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ORIGINAL ARTICLE

Resistin expression in pancreatic cancer

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Purpose: The aim of this study is to search the expression of resistin and its relation with other prognostic parameters in pancreatic ductal adenocarcinoma by immunohistochemical method.
Methods: A total of 26 patients who had a histopathological diagnosis of pancreatic ductal adenocarcinoma and were followed up in Antalya Education and Research Hospital were enrolled in the study. The immunohistochemically stained sections obtained from biopsy materials of these patients were evaluated and scoring was performed.
Results: Resistin was found negative in 20 (76.9%) and positive in 6 (23.1%) of the patients. No significant relationship was found between resistin expression and gender, age, stage and the type of surgery applied. When the factors affecting survival were investigated, there was no significant relationship between gender, performance score, stage, and survival. In addition, no significant relationship was found between resistin expression and survival.
Conclusion: Some previous studies established that adipocytokines like resistin, leptin, etc. that are derivated from adipose tissue may play a role in the development, progression and determination of prognosis of the cancer types. In our study, no significant relationship was found between resistin expression and the survival in pancreatic cancer. We suggest that more studies are needed to establish the effects of resistin, especially to reveal its effects on pancreatic cancer.
Keywords: Resistin, pancreatic cancer, immunohistochemistry.

Pankreas kanserinde resistin ekspresyonu

Amaç: Bu çalışmanın amacı pankreatik duktal adenokarsinomda resistin ekspresyonunu ve diğer prognostik parametrelerle ilişkisini immünohistokimyasal yöntemle araştırmaktır.
Yöntem: Çalışmaya, histopatolojik olarak pankreas duktal adenokarsinom tanısı konan ve Antalya Eğitim ve Araştırma Hastanesi'nde takip edilen toplam 26 hasta alındı. Bu hastaların biyopsi materyallerinden elde edilen immünohistokimyasal olarak boyanan bölümler değerlendirildi ve skorlama yapıldı.
Bulgular: Resistin hastaların 20'sinde (%76.9) negatif, 6'sında (%23.1) pozitif bulundu. Resistin ekspresyonu ile cinsiyet, yaş, evre ve uygulanan ameliyat tipi arasında anlamlı bir ilişki bulunmadı. Sağkalımı etkileyen faktörler araştırıldığında; cinsiyet, performans skoru, evre ve sağkalım arasında anlamlı bir ilişki gözlenmedi. Ayrıca, resistin ekspresyonu ile sağkalım arasında anlamlı bir ilişki bulunamadı.
Sonuç: Daha önceki bazı çalışmalar, yağ dokusundan üretilen resistin, leptin vb. gibi adipositokinlerin, farklı kanser türlerinin gelişiminde, ilerlemesinde ve prognozun belirlenmesinde rol oynayabileceğini göstermiştir. Çalışmamızda pankreas kanserinde resistin ekspresyonu ile sağkalım arasında anlamlı bir ilişki bulunmadı. Resistinin, özellikle pankreas kanserindeki etkilerini ortaya koymaya yönelik daha fazla çalışmaya ihtiyaç duyulduğunu düşünüyoruz.
Anahtar Kelimeler: Resistin, pankreas kanseri, immünohistokimya.

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Pancreatic cancer presents a high mortality and morbidity rate. It is the eighth most common reason of cancer-related deaths in the world [1]. The high rate of mortality of this cancer has led to search for some other prognostic factors.

Fat tissue functions as an endocrine organ that synthesizes and secretes many factors with various effects [2]. In recent years, it has been shown that adipocytokines secreted by the fat tissue such as tumor necrosis factor–alpha (TNF-α), interleukin (IL-6), type 1 plasminogen activator inhibitor, hepatocyte growth factor, adiponectin, leptin, resistin, visfatin, and apelin play a role in metabolic syndrome and carcinogenesis [3]. Adipocytokines play an important role in tissue homeostasis; obesity-related diseases such as non-alcoholic liver disease (NALD) and hepatocellular carcinoma as its complication [4]. Resistin is known as one of these adipocytokines. It is secreted by the fat tissue and is associated with obesity, insulin resistance, and inflammation [5]. All three of these factors are known as risk factors for pancreatic cancer. However, the relation between resistin and pancreatic cancer is not clearly understood yet.

