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The effect of ursodeoxycholic acid treatment on epidermal growth factor in patients with bile reflux gastritis

Ursodeoksikolik asit tedavisinin alkalen reflü gastritli hastalarda epidermal büyüme faktörü düzeyine etkisi

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Background/aims: *Study was performed to evaluate the effect of ursodeoxychlic acid treatment on epidermal growth factor, which is secreted in response to mucosal injury and is also a factor in the protection and healing ofgastric mucosal injury in patients with bile reflux gastritis following cholecystectomy.* ***Methods:*** *Thirty-one dyspeptic patients who had previously undergone cholecystectomy were included in the study. Upper gastrointestinal endoscopy was performed before and after a six week ursodeoxychlic acid treatment period and a biopsy was taken. Endoscopic biopsy materials were stained with epidermal growth factor (Zymed, supersensitive) immunohistochemical monoclonal kit.* ***Results:*** *The results of endoscopic examination prior to treatment were as follows: 24 cases (77%) had reflux gastritis, five cases (16%) antral gastritis, two cases (6.5%) diffuse gastritis and all cases had enterogastric reflux. In all but one case, epidermal growth factor was found to be positive at varning degrees. After ursodeoxychlic acid treatment, complete healing was observed at endoscopy in nine cases (29%) and partial healing at varning degrees was observed in all others. The degree of positivity of epidermal growth factor reduced significantly (p<0.001).* ***Conclusions:*** *A decrease in the degree of epidermal growth factor positivity was observed following ursodeoxychlic acid treatment. This can be explained by the decrease in epidermal growth factor release due to healing of mucosal injury following treatment. Further investigations are needed to clarify whether ursodeoxychlic acid has a direct effect on epidermal growth factor.*

Key words: Bile reflux gastritis, Ursodeoxycholic acid, Epidermal growth factor.

Amaç: *Bu çalışma, alkalen reflü gastrit tespit edilen hastalarda ursodeoksikolik asit tedavisinin mukozal hasara cevap olarak salınan, gastrik mukoza için koruyucu ve iyileştirici etkileri olan epidermal büyüme faktör düzeyine etkisinin araştırılması amacıyla düzenlendi.* ***Yöntem:*** *Epigastrik yakınmaları olan, daha önce kolesistektomi ameliyatı olmuş 31 hasta çalışmaya alındı. Tedavi öncesinde ve 6 haftalık ursodeoksikolik asit (UDKA) tedavisi sonrasında üst gastrointestinal sistem endoskopisi uygulandı ve biyopsi materyali alındı. Endoskopik biyopsi materyalleri EGF (Zymed, supersensitiv) monoklonal kit ile immunohistokimyasal boyama yapıldı.* ***Bulgular:*** *Tedavi öncesi yapılan endoskopik incelemede 24 (%77) hastada reflü gastritis, 5 (%16) hastada antral gastritis, 2 (%6.5) hastada diffüz gastritis ve tüm hastalarda enterogastrik reflü tespit edildi. Tedavi öncesinde bir hasta dışında tüm hastalarda EGF değişik derecelerde pozitifti. Tedavi sonrasında yapılan endoskopik incelemede 9 hastada tamamen düzelme, diğer hastalarda da değişen oranlarda düzelme saptandı. Tedavi sonrasında EGF pozitifliği değişen derecelerde anlamlı olarak azaldı (p <0,001).* ***Sonuç:*** *UDKA tedavisi sonrasında EGF düzeyinde azalma saptadık. Bu durum, ursodeoksikolik asid tedavisi sonrasında mukozal hasarın düzelmesine bağlı olarak EGF salınımının azalması ile açıklanabilir. UDKA' nın EGF üzerine direk etkisinin olup olmadığının belirlenmesi için ise yeni araştırmalara ihtiyaç vardır.*

Anahtar kelimeler: Alkalen reflü gastritis, ursodeoksikolik asit, epidermal büyüme faktörü.

