Fas and Fas Ligand Expression and Relationship with Clinicopathologic Parameters in Pancreas Cancer

Pankreatik Kanserde Fas ve Fas Ligand Ekspresyonu ve Klinikopatolojik Parametrelerle İlişkisi

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ABSTRACT Objective: Fas (CD95/APO-1) and Fas Ligand (FasL) are important in the process of apoptosis in the immune system. Hereby, we aimed to examine the expression of Fas/FasL in pancreatic adenocarcinoma and chronic pancreatic tissue and its relation with clinicopathological characteristics using immunohistochemical studies. **Material and Methods:** Pancreatic adenocarcinoma (n= 35) and chronic pancreatitis cases (n= 25) underwent assessment for Fas and FasL expression using immunohistochemistry. **Results:** The cancer group consisted of 24 female and 11 male patients, while the chronic pancreatitis group included 21 female and 4 male patients. All patients had been diagnosed with adenocarcinoma. According to the International Union Against Cancer (UICC) staging, 19 patients were classified as stage III (unresectable) and 16 patients had stage IV disease. All patients classified as stage III were revealed to have unresectable tumors intraoperatively. The loss of Fas expression was higher in pancreatic adenocarcinoma than in chronic pancreatitis (57.1% vs. 29.2%, p= 0.034). FasL over-expression was higher in pancreatic adenocarcinoma than in chronic pancreatitis (57.1% vs. 26.1%, p= 0.031). Cytoplasmic staining of Fas (77.1% vs. 22.9%, p= 0.016) and FasL (61.1% vs. 38.9%, p= 0.002) was higher than membranous staining. According to stages, loss of Fas expression was greater in stage IV when compared with stage III (73.9% vs. 25%, p= 0.01). There was no significant relationship between Fas and the stage of cancer (p> 0.05). Furthermore, there was no correlation between Fas/FasL staining and age and sex (p> 0.05). **Conclusion:** In pancreatic adenocarcinoma, loss of Fas expression and Fas L over-expression were statistically significant.

Key Words: Fas ligand protein; pancreatic neoplasms

ÖZET Amaç: Fas (CD95/APO-1) ve Fas Ligand (FasL) immün sistemde yer alan apoptozis için önemlidir. Biz bu çalışmada, immünohistokimyasal işlemler kullanarak, Fas/FasL'nin pankreas adenokarsinomu ve kronik pankreatit dokularındaki ekspresyonunu ve klinikopatolojik özellikler ile ilişkisini incelemeyi amaçladık. **Gereç ve Yöntemler:** Pankreatik adenokarsinom (n= 35) ve kronik pankreatit (n= 25) olguları immünohistokimyasal olarak Fas ve FasL ekspresyonu açısından incelendi. **Bulgular:** Kadın/erkek sayısı kanser grubunda 24/11 ve kronik pankreatit grubunda 21/4 idi. Tüm hastalar adenokarsinom tanısı almıştı. "International Union Against Cancer (UICC)" evrelemesine göre, 19 hasta evre III (anrezektabl), 16 hasta evre IV olarak belirlendi. Evre III olan tüm hastalar operasyon esnasında anrezektabl olarak değerlendirildi. Fas ekspresyonunun kaybı pankreatik adenokarsinomda kronik pankreatitte olduğundan daha fazla idi (%57.1'e karşı %29.2, p= 0.034). FasL'nin aşırı ekspresyonu, pankreatik adenokarsinomda kronik pankreatitte olduğundan daha fazla idi (%57.1'e karşı %26.1, p= 0.031). Fas (%77.1'e karşı %22.9, p= 0.016) ve FasL (%61.1'e karşı %38.9, p= 0.002) sitoplazmik boyanması, membranöz boyanmadan daha fazla idi. Evreye göre; Fas ekspresyonu kaybı; karşılaştırıldığında evre IV'te evre III'te olduğundan daha fazla idi (%73.9'a karşı %25, p= 0.01). FasL ile evre arasında belirgin bir ilişki gözlenmedi (p> 0.05).Yine Fas/FasL boyanması ile yaş ve cinsiyet arasında bir ilişki bulunmadı (p> 0.05). **Sonuç:** Pankreas adenokarsinomunda Fas ekspresyon kaybı ve FasL ekspresyon artışı istatistiksel olarak anlamlı bulunmuştur.

