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Fibroblast growth factor-21, epidermal growth factor receptor, interleukine-6, myeloperoxidase, lipid hydroperoxide, apoAI, apoB concentration, and lipid and lipoprotein ratios, as diagnostic serum biomarkers for gastric cancer

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Conflict of interest: none declared

Short title: FGF-21 level as a new marker in gastric cancer
INTRODUCTION

Gastric cancer (GC) is a malignant tumor characterized by high rates of morbidity and mortality which mainly results from a lack of specific symptoms presented in early stages. GC is classified in terms of histopathological type and Lauren classification [1]. For individual assessment of prognosis and type of treatment the histopathologic tumor grade along with evaluation of the clinical stage are used. In GC, surgical treatment remains the main therapeutic method. Research suggests that combination therapy improves the results of treatment although currently available chemotherapy regimens are characterized by a response to treatment observed in 40-60% of cases [2]. Searching for methods of identifying tumors sensitive to neoadjuvant treatment and examining the mechanisms responsible for oncological resistance is currently important. Scientific studies have shown that FGF-21 levels are closely related to lipid metabolism [3]. FGF-21 also plays a crucial role in the balance of the proinflammation/antiinflammation system [4]. Unfortunately, literature information about FGF-21 in gastric cancer was poor [3]. It has been shown that gastric epithelial cells stimulate numerous signaling pathways, including epidermal growth factor receptor (EGFR) activation [5]. Pro-inflammatory cytokines (TNF-α, IL-6) produced by tumor or host tissue due to tumor presence leads to both systemic and local inflammation in cancer [6]. The inflammatory microenvironment promotes gastrointestinal cancer development and invasion [6]. The relationship between lipids, lipoproteins, inflammation, oxidative stress, and FGF-1 and EGFR level is poorly understood [7-10].

The aim of our research is to determine the concentrations of FGF-21, EGFR, IL-6 (interleukine – 6), LPO (lipid hydroperoxide), MPO (myeloperoxidase), lipids, lipoproteins (apoAI, apoB), lipid and lipoprotein ratios and its relationship with GC tumor grade and stage. Understanding the metabolism of lipids and lipoproteins in GC can help to develop biomarkers for early diagnosis, monitoring and imploring clinical management.
PATIENTS AND METHOD: This study involved 30 patients with gastric adenocarcinoma, 4 females and 26 males, aged 39–74 years, hospitalized in the Second Department of General and Gastrointestinal Surgery and Surgical Oncology of the Alimentary Tract of the Medical University in Lublin, Poland, who were qualified for radical surgical treatment with the combination of preoperative chemotherapy. All patients were divided into groups: stage IIA+IIB; stage IIIA+IIIB; controls. The control group consisted of 18 healthy volunteers, 5 females and 13 males, aged 30–55 years. Written informed consents were obtained from all the participants. The study was approved by the Ethics Committee of the Medical University in Lublin, Poland (KE-0254/297/216) and carried out in accordance with the principles of the Helsinki Declaration.

The study material was blood serum collected from patients before preoperative chemotherapy. Routine laboratory and lipid parameters were determined in fresh serum on Cobas Integra 6000 analyzer. The remaining of the sera was aliquoted, frozen and stored at -80° C. Consequently the concentration of apoAI, apoB, MPO, IL-6, FGF-21, and EGFR was measured by ELISA R&D Systems kits and LPO using Cayman’s Chemical Lipid Hydroperoxide kit.

Statistical analysis. For comparison of more than two groups, the Kruskal-Wallis test was used. The relation between FGF-21 or EGFR levels and concentration of LPO, MPO, IL-6, lipid, lipoproteins and lipid and lipoprotein ratios were examined by Spearman’s correlation analysis.