INTRODUCTION

Gastritis is an inflammatory response of the gastric mucosa to damage. Alkaline reflux gastritis is caused by alkaline duodenal content which causes irritation of the gastric mucosa when it is regurgitated into the stomach. It is known that deoxycholic acid has a directly damaging effect on the gastric mucosa. In recent years, alkaline reflux gastritis has been treated with ursodeoxycholic

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Table 1. Effects of EGF

EGF increases:
Ion uptake,
Glycolysis,
Production of DNA,
Production of RNA,
Gastric blood flow,
Formation of Prostaglandins in the gastric mucosa.
EGF decreases:
Gastric acid secretion.
EGF has mitogenic effect on mesodermal and ectodermal cells.

acid (UDCA), a chenodoxycholic acid 7 beta hydroxyl epimer, and it has been considered as an addition to other treatment alternatives (1,2). The most important advantage of treatment with UDCA is that it has less toxicity and a smaller number of side-effects than chenodoxycholic acid (3). It also decreases the amount of colic acid, chenodoxycholic acid and deoxycholic acid in the refluxed bile. In addition, treatment with UDCA decreases the irritation of the gastric mucosa by taurin and conjugated bile acids. Furthermore, fortification of the bile with UDCA has been shown to result in a considerable improvement in clinical and endoscopic findings (4).

Epidermal growth factor (EGF), first derived from extracts of the submaxillary gland in rats thirty years ago (5), is a protein produced by secretory glands and gastrointestinal mucosa and is the most important member of the growth factor family (6,7). It is primarily secreted by salivary glands in human beings and is present in fluids of the digestive system such as saliva, gastric fluid and duodenal fluid. EGF is bound to specific EGF molecules on the cell surface, each of which has a molecular weight of 170 million Dalton, in both in vivo and in vitro cell cultures (8-11). Following binding to these receptors, both EGF itself and its receptors undergo endositosis. Binding of a ligand to EGF receptor activates cell signalization and in turn protein kinase is phosphorylated (12,13). EGF, shown in many tissues, has been reported to be mitogenic for mesodermal and ectodermal cells. It increases ion intake, glycolysis and production of RNA and DNA (14). The effects of EGF are shown in Table 1.

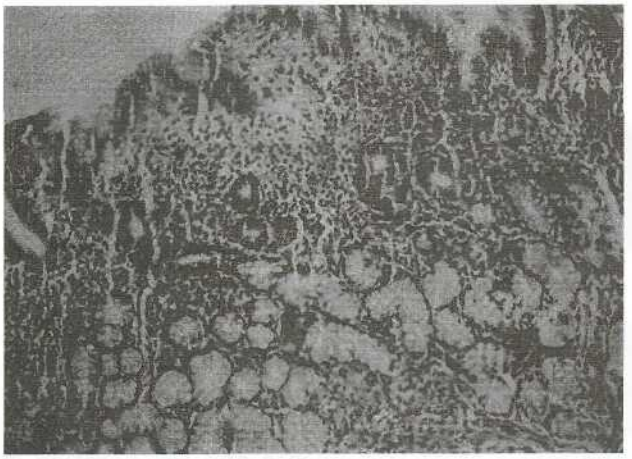
EGF is the most important growth factor and plays a role in the protection of gastric mucosa against acute damage (15). It stimulates migration and proliferation of mucosal cells and thus mucosal repair. It also protects intact intestinal mucosa against bacterial colonization (16). In addition, it has nonmitogenic functions. For example, it increases inhibition of gastric acid secretion, gastric blood flow and formation of prostaglandins (6). Furthermore, EGF plays a primary role in the preservation of mucosal integrity and repair of damaged mucosa (7).

The aim of this study was to investigate the effects of UDCA on EGF, which is secreted in response to gastric mucosal damage and which plays a role in the repair of gastric mucosa and regeneration of cells, in patients diagnosed with alkaline reflux gastritis based on endoscopic and pathological examinations.

MATERIAL AND METHODS

This case-control study included 31 patients referred to the gastroenterology outpatient clinic, Ankara Hospital, between November 1999 and December 1999. The patients had undergone cholecystectomy at least four months and at most 27 years previously. They were aged between 38 and 70 years (mean of 57.12±10.03 years) and there were nine males (29%) and 22 females (71%). Regardless of the time since operation, patients with epigastric complaints were included in the study but those with a history of peptic ulcus likely to cause epigastric complaints, treated in the past six months for a gastrointestinal disease, with systemic diseases such as diabetes mellitus, hypothyroiditis and pancreatitis, with chronic obstructive lung disease or coronary artery disease, likely to be intolerant of endoscopy and those with a history of treatment with nonsteroid anti-inflammatory drugs were not included. Approval for the study was obtained from the ethics committee.

