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# Caustic esophageal injury decreases the number of interstitial cells of Cajal in the rat esophagus

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**Aim:** To evaluate the effect of caustic esophageal injury (CEI) on the number of interstitial cells of Cajal (ICC).

**Materials and methods:** Wistar rats were divided into 3 groups: control, acid, and alkali induced CEI. CEI was performed by the injection of 1 mL of 10% hypochloric acid in the acid group, and 1 mL of 10% sodium hydroxide was injected into the alkali group. Distal esophageal segments were removed 24 h after injection. CEI was graded and the number of ICC were investigated (CD-117 staining). The number of ICC was compared in groups and correlated with CEI grades.

**Results:** Typical histopathologic features of CEI were encountered in acid (mean grade: 0.25 ± 0.15), and in alkali (median: 2, range: 1.25-3) groups. The number of ICC was decreased in both the acid (mean: 12.8 ± 9.2) and alkali (median: 2, range: 0.0-4.0) groups with respect to the control group (mean: 30.5 ± 6.5) ($P < 0.05$). In addition, alkali group had a decreased number of ICC, when compared to the acid group ($P < 0.05$). Histopathologic grading showed an inverse correlation with number of ICC in both the acid (rs: 0.678) and alkali (rs: 0.759) groups.

**Conclusion:** CEI decreases the number of ICC in the rat esophagus. Alkali caustics cause a higher grade of esophageal injury and a greater decrease in the number of ICC. Motility disorders after caustic ingestion may be related to the decreased number of ICC.

**Key words:** Caustic esophageal injury, interstitial cells of Cajal, esophagus

## Kostik özefagus zedelenmesi rat özefagusunda interstisiyel Cajal hücrelerini azaltmaktadir

**Amaç:** Kostik özefagus zedelenmesinin (KÖZ) interstisiyel Cajal hücreleri (İCH) sayısına etkisini değerlendirmek amacıyla deneysel bir çalışma yapılmıştır.

**Yöntem ve gereç:** Wistar sıçanlar kontrol, asit ve alkali KÖZ oluşturmak üzere üç gruba ayrıldı. KÖZ asit grupta 1 mL'lik % 10'luk hipoklorik asit, alkali grupta ise 1 mL'lik % 10'luk sodium hidroksit enjekte edilerek elde edildi. Distal özefagus segmentleri enjeksiyondan 24 saat sonra çıkartıldı. KÖZ evrelendi ve ICH sayıları incelendi (CD-117 boyaması). Gruplardaki ICH sayıları gruplar arasında karşılaştırılmış ve KÖZ evreleri ile korale edilmiştir.

**Bulgular:** KÖZ'e ait tipik histopatlojik bulgular asit (ortalama evre: 0,25 ± 0,15) ve alkali (ortanca: 2, aralık: 1,25-3) gruplarda izlendi. ICH sayıları kontrol grubuna (ortalama: 30,5 ± 6,5) oranla asit (ortalama: 12,8 ± 9,2) ve alkali (ortanca: 2, aralık: 0,0-4,0) gruplarda azalmıştı ($P < 0,05$). Ayrıca alkali grupta asit gruba oranla IHC sayısı azalmış bulundu ($P < 0,05$). Histopatolojik evrelemede, IHC sayısı ile hem asit (rs: 0,678) hemde alkali (rs: 0,759) gruplar arasında ters korelasyon tespit edildi.

**Sonuç:** KÖZ, sıçan özefagusunda İCH sayısını azaltmaktadır. Alkali kostikler daha yüksek evreli özefagus zedelenmesi ve daha çok İCH sayısında azalmaya neden olmaktadır. Kostik alımı sonrası gözlenen motilite sorunları azalmış İHC sayıları ile ilişkilendirilebilir.

**Anahtar sözcükler:** Kostik özefagus zedelenmesi, intertisiyel Cajal hücreleri, özefagus

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## Introduction

Ingestion of caustic substances remains a major health hazard in children (1). The most striking aspect is that the majority of caustic ingestions occur in children under 3 years of age, and are extremely preventable (1). The extent and severity of injury depend on the concentration and form of the ingested substance. Caustic esophageal injury (CEI) results in serious problems, such as esophageal dysmotility, with lifelong consequences.

Interstitial cells of Cajal (ICC) are specialized cells, generating electric slow waves that are responsible for paced contraction of gastrointestinal musculature (2). They have been considered as being not only generators of spontaneous pacemaker activity in smooth muscle, but also involved in the process of neurotransmission (2).

