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Clinical significance of cathelicidin antimicrobial peptide in patients with pancreatic cancer**

Kliniczne znaczenie katelicydyny u chorych na raka trzustki

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Keywords

pancreatic cancer, cathelicidin, antimicrobial peptide, LL-37

Słowa kluczowe

rak trzustki, katelicydyna, peptyd antydrobnoustrojowy, LL-37

Summary

Introduction. Cumulative evidence from tumor biology studies indicates that cathelicidin, a multifunctional host defence peptide, might play an important role in carcinogenesis.

Aim. The aim of our study was to assess the clinical significance of cathelicidin antimicrobial peptide (CAMP) in patients with pancreatic cancer.

Material and methods. The study included 139 patients: 101 with pancreatic cancer and 38 gender and age-matched healthy volunteers. The serum concentrations of CAMP were measured by an enzyme-linked immunoassay (USCN life science, INC. Houston, USA). The associations of the analysed cathelicidin and clinical data at diagnosis have been evaluated. The consent of the bioethical committee od Medical University of Łódź, as well as written consent from all the subject were obtained.

Results. Serum levels of cathelicidin were higher in patients with pancreatic cancer compared to control group – 2519.1 vs 249.2 ng/ml respectively ($p < 0.001$). Increased CAMP levels were associated with with high bilirubin and low hemoglobin levels in PC patients ($p < 0.05$). The current study failed to show the correlation between cathelicidin and tumor size, its histologic grade and the presence of regional or distant metastases. The CAMP levels were also unrelated to other analysed clinical data of PC patients.

Conclusions. In conclusion, in our preliminary study we suggested the possible role of cathelicidin in pancreatic carcinogenesis however further studies are needed to ultimately define the clinical significance of this peptide.

Streszczenie

Wstęp. Dane z badań podstawowych wskazują, że katelicydyna, wielofunkcyjny peptyd antydrobnoustrojowy, może odgrywać istotną rolę w procesie karcynogenezy w trzustce.

Cel pracy. Celem pracy była ocena znaczenia klinicznego katelicydyny u chorych na raka trzustki (RT).

Materiał i metody. Do badania zakwalifikowano 139 chorych: 101 z rozpoznanym RT i 38 zdrowych ochotników. Stężenie katelicydyny w surowicy było mierzone metodą immunoenzymatyczną z użyciem testu USCN life science, INC Houston, USA. Przeanalizowano związek między badanym parametrem a danymi klinicznymi chorych na RT. Uzyskano zgodę komisji bioetycznej Uniwersytetu Medycznego w Łodzi oraz pisemne zgody pacjentów na przeprowadzenie badania.

Wyniki. Stężenie katelicydyny u chorych na RT było wyższe w porównaniu z grupą kontrolną (odpowiednio 2519,1 vs 249,2 ng/ml ($p < 0,001$)). Wysokie stężenie katelicydyny wiązało się ze wzrostem bilirubiny i niskim poziomem hemoglobiny u chorych na RT ($p < 0,05$). Nie wykazaliśmy z kolei zależności między katelicydyną a rozmiarem guza, zróżnicowaniem histopatologicznym guza oraz obecnością przerzutów odległych i do węzłów chłonnych. Stężenie katelicydyny nie było też związane z żadnymi innymi danymi klinicznymi chorych.

Wnioski. Podsumowując, wstępne wyniki naszej pracy sugerują prawdopodobną rolę katelicydyny w procesie karcynogenezy w trzustce, potrzebne są jednak dalsze badania, aby jednoznacznie potwierdzić jej rolę.

Conflict of interest
Konflikt interesów

None
Brak konfliktu interesów

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INTRODUCTION

The searching of links between cancer and the human immune system becomes particularly important issue. The cathelicidin is a multifunctional host defence peptide, the full significance of which in the human immune defences is only beginning to be fully recognized (1, 2). The only human cathelicidin peptide, known as LL-37, is a free peptide part of the precursor protein hCAP18, acting as the active form of cathelicidin antimicrobial peptide (CAMP). It is encoded by the CAMP gene, located on chromosome 3p21 (3, 4). Cathelicidin has been discovered to mediate many host responses, including antimicrobial activity, angiogenesis, and activation of chemokine secretion. This peptide is secreted by bone marrow cells, circulating leukocytes, and numerous types of epithelial tissues, such as skin and gastrointestinal mucosa (2, 5). Cathelicidin is also a promoter of islet paracrine signaling that enhances islet function and glucoregulation (6).