The patients were prescribed UDCA 10mg/kg daily for six months and they underwent endoscopy with sedation when required, of the upper gastrointestinal system before and after the treatment period. Informed consent was obtained from the patients before endoscopy, which was performed with a GIF-K-30 OLYMPUS endoscope. Four biopsy specimens were taken, two from the prepyloric antrum and two from the corpus. The investigators, blinded to the diagnosis of alkaline reflux gastritis, performed histopathological examination of the specimens.



Figüre 1. H-E staining of gastric mucosa

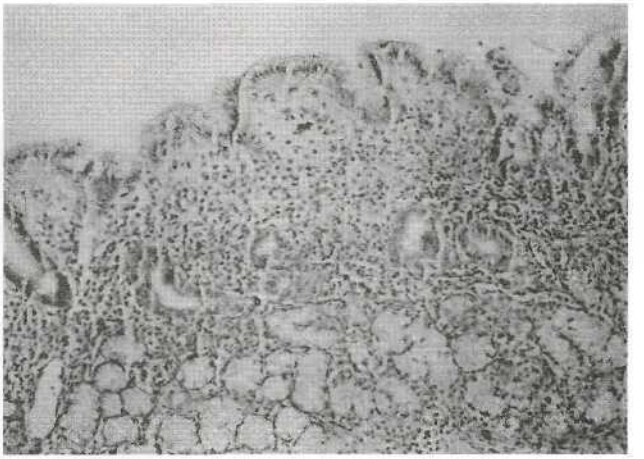


Figure 2. EGF immunohistochemistry: negative staining

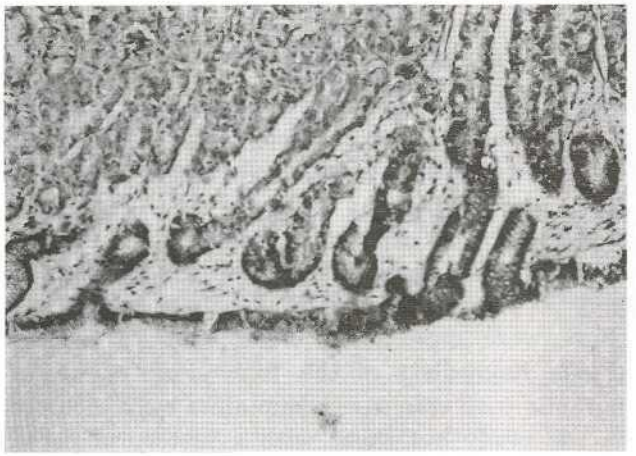


Figure 3. EGF immunohistochemistry: positive staining

For histopathological examination, biopsy materials were placed in 10% formalin and sent to the pathology laboratory. The materials were embedded in paraffin blocks and sections of 5 micron were then obtained from the blocks. Finally, they were stained with Hematoxylene-Eosin and examined under a light microscope (Figure 1). Later, the sections were transferred onto slides with lysine and subjected to immunohistochemical staining using EGF (Zymed, supersensitive) monoclonal kit.

The sections stained with Hematoxylene Eosin were evaluated according to Sydney's classification. The sections stained with EGF were evaluated based on the staining percentages of foveolar epithelium. When there was no staining, EGF was considered negative (-) (Figure 2). When 25% of the epithelium was stained, EGF was considered as slightly positive (+), 25-49% was moderately positive (++) and 50% was highly positive (+++) (Figure 3).

Statistical Analysis

Chi-square and Wilcoxon Signed Rank tests were used to compare the parameters obtained before treatment with those obtained after treatment.

Statistical Package program for the Social Sciences (SPSS, version 9.0) was used for statistical analysis. $P<0.05$ was considered significant. Data were expressed in mean ± SD.

