based on the quantity of target protein, substrate was consumed by HRP and blue color was formed. Finally, the reaction was stopped by the addition of stop solution and the blue color turned to yellow. The light absorption the product was read by ELISA Reader at 450/630 nm and progastrin concentration in samples was determined and analyzed based on the standard curve resulting from the light absorption of standard samples.

Statistical analysis

The data were analyzed used SPSS16 (SPSS Inc., Chicago, IL, USA). Parametric t-test was used to compare the concentration in case and control groups. When progastrin concentration was measured, Kruskal-Wallis and Mann Whitney non-parametric tests were used at 95% confidence level. Moreover, Pearson's correlation coefficient was used to analyze the relation between the variables.

Results

Table 1 shows that there were 25 men (62.5%) and 15 women (37.5%) in case group. In control group there were 25 men (59.5%) and 17 women (40.5%), revealing no significant difference between groups (P = 0.824). Moreover, the mean age was 63.48 ± 13.37 for patients with gastric cancer and 62.60 ± 16.61 for the control group, showing no statistically significant difference (P = 0.79).

In Table 2, the mean of progastrin concentration in the patients' serum and control samples is demonstrated. The table shows that the mean of progastrin concentration in patients with gastric cancer was 170 pg/mL while it was 36.19 pg/mL in healthy participants, indicating a significant difference in progastrin serum between case and control group (P = 0.035). The analysis of progastrin serum level in the subgroups of patients with gastric cancer showed that there was no significant relation between the serum level of this biomarker and clinicopathological

Table 1. Demographic Characteristics of Gastric Cancer Patients and Controls Participants

Variable	Case group	Control group	P-value
Sex, number (percentage)			
Male	25 (62.5)	25 (59.5)	0.824
Female	15 (37.5)	17 (40.5)	
Age, mean (years)	63.48 ± 13.37	62.60 ± 16.61	0.793

Table 2. Comparison of Progastrin Serum Concentration in Patients with Gastric Cancer and Healthy Individuals

Variable	Median	Mean±Standard deviation	P-value
Case group (40)	28	170±323.464	0.035
Control group (42)	23	36.19±37.311	

Table 3. Frequency Distribution of Patients with Gastric Cancer based on Malignancy Indices and Progastrin Serum Concentration

Malignancy indices	Number	Median	Mean±Standard deviation	P-value
Region of tumor involvement in stomach				
Cardia	8	45.5	154.25±342.181	0.816
Non-cardia	30	37.5	151.30±295.897	
Total	38	38.0	151.92±301.2293	
Tumor invasion on stomach wall				
T1/ T2	3	9.0	18±16.46	0.196
T3/ T4	20	29.0	106.05±266.11	
Total	23	28.0	94.57±212.369	
Involvement of lymph nodes				
N0	6	28.5	37.33±38.469	0.940
N1	3	36.0	29±13	
N2	8	20.0	144.63±346.012	
N3	4	22.5	32±22.106	
Total	21	27.0	76±213.107	
Distant metastasis				
M0	1	37.0	37	1.000
Mx	4	32.5	95.25±143.381	
Total	5	37.0	83.60±126.875	
Involvement stage				
Stage II	9	38.0	68.67±81.126	0.165
Stage III	16	22.5	164.44±333.608	
Total	25	30.0	118.44±270.563	
Gastric adenocarcinoma type of pathology				
Diffuse	15	41.0	339.78	0.971
Intestinal	18	43.5	203.72±367.809	
Total	33	41.0	199.36± 349.861	
Tumor differentiation				
Well	3	54.0	361.33±553.232	0.663
Moderately	8	51.0	279.88±445.891	
Poorly	13	38.0	179.31±364.584	
Total	24	39.5	235.58±401.260	
Lymphatic invasion				
With invasion	15	23.0	41.93±65.312	0.110
Without invasion	3	64.0	68.67±41.199	
Total	18	28.5	46.39±61.787	
Perineural invasion				
With invasion	10	28.5	53.20±78.268	0.671
Without invasion	4	36.5	49.75±42.836	
Total	14	33.0	52.21±63.316	

findings related to survival including stage, grade, distant metastasis, metastasis to lymph nodes, and perineural and lymphatic involvement (Table 3).

