Late Complications of Corrosive Esophagitis May Be Predicted Using Biochemical Methods: Preliminary Report

Koroziv Özofajitin Geç Komplikasyonları Biyokimyasal Yöntemlerle Öngörülebilir: Ön Rapor

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ABSTRACT Objective: High tissue levels of free oxygen radicals (FORs) have been reported in corrosive esophagitis (CE). The present study aimed to determine whether prediction of injury and late complications in CE is possible based on blood levels of FORs, Interleukin (IL)-6, Tumor necrosis factor (TNF)-α, and IL-10. **Material and Methods:** Forty-two male Wistar albino rats were randomly divided into 2 main groups (A and B), each consisting of 3 subgroups (A1, A2, A3, B1, B2, and B3) of equal number. To induce experimental CE 17.5% NaOH was used in subgroups A2 and B2, and 37.5% NaOH in subgroups A3 and B3. The sham groups (A1 and B1) received 0.9% NaCl. Blood levels of lipid hydroperoxide (LPO) and glutathione peroxidase (GPx) were measured in group A. Blood levels of IL-6, IL-10, and TNF-α were measured in group B. Esophageal samples for histopathological evaluation were obtained following scarification after 3 weeks. **Results:** There were significant differences between the sham subgroups and CE subgroups, regarding histopathological scores ($p< 0.05$). There was no significant difference between the groups in blood levels of LPO, GPX, TNF-α or IL-10 ($p> 0.05$). There were significant differences between severe CE subgroups and the sham subgroups, based on IL-6 values ($p= 0,007$). **Conclusion:** In contrast to previous reports, which demonstrated increased tissue levels of FORs, blood levels of FORs were not high in this study. Nonetheless, in the light of the observed differences in IL-6 levels between the subgroups, we suggest that injury and late complications of CE may be predicted following additional experimental and clinical research using biochemical methods.

Key Words: Caustics; esophagitis; complications; cytokines

ÖZET Amaç: Koroziv özofajitte, yaralanmanın ve geç komplikasyonların öngörülmesinde çeşitli yöntemler bildirilmiştir. Yapılan çalışmalar, akut dönemde, dokudaki serbest oksijen radikalleri (SOR) düzeylerinin artmış olduğunu ortaya koymuştur. Çeşitli travmalarda interlökin (IL)–6, tümör nekroz faktörü (TNF)-α ve IL-10 gibi proenflamatuar sitokin (PES) ve anti-enflamatuar sitokin (AES) düzeylerinde değişiklikler olduğu bilinmektedir. Bu bilgiler ışığında koroziv özofajit modelinde SOR, PES ve AES'lerin kan düzeylerinin, doku hasarının ve striktür gelişiminin öngörülmesinde etkili olup olamayacağının araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmada 42 erkek Wistar Albino sıçan kullanıldı. Sıçanlar, A ve B grupları şeklinde iki ana gruba ayrıldıktan sonra, bu iki ana grup da her birinde eşit sayıda sıçan olacak şekilde üçer alt gruba ayrıldı (A1, A2, A3, B1, B2 ve B3). A2 (n= 7) ve B2 (n= 7) gruplarında %17,5'luk sodyum hidroksit (NaOH), A3 (n= 7) ve B3 (n= 7) gruplarında ise %37,5'luk NaOH kullanılarak özofajit oluşturuldu. Kontrol gruplarını oluşturan A1 (n= 7) ve B1 (n= 7) gruplarında izotonik kullanıldı. A gruplarında SOR'den lipid hidroperoksitin (LPO), anti-oksidanlardan glutatyon peroksidaz (GPX), B gruplarında ise IL-6, IL-10 ve TNF-α'nın kan düzeyleri ölçüldü. Üçüncü haftanın sonunda denekler öldürülerek, yanık oluşturulan distal özofagus histopatolojik yönden değerlendirildi. **Bulgular:** Ağır ve orta şiddette özofajit oluşturulan gruplar ile kontrol grupları arasında darlık endeksi ve histopatolojik skorlama sonuçları bakımından istatistiksel olarak anlamlı fark bulundu. Kandaki LPO, GPX, TNF-α ve IL-10 düzeyleri açısından gruplar arasındaki farklar istatistiksel açıdan anlamlı bulunmadı. IL-6 düzeyleri açısından, ağır özofajit oluşturulan grup ile kontrol grubu ve orta şiddette özofajit oluşturulan gruplar arasında istatistiksel olarak anlamlı fark ($p= 0,007$) bulundu. **Sonuç:** Daha önce yayımlanmış çalışmalarda, akut dönemde dokuda SOR düzeylerinin yüksek olduğu bildirilmiş, ancak bu çalışmada kanda yüksek düzeyler ölçülmemiştir. Bununla birlikte, IL-6 düzeyleri arasındaki anlamlı farktan yola çıkarak, yapılacak geniş deneysel ve klinik çalışmalar ile geç komplikasyonların öngörülmesini sağlayacak veriler elde edilebileceğini düşünmekteyiz.