Multiple ridge stepwise forward regression analysis was used to investigate the relationship between FGF-21 as dependent variable and EGFR, LPO, MPO, IL-6, lipids, apoAI, apoB concentrations, lipids, and lipid and lipoprotein ratios as non-dependent variable. In the model of multiple regression analysis, high correlations between predictor variables result in inadequate regression coefficients. In such cases, multiple ridge stepwise forward regression
analysis improves the accuracy of the model. In the model of multiple ridge forward stepwise regression analysis, FGF-21 or EGFR was selected as the dependent variable and LPO, MPO, IL-6, lipids, lipoproteins as the non-dependent variable, and for each of the non-dependent 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 multiple ridge stepwise forward regression (\( \beta \)), which provides information about the relationship between the dependent FGF-21 and the non-dependent variables.

The statistical significance of all variables was established at \( P<0.05 \).

RESULTS

The results indicated that GC patients with lower GC stage had a beneficial concentration of lipids and apoAI level, HDL/apoAI ratio, but not apoB, and FGF-21 and IL-6 level and apoB/apoAI ratio. The patients with higher GC stage showed worse HDL-C, apoAI, apoB, FGF-21, MPO, IL-6 level and HDL-C/apoAI, apoB/apoAI, MPO/apoAI and MPO/HDL-C ratios (table 1).

The Spearman’s analysis showed correlation between:

- FGF-21 and HDL-C level (\( R=-0.496, p=0.010 \));
- EGFR and HDL-C/apoAI (\( R=0.438, p=0.019 \));
- FGF-21 and EGFR level (\( R=-0.438, p=0.028 \));
- FGF-21 and LDL-C/HDL-C ratio (\( R=0.422, p=0.044 \));
- EGFR and HDL-C level (\( R=0.509, p=0.010 \));
- MPO and LDL-C level (\( R=0.405, p=0.049 \));
- MPO level and TC/HDL ratio (\( R=0.446, p=0.019 \));
- LPO and LDL-C level (\( R=0.405, p=0.049 \));
- IL-6 level and apoB/apoAI ratio (\( R=0.430, p=0.031 \));
- MPO and HDL-C level (\( R=-0.663, p=0.025 \));
Multiple ridge stepwise forward regression analysis showed that FGF-21 ($R^2=0.395$) level depends significantly negatively on the concentration of EGFR ($\beta=-0.590$, $p=0.007$), but EGFR level ($R^2=0.596$) depends’ significantly negatively on IL-6 concentration ($\beta=-0.540$, $p=0.016$), which suggested that EGFR level, in part, resulted in decrease in FGF-21 concentration, and that IL-6 ($R^2=0.594$, $\beta=-0.540$, $p=0.016$), in part, resulted in decrease of EGFR level.

DISCUSSION

In the study groups lipid and lipoprotein levels varied from decreased to increased, suggesting dyslipidemia and dyslipoproteinemia of the study subject despite the unquestioned concentration of TC, LDL-C, TG. The concentration of apoAI, HDL-C and HDL-C/apoAI ratio remained unchanged, but FGF-21, IL-6, apoB level and apoB/apoAI ratio increased in IIA+IIB GC staging in contrast to that reported by Shi F, et al. [10]. Furthermore, these disorders were worsened considerably in the IIIA+IIIB group, and we observed a significant decrease in HDL-C, apoAI level and HDL-C/apoAI ratio and significantly increased apoB concentration and apoB/apoAI and lipid ratios, which was confirmed by other studies [7]. These disturbances were accompanied by increased of FGF-21, IL-6, MPO levels and MPO/apoAI and MPO/HDL-C ratios, and were significantly worsening as compared to IIA+IIB group.

For the first time we showed that GC patients had disturbed apoAI, HDL-C, apoB, MPO, IL-6 and FGF-21 levels and HDL-C/apoAI and apoB/apoAI, MPO/apoAI and MPO/HDL-C ratios which were worsening together with stage of GC, and that increasing FGF-21 concentration is a marker which differentiated GC patients depending on the stage of gastric cancer.

