RESEARCH LETTER

Fibroblast growth factor 21, epidermal growth factor receptor, interleukin 6, myeloperoxidase, lipid hydroperoxide, apolipoproteins A-I and B, as well as lipid and lipoprotein ratios as diagnostic serum biomarkers for gastric cancer

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Introduction Gastric cancer (GC) is a malignant tumor characterized by high rates of morbidity and mortality, which mainly results from the absence of specific symptoms at early stages. Gastric cancer is classified according to a histologic type and the Lauren classification.[1] For an individual assessment of prognosis and type of treatment, the histologic tumor grade along with evaluation of the clinical stage is used. In GC, surgical treatment remains the main therapeutic option. Research suggests that combination therapy improves the outcomes of treatment, although with current chemotherapy regimens, response to treatment is observed in 40% to 60% of cases.[2] It is important to search for methods that would identify tumors sensitive to neoadjuvant treatment as well as examine the mechanisms responsible for resistance to oncologic treatment. Studies have shown that fibroblast growth factor 21 (FGF-21) levels are closely related to lipid metabolism.[3] It also plays a crucial role in maintaining proinflammatory/anti-inflammatory balance.[4]

There are scarce literature data on FGF-21 in GC.[3] It has been shown that gastric epithelial cells stimulate numerous signaling pathways, including epidermal growth factor receptor (EGFR) activation.[5] Production of proinflammatory cytokines (tumor necrosis factor α and interleukin 6 [IL-6]) by tumor or host tissue due to tumor presence leads both to systemic and local inflammation in cancer.[6] The inflammatory microenvironment promotes GC development and invasion.[6] The relationship between lipids, lipoproteins, inflammation, oxidative stress, as well as FGF-1 and EGFR levels is poorly understood.[7-10]

The aim of our study was to determine the concentrations of FGF-21, EGFR, IL-6, lipid hydroperoxide (LPO), myeloperoxidase (MPO), lipids, lipoproteins (apolipoproteins A-I [apoA-I] and B [apoB]), as well as lipid and lipoprotein ratios and to examine their associations with GC grade and stage. A better understanding of lipid and lipoprotein metabolism in GC might help develop biomarkers for early diagnosis and monitoring of this cancer as well as for improving clinical management of patients.

Patients and methods This study included 30 patients with gastric adenocarcinoma (4 women and 26 men; age range, 39–74 years), who were hospitalized in the 2nd Department of General and Gastrointestinal Surgery and Surgical Oncology of the Alimentary Tract at Medical University in Lublin (Poland) and who were referred for radical surgical treatment in combination with preoperative chemotherapy. Patients were divided into groups: patients with GC stage IIA+IIB, those with GC stage IIIA+IIIB, and controls. The control group consisted of 18 healthy volunteers (5 women and 13 men; age range, 30–55 years).

Blood serum was collected from patients before preoperative chemotherapy. Routine laboratory and lipid parameters were determined in fresh serum, using a Cobas Integra 6000 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). The remaining serum was aliquoted, frozen, and stored at a

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TABLE 1 Differences in laboratory parameters between patients with gastric cancer by stage, in all patients with gastric cancer, and in controls

Parameter	GC stage IIA+IIB (n = 11)	GC stage IIIA+IIIB (n = 19)	All GC patients (n = 30)	Controls (n = 18)
Age, y	57 (39–71)	59 (48–74)	59 (39–74)	53 (31–57)
BMI, kg/m^2	24 (21–30)	26 (20–31)	25.4 (20–31)	24 (21–27)
TC, mmol/l	4.76 (3.63–6.39)	4.56 (2.64–5.83)	4.63 (2.64–6.39)	5.13 (2.82–5.18)
LDL-C, mmol/l	2.95 (2.15–4.29)	2.77 (1.40–4.40)	2.82 (1.40–4.40)	3.03 (1.11–5.18)
HDL-C, mmol/l	1.30 (0.62–1.37)	1.01 (0.62–1.37)[c,d]	1.08 (0.62–53)[c]	1.48 (1.14–1.63)
TG, mmol/l	99 (44–212)	124 (44–286)	119 (44–1.37)	104 (30–239)
apoA-I, g/l	1.57 (1.14–2.73)	1.30 (0.90–1.87)[b,d]	1.46 (0.90–2.73)[a]	1.58 (1.15–1.99)
apoB, g/l	1.07 (0.68–1.61)[a]	0.99 (0.74–1.42)[a]	1.02 (0.68–1.61)[a]	0.70 (0.41–1.17)
TC/HDL-C ratio	3.80 (2.78–619)	4.69 (2.30–7.01)[b]	4.25 (2.30–7.01)[b]	3.47 (1.80–4.41)
LDL-C/HDL-C ratio	2.45 (1.23–4.51)[a]	3.03 (1.20–5.53)[b]	2.70 (1.20–5.53)[b]	2.01 (0.71–2.80)
TG/HDL-C ratio	2.11 (1.76–8.40)	3.18 (1.76–7.15)[b]	2.83 (1.76–8.17)[a]	1.70 (0.55–4.9)
apoB/apoA-I ratio	0.71 (0.12–0.99)[b]	0.75 (0.36–1.34)[b]	0.73 (0.12–1.34)[b]	0.46 (0.27–0.74)
HDL-C/apoA-I ratio	0.31 (0.25–0.42)	0.26 (0.19–0.43)[c,d]	0.28 (0.25–0.43)[b]	0.35 (0.32–0.56)
MPO, pg/ml	56 (17–266)	100 (40–435)[a]	78 (17–435)	46.0 (14–102)
LPO, nmol/l	150 (97–352)	153 (82–458)	151 (82–458)	134 (80–235)
FGF-21, pg/ml	223 (103–556)[b]	289 (104–748)[c,d]	255 (103–748)[b]	90 (40–165)
EGFR, pg/ml	50 (36–65)	51 (33–59)	50 (33–65)	46 (39–54)
IL-6, pg/ml	1.42 (0.80–10.63)[a]	3.10 (0.90–45) [c,d]	2.47 (0.80–45)[b]	0.74 (0.2–1.4)
MPO/apoA-I ratio	0.37 (0.15–2.31)	0.77 (0.35–4.80)[a]	0.53 (0.15–4.80)	0.30 (0.15–1.27)
MPO/HDL-C ratio	1.12 (0.56–8.86)	2.56 (2.10–8.20)[c]	1.86 (0.56–8.86)[b]	0.80 (0.44–8.37)