Anahtar Kelimeler: Kostikler; özofajit; komplikasyonlar; sitokinler

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Corrosive esophagitis (CE), which can cause esophageal stricture, remains an important medical problem in developing countries.[1] It usually occurs as a result of accidental ingestion of uncontrolled and inexpensive cleaning solutions that are sold in uncontrolled markets.[1,2] Patients usually present with nausea, vomiting, drooling, dysphagia or odynophagia, mucosal bleeding, and respiratory distress associated with aspiration.[3,4] Several diagnostic trials were conducted to identify esophageal injury in CE using radiocontrast esophagography, scintigraphy, and esophageal ultrasound; however, the usefulness and prognostic value of these methods remain controversial.[5,6] Currently, endoscopy, which is performed within 24-48 hours of ingestion, is recommended as the most effective diagnostic method for assessing the depth and extent of injury, and consequently the potential for complications.[1] Recently, however, some studies that investigated the use of endoscopy reported that endoscopy revealed important findings about injury, but was insufficient for predicting late complications in some cases.[3,4]

Alkaline injury causes liquefaction necrosis, while acidic injury causes coagulation necrosis.[1,7] Ischemic tissue injury occurs during the acute necrotic phase, which is defined as the first 1-4 days. Additionally, an increase in free oxygen radicals (FORs) leads to tissue damage during the early phase of corrosive esophageal injury.[3,7,8] Fibrosis, which is characterized by severe inflammation and increased collagen deposition, occurs due to this tissue damage.[9]

Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) are known as pro-inflammatory cytokines (PICs). Their levels increase in response to trauma and septic conditions. They modulate the inflammation and defense mechanisms of an organism;[10,11] however, IL-10, which is known as an anti-inflammatory cytokine (AIC) restricts inflammation via inhibition of many PICs.[12,13] Various traumatic and infectious conditions occur in CE. As such, we think that the levels of PICs may increase in CE. The present study aimed to determine if injury and late complications in CE can be predicted based on blood levels of FORs, IL-6, TNF-α, and IL-10.

MATERIAL AND METHODS

ANIMAL CARE

The study protocol (No. 08/23) was approved by the Başkent University Faculty of Medicine and the Animal Experiments Ethics Committee. The study was run in accordance with the Başkent University Department of Laboratory Animal Science (Ankara, Turkey) guidelines for animal experimentation. All animals in the study received care according to the *Principles of Laboratory Animal Care* (National Society of Medical Research) and *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences) published by the National Institutes of Health (NIH publication No. 85-23, revised, 1996). Animals were housed in a controlled environment at 18 ± 1°C and 50%-70% relative humidity, with a 12-h light/dark cycle. The animals had ad libitum access to food pellets and water. The study included 42 male Wistar albino rats weighing 200-250 g that were randomly divided into 2 main groups (A and B), each consisting of 3 subgroups (A1, A2, A3, B1, B2, and B3) of equal number (n= 7).

The amount of serum required for measurement of study parameters was calculated to be 1-1.5 cc. The mean hemotocrit values of rats were changing between 40% and 50%. Therefore the volume of blood should be at least 2 or 2,5cc to obtain adequate serum amount. Under these circumstances, the difficulty of collecting that amount of blood from the rats and the high risk of mortality due to the venipuncture procedure necessitated the establishment of two groups of rats for measurement of different parameters. Thus, the rats were randomly assigned to two main groups (A and B). Blood sampling was done only once.