The Spearman’s correlation and the multiple stepwise forward regressions showed that FGF-21, EGFR, LPO, MPO and IL-6 concentration modified lipids and lipoproteins. Our results
showed disturbances in the metabolism, composition and concentration of lipids and apoB in LDL particles, as well as disturbed metabolism, composition and concentration of apoAI and HDL-C in HDL particles depending on inflammation and oxidative stress. A higher MPO and IL-6 concentration resulted in deterioration of apoAI concentration and a significant increase in MPO/apoAI and MPO/HDL-C ratios. The inflammation induces increase MPO concentration which decreases apoAI and HDL-C level and HDL particle and losing slowly its properties [11]. Huang Y, et al. [12] reported that both HDLs and its structural protein, apo A-I, are dysfunctional and are grossly oxidized by MPO. The presented studies are focused in the recently reported studies of other authors. [7-12]. Zamanin-Daryou M, et al. [8] reported that in gastric cancer, homeostasis of lipids and cholesterol is dysregulated which makes easier cancer cells to proliferate and avoid apoptosis. However, apoAI/HDL composition showed anti-tumor effects, and in gastric cancer can modulate cholesterol content in immune and tumor cell membrane lipid rafts and influence signaling pathways [8]. The lipid rafts play the role of a platform for biologically active lipids and proteins that may impact the immune response, the communication between the tumor surrounding stromal cells [8]. Antitumor function ApoA-I/HDL appears to modulate immune response. ApoAI/HDL composition modulates macrophages from pro-tumor M2 to anti-tumor M1 phenotype for tumor rejection [8]. Tumor-associated macrophages (TAMs) are the essential part of the tumor microenvironment and promote the cancer invasion [6]. Higher FGF-21 levels could be used as an early biomarker in early-stage breast cancer patients, and the monitoring of FGF-21 levels could estimate prognosis [3]. EGFR activation enhances cell growth, differentiation, proliferation and can promote the development of malignancies [5]. Sierra JC, et al. [5] suggests that activation of EGFR can lead to gastric cancer.
We investigated new markers of GC. Our results indicated that FGF21 can be a candidate for a biomarker for early GC stage. However, the study must be conducted in larger group of patients with grading GC depending on staging cancer.

CONCLUSIONS. This study suggests that relation between FGF-21, EGFR and IL-6 levels in GC patients affects immune and tumor cell membrane lipid raft. ApoAI and HDL function play an important role in gastric cancer reject in early stage and can help to assess progression of GC stages. In GC patients the concentration of FGF-21, EGFR and inflammatory, oxidative stress and disorders of LDL and HDL particles metabolism can lead to progression of GC. Moreover, FGF-21 can be used as a biomarker in early GC staging.

REFERENCES


Table 1. Lipids, lipoproteins, myeloperoxidase, lipid hydroperoxide, interleukine - 6, fibroblast growth factor-21, epidermal growth factor receptor level and lipid lipoprotein ratios in stage gastric cancer (GC) patients, and controls, median (min-max).