Anahtar Kelimeler: Fas ligand; pankreas kanseri

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Tumor cells begin to proliferate inappropriately when apoptotic pathways maintaining the cellular integrity of organisms decompose.[1] The transporter protein named 'Fas' also functions as a receptor. It binds to Fas-ligand and causes programmed cell death by transporting an intracellular signal. Fas-L, a member of tumor necrosis factor receptors and the ligand family, is a 37-40 kDa type II membrane protein.[2-5] The Fas cell death receptor ligation transfers a 'death signal', which soon starts and maintains the apoptotic pathway with the help of either activating antibody or FasL.[6] There is a pathway regulating the continuity of the immune system called the 'apoptotic pathway'. The Fas receptor is a part of this pathway. Tumor cells may capture the surveillance of immunity and this can occur by the loss of Fas expression with tumor cells.[7] Various studies have shown that when compared to Fas-negative tumors, it has been shown that the survival rate of Fas-positive tumors in the breast, liver and the lungs is much higher.[1,8,9] FasL is primarily expressed in activated T-lymphocytes and it appears to play an important role in T-cell mediated cytotoxicity by inducing apoptosis in target cells expressing Fas.[10] FasL expression by tumor cells is postulated to "counter-attack" the immune system by inducing apoptosis in Fas-expressing anti-tumor T lymphocytes, acting in a similar fashion to epithelial cells in immune-privileged sites.[11] There are many studies on the subject and some of those suggested that FasL might be expressed by cell lines of the colon, melanoma, and hepatocellular carcinomas.[11-13] When the relationship of cancer and Fas/FasL is evaluated in pancreatic carcinoma, pancreatic adenocarcinoma cell lines include Fas and FasL, but data regarding the clinical characteristics and correlations are limited.[14-17] Thus, in this study, we aimed to evaluate the expression of Fas/Fas L in pancreatic adenocarcinoma and chronic pancreatic tissue and its relation with clinicopathological characteristics using immunohistochemical studies.

MATERIAL AND METHODS

The study included 35 patients who had pancreatic adenocarcinoma and 25 patients with chronic pancreatitis in the department of Medical Oncology, University of Uludağ, Turkey, between 2000 and 2004. Approval for the study was obtained from the local ethics committee. Demographic data were obtained from medical charts. Clinical follow-up was carried out by reviewing the clinical charts and/or by contacting the patients' physicians. Staging was based on radiological assessment according to the AJCC/UICC (American Joint Committee on Cancer /International Union Against Cancer).

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Immunohistochemical staining was carried out on 5-µm-thick sections on lysinated slides from routinely fixed paraffin-embedded blocks. Paraffin-embedded sections of pancreatic tumors were de-paraffinized in xylene and were re-hydrated in graded alcohol. The sections were pre-treated in a microwave in 10 mM sodium citrate (pH 6.0) for 20 minutes and were incubated in 3% H_2O_2 for 15 min. The slides were then washed with PBS buffer three times for 5 minutes. After treatment with blocking serum for 10 minutes, the sections were incubated for 60 minutes with primary monoclonal antibodies against Fas (CD95) and FasL, clone GM30, dilution 1:40 and clone 5D1, dilution 1:25, respectively (Novocastra Laboratories, Newcastle, United Kingdom). The immunohistochemical staining was performed using the streptoavidin-biotin kit (Universal Quick kit, Novocastra) by the avidin-biotin-peroxidase method, according to the manufacturer's instructions. Novocastra DAB chromogen was used as the chromogen, and the slides were counter-stained with Mayer's hematoxylin. Formalin-fixed, paraffin-embedded sections of human prostate were used as positive controls of FasL; sections of the human small intestine served as positive controls for Fas. Sections without primary antibodies, as well as those with non-immunized mouse serum for FasL and Fas served as negative controls. All histological slides were examined by a pathologist who was blinded to clinical data or the disease outcome. Fas and FasL staining were scored independent from the localization of membrane or cytoplasmic staining. Depending on the proportion of positive cells, immunostaining classification is

negative (<5%), weak (5%-30%), moderate (30%-60%), or strong (>60%). However, since the number of cases were low, they were classified as negative (<5%) and positive (>%5).

