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Evaluation of concentrations of pro/anti-inflammatory cytokines after complication-free ECRP in cholangiocarcinoma

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Original Article

ABSTRACT

Background/Aims: Variations in pro and anti-inflammatory cytokine levels occur commonly after ERCP procedure complications, such as in post-ERCP pancreatitis. Besides, the relationship between increased cytokine levels and multidrug resistance has been shown in cholangiocarcinoma patients. Our aim was to investigate the impact of cytokine level changes on treatment strategy after uncomplicated ERCP procedures in cholangiocarcinoma patients.

Materials and Methods: Of 75 patients enrolled in this study, 25 were cholangiocarcinoma, and 50 were choledocholithiasis patients. Levels of serum IL-1β, IL-6, IL-8, IL-10, and TNF-α were evaluated 2 hours before and 12 hours after complication-free ERCP, and statistical analysis of the results was obtained; if p value <0.05, it was accepted as statistically significant.

Results: There was no statistically significant difference in the distribution of age (23-87 years; range: 59.8±16.6), gender (37 males vs 38 females), and levels of pre- and post-ERCP serum IL-1β, IL-6, IL-8, IL-10, and TNF-α in both patient groups, despite the presence of some change in test means (p:0.179, 0.445, 0.522, 0.937, and 0.065, respectively). However, significantly decreased levels of TNF-α were observed in the benign group, when comparing pre- and post-ERCP period (p<0.05).

Conclusion: Serum concentrations of IL-1β, IL-6, IL-8, IL-10, and TNF-α evaluated after complication-free ERCP performed in patients with cholangiocarcinoma do not cause any change in treatment planning that would affect multidrug resistance.

Keywords: Cytokine, ERCP, cholangiocarcinoma, interleukin

INTRODUCTION

Cytokines, such as IL-1β, IL-6, IL-8, IL-10, and Tumor necrosis factor-alpha (TNF-α), play roles in inflammation. Cytokine level changes are observed frequently among benign and malignant etiologies that require Endoscopic retrograde cholangiopancreatogram (ERCP). Increases levels of cytokines, like IL-6, IL-1α, and TNF-α, in menopausal women with cholelithiasis and the obvious role of IL-8 in pancreatitis and the role of macrophages in illnesses have been shown (1,2). Carcinogenesis in cholangiocarcinoma (CCA) is a consequent process frequently requiring cholestasis and chronic inflammation. Secretion and interaction of IL-6, transforming growth factor-beta (TGF-β), TNF-α, and platelet-derived growth factor (PDGF) are necessary for proliferation of cholangiocytes (3). Higher levels of IL-6 have been found (221-fold higher) in CCA patients than control volunteers (4). It has been found that peritumoral chronic inflammation is associated with the migration and invasion of malignant cells and that LPS-active macrophages are secreting inflammatory cytokines, such as IL-4, IL-6, IL-10, TNF-α, and TGFβ1 (5).

While benign and malign diseases of the bile duct cause changes in the levels of cytokines, on the other hand, acute pancreatitis developing after ERCP causes an increase in the levels of TNF-α and IL-6 (6). Studies related to cytokines are generally performed for prediction of

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post-ERCP pancreatitis and for investigation of its relationship with the prognosis, and serum levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α have been frequently found to be higher. Another investigator has shown that levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α are increased 8 and 24 hours after intervention in patients with post-ERCP pancreatitis, and binary logistic regression analysis revealed that the sensitivity of IL-6 was 100% and the specificity was 87% 8 hours after intervention (7,8). Pro-inflammatory (ie, TNF-α) and anti-inflammatory (eg, IL-6, IL-10) cytokines have found to be balanced in acute pancreatitis patients. Functional disintegration of T helper lymphocytes into subtypes of Th1 and Th2 by polarization plays an important role in this balance (9). Th1 secretes inflammatory cytokines, and Th2 secretes anti-inflammatory cytokines (10). These cytokines control subtypes of T-helpers; for example, IL-10 blocks the Th1 subtype. Both T-helper subtypes are secreting TNF-α (6,11).

