RESEARCH ARTICLE

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Significance of Serum Apelin Levels as a Biomarker for Gastric Cancer Diagnosis

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Abstract

Objectives: Gastric cancer (GC) is one of the common lethal disease and the most common cancers worldwide and in Iran. North of Iran is known as a common area of gastric cancer and a high-risk zone in Iran. Apelin is a biomolecule that plays roles in various types of cancers. This study was designed to investigate the serum apelin-12 levels in patients with gastric cancer as a predictive marker and affordable noninvasive alternative. **Methods:** In this case-control study, the case group included 42 patients with gastric cancer who were diagnosed by endoscopy and pathological findings. The participants in the case group were compared with the control group including 43 healthy individuals with no history of gastric cancer in their first-degree relatives and visiting the lab for routine tests. Apelin-12 serum level was assessed using ELISA kit. Data were analyzed in SPSS V16.0 applying Fisher's exact test, Mann-Whitney U test, and t-test. **Results:** Serum apelin-12 in patients with gastric cancer was found to be statistically lower than that in healthy individuals ($p< 0.05$). There were no significant differences between clinicopathological characteristics and apelin-12 expression. The median survival time in experimental and control groups was 16.0 months. **Conclusions:** In the current study, serum levels of apelin were significantly different between cases and controls.

Keywords: Gastric cancer- apelin- biomarker- serum- ELISA

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Introduction

Apelin is a biomolecule that is produced from a 77 amino acid protein of preproapelin encoded by the APLN gene on the long arm of chromosome X (Lee et al., 2000). This precursor synthesizes various shorter isoforms with an identical sequence in C-termini. Apelin-12 is the shortest isoform that actively induces different physiological effects via signaling pathways (Chapman et al., 2014). The width distributed of apelin and APJ in the body represents their contribution to biological functions and activities (Lv et al., 2013). Different roles of apelin are mediated by its binding to GPCR receptor APJ and regulating diverse physiological processes (Lv et al., 2017). Apelin is associated with adhesion molecules, chemokines, and inflammatory processes via NF-ƘB and PI3K pathways (Lv et al., 2013). Furthermore, the PI3K pathway leads to vascular proliferation and angiogenesis (Lv et al., 2013). The relationship between apelin and different types of cancers has been investigated in some studies (Podgórska et al., 2019a; Podgórska et al., 2018b; Marta B. Wysocka et al., 2018b). Apelin is believed to be related to different types of cancers including cholangiocarcinoma (CAA), none- small cell lung cancer, prostate, ovarian, oral and squamous cell carcinoma, and gastric cancer (GC) (Antushevich et al., 2018; Wysocka et al., 2018a). Today, cancer, like cardiovascular disease, is a common cause of death (Hedayatizadeh-Omran, 2014). Gastric cancer is becoming a serious health issue owing to its high frequency, aggressive nature, and low cure rate (Patru et al., 2013). It is considered as a third leading cause of cancer death and the fifth most common cancer worldwide (Bray et al., 2018). Also, GC is reported as a highly frequent cancer in Iran (Darabi et al., 2016; Rastaghi et al., 2019; Zarea et al., 2017). According to different researches, gastric cancer is more prevalent in Mazandaran province as a high-risk zone in Iran (Darabi et al., 2016; Farhood et al., 2018; Mousavi et al., 2015; Rastaghi et al., 2019). High incidence and mortality rate of gastric cancer and poor prognosis of GC patients cause a serious health concern (Hu et al., 2019; Matsuoka et al., 2018). Early detection of gastric carcinoma leads to appropriate therapeutic interventions and effective treatment decisions (Wu et al., 2014). The development of noninvasive and inexpensive methods for GC diagnosis and preventive screening seems to be clinically important that minimizes the patient's burden (Wu et al., 2019). Finding accurate, discriminative, prognostic, and validated biomarkers

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such as DNA, RNA, miRNA, and protein markers could be more reliable for disease prediction and personalized medicine (Abbas et al., 2018). High incidence of gastric cancer in Iran especially in Mazandaran province as a hot zone region (Khademloo, 2018), calls for improving detection and screening methods. This study was designed to evaluate the expression profile of serum AP-12 levels in gastric cancer patients compared with healthy controls to be considered as a predictive marker. Although gastric tumor apelin-12 level has been previously reported to be used as a marker for diagnosis (Feng et al., 2016), the purpose of this study was to make access easy and assess the serum AP-12 level in gastric cancer patients as a fast, cost-effective, and predictive biomarker.

Materials and Methods

Patients

We registered 42 patients diagnosed with gastric cancer confirmed by histological examination in Imam Khomeini Hospital, Sari, Iran. Blood samples of the patients were collected using tubes containing clot activator. The study included 43 healthy individuals who referred to perform routine blood tests for a regular checkup as a control group. All serum samples were prepared by centrifuge of the clotted whole blood (5 ml) at 800 g for 20 min. The serum was then transferred to sterile cryotubes and stored in -80°C refrigerator. All patients and controls provided and assigned the written informed consent.