<table>
<thead>
<tr>
<th></th>
<th>IIA+IIBGC patients n=11</th>
<th>IIA+IIIBGC patients n=19</th>
<th>All GC patients n=30</th>
<th>Controls n=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages, years</td>
<td>57(39-71)</td>
<td>59(48-74)</td>
<td>59(39-74)</td>
<td>53(31-57)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24(21-30)</td>
<td>26(20-31)</td>
<td>25.4(20-31)</td>
<td>24(21-27)</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.76(3.63-6.39)</td>
<td>4.56(2.64-5.83)</td>
<td>4.63(2.64-6.39)</td>
<td>5.13(2.82-5.18)</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.95(2.15-4.29)</td>
<td>2.77(1.40-4.40)</td>
<td>2.82(1.40-4.40)</td>
<td>3.03(1.11-5.18)</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.30(0.62-1.37)</td>
<td>1.01(0.62-1.37)↓</td>
<td>1.08(0.62-5.3)***</td>
<td>1.48(1.14-1.63)</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>99(44-212)</td>
<td>124(44-286)</td>
<td>119(44-137)</td>
<td>104(30-239)</td>
</tr>
<tr>
<td>apoAI, g/L</td>
<td>1.57(1.14-2.73)</td>
<td>1.30(0.90-1.87)↓</td>
<td>1.46(0.90-2.73)*</td>
<td>1.58 (1.15-1.99)</td>
</tr>
<tr>
<td>ApoB, g/L</td>
<td>1.07(0.68-1.61)*</td>
<td>0.99(0.74 – 1.42)*</td>
<td>1.02(0.68-1.61)*</td>
<td>0.70 (0.41-1.17)</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>3.80(2.78-619)</td>
<td>4.69(2.30-7.01)**</td>
<td>4.25(2.30-7.01)**</td>
<td>3.47 (1.80-4.41)</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>2.45(1.23-4.51)*</td>
<td>3.03(1.20-5.53)**</td>
<td>2.70(1.20-5.53)**</td>
<td>2.01 (0.71-2.80)</td>
</tr>
<tr>
<td>TG/HDL-C</td>
<td>2.11(1.76-8.40)</td>
<td>3.18(1.76-7.15)**</td>
<td>2.83(1.76-8.17)*</td>
<td>1.70(0.55-4.9)</td>
</tr>
<tr>
<td>apoB/apoAI</td>
<td>0.71(0.12-0.99)**</td>
<td>0.75(0.36-1.34)**</td>
<td>0.73(0.12-1.34)**</td>
<td>0.46 (0.27-0.74)</td>
</tr>
<tr>
<td>HDL-C/apoAI</td>
<td>0.31(0.25-0.42)</td>
<td>0.26(0.19-0.43)***↓</td>
<td>0.28(0.25-0.43)***</td>
<td>0.35 (0.32-0.56)</td>
</tr>
<tr>
<td>MPO, pg/ml</td>
<td>56(17-266)</td>
<td>100(40-435)*</td>
<td>78(17-435)</td>
<td>46.0 (14-102)</td>
</tr>
<tr>
<td>LPO, nmol/L</td>
<td>150(97-352)</td>
<td>153(82-458)</td>
<td>151(82-458)</td>
<td>134(80-235)</td>
</tr>
<tr>
<td>FGF-21, pg/ml</td>
<td>223(103-556)**</td>
<td>289(104-748)***↓</td>
<td>255(103-748)**</td>
<td>90(40-165)</td>
</tr>
<tr>
<td>EGF R, pg/ml</td>
<td>50(36-65)</td>
<td>51(33-59)</td>
<td>50(33-65)</td>
<td>46(39-54)</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>1.42(0.80-10.63)*</td>
<td>3.10(0.90-45)**↓</td>
<td>2.47(0.80-45)**</td>
<td>0.74 (0.2–1.4)</td>
</tr>
<tr>
<td>MPO/apoAI</td>
<td>0.37(0.15-2.31)</td>
<td>0.77(0.35-4.80)*</td>
<td>0.53(0.15-4.80)</td>
<td>0.30(0.15-1.27)</td>
</tr>
<tr>
<td>MPO/HDL-C</td>
<td>1.12(0.56-8.86)</td>
<td>2.56(2.10-8.20)***</td>
<td>1.86(0.56-8.86)**</td>
<td>0.80(0.44-8.37)</td>
</tr>
</tbody>
</table>

*- 0.05 p, **- 0.01p, ***- 0.001 p vs controls; ↓- 0.05 vs IIA+IIB group, BMI – body mass index; MPO – myeloperoxidase; LPO - lipid hydroperoxide; EGF-21 – fibroblast growth factor-21; IL-6 – interleukine-6; FGFR- epidermal growth factor receptor