Abstract

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Title of the dissertation: Assessment of the relationship between melatonin, secretion of selected adipokines, and markers of inflammation and oxidative stress in the course of neuroendocrine tumors. New potential diagnostic markers.

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Neuroendocrine neoplasms (NENs) are a heterogeneous group of neoplasms originating from a disseminated system of neuroendocrine cells. Apart from the genetic etiology, the cause of NENs is not fully known.

The most common NENs include gastroenteropancreatic (GEP-NETs) and lungs (L-NENs) tumors. They are still a big diagnostic problem due to the lack of characteristic symptoms and specific markers helpful in their diagnosis. Their final diagnosis often takes place at the stage of advanced disease or at the time of metastasis, which significantly delays effective therapy and is associated with the risk of metastases. Among non-specific markers useful in the diagnosis of NENs, chromogranin A (CgA), neuron-specific enolase (NSE) and gonadotropins should be mentioned, while specific markers include insulin, glucagon, gastrin or somatostatin, which also manifest the clinical feature in a given type of NENs.

The aim of this study was to determine a number of parameters related to oxidative stress, endocrine function of adipose tissue and inflammation in the blood of patients with NENs of various locations and in healthy people, as well as to identify potential new diagnostic markers for NENs.

The study included 86 patients of the Oncology Center Prof. Franciszek Łukaszczyk in Bydgoszcz, Poland diagnosed with NENs of various locations (pancreas, gastrointestinal tract, lung, other locations). The patients were divided according to the tumor location: pancreas – pNENs (n=22), gastrointestinal tract – GI-NENs (n=34), lungs – L-NENs (n=12), other location – o-NENs (n=18). The control group consisted of 35 healthy people. The exclusion criteria for the NEN patients included other cancers and acute and chronic diseases, while the control group excluded the presence of acute and chronic diseases (cancer, diabetes, obesity), autoimmune and cardiometabolic disorders. The material for the study was venous blood

samples collected once, in the morning, on an empty stomach by qualified personnel. For the planned tests, 9 ml of blood sample was collected into a tube with anticoagulant for the plasma and erythrocyte isolation, while 9 ml of blood sample was collected into a tube without anticoagulant, containing clotting activator and separating gel, for the serum isolation. The research was carried out with the consent of the Bioethics Committee at Collegium Medicum in Bydgoszcz of the Nicolaus Copernicus University in Toruń: KB 423/2020. Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activities were measured in erythrocytes, while malonyldialdehyde (MDA) level was assessed in both the erythrocytes and blood plasma. Spectrophotometric methods and kinetic measurement were used to measure the above-mentioned parameters. CgA, melatonin and omentin-1 concentrations were determined using ready-made ELISA kits. The concentration of selected adipokines and a number of inflammatory parameters were determined using ready-made multiplex ELISA kits.

As a part of the statistical analysis, the mean and standard error, as well as the median with the first and third quartiles were determined for each group. A one-way ANOVA or its equivalent, the Kruskal-Wallis test, was also performed among four groups of NEN patients with NENs. Correlation coefficients between the CgA values and the other parameters were determined. For the selected parameters, an analysis was performed to plot the ROC (receiver operating characteristic) curves with cut-off points, as well as the sensitivity and specificity of the variables, and a logistic regression model was determined to determine the probability of the disease.

Increased plasma MDA concentration in the NEN patients was observed, regardless of the location. In the case of the erythrocytic MDA concentration, a significant change concerned NENs, L-NENs and o-NENs compared to the control group, which indicates that an increased lipid peroxidation process accompanies the course of NENs. The SOD activity decreased significantly in all study groups compared to the control group, while no changes were noted in the case of CAT. The GPx activity increased significantly in pNENs, while, in GI-NENs, its activity decreased compared to the control group. Melatonin concentration was significantly lower in all groups of NENs.

In the case of diabetic parameters, statistically significant differences in the group of patients with NENs concerned the concentration of glucose-dependent insulinotropic peptide (GIP), insulin, glucagon and plasminogen activator inhibitor 1 (PAI-1) compared to with the control group. A decreased concentration of ghrelin was noted in the group of patients with

L-NENs. However, among the tested adipokines, an increase in the concentration of visfatin and a decrease in the concentration of resistin were observed in all locations of NENs compared to the control group. The concentration of omentin-1 increased in all groups except for o-NENs, while the level of leptin showed a difference only in the group of patients with o-NENs. The CgA concentration was statistically significantly higher in NENs, pNENs and L-NENs.

Among the parameters of inflammation, a statistically significant increase in the concentration of the following parameters was observed in all tested groups in relation to the control group: CTACK (cutaneous T-cell-attracting chemokine), eotaxin, G-CSF (granulocyte colony-stimulating factor), GM-CSF (granulocyte-macrophage colony-stimulating factor), GRO-α (growth regulated oncogene), HGF (hepatocyte growth factor), IFN-α2, IFN-γ (Interferone γ), IL-1α (IL), IL-1β, IL-1ra, IL-2, IL-2Rα, IL-4, IL-5 , IL-6, IL-7, IL-8, IL-10, IL-15, IL-16, IL-17A, LIF (leukemia inhibitory factor), MCP-1 (monocyte chemotactic protein 1), MCP-3, M-CSF (macrophage colony stimulating factor), MIF (macrophage migration inhibitory factor), MIG (monokine induced by interferon-γ), β-NGF (nerve growth factor β), RANTES (regulated on activation, normal T-cell expressed and secreted), SCF (factor stem cell factor), SCGF-β (stem cell growth factor), TNF-α (tumor necrosis factor), VEGF (vascular endothelial growth factor). In turn, the decrease in the concentration of TNF-β and PDGF-BB (platelet-derived growth factor) was observed in all tested groups compared to the control group. There were no statistically significant changes in IL-13 and IP-10 (interferon γ-induced protein 10 kDa levels in all groups of patients.

In addition, a positive correlation between the concentration of CgA and such parameters of oxidative stress and inflammation as GPx, GLP-1 (glucagon-like peptide-1), M-CSF, GM-CSF, CGF- β, HGF, GRO-α, IFN-γ, IL-1α, IL-2Rα, IL-5, IL-9, IP-10, MIP-1β (macrophage inflammatory protein 1 beta), TNF-β, glucagon, leptin, visfatin, was shown, while the omentin-1 level was negatively correlated with the concentration of CgA.

The analysis of the ROC curves allowed to indicate the most sensitive and specific parameters, which were also used for the logistic regression analysis in order to determine the disease probability pattern. The formula was created based on the concentration of GRO-α and TNF-β, which allowed for the extreme differentiation the groups of NENs. The sensitivity and specificity for GRO-α and TNF-β were 83% and 11% and 93% and 5%, respectively. Verifying the ROC curve for CgA, it was noted that the sensitivity for this parameter was 72%, and the specificity was 26%.

The results of the study indicate that oxidative stress, inflammation and impaired adipose tissue function are involved in the course of NENs. Parameters with greater sensitivity than the CgA level, which could be used as markers in the diagnostics and maybe also in the prognosis or therapeutic process of NENs, were identified. The observed significantly lower concentration of melatonin may suggest its influence on the cancer pathogenesis or its secondary deficiencies associated with the process of carcinogenesis. The predictive formula for NENs determined with the use of GRO-α and TNF-β concentrations allows for the confirmation or exclusion of those neoplasms with an accuracy of 94.5%.

Undoubtedly, further research is needed on the role of the indicated new potential NEN markers not only in the diagnosis, but also in the progression of the disease and response to therapy.

Keywords: adipokine, inflammation, melatonin, neuroendocrine tumors, oxidative stress