Determining apelin-12 concentration by ELISA

Circulating apelin-12 was measured by immunoenzymatic assay using commercially available ELISA kits (abx150697, Abbexa, UK), according to the manufacturer's instructions. A competitive binding enzyme-linked immune-sorbent assay was used for the quantitative detection of apelin-12. An antibody specific to apelin-12 was pre-coated onto a 96 well plate. A competitive inhibition reaction was launched between biotin labeled apelin-12 and unlabeled apelin-12 with the pre-coated antibody specific to apelin-12. The unbound conjugates were washed away, avidin conjugated to horseradish peroxidase was added to each microplate well and incubated. Then the TMB substrate solution was added, the color change appeared only in wells containing apelin-12 by adding the acidic stop solution. The Optical Density (O.D). Absorbance was measured spectrophotometrically at 450 nm wavelength in a microplate reader Synergy™ HTX Multi-Mode Microplate Reader (BioTek; USA) equipped with specialized software Gen5 (BioTek; the USA). The concentration of apelin-12 was calculated based on the standard curve.

Statistical analysis

Data were analyzed in SPSS V16.0 applying Fisher's exact test, Mann-Whitney U test, and t-test. The survival rate was explained by Kaplan-Meier calculations. The Spearman's correlation let us know the association between the concentration of apelin-12 and survival rate. Moreover, this association was evaluated by Partial correlation after the removal of the effects of age and sex. The Univariate analysis was used to eliminate the effect of age and sex on differences of apelin-12 between the case and control groups.

Results

Forty-two gastric cancer patients and 43 healthy individuals without a family history of gastric cancer were studied. The mean age of patients was 67 and for controls, it was 61.02 years old. Male patients included 76.2% in the case group (P= 0.024). The demographic characteristics of the study population are presented in Table 1. The clinicopathological features of the GC patients are compared in Table 2. Kaplan–Meier survival analysis showed that the median survival time in the experimental group was 16.0 months (95% CI, 13.618- 18.382). The distribution of apelin-12 in both case and control groups are summarized in Table 3. The concentration of serum AP-12 levels was significantly higher in healthy controls (p= 0.024). There were significant differences in sex and age between the two groups and Univariate analysis demonstrated age and sex as independent prognostic factors (p= 0.879 and p= 0.073, respectively) that had no effect on apelin differences between the two groups. Apelin concentration exhibited no significant correlation with the survival rate of gastric cancer patients (p= 0.717, Spearman's- rho= 0.058). Also, the Spearman's rho correlation was not significantly different after the removal of sex and age by partial correlation (p= 0.358, spearman's- rho= -0.149).

Discussion

The apelin peptide is expressed in different types of organs such as the gastrointestinal tract, heart, lung, breast, brain, and liver (Heo et al., 2012). Apelin is linked to cell growth, migration, metastasis, and carcinogenesis via different mechanisms including impact on actin-binding protein, cofilin, regulating the cell adhesion and migration by remodeling of actin cytoskeleton (Podgórska et al., 2018b); involved in angiogenic responses via apelin/APJ, AMPK/eNOS and PI3K/Akt/eNOS signaling pathways and up-regulation of HIF-1, VEGF, and VEGFR (Kunduzova et al., 2008; Marta B. Wysocka et al., 2018b); regulation of angiogenesis by stimulation of proliferation, migration, and vascular formation (Kidoya et al., 2012; Wysocka et al., 2018; Yang et al., 2016). Apelin and its receptor adjust numerous pathways that lead to proliferation signals, evasion of apoptosis, sensitivity to blocking antibodies, and sustaining angiogenesis to promote invasion and metastasis (Arvin et al., 2018). This data confirm the important role of apelin in growth, proliferation, and metastasis. This study investigated the expression levels

Table 1. Demographic Characteristics of the Study Population

Sex	Control N (%)	Case N (%)	P-value
Male	22 (51.2)	32 (76.2)	0.024
Female	21 (48.8)	10 (23.8)	
Total	43 (100)	42 (100)	

Table 2. Frequency of Clinicopathological Features of Gastric Cancer Patients

Characteristics	Frequency (percent)
Tumor location	
Cardia	6 (14.6)
Fundus	5 (12.2)
Body	8 (19.5)
Antrum	13 (31.7)
Overlap	9 (22.0)
Grade	
1	5 (12.2)
2	23 (56.1)
3	13 (31.7)
Lymphatic invasion	
Yes	25 (61.0)
No	16 (39.0)
Perineural invasion	
No	15 (36.6)
Yes	26 (63.4)
T	
2	16 (39.0)
3	21 (51.2)
4	4 (9.8)
N	
0	11 (26.8)
1	18 (43.9)
2	10 (24.4)
3	2 (4.9)
M	
0	29 (70.7)
1	12 (29.3)
Stage	
I	7 (17.1)
II	15 (36.6)
III	9 (22.0)
IV	10 (24.4)
Tumor type	
Diffuse	26 (63.4)
Intestinal	15 (36.6)