Data are presented as median (min–max).

a $P < 0.05$ vs controls; **b** $P < 0.01$ vs controls; **c** $P < 0.001$ vs controls; **d** $P < 0.05$ vs IIA+IIB group

Abbreviations: ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; BMI, body mass index; GC, gastric cancer; EGFR, epidermal growth factor receptor; FGF-21, fibroblast growth factor 21; HDL-C, high-density lipoprotein cholesterol; IL-6, interleukin 6; LDL-C, low-density lipoprotein cholesterol; LPO, lipid hydroperoxide; MPO, myeloperoxidase; TC, total cholesterol; TG, triglycerides

temperature of –80°C. The levels of apoA-I, apoB, MPO, IL-6, FGF-21, and EGFR were measured by enzyme-linked immunosorbent assay kits (R&D Systems, Inc, Minneapolis, United States), and the levels of LPO, by Lipid Hydroperoxide (LPO) Assay Kit (Cayman Chemical, Ann Arbor, Michigan, United States).

Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of Medical University in Lublin (KE-0254/297/2016) and conducted in accordance with the principles of the Helsinki Declaration.

Statistical analysis For a comparison of more than 2 groups, the Kruskal–Wallis test was used. The associations between FGF-21 or EGFR levels and LPO, MPO, IL-6, lipid, apoA-I, and apoB concentrations as well as lipid and lipoprotein ratios were examined by the Spearman correlation analysis. A forward stepwise multiple regression analysis was used to assess the relationship between FGF-21 as a dependent variable and EGFR, LPO, MPO, IL-6, lipid, apoA-I, and apoB concentrations as well as lipid and lipoprotein ratios as independent variables. In the model of multiple regression analysis, high correlations between predictor variables result in inadequate regression coefficients. In such cases, a forward stepwise multiple regression analysis improves the accuracy of the model. In this model, FGF-21 or EGFR was selected as the dependent variable and LPO, MPO, IL-6, lipids, and lipoproteins as independent variables, and for each of the independent variables, parameters were calculated according to the equation: $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n$. The relationship between the dependent variables is expressed by the coefficient of forward stepwise multiple regression (β), which provides information about the relationship between the dependent variable (FGF-21) and independent variables.

The significance level for all variables was set at a P value of less than 0.05.

Results Patients with GC at a lower stage had beneficial lipid and apoA-I levels as well as the ratio of apoA-I to high-density lipoprotein cholesterol, but not apoB, FGF-21, and IL-6 levels or the apoB/apoA-I ratio. Patients with a higher tumor stage showed lower HDL-C and apoA-I levels as well as HDL-C/apoA-I ratio, and higher apoB, FGF-21, MPO, and IL-6 levels as well as apoB/apoA-I, MPO/apoA-I, and MPO/HDL-C ratios (TABLE 1).

The Spearman analysis showed correlations between FGF-21 and HDL-C (R = –0.5, P = 0.01); EGFR and HDL-C/apoA-I (R = 0.44, P = 0.02); FGF-21 and EGFR (R = –0.44, P = 0.03); FGF--21 and LDL-C/HDL-C (R = 0.42, P = 0.04); EGFR and HDL-C (R = 0.51, P = 0.01); MPO and LDL-C (R = 0.41, P = 0.049); MPO and the ratio of HDL-C to total cholesterol (TC) (R = 0.45, P = 0.02); LPO and LDL-C (R = 0.41, P = 0.049); IL-6 and apoB/apoA-I (R = 0.43, P = 0.03); MPO and HDL-C (R = –0.66, P = 0.03); and IL-6 and HDL-C (R = –0.6, P = 0.04).