EXPERIMENTAL PROTOCOL

General anesthesia was induced by intraperitoneal injection of 60 mg kg^{-1} of ketamine hydrochloride (Bayer Anima Healthcare, Kansas, USA) and 8 mg kg^{-1} of xylazine hydrochloride (Richter Pharma, Wels, Austria) after 12 hours of fasting. All rats were fixed, following routine

shaving, disinfection, and draping. Corrosive esophageal injury was produced by modification of a previously reported experimental model.[3,8,11] The abdominal cavity was entered through a midline incision and the stomach was exteriorized from the abdomen. A 1.5-2-cm abdominal segment of the esophagus was exposed. A 5F feeding tube was placed into the distal esophageal segment via the oral route.

The distal esophageal segment was isolated and tied with 2/0 silk sutures. The isolated esophageal segment was distended by administration of 0,9% saline in the sham subgroups (A1 and B1), 17,5% sodium hydroxide (NaOH) in the A2 and B2 subgroups, and 37,5% NaOH in the A3 and B3 subgroups until dilatation and slight translucency of the esophageal wall were noted for 2 minutes. Afterwards, the isolated esophageal segment was rinsed for 1 minute with distilled water, and the silk sutures were removed. Then, the abdominal layer and skin were repaired by layers with 4/0 silk suturing and wound closure was accomplished. For the first 3 days following surgery, 10 mL of isotonic saline was administered subcutaneously to all animals to prevent dehydration. All animals were kept on a standard rhodent pellet diet and tap water (ad libitum) after surgery. At 24 hours post-surgery, blood samples were collected from tail veins for biochemical analysis. The rats were sacrificed 21 days post surgery with 150 mg kg^{-1} of thiopental sodium (Pental Sodyum 0.5 g İ.E. Ulagay İlaç Sanayi Türk AŞ. Topkapı, İstanbul, Turkey) injected intraperitoneally. The burned area of the esophagus was excised from each rat for histopathological assessment.

BIOCHEMICAL ANALYSIS

Blood samples were collected to determine the level of lipid hydroperoxide (LHP), which is among the primary oxygenated products of polyunsaturated fatty acids and is known as a FOR, and the activity of glutathione peroxidase (GPx), which is an anti-oxidant enzyme that protects tissues from oxidative damage, in group A. IL-6, TNF-α, and IL-10 levels were measured in group B.

LIPID HYDROPEROXIDE DETERMINATION

A colorimetric assay kit (Cayman Chemical Company Lipid Hydroperoxide Assay Kit

Ann Arbor, MI, USA) was used to measure LHP levels.

GLUTATHIONE PEROXIDASE (GPX) ACTIVITY DETERMINATION

The Cayman chemical glutathione (GSH) peroxidase assay kit (Cayman Chemical, Ann Arbor, MI, USA), which measures GPx activity indirectly via a coupled reaction with glutathione reductase (GR) was used. Oxidized glutathione (GSSG), produced upon reduction of hydroperoxide by GPx is recycled to its reduced state by GR and reduced nicotinamide adenine dinucleotide phosphate (NADPH):

$$R\text{-}O\text{-}O\text{-}H + 2\,GSH \xrightarrow{GPx} R\text{-}OH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2\,GSH + NADP^+$$

Oxidation of NADPH to nicotinamide adenine dinucleotide phosphate (NADP) is accompanied by a decrease in absorbance at A_{340} nm. Under conditions in which GPx activity is rate limiting, the rate of decrease in A_{340} is directly proportional to GPx activity in the sample.

TNF-α, IL-6, AND IL-10 MEASUREMENT

Enzyme-linked immune sorbent assay kits of TNF-α, IL-10 (Invitrogen, Camarillo, USA) and IL-6 (Bender MedSystem, Vienna, Austria) were used to measure PIC and AIC levels.