Although studies focused on the physiological role of ICC are preliminary, their potential role in the pathophysiology of gastrointestinal motor disorders has already been identified. The distributions of ICC in CEI have not been previously evaluated. Our aim was to evaluate the effect of CEI on the number of ICC, and to put forward the possible role of ICC in CEI related esophageal dysmotility.

## Materials and methods

Wistar albino rats (body weights: 190-250 g) were divided into 3 study groups: control (n: 12), acid (n: 12), and alkali (n: 12) caustic ingestion groups.

### Surgical Procedure

All surgical procedures were performed under general anesthesia with intramuscular ketamine hydrochloride (40 mg/kg, Ketelar®, Eczacıbaşı, İstanbul, Turkey). CEI was performed as described by Gehonno and Guedon (3). From a median laparotomy incision, a 1.5 cm abdominal esophageal segment was isolated and removed in the control group. The abdominal esophagus segments were isolated and tied from both the upper and lower ends with 2/0 catgut suture in the acid and alkali caustic groups. One milliliter of 10% hydrochloric acid was instilled in the acid group, and 1 mL of 10% sodium hydrochloride was instilled in the alkali group. Caustics were applied via 24 F catheter for 3 min for both groups. Esophageal segments were rinsed with 1 mL of distillated water. All animals were harvested 24 h after the surgical procedure, and 1.5 cm abdominal esophageal segments were removed.

All esophageal segments in the control, acid, and alkali caustic injury groups were evaluated for histopathological and immunohistochemical analysis to determine the grade of CEI and number of ICC.

### Histopathologic Study

The esophageal samples were inflated and fixed with 10% formalin. Then all esophageal segments were embedded in paraffin. Tissues were sectioned into 6 μm pieces and stained with routine hematoxylin and eosin stain. As c-kit immunohistochemistry recognizes both ICC and mast cells, all sections were stained with 0.5% toluidine blue, which is a specific mast cell stain. The ICC quantification was assessed after subtracting the sections stained with toluidine blue. The most distal section of specimens was examined by the same pathologist, who was blind to the study. The injury was graded according to depth of the ulcer as reported by Ozel et al. (4) (Table 1).

Table 1. Histopathologic grading of esophageal segments in CEI.

| Grade | Histopathologic finding |
|---|---|
| 0 | No ulcer |
| I | Ulcer involving only in epithelium |
| II | Ulcer involving epithelium and muscularis mucosa |
| III | Ulcer proceeding to submucosa |
| IV | Ulcer proceeding to inner circular muscle layer and full thickness ulcer development |

**Immunohistochemical Analysis**

The distal section of the specimen with 6 μm thickness was obtained by microtome, transferred to adhesive slides, and dried in an autoclave at 37 °C overnight, and then at 60 °C for 30 min. They were deparaffinized and dehydrated by immersion into xylene twice for 10 min, and alcohol twice for 2 min. The specimens were placed in 3% $H_2O_2$ for 5 min to inhibit endogenous peroxidases. The preparations were transferred into citrate-based antigen retrieval solution (pH 6) for the c-kit (CD-117, DakoCytomation Denmark A/S) and placed in a microwave oven twice for 7 min. By using the Shandon Sequenza™ manual staining device for standardization, classical avidin-biotin-peroxidase method and DAB (Diaminobenzidine) chromogen were applied for 20 min for immunohistochemical analysis of the c-kit. Positive controls for the c-kit antibody were the small intestine. Non-immune mouse serum served as a negative control, and Mayer's hematoxylin was used as a counter-stain.

The c-kit positive, spindle-shaped cells adjacent to esophageal smooth muscle cells were evaluated as ICC. The number of stained ICC was investigated in selected esophageal sections.

**Statistical Analysis and Ethics**

The Kruskal-Wallis test was used to detect differentiation among groups. The numbers of ICC in the control, acid, and alkali groups were compared using paired samples from the t-test, and Mann-Whitney U test. CEI grades were also correlated with the number of ICC by Pearson and Spearman's correlation (SPSS 12.0). P values lower than 0.05 were considered significant.

This study was approved by the Local Ethical Committee (KU. 2007/010). All animals received humane care in compliance with European Convention on Animal Care.