Multi-drug resistance is observed in many cancers, and it is a condition occurring by accumulation of cytotoxic drugs inside the cell via the transmembrane multidrug transporter P-glycoprotein (P-gp) coded by multidrug resistance 1 gene (mdr1) in the resistance to chemotherapeutic agents (12,13). Increased IL-6 expression has been shown in cases with multidrug resistance (14). CCA is the most chemotherapeutic-resistant type of cancer, and it has higher levels of IL-6. It has been observed that side effects, like fatigue, sleeplessness, loss of appetite, depression, and cognitive alterations related to treatment, in patients having cytotoxic chemotherapeutic drug therapy are caused by stimulation of IL-1β from immunity cells. These side effects are the remarkable markers of IL-1β-mediated inflammation of the central nervous system with increased plasma concentrations of IL-6, IL-1 receptor antagonist, and water-soluble TNF receptor-I/II (15).

This study is conducted because there is no knowledge in the literature about whether complication-free ERCP is causing any significant alterations in serum levels of IL-1β, IL-6, IL-8, IL-10, and TNF-α or not and if this condition affects treatment decisions in clinical practice despite increases in the levels of cytokines in some benign (for example, acute pancreatitis and cholangitis) and malignant CCA disorders.

MATERIALS AND METHODS

Ethics

The study protocol was approved by the institutional review board (B.30.2.BAV.0.05.05/398-11.07.2012 and 71306642/050-01-04/105). All procedures were in accordance with the ethical standards of the committee on human experimentation of our institution and with the Declaration of Helsinki. Written informed consent was taken from all patients before participating in this study.

Patients

Patients who were referred to our clinic for ERCP were enrolled in this study. A total of 75 (37 males and 38 females) patients, including 34 CCA and 68 choledocholithiasis patients. Diagnosis of choledocholithiasis was based on patient history (right upper quadrant and epigastric pain, vomiting, fever), physical examination (Murphy sign), laboratory tests (elevated AST, ALT, alkaline phosphatase, GGT, and total and direct bilirubin concentrations), ultrasonography, MRCP, upper abdominal MRI, ERCP, and/or EUS. Diagnosis of CCA was based on patient history (icterus, weight loss), physical examination (silent jaundice), imaging methods (CT, MRI, and PET), laboratory tests (elevated AST, ALT, alkaline phosphatase, GGT, total and direct bilirubin, CEA, and CA 19-9 concentrations), and histopathologic examinations.

Exclusion criteria

Patients with sepsis; severe heart, renal, or liver failure; acute pancreatitis; or acute cholangitis, as well as those ineligible for ERCP due to bleeding tendency and pregnant women were excluded from the study. Patients who had complications during ERCP procedure were also excluded from this study.

Sample collection

Blood samples were collected 2 hours before and 12 hours after the ERCP procedure from all patients and were stored at -80°C. Levels of serum inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, and TNF-α) were measured by ELISA (Boehringer Mannheim, Germany). ELISA assays were analyzed in the Department of Biochemistry, İstanbul Cerrahpaşa Medical Faculty.

ERCP procedure

Before ERCP, local pharyngeal 10% lidocaine spray, propofol 200 mg, and dormicum 5 mg were used for sedation. Butylscopolamine (Buscopan, Boehringer Ingelheim) was used for relaxation of the duodenum. The equipment used was a side-sighted Duodenoscope Pentax A-120227 (Pentax, Made in Germany). A Siemens Siremobile Compact was used to obtain radiologic images. For antibiotic prophylaxis, 1 gr ceftriaxone was applied intravenously to patients undergoing ERCP. None of the patients used NSAIDs in order not to affect the cytokines in the study. During the ERCP process, sphincterotomy (sphincterotome, needle knife) was performed in all patients. All patients were followed up clinically, radiologically, and in terms of laboratory tests for 48 hours after ERCP for development of any complication (post-ERCP pancreatitis, cholangitis, sepsis, bleeding, perforation, etc.).