Cumulative evidence from tumor biology studies indicates that cathelicidin plays a prominent role in carcinogenesis. An increasing amount of evidence suggests that LL-37 can have two different and contradictory effects and may act either as a pro-tumorigenic or anti-cancer agent, dependently on tumor biology (7). Expression of cathelicidin is upregulated in various ovarian cancer types, including serous adenocarcinomas, mucinous adenocarcinomas and granulosa cell tumors when compared with normal ovarian tissues (8). It has been also shown that LL-37 stimulates proliferation and independent growth of cultured lung cancer cells as well as promotes tumorigenicity and formation of larger tumors in a lung cancer xenograft model (9).

On the other hand the expression of cathelicidin was remarkably downregulated in human colon cancer tissues whereas exogenous LL-37 induced apoptotic cell death in cultured colon cancer cells (10). Cheng et al. also reported that cathelicidin inhibits colon cancer development by interfering with epithelial-mesenchymal transition and cancer associated fibroblast (11). Results of other study show that during progression from atrophic gastritis to adenocarcinoma, the expression of LL-37 is reduced. It has been observed that this peptide is absent or expressed at very low levels in gastric hyperplastic polyps, tubular adenomas, and adenocarcinomas (12).

Little is known about role of cathelicidin in pancreatic carcinogenesis. Recently, Sainz et al. have reported that hCAP-18/LL-37 was strongly expressed in the stroma of advanced primary and secondary pancreatic duct adenocarcinomas and was secreted by immune cells of the stroma in response to tumour growth factor-β1 (13). To the best of our knowledge there are no other studies concerning the role of this peptide in pancreatic cancer.

AIM

The aim of our study was to assess the clinical significance of cathelicidin antimicrobial peptide in patients with pancreatic cancer.

MATERIAL AND METHODS

The study included 139 patients: 101 with pancreatic adenocarcinoma (48 men and 53 women aged 51-83) and 38 gender and age-matched healthy volunteers. Analysed patients were hospitalized in Department of Digestive Tract Diseases of Medical University of Łodź or Department of Digestive Tract Surgery of Silesian Medical University between 2012 and 2014. The pathologic diagnosis of ductal pancreatic adenocarcinoma were confirmed in all cases. Nineteen patients (18.8%) with PA underwent Whipple resection or distal pancreatectomy, 25 patients (24.8%) – palliative surgery and 57 patients (56.4%) – palliative chemotherapy and/or palliative endoscopic treatment.

The associations of the cathelicidin levels and clinical data at diagnosis have been evaluated. The following demographic and clinical data have been analysed: age, tumor size, lymph node involvement, histological grade, distant metastases, history of smoking, weight loss > 10% as well as selected laboratory parameters: Ca 19-9, total bilirubin, hemoglobin and glucose serum levels.

Peripheral venous blood samples were obtained from all analysed patients at the time of hospital admission. All subjects were free of known infection or renal disease and were not taking immunosuppressive medications. Sera were separated by centrifugation at 3000 revolutions per minute and were stored at -80°C until the levels of analysed markers were assessed. The serum concentrations of CAMP were measured by an enzyme-linked immunoassay (USCN life science, INC. Houston, TX, USA), according to the manufacturer's recommendations. The minimum detectable dose of human CAMP was less than 46 pg/mL. The sensitivity of this assay, or lower limit of detection was defined as the lowest protein concentration that could be differentiated from zero.

Statistical analysis comprised arithmetical mean, median and standard deviation. To determined differences between groups Mann-Whitney t-test were used. Association between continues variables was analyzed with Pearson's correlation test. P-values < 0.05 were considered to be significant.

The consent of the bioethical committee od Medical University of Łódź, as well as written consent from all the subject were obtained.

RESULTS

Mean ages of analysed patients were not significantly different for those with pancreatic cancer (mean 63.7 ± 3.8) and controls (60.2 ± 4.1; $p > 0.05$). In patients with pancreatic adenocarcinoma the tumor size ranged from 1.5 to 6.2 cm (mean 3.4 ± 2.3). For histological differentiation 29, 32 and 33 patients were classified into G1, G2 and G3 respectively, whereas 6 patients had missing data. Lymph nodes metastases were observed in 52 patients with pancreatic cancer (51.9%) and liver metastases – in 21 of them (20.8%). Twenty nine patients (28.7%) presented weight loss > 10% with the mean weight loss 8.4 ± 0.9 kg during 6 months.