The forward stepwise multiple regression analysis showed that FGF-21 (R^2 = 0.4) levels were negatively correlated with EGFR levels (β = –0.59, P = 0.01), while EGFR levels (R^2 = 0.6), with IL--6 levels (β = –0.54, P = 0.02). This suggests that elevated EGFR levels, in part, resulted in a decrease of FGF-21 levels, and that IL-6 (R^2 = 0.6, β = -0.54, P = 0.02), in part, resulted in a decrease of EGFR levels.

Discussion Our patients had abnormal lipid and lipoprotein levels, either too low or too high, suggesting that they had dyslipidemia and dyslipoproteinemia despite normal concentrations of TC, LDL-C, and triglycerides. The levels of apoA-I and HDL-C as well as the HDL-C/apoA-I ratio remained unchanged, but FGF-21, IL-6, and apoB levels as well as the apoB/apoA-I ratio increased in patients with GC stage IIA+IIB. This result is in contrast to that reported by Shi et al.[10] Furthermore, these abnormalities were considerably worse in the IIIA+IIIB group, and we observed a significant decrease in HDL-C and apoA-I levels as well as the HDL-C/apoA-I ratio and a significant increase in apoB levels as well as apoB/apoA--I and lipid ratios, which is in line with a study by Ma et al.[7] These disturbances were accompanied by increased FGF-21, IL-6, and MPO levels as well as MPO/apoA-I and MPO/HDL-C ratios and were significantly worse compared with the results for the IIA+IIB group.

For the first time, we showed that GC patients had abnormal MPO, IL-6, and FGF-21 levels as well as MPO/apoA-I and MPO/HDL-C ratios, and that the disturbances were more pronounced with the increasing stage of GC. Moreover, increased FGF-21 concentrations were shown to differentiate between different stages of GC.

The Spearman correlation and the forward stepwise multiple regression showed that FGF--21, EGFR, LPO, MPO, and IL-6 concentrations modified lipid and lipoprotein levels. Our study revealed disturbances in the metabolism, composition, and concentration of lipids and apoB in low-density lipoprotein (LDL) particles, as well as disturbances in the metabolism, composition, and concentration of apoA-I and HDL-C in high-density lipoprotein (HDL) particles depending on inflammation and oxidative stress. Higher MPO and IL-6 concentrations resulted in a reduction of apoA-I levels and a significant increase of MPO/apoA-I and MPO/HDL-C ratios. Inflammation induces an increase in MPO concentrations, which decreases apoA-I and HDL-C levels, and consequently, the HDL particle gradually loses its properties.[11] Huang et al[12] reported that both HDL and its structural protein, apoA--I, are dysfunctional and are oxidized to a large extent by MPO.

Our results are in line with those reported recently by other authors.[7-12] Zamanin-Daryoush et al[8] noted that lipid and cholesterol homeostasis is dysregulated in GC, which makes it easier for cancer cells to proliferate and avoid apoptosis. However, the apoA-I/HDL ratio showed antitumor effects, and in GC, it can modulate cholesterol content in immune and tumor cell membrane lipid rafts and influence signaling pathways.[8] The lipid rafts serve as a platform for biologically active lipids and proteins that may impact the immune response and the communication between the tumor surrounding stromal cells.[8] Antitumor function of ApoA-I/HDL appears to modulate the immune response. The appropriate composition of the ApoA-I/HDL ratio is associated with the conversion of macrophages from protumor M2 to antitumor M1 phenotype.[8] Tumor--associated macrophages are the essential part of the tumor microenvironment and promote cancer invasion.[6] It was reported that higher FGF-21 levels may serve as a potential biomarker of early--stage breast cancer, and that the monitoring of FGF-21 levels could help determine the prognosis.[3] Activation of EGFR enhances cell growth, differentiation, and proliferation and can promote the development of malignancies.[5] Sierra et al[5] suggested that EGFR activation can lead to GC.

We investigated new markers of GC. Our results indicated that FGF-21 can be a candidate biomarker for early-stage GC. However, further studies should be conducted on a larger group of patients, with GC grading depending on cancer stage.

In conclusion, this study suggests that the relationship between FGF-21, EGFR, and IL-6 levels in patients with GC affects the immune response and tumor cell membrane lipid raft. In patients with GC, the concentrations of FGF-21 and EGFR, as well as inflammation and oxidative stress connected with the disorders of metabolism of LDL and HDL particles, can lead to cancer progression. Moreover, FGF-21 can be used as a biomarker of early-stage GC.

ARTICLE INFORMATION

CONFLICT OF INTEREST None declared.

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