Statistical analysis

All statistical analyses were performed by using Statistical Package for the Social Sciences (SPSS) for Windows 15.0 software. Quantitative data were reported as mean±SD deviation; normally distributed parameters were compared by using student's t-test, and abnormally distributed parameters were compared by using Mann-Whitney U-test. Paired-sample t-test was used for intra-group comparisons of normally distributed parameters, and continuity correction (Yates) test was used for the comparison of qualitative data. A $p<0.05$ was considered statistically significant.

Table 1. Demographic characteristics of the study groups patients

	Malignant mean±SD	Benign mean±SD	[1]p
Age	64.7±15.6	58.6±17.1	0.134
Gender	n (%)	n (%)	[2]p
Female	13 (%52)	25 (%50)	1,000
Male	12 (%48)	25 (%50)	

[1]Student t-test, [2]Continuity Correction (Yates) test
SD: standard deviation

RESULTS

Demographic characteristics of the patients are shown in Table 1. Mean age of the patients was 59.8±16.6 (*R: 23-87*). The study continued with a total number of 75 patients. Of them, 25 were CCA (33.3%) and 50 were choledocholithiasis (66.7%) (Table 1); 27 patients (9 with CCA, 18 with choledocholithiasis) were excluded from the study because of complications (infection, bleeding) during the ERCP process. The tumor was localized distally in 18 patients and localized medially in 7 patients out of 25 CCA patients.

Effect of ERCP on inflammatory cytokine levels in benign and malignant etiologies

No statistically significant difference was detected in the levels of serum IL-1β, IL-6, IL-8, IL-10 ,and TNF-α during the pre- and post-ERCP periods in both patient groups. However, the decline in the level of TNF-α in the benign group was more than in the malignant group after ERCP compared to levels obtained before ERCP; this difference was very close to significance, but no statistically significant difference was found (p>0.05) (Table 2).

DISCUSSION

In this study, we investigated the changes in serum levels of both inflammatory and anti-inflammatory cytokines before and after ERCP in a CCA group.

The frequency of occurrence of pancreatitis clinically is about 5%-10%, although transient increases in pancreatic enzymes are seen at up to 75% frequency after ERCP in general (16). In this study, no significant changes were observed in the levels of serum IL-6 and TNF-α in the pre- and post-ERCP procedures in CCA patients. This observation was consistent with previous studies, which reported that IL-6 and TNF-α were evaluated in cases with ERCP-related pancreatitis and found that the level of TNF-α was not changing, even if pancreatitis developed, and that a significant increase in IL-6 was starting after the sixth hour (17).

IL-10 is a potent inhibitor of cytokines and has been shown to attenuate pancreatitis, because increased inflammatory cytokine levels are shown in post-ERCP pancreatitis in animal models and pilot human studies (18). The presence of normal IL-10 levels in our study showed that no change in cytokine levels existed to cause an increase in İL-10 levels related to complicated ERCP, such as post-ERCP pancreatitis.

Although secretion of IL-8 and IL-1-RA (antagonist of IL-1) occurs at the beginning phase of pancreas destruction during post-ERCP pancreatitis, IL-1β was not found in circulation (19). In the study by Chen et al. (7), post-ERCP pancreatitis developed in 7 patients out of 78; increased levels of TNF-α, IL-6, IL-8, and IL-10 have been shown besides IL-1β levels at 8 and 24 hours after intervention, and it was determined that the sensitivity of IL-6 was 100% and specificity was 87% in diagnosing post-ERCP pancreatitis when the cut-off value was accepted as 36 pg/mL at 8 hours after intervention (high diagnosing value) (8).

Chronic infection frequently seen in CCA leads to secretion of various cytokines from cholangiocytes and inflammatory cells. IL-6, being a key cytokine in the carcinogenesis, upregulates the survival of CCA cells with potent anti-apoptotic protein Mcl-1 (20-22). This results in activation of epidermal growth factor induction with biliary acid (23) and stopping of aging with telemorase induction (24). It has been shown that the sensitivity is 71.1% and specificity is 90% for detectable IL-6 levels (0.18 ng/mL in the differential diagnosis of CCA and benign bile duct diseases) (25). It has also been shown that there is increased IL-6 expression in tumors resisting chemotherapeutic agents (26). Somehow, patients who have a CCA diagnosis encounter with an ERCP procedure for diagnosis or treatment aims. It seems that insignificant changes in the IL-6 level after ERCP procedure in patients with CCA (one of the most therapy-resistant types of cancer) do not affect the drug choice during treatment planning, especially for the multidrug resistance issue.