In this study, the resistin expression was searched in pancreatic ductal cancer by immunohistochemical method.

METHODS

Patient selection

This study included patients who were histopathologically diagnosed pancreatic ductal adenocarcinoma (Figure 1) and in follow-up in Antalya Education and Research Hospital between January 2013 and October 2013. Patients who were staged according to radiological and clinical findings were restaged according to the 7th edition of the American Joint Committee on Cancer (AJCC). Finally, a total of 26 patients with appropriate tissue samples were enrolled in the study. Demographic data such as age, gender, and information about the stage of the disease and the treatment applied were obtained by searching the patient files.

The study protocol was approved by the Ethics Committee of the Antalya Education and Research Hospital. Ethics committee number is 2017-027.

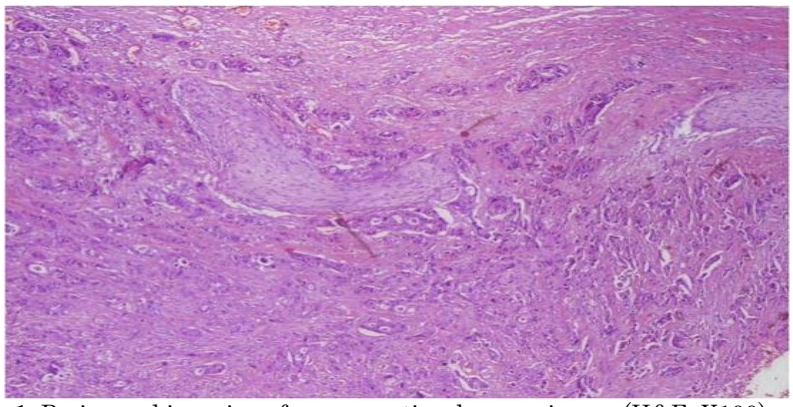

Figure 1. Perineural invasion of a pancreatic adenocarcinoma (H&E, X100)

Tissue preparation and immunohistochemical staining

Biopsy materials obtained for histopathological diagnosis were placed in 10% formaldehyde immediately after the process and fixed for 24 hours. After the fixation, materials were grossly examined and appropriately sampled. Tissue samples were embedded in paraffin after routine tissue procedure. Immunohistochemical staining was applied on cross-sections obtained from the tissue samples with tumor chosen after evaluating the hematoxylin and eosin-stained slides. Cross-sections of 4-µm thickness prepared for immunohistochemical staining were deparaffinised in an oven at 60°C for 2 h. Subsequently, they were kept in xylene for 30 min and 100% alcohol for 30 min, and then washed with water. Slides were kept in a solution buffered with 10% citrate in the microwave at maximum power (800 watts) for 15 min. Afterward, the power was decreased by half for an additional

20 min in the microwave. Slides brought out of the microwave were kept at room temperature for 20 min. Endogenous peroxidase activity was removed by being kept in 3% hydrogen peroxide for 10 min. Slides that were washed with Phosphate buffered saline (PBS), were processed with 3x5 PBS and then kept in protein blockage for 10 min. After being kept in primary antibody Anti-resistin mouse monoclonal antibody (Anti-Resistin antibody (ab136877), Abcam, England) for 60 min, they were washed in PBS for 5 min. Afterward, they were treated with biotinylated secondary antibody (Novocastra peroxidase kit- Newcastle) for 20 min and washed with PBS for 5 min. They were kept with peroxidase-conjugated antibody for 20 min. Then they were washed in PBS for 5 min. They were kept in chromogenic Diaminobenzidine (DAB) for 5 min. Slides were washed under tap water and then counterstained with hematoxylin. They were dehydrated, dried, and mounted with Entellan.

Evaluation of immunohistochemically stained sections

Resistin expression in the tumor cells was evaluated by 2 pathologists (ASA, DS) who were unaware of the clinicopathological information of the patients. The cytoplasmic staining was evaluated and the scoring was performed according to the study of Lee YC et al., in which they searched the expression of resistin in breast cancer [6]. The scoring system is as follows: Score 0: no stained cell is observed, score 1: staining is observed in 25% and less of the tumor cells, score 2: staining is observed in 26-50% of the tumor cells, score 3: staining is observed in 51-75% of the tumor cells, and score 4: staining is observed in 76% and more of the tumor cells. Score 0 cases were evaluated as negative, whereas cases with score 1,2,3, and 4 were evaluated as positive.