Serum levels of CA19-9 as well as bilirubin levels were higher in patients with pancreatic cancer

compared to control group ($p < 0.001$; respectively 201.3 ± 17.4 U/ml versus 17.9 ± 4.2 U/ml for CA19-9 and 4.1 ± 1.4 mg/dl versus 0.8 ± 0.2 mg/dl for bilirubin). Moreover, statistically significant difference was also found between mean hemoglobin and glucose levels in pancreatic cancer patients compared to healthy volunteers. The mean hemoglobin level was lower in analysed patients (10.2 ± 0.2 mg/dl) versus control group (13.1 ± 0.4 mg/dl; $p < 0.05$) and glucose levels higher (137 ± 4.3 mg/dl) in PC group versus 85.1 ± 2.1 mg/dl in healthy volunteers ($p < 0.05$).

Serum levels of cathelicidin were also statistically higher in patients with pancreatic cancer compared to control group (fig. 1). In PC patients the median CAMP levels were 2519.1 ± 41.2 ng/ml, and in the control group 249.2 ± 13.7 ng/ml ($p < 0.001$). The potential correlation between CAMP levels and clinical data of PC patients was investigated. Increased CAMP levels were associated with decreased hemoglobin results and severe anemia in PC patients ($p < 0.0003$; fig. 2). The positive correlation between CAMP and bilirubin serum levels was also observed un this group of patients ($p < 0.05$; fig. 3). The current study failed to show the relationship between analysed cathelicidin and CA 19-9 results, patients age, weight loss, tumor size and the presence of regional or distant metastases (fig. 4-7). The CAMP levels were also unrelated to the tumor histologic grade (data not shown).

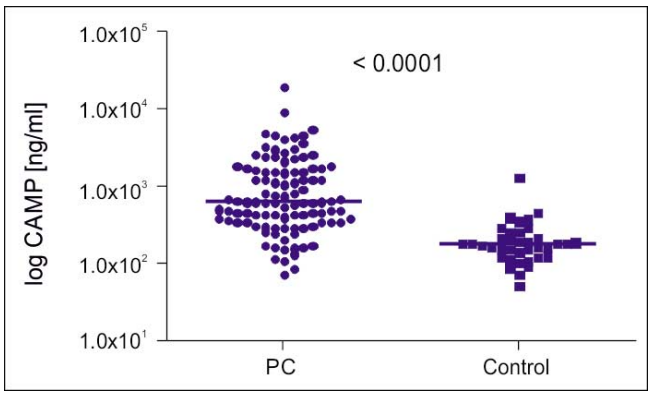

Fig. 1. Comparison of CAMP serum levels in patients with pancreatic cancer (PC) and control group

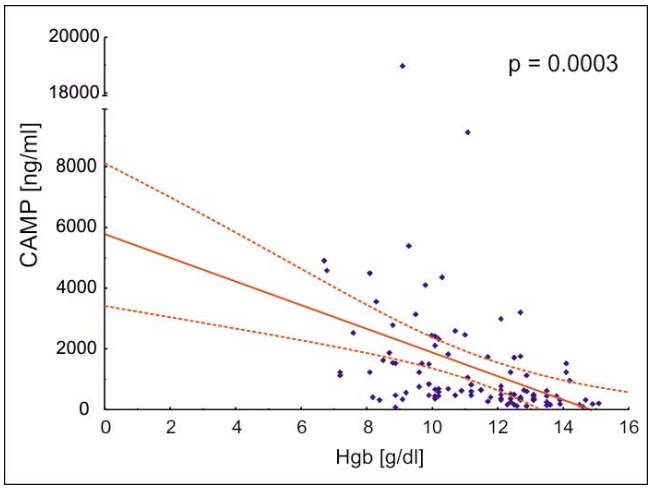

Fig. 2. The correlation between CAMP and hemoglobin serum levels in patients with pancreatic cancer

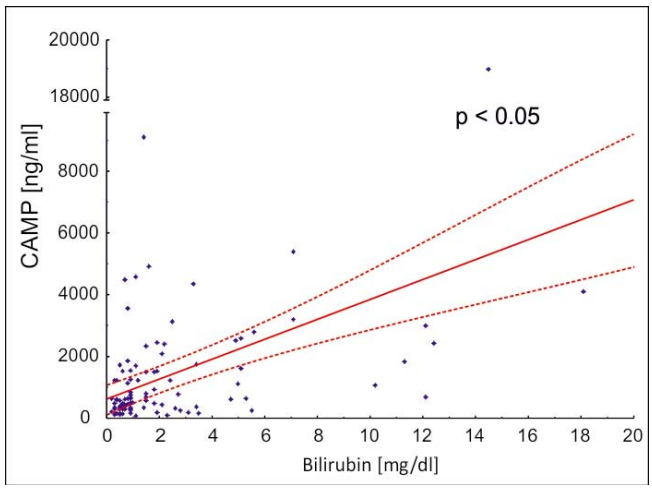

Fig. 3. The correlation between CAMP and bilirubin serum levels in patients with pancreatic cancer