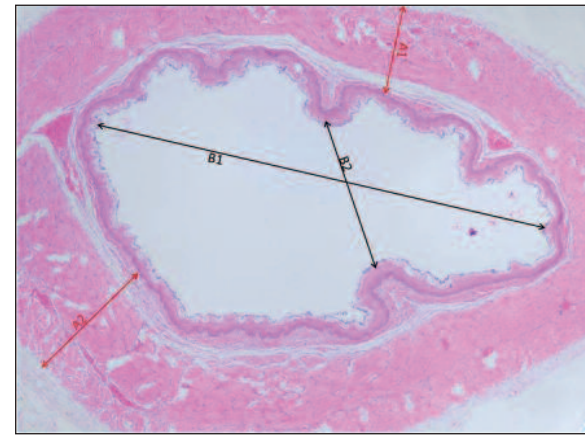

FIGURE 1: Hematoxylin-eosin staining of esophageal section in the control group. A1 and A2, wall thickness; B1 and B2, lumen diameter (x100).
(See for colored form http://tipbilimleri.turkiyeklinikleri.com/)

HISTOPATHOLOGIC ASSESSMENT

Light Microscopic Examination

Distal esophageal segments were fixed in 10% buffered formalin. The specimens were embedded in paraffin, and 4-μm sections were prepared by cutting transversely at the level of injury and they were placed on glass slides. Four slides were evaluated for each rat. Each slide was stained with hematoxylin-eosin (H&E) and Masson's trichrome. The pathologist grading the specimens was blinded to which specimens were treated and untreated. Histological examination was performed with a millimetric ocular microscope (Olympus BX-50, Tokyo-Japan). The thickness of the esophageal wall and the diameter of the lumen were measured by Olympus DX2 digital imaging system for calculation of the stricture index [wall thickness ($\alpha1 + \alpha2$)/2]/[lumen diameter ($\beta1 + \beta2$)/2] (Figure 1). Masson's trichrome-stained sections were histopathologically evaluated and were scored semi-quantitatively; the results were presented in Table 1.[2]

STATISTICAL ANALYSIS

All values were presented as mean ± standard deviation (SD). Statistical analysis of the stricture index, histological scores and biochemical results was performed using the Mann-Whitney U test. All statistical analyses were performed using SPSS v.15.0. Statistical significance was considered $p< 0.05$.

RESULTS

In total, 37 rats survived until the end of the study. Necropsy was performed in 5 rats that died during the study. Mediastinitis and esophageal perforation were observed in 2 and 3 rats, respectively. We could not obtain sufficient esophageal tissue for histopathological examination. We could not measure the IL-6 level in one rat in subgroup B1 nor the TNF-α level in two rats in each of subgroups B2 and B3. Biochemical, histopathological, and statistical results were summarized in Tables 2 and 3.

HISTOPATHOLOGICAL RESULTS

There were no differences in the stricture index ratio ($p> 0.05$) between the sham and corrosive-injured subgroups of group A. There were significant differences in histopathological findings ($p= 0.001$) between the sham and corrosive-injured subgroups of group A. Although the difference in the stricture index was significant between subgroups A2 and A3 ($p= 0.038$), there was no difference in histopathological findings ($p= 0.18$) (Table 2). There were no differences in the stricture index between the three subgroups of group B ($p> 0.05$); however, statistically significant differences in histopathological findings were observed between the sham and corrosive-injured subgroups of group B ($p< 0.05$) (Table 3 and Figure 2).

TABLE 1: The histopathological scoring system.

Histopathological findings
Submucosal collagen deposition
None (0)
Mild (submucosal collagen at least twice the thickness of the muscularis mucosa) (1)
Marked (submucosal collagen more than twice the thickness of the muscularis mucosa) (2)
Muscularis mucosa injury
None (0)
Yes (1)
Muscular layer injury and collagen deposition
None (0)
Mild (collagen deposition around the smooth muscles) (1)
Marked (collagen deposition around smooth muscles and replacement of muscles with collagen) (2)

TABLE 2: Biochemical, histopathological, and statistical results in the A subgroups.

				p value		
	A1	A2	A3	A1 vs. A2	A1 vs. A3	A2 vs. A3
LHP	4.01 ± 1.04	4.13 ± 2.13	4.02 ± 1.89	0.91	0.99	0.93
GSHPx	8.07 ± 4.79	11.37 ± 3.88	12.10 ± 8.21	0.18	0.28	0.83
Stricture index	0.58 ± 0.3	0.47 ± 0.2	0.78 ± 0.27	0.447	0.242	0.038*
Histopathological score	0	4.14 ± 1.46	5.67 ± 1.86	0.001*	0.001*	0.181

* Statistically significant.
LHP: Lipid hydroperoxide; GSHPx: Glutathione peroxidase.