Discussion

Gastric cancer is a malignant tumor with high prevalence and death at global level. Advances in discovering biomarkers have helped improvement of early and accurate diagnosis of gastric cancer and prognosis and treatment of different diseases including cancer. When diagnosed, most cases of gastric cancer are either at moderate or severely advanced stage. Nonetheless, using biomarkers can improve early diagnosis of gastric cancer (Ye et al., 2020). In this case-control study, the mean of progastrin serum concentration was 170 pg/L in cancer patients and 36.19 pg/L in healthy participants, indicating significant difference between the groups (P = 0.035). Konturek et al. examined progastrin serum level with interleukin 8 using ELISA. In their study, progastrin

serum level was significantly higher in patients with gastric cancer compared with the control group, which was congruent with the results of this study (Konturek et al., 2003). Iwase et al., (1997) studied the increase of gastrin expression at adenocarcinoma gastric cell line (AGS) and showed that gastrin stimulates cell growth and proliferation at gastric cancer grade through CCK-B receptor. Prieur and Joubert (2017) examined gastrin serum biomarkers in diagnosis gastric cancer. The results of their study showed that in 673 studied serum samples, progastrin was significantly higher in patients with cancer compared with normal individuals, which was congruent with this study. In a descriptive-analytical study, Wu et al., (2018) studied the role of PRO-GRP in diagnosis and response to treatment in small cell carcinoma; 75 patients with SCLS, 234 patients with NSCLC, and 264 patients with benign lesions were examined. Their results revealed that PRO-GRP expression was higher in patients with SCLC compared with patients with NSCLC and benign pulmonary lesions. Moreover, in congruence with the results of this study, Stanislaw et al., (2003) state the effectivity of progastrin serum marker in patients with gastric cancer. This further reinforces the finding that progastrin is significantly higher in the serum of patients with gastric cancer compared with normal people. Furthermore, Wojcik and Kulpa, (2017) studied the relation between progastrin serum biomarker and diagnosis of pulmonary cancer using ELISA. They found that the marker increased in lung cancer and could be used as a diagnostic marker.

Li et al., (2020) examined the pro-gastrin releasing peptide serum level in 50 healthy individuals in comparison with 66 patients with benign gastric lesions and 150 patients with gastric cancer using ELISA. The results reported progastrin had significant relationship with tumor size, metastasis to lymph nodes, invasion depth, differentiation, and stage. The progastrin serum level in this study was similar to that of the study by Li; however, there was no significant relation between progastrin level and tumor size, metastasis to lymph nodes, invasion depth, differentiation, and stage. Wu et al., (2018) showed that PRO-GRP expression had a relation with stage of disease, but had no relation with prognostic factors such as stage, grade, invasion depth, perineural and lymphatic involvement, and distant metastasis in gastric cancer, which was congruent with the results of the present study. One of the limitations of this study was the small number of samples which was due to the small number of patients at the time of coronavirus disease.

In sum, the results of this study demonstrated that progastrin serum level was higher in patients with gastric cancer, but this biomarker had no role in prognosis and clinicopathological findings related to survival including stage, grade, distant metastasis, metastasis to lymph nodes, and perineural and lymphatic involvement. Future studies are recommended to examine progastrin expression in larger sample size and on both blood and tissue samples. In addition, the level of this biomarker in patients with peptic ulcer and premalignant lesions and the relation between inflammatory factors such as cytokines and progastrin expression in patients with gastric cancer could be examined in future studies.

Author Contribution Statement

All authors contributed equally in this study.

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Ethical approval

This study was approved by the Research Ethics Committees of Mazandaran University of Medical Sciences (IR.MAZUMS.IMAMHOSPITAL.REC.1397.3534).

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