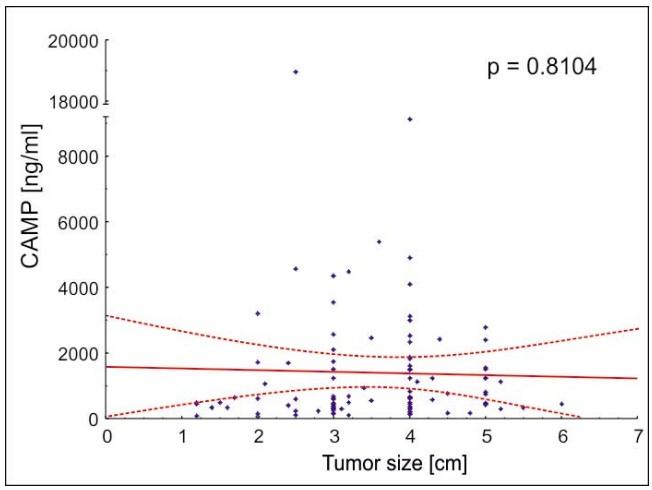

Fig. 4. The correlation between CAMP serum levels and tumor size of patients with pancreatic cancer

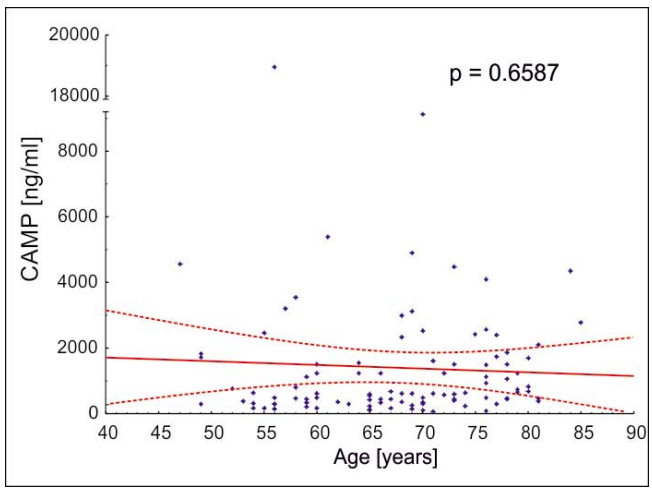

Fig. 5. The correlation between CAMP serum levels and age of patients with pancreatic cancer

DISCUSSION

To our knowledge, our study is the first report of systemic serum levels of cathelicidin in pancreatic diseases patients. However, in recently published data the role of cathelicidin in microenvironment of pancreatic

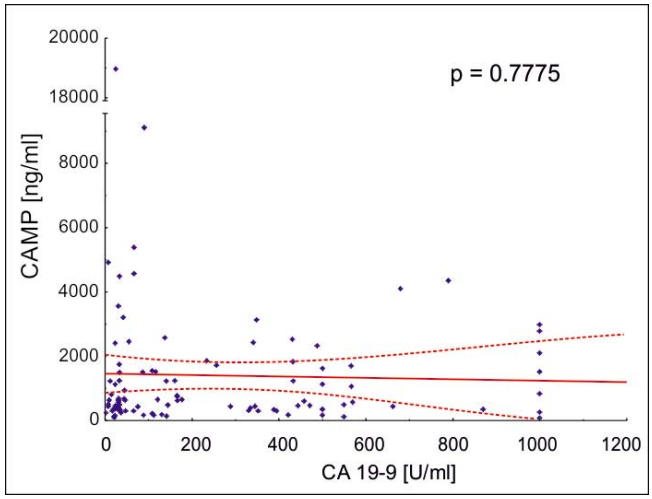

Fig. 6. The correlation between CAMP and CA19-9 serum levels in patients with pancreatic cancer

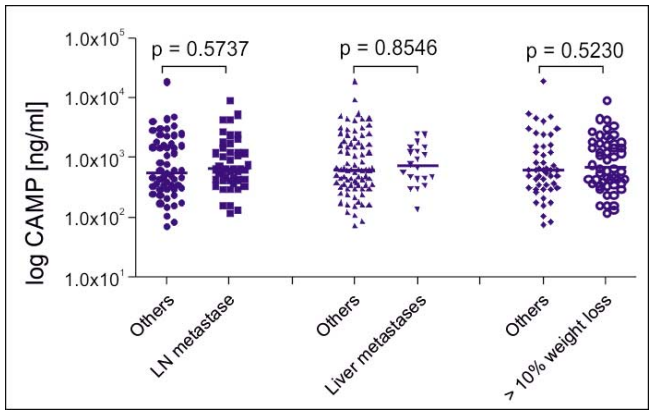

Fig. 7. The CAMP serum levels in patients with weight loss, lymph node (LN) and liver metastases