**Results**

In the acid and alkali caustics ingestion groups, hematoxylin and eosin staining findings revealed typical histopathologic features of CEI. The mean histopathologic grades of acid and alkali CEI were 0.25 ± 0.15 and 2 (range: 1.25-3), respectively. Although immunohistochemical analysis with the c-Kit showed a normal distribution of ICC in the control group (Figure 1), a decreased number of ICC was detected in the acid and alkali ingestion groups (Figure 2).

Histopathologic grades and the number of ICC in the control, acid, and alkali caustic ingestion groups are summarized in Table 2.

The number of ICC decreased significantly in both acid (mean: 12.8 ± 9.2) and alkali (median: 2, range: 0.0-4.0) caustic ingestion groups with respect to the control group (mean: 30.5 ± 6.5) ($P < 0.05$). In addition, the alkali caustic ingestion group had a decreased number of ICC, when compared to the acid caustic ingestion group ($P < 0.05$).

When the relationship between the histopathologic grading and the number of ICC was

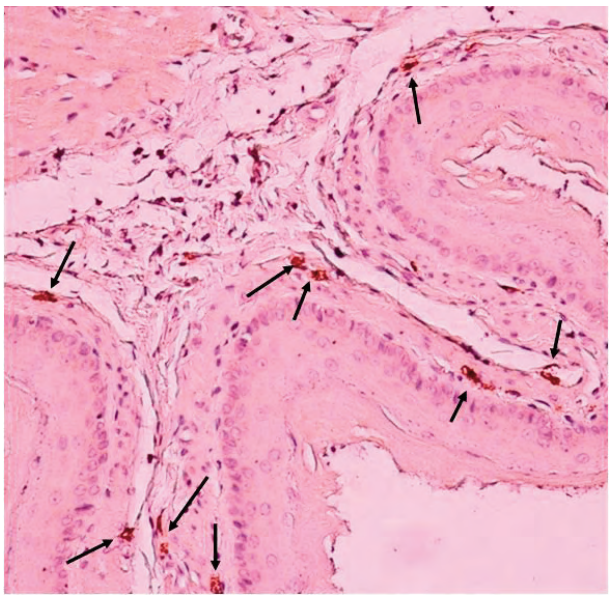

Figure 1. Immunohistochemical staining of ICC demonstrating spindle-like cells (arrows) in esophageal submucosa in the control group (CD-117 ×200).

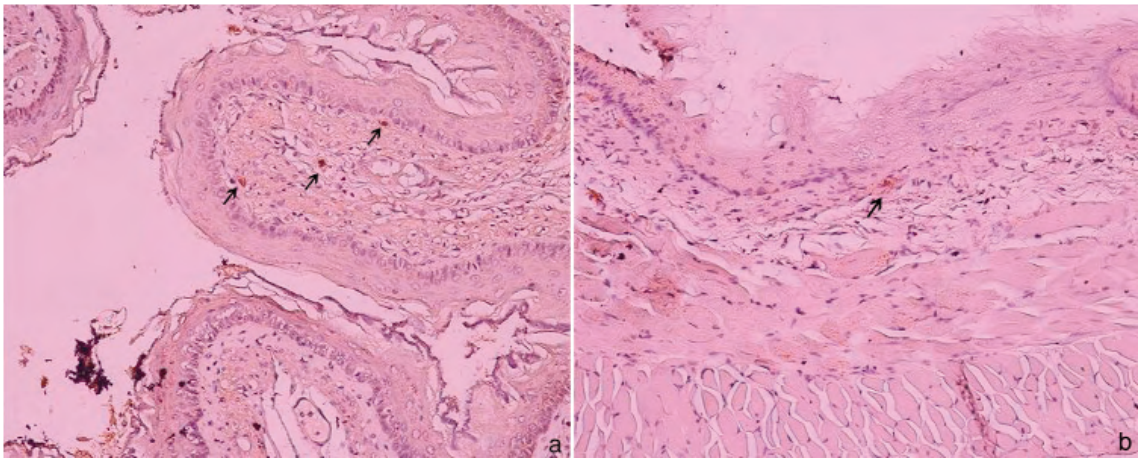


Figure 2. Acid (a) and alkali (b) caustic ingestion groups with different CEI grading showed decreased number of ICC in esophageal submucosa (CD-117 ×200).