Statistical Analysis

Statistical analysis was performed using the SPSS version 10.05. The Fisher's exact-test and the Pearson's-Chi-Square test were used to compare groups. P values of <0.05 were considered statistically significant. The correlation between Fas/FasL staining, and age and gender was assessed using the Spearman's Correlation test. Again, the relation between the staining rates and the stage was analyzed using the Fischer's Exact-test.

RESULTS

The cancer group consisted of 24 female and 11 male patients, while the chronic pancreatitis group comprised 21 female and 4 male patients. All patients had adenocarcinoma. According to the UICC staging, 19 patients were classified as stage III (unresectable) and had stage IV disease (Table 1). Intraoperative evaluation revealed that all stage III patients had unresectable tumors. The loss of Fas expression was higher in pancreatic adenocarcinoma than in chronic pancreatitis (57.1% vs. 29.2%, p= 0.034). FasL over-expression was higher in pancreatic adenocarcinoma than in chronic pancreatitis (57.1% vs. 26.1%, p= 0.031.) (Table 2) (Figure 1). Cytoplasmic staining of Fas (77.1% vs. 22.9%, p= 0.016) and FasL (61.1% vs. 38.9%, p= 0.002) was more evident and significant than membranous staining. According to the stages, loss of Fas expression was greater at stage IV than at stage III (73.9% vs. 25%, p= 0.01) (Table 3). There was no significant relationship between FasL over-expression and stage of cancer (p= 0.603). Furthermore, there was no correlation between Fas/FasL staining and age (p= 0.218; rs= 0.163) and between Fas/FasL staining and sex (p= 0.887; rs= 0.019).

TABLE 1: Demographic features of patients with pancreatic cancer and chronic pancreatitis.

	Pancreatic cancer Mean ± SD	Chronic pancreatitis Mean ± SD
Age (Mean) yr	56.5 ± 8.5	55.3 ± 8.2
Sex (F/M)	24/11	21/4
Tumor stage		
III	19 pts	
IV	16 pts	

YR: Year, F: Female, M: Male, pts: Patients

TABLE 2: Immunohistochemical staining for Fas/FasL in chronic pancreatitis and adenocarcinoma tissues.

Immunoreactivity	Chronic pancreatitis n	%	Adenocarcinoma n	%	p value
Fas positive	17	70.8	15	42.9	
Fas negative	7	29.2	20	57.1	0.034
Fas L positive	6	26.1	19	57.1	
Fas L negative	17	73.9	16	42.9	0.031
Distribution					
Cytoplasmic (Fas)	24	100	27	77.1	
Membranous (Fas)	-		8	22.9	0.016
Cytoplasmic (Fas L)	21	100	11	61.1	
Membranous (FasL)	-		7	38.9	0.002

CONCLUSION

The Fas/FasL system has a primary role in the induction of apoptotic cancer growth in the balance of pro-apoptotic and anti-apoptotic programs.[18] Over- and under-expression of Fas or FasL have been shown in renal, lung and colon carcinomas, in addition to brain tumors.[19-22] Loss of Fas expression has also been observed in lung, esophagus, and colon adenocarcinomas.[7,11,23,24] Leveugle et al. reported decrease in Fas expression in small cell lung carcinoma.[25] Leveugle's study indicated Fas expression with sensitivity of cells to Fas-induced apoptosis. Loss of Fas expression was suggested to be involved in lung tumorogenesis. In the study of Pernick et al, Fas negativity in pancreatic adenocarcinoma was 81 and Fas positivity was 19, respectively.[10] Similar to the results of these two reports, loss Fas expression was higher in cancer cases in our study also. Fas negativity was 57.1% in our study. Loss of Fas has been shown to be related to resistance to apoptosis and its significance in tumor pathogenesis depends on its role in immune escape.[26,27] FAP (Fas-associated phosphatase)-1 expression can block the apoptotic function