tumors was confirmed. In the unique study concerning pancreatic cancer, Sainz et al. observed that cathelicidin not only provide structural support for pancreatic tumour development, but more importantly may provide cues to cancer stem cells that regulate their self-renewal and metastatic potential. They proved that treatment of pancreatic cancer stem cells with recombinant LL-37 increased pluripotency-associated gene expression, self-renewal, invasion and tumourigenicity (13).

In the present study we observed higher serum levels of cathelicidin in patients with pancreatic cancer compared to control group. Earlier increased LL-37 serum level were shown in patients with psoriasis, Behçet's disease, systemic bacterial infection and hepatitis B and C virus disease (14-17). In the recently published study increased serum cathelicidin levels were also observed in patients with inflammatory bowel diseases, without association with duration of diseases as well as age and gender of patients (18).

Some studies reported the prognostic significance of cathelicidin isolated from peripheral blood. Gombart et al. observed that its lower baseline levels is associated with increased risk of death attributable to infection in hemodialysed patients. Individuals with the lower serum levels of cathelicidin had an approximately 3-fold increase 1-year mortality due to infectious causes (19). Similarly, in adults hospitalized with community-acquired pneumonia, there was a trend towards increased 30-day mortality with lower cathelicidin (16). Moreover, lower cathelicidin levels were also independently associated with the necessary of bronchiolitis hospitalization ≥ 24 hours (20).

It was also observed that cathelicidin level negatively correlates with systemic lupus erythematosus disease activity index and clinical disease activity of patients with Crohn's disease, especially with stricture formation (18, 21). The authors suggested that based on the anti-fibrogenic effect of cathelicidin in animal models, it is reasonable to expect that low cathelicidin level correlates with an elevated risk of intestinal stricture (18).

The source of circulating cathelicidin is still not definitively known. It is known that plasma LL-37 levels might depend on neutropenic conditions and might constitute an indicator of myelopoietic activity. Ye et al. assessed the value of the plasma protein hCAP-18 in patients with neutropenia lasting more than two months. They suggested that the plasma protein hCAP-18 might be a marker to discriminate neutropenia of various etiologies. Benign forms of primary chronic neutropenia could thus be distinguished from chronic neutropenia with underlying severe diseases (22). Further assessment of cathelicidin will benefit from the identification of agents that can modulate its systemic levels. Identification of cathelicidin levels in other groups of patients is essential to assess its full clinical significance.

Moreover, according to published data the cathelicidin may be involved in metastasis development. It may facilitate tumor progression through multiple mechanisms, including increased proliferation, migration and invasion (23-25). Weber et al. reported that treatment with LL-37 peptide significantly stimulated the migration of breast cancer cells and their colonies acquired a dispersed morphology indicative of increased metastatic potential (26). Similarly it was prouved that LL-37 increased tumor cell proliferation, migration and invasion of A375 and A875 malignant melanoma cell lines (27). It was also demonstrated that LL-37 is overexpressed in bone metastasis of prostate cancer what suggest it importance during neoplastic progression (28).

However, the data from literatures about the role of cathelicidin in carcinogenesis are not uniform. Some reports have shown that LL-37 may even induce cell death in many tissues (10, 11, 29). It is suggested that the anticancer effect of LL-37 might be associated with its bactericidal properties. The relationship between bacterial infections and cancer has been proven, and a reduction of bacterial infections can have a direct impact on the development of cancer (30). In contrast,

Li et al. found that levels of cathelicidin were increased in colon cancer tissues and cathelicidin was mainly expressed in immune cells. Moreover, the neutralization of this peptide, significantly reduced the proliferation of tumor cells, resulting in an inhibition of tumor growth (31). We belived that this issue merit further evaluation, based on prospective observations of oncologic patients. Thus, we plan the next investigation concerning the analysis of cathelicidin expression in pancreatic cancer tissue compared with clinical data of patients.

CONCLUSIONS

In conclusion, in our preliminary study we confirmed the possible role of cathelicidin in patients with pancreatic cancer. We suggest that serum levels of cathelicidin are worth determining in different oncologic patients. However, our study may be difficult to compare because there is no data about this peptide serum level in patients with pancreatic diseases. Further studies on the biological activity of the cathelicidin are needed to ultimately define the role of this peptide in pancreatic carcinogenesis.

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