Table 2. Effects of UDCA Treatment on EGF Levels

	EGF Levels Before Treatment		*EGF Levels After Treatment*	
	Number of Patients	*%*	*Number of Patients*	*%*
Negative	1	(3.2)	8	(25.8)
Slightly positive	10	(32.3)	20	(64.5)
Moderately Positive	16	(51.6)	3	(9.7)
Highly Positive	4	(12.6)	0	(0)

RESULTS

The 31 patients, {nine male (9%), 22 female (71%)} were aged between 38 and 70 years with a mean of 57.1±10.0 years. On endoscopy, all patients had enterogastric reflux, 24 (77%) reflux gastritis, five (16%) antral gastritis, and two (6.5%) diffuse gastritis. Examination of biopsy specimens obtained from the prepyloric antrum and the corpus revealed that 26 patients (83.9%) had chronic gastritis, three (9.7%) erosive gastritis and two (6.55) superficial gastritis. Histopathological examination performed before treatment showed various degrees of foveolar hyperplasia edema and proliferation of smooth muscle in the lamina propria in all patients. Histopathological examination performed after treatment revealed the same findings although the severity was decreased. All but one patient showed various degrees of EGF positivity. H. pylori was positive in 13 patients (41.9%).

Following six weeks UDCA therapy, endoscopy revealed complete recovery in nine of 31 patients and various degrees of recovery in the rest of the patients. Treatment with UDCA caused a decrease in H. pylori positivity, being positive in 13 patients (41.9%) before treatment and in ten patients (32%) after treatment. However, the difference was not significant ($p>0.05$). The degree of EGF positivity decreased in 23 patients (74.2%), but it did not change in seven patients (22.6%) and increased in one patient (3.2%) (Table 2). There was a significant difference in EGF levels before and after treatment ($p<0.001$). However, there was no relationship between the change in EGF levels and the diagnosis on endoscopy and on pathological examination and H. pylori positivity following treatment ($p>0.05$).

DISCUSSION

Although reflux gastritis is encountered in people who have not undergone surgery on their stomachs and gallbladders, it is more frequently seen those who have (17,18). In a study by Baren et al, the rate of bile reflux reached 100% after a gastric operation (19). Consistent with the findings in the literature, we found alkaline reflux in 100% of patients on endoscopy and alkaline reflux gastritis in 90% of the patients.

Diagnostic procedures for alkaline reflux gastritis include analysis of bile salts in the gastric fluid, hepatobiliary scintigraphy, endoscopic examination of the upper gastrointestinal tract and endoscopic biopsy. At endoscopy, greenish gastric fluid on hemorrhagic, vulnerable mucosa indicates bile reflux. Histopathological examination of the biopsy specimen taken during endoscopy allows a firm diagnosis (20- 23).

The most important member of growth factor family, is EGF, which exists in a number of tissues. It increases mitosis and regeneration of cells and therefore plays a role in protection of the gastric mucosa from various types of damage integrity of mucosa and in repair of the damaged mucosa (15, 7).

Konturek et al, in their experimental study on 150 Wistar rats, found increased levels of EGF in the mucosa, with ulceration and gastritis, using immunochemical methods (24). Consistent with their finding, we observed various degrees of EGF positivity in 93% of patients (30/31).

Although the histological features of alkaline reflux gastritis are almost the same as those of H. pylori gastritis, foveolar hyperplasia, presenting with proliferation and with a dilatation of foveolar epithelium and the convoluted appearance of the epithelium, is an important sign of gastritis due to bile reflux or caused by non steroid anti-inflammatory drugs. Unlike H. pylori gastritis, the intensity of inflammatory cells is normal or slightly abnormal (25, 26) in alkaline reflux gastritis. The presence of lymphoid follicle is an important diagnostic sign of H. pylori gastritis (27).

Infection with H. pylori is a factor affecting EGF levels. Some studies revealed an association between H. pylori infection and increased EGF levels in gastric mucosa (28). Walter et al, in their study on 28 H. pylori positive patients with duodenal ulcer and 16 H. pylori negative patients with duodenal ulcer, showed a two-fold increase in mucosal EGF levels in H. pylori negative patients using immunohistochemical methods (7). In this study, however there was no significant difference in EGF levels between H. pylori positive and negative patients. We thought that increased EGF levels were secondary to alkaline reflux gastritis rather than increased intensity of H. pylori.

To our knowledge, there is no study which has investigated the effect of UDCA on EGF levels, although many studies have shown that UDCA may be used in alkaline reflux gastritis with positive effects (1-3). We found that UDCA therapy facilitated a complete recovery in nine of 31 patients and various degrees of recovery in the

rest of the patients. However, the treatment decreased EGF levels. This can be explained by the fact that in patients with alkaline reflux gastritis, EGF increased in response to the irritant effect of the bile and then decreased as the mucosal damage healed following UDCA treatment. Further studies may reveal whether UDCA has a direct effect on EGF.

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