Multidrug resistance, the principal mechanism by which many cancers develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapy. Just studying the cytokines will not be used for the justification of multidrug resistance in CCA; other studies can also be used with cytokine level changes in patients. The MDR1a gene, which encodes P-glycoprotein, is expressed at significant levels in about half of all human cancers. Although most of the studies have been completed to evaluate post-ERCP pancreatitis up to now, it has been shown that ERCP intervention does not cause any significant alteration in cytokine levels in patients with CCA in our study. In addition to exclusion of patients with cholangitis and pancreatitis from the study (in accordance with the methodology), it is important that no statistically significant result was detected, despite differences in mean levels of IL-6 before and after ERCP. Unchanged cytokine levels after ERCP are another indicator of the having no complication, such as inflammatory and infectious pathologies, in CCA patients.

Cons of the study

1. Limited number of patients
2. There is no healthy control group, since applying an invasive intervention like ERCP to healthy individuals is ethically wrong.

Table 2. Effect of ERCP on tests according to benign and malignant etiologies

Tests	ERCP	Malignant group mean±SD	Benign group mean±SD	p[1]
IL-1β	Before	63.17±17.13	59.72±21.66	0.490
	After	62.51±17.21	60.85±20.58	0.729
	Before- after [2]p	0.246	0,434	
	[3]Difference	-0.66±2.78(-0.6)	1.12±10.07 (0.4)	0.179
IL-6	Before	66.19±16.29	68.53±15.36	0.544
	After	67.75±17.05	68.79±15.58	0.791
	Before- after [2]p	0.310	0.702	
	[3]Difference	1.56±7.52 (-0.1)	0.27±4.96 (0.3)	0.445
IL-8	Before	72.07±16.69	75.89±19.88	0.411
	After	72.72±18.96	76.29±20.27	0.464
	Before- after [2]p	0.587	0.704	
	[3]Difference	0.65±5.88 (0.5)	0.40±7.39 (-0.55)	0.522
IL-10	Before	5.29±0.57	5.26±0.71	0.864
	After	5.22±0.65	5.21±0.66	0.970
	Before- after [2]p	0.210	0.382	
	[3]Difference	-0.06±0,26 (-0.1)	-0.04±0.37 (0)	0.937
TNF-α	Before	96.28±20.86	98.39±22.09	0.693
	After	95.84±21.69	96.33±21.21	0.926
	Before- after [2]p	0.653	0.015 (p<0.05)	
	[3]Difference	-0.44±4.83 (0.1)	-2.05±5.75 (-1.3)	0.065

[1]Student t-test, [2]Paired-samples t-test, [3]Mann-Whitney U-test
ERCP: Endoscopic retrograde cholangiopancreatogram; SD: standard deviation; TNF-α: Tumor necrosis factor-alpha

Pros of the study

1. Although it is recommended that increased IL-6 levels may help in the diagnosis of post-ERCP pancreatitis in patients with choledocholithiasis and of CCA, no significant results were obtained in our study after ERCP that would help researchers who are willing to study in this field.
2. During the treatment planning phase, drug choice could be performed freely in CCA patients, since no changes in levels of IL-6 and the other cytokines occur due to ERCP procedures.

Conclusion

The presence of increased pro-inflammatory and anti-inflammatory cytokine levels after ERCP procedures requires an understanding of the underlying pathology, such as pancreatitis and other infections, etc. Although increased cytokine levels are generally present in CCA, our study showed that uncomplicated ERCP procedure performed for patients with CCA did not stimulate serum pro-inflammatory and anti-inflammatory cytokine discharge significantly in the earlier stage. Thus, it may also be considered that the ERCP procedure has no additional role in other pathologies related with increased cytokine levels. Large and long-term studies are needed in order to explain the relationship between cytokines and their responsibility in tissue damage and complications.

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