of serum apelin-12 in gastric cancer patients compared with healthy controls. Our data showed that serum apelin-12 level was statistically lower in patients than that in healthy individuals ($p< 0.05$). We found no significant differences between clinicopathological characteristics and apelin-12 expression. Also, survival analysis showed that the median survival time in experimental and control groups was 16.0 months. Nevertheless, further studies with a larger sample size should be carried out to predict patient survival. Evidence shows that tumor overexpression of apelin is linked to advanced clinical features and poor outcomes in gastric cancer by interaction with various cytokines such as IL-1, IL6, MMP1, MMP9, and BMP-2 (Feng et al., 2016). Immunohistochemical staining also showed high expression levels of apelin in the cytoplasm and endothelial cells of tumor sample (Feng et al., 2016). Increased levels of secreted apelin are confirmed in colon cancer. Apelin was found to potentiate colon cancer cells to invade and migrate during carcinogenesis (Podgórska et al., 2018b). In an investigation on the angiogenic role of apelin in human non-small cell lung cancer (NSCLC), both mRNA and protein of apelin were seen to be highly expressed in lung cancer patients and cell lines. Apelin was also assumed as a novel angiogenic factor contributing to poor survival (Berta et al., 2010). The significantly increased levels of apelin were detected in non-smoker lung cancer patients in Iran (Gholamnejad et al., 2019). It is worth considering the apelin-12 levels in different types of lung malignancies. The analysis of both tumor and serum apelin revealed that the expression of apelin and its receptor in tumor tissue and sera of colorectal patients were significantly higher than those of the control samples. Apelin level is also reported to be associated with lymph node and distant metastasis (Podgórska et al., 2019b). According to immunohistochemical findings, both invasive ductal and lobular cells in breast cancer expressed a similar level of immunoreactive apelin compared with normal breast cells (Wang et al., 2008). The expression profile of apelin in hepatocellular carcinoma showed overexpression of apelin/APJ system in moderately and poorly differentiated tumors compared with well-differentiated ones. This may cause vascularization and angiogenesis in hepatocellular carcinoma (Muto et al., 2014). This system is also involved in a proliferation signaling pathway which leads to carcinogenesis. Apelin can promote and activate Pi3K/Akt cascade, transcription, and cell growth. Therefore, apelin can play a prognostic role in hepatocellular carcinoma (Chen et al., 2019). Some studies have proven the role of apelin in solid tumor spread. Rayalam et al reviewed the importance of apelin inhibition in the blockade of the angiogenic signaling pathway. They reported that bevacizumab, anti-VEGF monoclonal antibody, restrict new blood vessels formation via apelin-mediated pathways. Therefore, it is routinely employed as a drug to treat colorectal, lung, breast, glioblastoma, and kidney tumors (Srujana et al., 2011). The expression changes of the apelin were reported and confirmed in different types of cancers as mentioned above. It was clearly demonstrated that the expression levels of apelin in cancer samples significantly differed from those in

Table 3. Comparison of Apelin Serum Concentration in Patients with Gastric Cancer and Healthy Individuals

Case/ Control	Mean	Median	IQR	p- value *
Case	3614.7	1896.3	821.5- 4836	0.024
Control	6701.2	4086.3	1464.24- 9834.2	

*P-value refers to median comparison using Mann-Whitney test

normal subjects. The expression changes of apelin in both serum and tissue samples should be noted and their association with clinicopathological features have also to be evaluated. To consider apelin as a prognostic biomarker more experiments should be done on different samples. Although the role of apelin in carcinogenesis processes including inflammation, angiogenesis, proliferation, and metastasis was studied (Lugano et al., 2019; Podgórska et al., 2018a; Wysocka et al., 2018b), the expression profile of apelin must be clearly clarified.

In conclusion, here, we quantitatively discussed the expression levels of serum apelin in patients with gastric cancer. In conclusion, our findings showed significantly different serum levels of apelin between cases and controls. We would recommend some suggestions that are also our probable limitations and could be considered in further studies; larger sample sizes from the diverse geographical region may influence statistical results and may have the use of both tissue samples with serum samples to more reliable results. In addition, apelin monitoring prior to intervention and after treatment may give beneficial outcomes.

Author Contribution Statement

Akbar Hedayatizadeh-Omran: Concepts, Definition of intellectual, Literature search, Experimental studies, Manuscript editing. Ramin Shekarriz: Definition of intellectual, Clinical studies, Guarantor. Omolbanin Amjadi: Literature search, Experimental studies, Data acquisition, Manuscript preparation, Manuscript review. Reza Alizadeh-Navaei: Data acquisition, Data analysis, Statistical analysis, Manuscript review. Shahrzad Babamohammadi: Experimental studies, Manuscript preparation

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Ethical Declaration

The research was approved by the research ethics committee Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.REC.1396.39).

Conflicting Interest

The authors declare that they have no conflict of interest.

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