Statistical analysis

Statistical analyses were carried out by SPSS software for Windows 15.0. Suitability of variables to normal dispersion was observed by using visual (histograms and probability graphics) and analytical (Kolmogorov-Smirnov, and Shapiro-Wilk test) methods. In Kolmogorov-Smirnov testing, p values above 0.05 are considered as normal dispersion. Differences between groups were observed by using the chi-square and Mann-Whitney U test and by Student's t-tests in the case of normal age dispersion. Kaplan-Meier survival analysis was performed for the relation of each immunohistochemical positive and negative result with survival. Statistical differences were confirmed by the log-ranking test. P values less than 0.05 were considered to be significant.

RESULTS BULGU

The study included 11 (42.3%) female and 15 (57.7%) male patients. The mean age of the patients was 69.1±8.6 (range:51-89). In means of the stage of the disease, 3 (11.5%) patients were in stage II, 7 (26.9%) patients were in stage III and 16 (61.5%) patients were in stage IV. Palliative surgery was applied in 3 and curative surgery in 8 patients whereas surgery was not applied in 15 of the patients, who were diagnosed by histopathologic evaluation of the biopsy material. According to the ECOG performance score, the ECOG score was found as 0,1,2, and 3 in 6 (%23.1), 11 (%42.3), 8 (%30.8), and 1 (%3.8) of the patients, respectively.

Resistin was found negative in 20 (76.9%) and positive in 6 (23.1%) of the patients (Figure 2,3 and 4). No significant relation was found between resistin expression and gender, age, stage and the type of surgery applied (p:0.664, p:0.217, p:0.773, p:0.599). The median follow-up was 5 (range:0.43-50.5) months. The 12-month and 24-month survival rates of the patients were found as was 23%, and 14%, respectively. Median survival was found as 4.4±2 (%95 Confidence Interval:0.38-8.42). When factors affecting survival were searched; no significant relation was observed between gender, performance score, stage and survival (p:0.228, p:0.835, p:0.16). There was no significant relationship between resistin expression and survival (p:0.685).

DISCUSSION

In our study, resistin expression was found positive in 23.1% of the pancreatic ductal adenocarcinoma cases, by immunohistochemical method. A significant relation was not found between resistin expression and other prognostic factors.

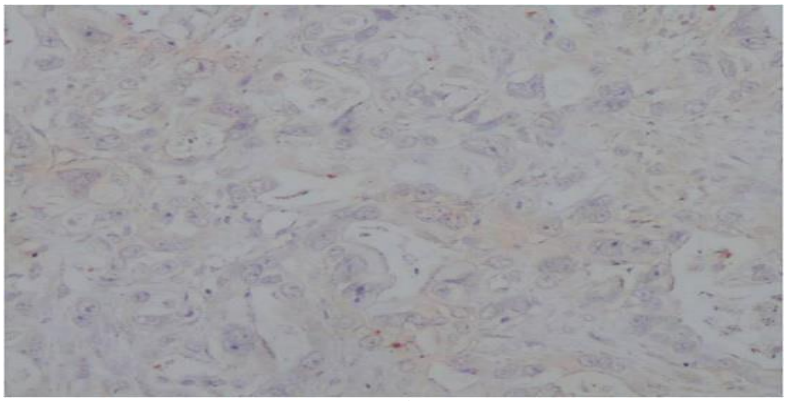

Figure 2. No staining with resistin in tumour cells (Immunohistochemistry,Resistin Antibody, X400)

Resistin is an adipocytokine derived from the adipose tissue that play role in lipid and glucose metabolism, energy balance, regulation of body homeostasis, inflammatory processes, and malignancies [7-9].

Among the studies related with adipocytokines, it is established that they play a role in the development and progression of cancer and determining the prognosis of the patients [10-12]. It is shown that an adipocytokine- resistin has a role in some cancer processes [11]. Increased expression of resistin was associated with tumor invasion, lymph node metastasis, and tumor differentiation in intestinal type gastric carcinomas [12].