Table 2. Histopathologic grades and number of ICC in control, acid and alkali caustic ingestion groups.

| No. of Exp. | Control Group | | Acid CI Group | | Alkali CI Group | |
|---|---|---|---|---|---|---|
| | Grade | No. of ICC | Grade | No. of ICC | Grade | No. of ICC |
| 1 | - | 36 | 0 | 14 | 1 | 8 |
| 2 | - | 24 | 0 | 2 | 2 | 1 |
| 3 | - | 28 | 0 | 27 | 1 | 12 |
| 4 | - | 33 | 1 | 4 | 2 | 0 |
| 5 | - | 36 | 0 | 14 | 1 | 4 |
| 6 | - | 37 | 0 | 21 | 3 | 3 |
| 7 | - | 25 | 0 | 18 | 2 | 4 |
| 8 | - | 32 | 0 | 23 | 2 | 4 |
| 9 | - | 30 | 0 | 15 | 3 | 0 |
| 10 | - | 26 | 1 | 1 | 2 | 0 |
| 11 | - | 18 | 0 | 6 | 4 | 0 |
| 12 | - | 41 | 1 | 0 | 4 | 0 |
| Mean/Medium | - | 30.5 ± 6.5 | 0.25 ± 0.15 | 12.8 ± 9.2 | 2; 1.25-3 | 2; 0.0-4.0 |

Abbreviations: Exp: Experiment, CI; Caustic ingestion, ICC; Interstitial cells of Cajal

evaluated, the histopathologic grading showed an inverse correlation with the number of ICC in both the acid and alkali caustic ingestion groups ($r_s$: 0.678, and $r_s$: 0.759, respectively, Pearson correlation).

## Discussion

Caustic esophageal injury (CEI) remains a major health problem during childhood. A majority of caustic injuries occur in children under the age of 3

and are usually accidental (1). Esophageal injury, due to caustics, varies depending on composition and concentration of the ingested substances. According to an extension of CEI, esophageal stricture, gastroesophageal reflux, esophageal perforation and dysmotility can be long term consequences.

In 1893, Raymond J. Cajal described non-neuronal spindle-like cells, known as interstitial cells of Cajal (ICC). These specialized cells are located in the myenteric region between the circular and muscular layer of the gastrointestinal tract (5). ICC share precursors with smooth muscle cells, and are of mesenchymal origin (6). They form networks that are widely distributed within the submucosal, intramuscular, and intermuscular layers of the gastrointestinal tract from the esophagus to the internal anal sphincter (7). ICC express the proto-oncogene c-kit. The antibodies for its gene product are available as an immunohistochemical label for ICC (6).

ICC act as a source of electric activity by generating slow waves that synchronize the circular muscle contractions along the longitudinal axis of the gastrointestinal tract, and drive the propagation of contractions in the aboral direction (6). The spread of electric signals from ICC to smooth muscle cells suggest that ICC could play a role in reception, transduction, and/or conduction of inputs from enteric motor neurons (8).

ICC are considered as being not only the pacemaker cells that trigger slow wave activity, but also involve the process of neurotransmission (2,9).

As the ICC network becomes impaired, slow waves disappear. Several studies have found altered structural arrangements of ICC associated with motility dysfunction (10). The absence or lowered density of ICC was detected in infantile hypertrophic pyloric stenosis, Hirschprung's disease, and constipation in anorectal malformations (10). Shafik et al. also reported that ICC are absent or deficient in the esophagogastric junction in patients with gastroesophageal reflux (2). Ischemia and acid exposure may cause structural and functional ICC deficiency. However, the effect of CEI on the distribution of ICC has not been previously evaluated.

ICC are particularly numerous in the lower esophageal sphincter of the esophagus, especially in its esophageal part (6). The resting electric activity of the esophagus appears to be controlled by the constant firing of ICC (2). According to our results, the number of ICC was decreased in the distal esophagus with CEI. We postulate that caustic substances penetrate the mucosal and muscular layers of esophagus and injure the ICC. In alkali CEI, the number of ICC was found to be lower when compared to the acid CEI. Moreover, higher histopathologic grades in the alkali CEI group show that the deeper esophageal injury results in a lower number of ICC.

Ingestion of caustic substances may produce severe chronic esophageal damage that leads to disturbances of esophageal motility (1,11). We suggest that the reduced number of ICC should also be considered as a causative factor in esophageal dysmotility in CEI. Further studies concerning the ICC function in CEI are needed to support our claim.

In conclusion, CEI decreases the number of ICC in the rat esophagus. Alkali caustics cause higher grades of esophageal injury, and result in a decreased number of ICC. Motility disorders after caustic ingestion may be related to a decreased number of ICC.

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