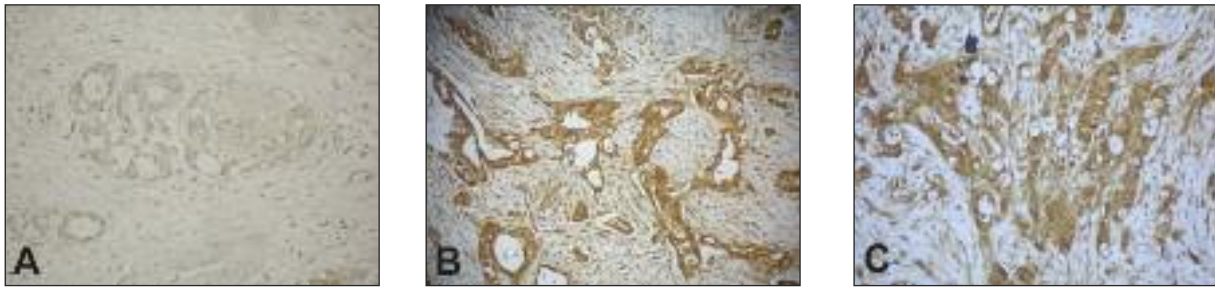

FIGURE 1: Fas and FasL immunostaining of tumor tissues. (A) Loss of Fas expression in pancreatic adenocarcinoma. (B,C) Over-expression of FasL in pancreatic adenocarcinoma (HE, x200).

of Fas and is correlated with resistance to Fas-mediated apoptosis.[28]

FasL expressing tumor cells may induce Fas killing of Fas-expressing normal tissue cells, in addition to engagement of endothelial cells in apoptosis. FasL-expressing tumors such as colon carcinoma, melanoma, and hepatocellular cell lines are able to kill Fas-expressing Jurkat lymphocytes in a Fas-mediated manner.[11-13] In our study, FasL over-expression in the tumor group was highly significant, while Pernick et al did not find any significant difference in pancreatic adenocarcinoma cases.[10] Furthermore, while Fas negativity was higher in men than in women in the same study there was no difference between men and women in our study. This difference may be attributed to the small number of patients and the heterogeneity of patients in the other study when compared to our patients.

The Fas/FasL clinical relationship was examined in tumors in several studies. Bernstorff et al concluded that in patients with pancreatic tumors with loss of Fas, there was a poorer differentiation and that the survival was shorter.[7] In our study, the loss of Fas expression was higher in stage IV patients than in stage III patients. The loss of Fas may be aassociated with a poorer clinical course in pancreatic cancer. In other studies resembling ours, FasL over-expression seemed to take over in the case of Fas loss in esophageal, gastric and colon cancer.[29-31] Boltze et al found lower Fas expression and higher FasL in chronic pancreatitis than in the tumor cells in adenocarcinoma, and this was concordant with the results of our study.[32] With tumor-specific

TABLE 3: Correlation of Fas/ FasL expression with stage of pancreatic adenocarcinoma.

	Stage III		Stage IV		
	n	%	n	%	p
Loss of Fas expression (+)	3	25	17	73.9	
Loss of Fas expression (-)	9	75	6	26.1	0.01
Fas cytoplasmic staining (+)	6	50	21	91.3	
Fas cytoplasmic staining (-)	6	50	2	8.7	0.08
FasL over-expression (+)	5	41.7	10	43.5	
FasL over-expression (-)	7	58.3	13	56.5	0.60
FasL cytoplasmic staining (+)	4	57.1	7	63.6	
FasL cytoplasmic staining (-)	3	42.9	4	36.4	0.58

T-lymphocytes, which stimulate apoptosis, the escape of host immune surveillance can be observed simultaneously with loss of Fas expression and expression of functional FasL by tumor cells.[33] Loss of Fas expression, which is an essential fragment of pancreatic tumors, is the access for tumors to escape from Fas-mediated apoptosis. The Fas killing of Fas-expressing normal tissue cells may be stimulated by FasL-expressing tumor cells and they may engage endothelial cells into apoptosis.[34,35] By stimulating apoptosis in tumor-attacking lymphocytes, pancreatic adenocarcinoma escapes immune surveillance and FasL may have a significant role in this function.[25] Briefly, the main result of our study is that in pancreatic adenocarcinoma, not only the loss of Fas expression may demonstrate a poorer prognosis, but also in the future, Fas correction and/or FasL inhibition may be adjuncts in therapy plans with regard to apoptosis.

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