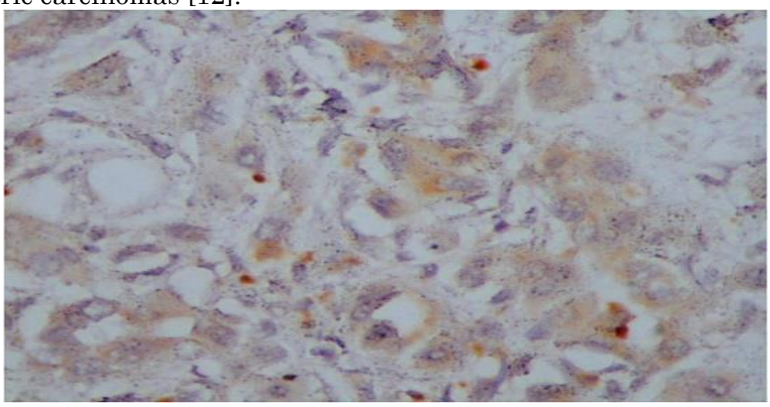

Figure 3. Cytoplasmic staining of resistin in tumour cells (Immunohistochemistry,Resistin Antibody, X400)

It has been shown that resistin plays a role in obesity and gastric cancer development with other adipocytokines such as TNFα, IL-6, adiponectin, leptin, visfatin that are secreted primarily secreted from the visceral fat tissue [13].

Yung-Yu Hsieh et al. showed that stromal cell-derived factor-1 (SDF-1) activated by resistin inhibits Toll-like receptor 4 (TLR4) in a series of gastric cancer cells. They suggested that the interaction between resistin activated SDF-1 and TLR4, p38 MARK, NF-KB may explain the relation of resistin with obesity and gastric cancer [14].

It is suggested that resistin can be effective in carcinogenesis is the angiogenic pathways. It is established that resistin can induce endothelial cell proliferation and migration. Resistin plays a role in the expression of VEGFR and MMP's and activates the ERK1/2 and p38 pathways, and as a result plays an important role in angiogenesis-related vascular diseases [15].

Pang et al. suggested that resistin has a role in the regulation of VEGF and angiogenic processes in ovarian cancer. In their study, they found that (HO-8910) resistin increased in VEGF protein and mRNA expression according to concentration, in ovarian epithelial carcinoma cells. When resistin was added to the cell culture formed by HO-8910 cells, a tube formation was observed and it was removed by anti-VEGF antibodies [16].

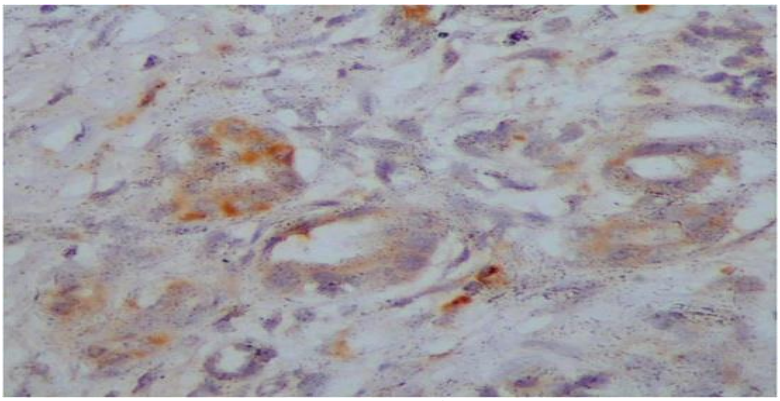

Figure 4. Cytoplasmic staining of resistin in tumour cells (Immunohistochemistry,Resistin Antibody, X400)

Diakowska et al. investigated resistin expression in normal and tumoral mucosal samples of gastroesophageal cancer patients and found that it was higher in tumoral mucosa than normal mucosa [11]. The highest resistin levels were observed among patients with cachexia and distant metastases; they suggested that resistin is an adipocytokine that is associated with cancer cachexia and metastatic processes. Their study was similar to our study stating that they found no relation between clinicopathological parameters.

Nakajimaan et al. evaluated the serum levels of adiponectin, resistin, visfatin, and leptin in their study and suggested that resistin and visfatin are appropriate biomarkers for evaluating gastric cancer [17]. In their study, it was found that resistin and visfatin have a significant correlation with BMI in the patient or control groups. They established that there was a relation between an increase in resistin and visfatin levels and stage, in contrast to our study.

In a study of Kumor et al. searching patients with an adenomatous polyp and colorectal cancer, serum levels of resistin were found higher in patients with colorectal cancer than the control group, but not significantly different from the patients with adenomatous polyp [18].

In a study evaluating the levels of adiponectin, visfatin and resistin in different groups of patients with colorectal cancer (CRC), colorectal polyp, inflammatory bowel disease (IBD) and in healthy control groups by ELISA method, different results were obtained. Resistin level was found highly elevated in the IBD group compared to the other groups, but no significant difference was found between CRC and control group [19].

In another study investigating the relationship between the serum levels of resistin and colorectal cancer, a higher resistin level was found in the group with cancer than the control group and that as the stage of the tumor increased the resistin level increased gradually. Besides, a significant relation was found between the levels of resistin and CRP pointing to the association with inflammatory processes [20]. A meta-analysis study summarising the studies searching the relation between the obese-related adipokines and colorectal cancer risk suggested that an increase in resistin levels upregulates the inflammatory cytokines that can increase the resistin levels afterward and increase the risk of cancer via NF-κB signaling pathway [21].

Serum resistin levels and insulin resistance were measured in patients diagnosed HCV related liver cirrhosis with hepatocellular carcinoma (HCC) and without HCC compared with healthy controls. It was found that HCC patients had higher mean values of HOMA-IR and resistin than cirrhotic patients and the control group. The study suggested that resistin and HOMA-IR can be considered as independent risk factors in the development of HCC and therfore can be used for early identify the HCV-related cirrhotic patients who are at increased risk for HCC [22].

Resistin has been established to play a role in the development of other types of cancer such as breast cancer, prostate cancer, endometrial cancer, and multiple myeloma [23-26]. In a study of Ilhan et al; while the serum level of resistin showed no significant difference between endometrial cancer patients and controls, high levels of resistin were found to be associated with lymph node metastasis in patients with endometrial cancer [27]. In a study searching the resistin expression in breast cancer

and adjacent normal breast tissue by immunohistochemical method, a high ressistin expression was found in association with a more malignant clinicopathological status and poorer survival [6].
Resistin is a cancer target as well. In a study searching the effects of the interaction between tumor and the tumor microenvironment on cancer progression and tumor-associated modulation, it was established that the tumor-associated dendritic cells (TADCs), which are the source of resistin, enhances the transition from epithelial to mesencyhmal in lung cancer and resistin in cell culture environment increases cell migration and invasion [28]. Neutralization of the environment from resistin has prevented advanced malignancy. In this study, in addition to the cell culture, an anti resistin antibody was given to the mice and a decrease in development and metastasis of lung cancer was observed.

Zyromski et al searched the effect of obesity on pancreatic cancer in their study with mice and found that larger tumors develop in obese mice and higher rates of metastasis and mortality [29]. They concluded that insulin resistance in obesity affects the tumor microenvironment directly and causes the development and progression of pancreatic cancer in this way.

In a study of Jiang et al, resistin expression was searched among 45 patients diagnosed with pancreatic ductal adenocarcinoma by the immunohistochemical method. The relation of resistin expression with clinicopathological features and prognosis was evaluated [30]. Resistin was found positive in 22 (48.9%) of the patients. They found a significantly higher rate of resistin expression in stage III-IV patients than stage I-II patients according to the Japan Pancreas Society Staging. In our study, we found the resistin positivity rate of 23% in patients; however, we did not find a significant relation between stage and resistin expression.

Limitations

The most important limitation of our research is that the number of patients is relatively low.

CONCLUSION

The most important limitation of our study is the number of cases. Thus, we were not able to establish the relation of well known prognostic factors such as performance score and stage with survival. Furthermore, all of the biopsy materials that were searched for the resistin expression did not include radical resection specimens as in the other studies. We searched the resistin expression in very small biopsies in 15 of the cases, that the evaluation of immunohistochemical staining had to be performed in a few numbers of cells. This situation may be more significant in more heterogeneous tumors, such as breast cancer that the expression of the immunohistochemical marker may differ from area to area in the tumor. Nevertheless, our findings suggest that resistin does not have a prognostic role in pancreatic cancer. More studies are in need to establish the effects of resistin in cell proliferation, differentiation, and pancreatic cancer.

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