TABLE 3: Biochemical, histopathological, and statistical results in the B subgroups.

	P value					
	B1	B2	B3	B1 vs. B2	B1 vs. B3	B2 vs. B3
TNF-α	5.68 ± 3.56	9.52 ± 7.63	6.0 ± 1.37	0.25	0.84	0.29
IL-6	80.3 ± 31.6	69.4 ± 34.4	186.6 ± 155	0.53	0.046*	0.007*
IL-10	11.68 ± 3.71	8.74 ± 4.07	7.24 ± 4.32	0.18	0.06	0.52
Stricture index	0.47 ± 0.26	0.54 ± 0.3	0.4 ± 0.16	0.644	0.591	0.332
Histopathological score	0.29 ± 0.49	3.57 ± 3.1	5.5 ± 2.07	0.017*	0.001*	0.295

* Statistically significant.
TNF-α: Tumor necrosis factor alpha; IL-6 and 10: Interleukin-6 and 10.

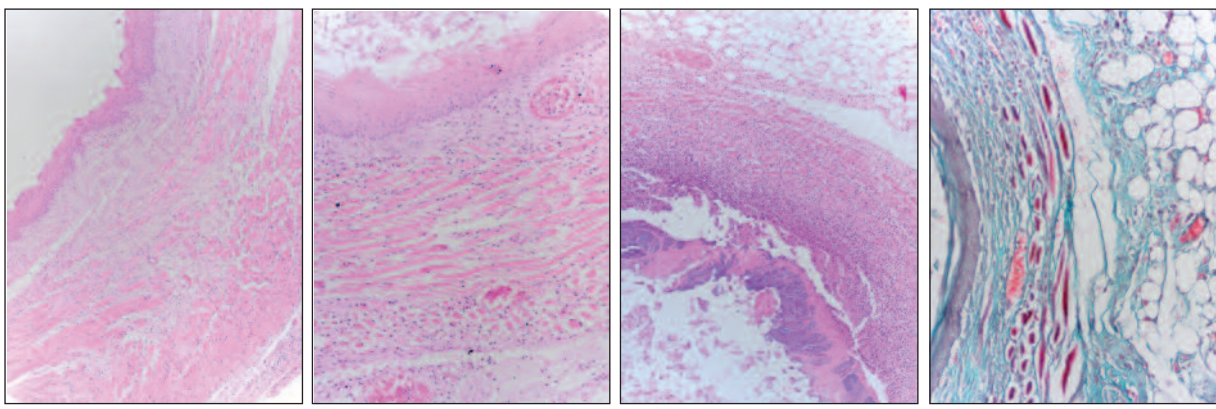

FIGURE 2: A, B: Hematoxylin-eosin (H&E) staining of esophageal section in injured rat with 17.5% NaOH. Smooth muscle atrophy and slight increase in fibrotic activity (Ax200) (Bx400). C- H&E staining of esophageal section in injured rat with 37.5% NaOH. Marked increase in inflammation and mucosal necrosis (x200) D- Masson's trichrome staining of esophageal section in injured rat with 37.5% NaOH. Abundant fibrosis and increased fibrous tissue with severe smooth muscle atrophy (x400).
(See for colored form http://tipbilimleri.turkiyeklinikleri.com/)

BIOCHEMICAL RESULTS

Bichemical and statistical results were summarized in Table 1 and Table 2. Blood levels of LHP and GPx were not significantly different between the three subgroups of group A (p> 0.05). There was no significant difference in TNF-α level in the three subgroups of group B (p> 0.05). IL-6 levels were significantly higher in subgroup B3 than in subgroup B2 (p< 0.007) and subgroup B1 (p= 0.046). Although the difference in IL-10 between the three subgroups of group B was insignificant (p> 0.05), IL-10 levels were higher in subgroup B1 than in subgroups B2 and B3.

DISCUSSION

Corrosive gastrointestinal injury, which develops after ingestion of acid or alkaline substances, usually occurs in the oral cavity or esophagus. Such injury may sometimes extend to the stomach, penetrate deep tissues, or present with perfora-

tion.[1,7] The incidence of stricture formation, which is the most common late complication, is between 2% and 63% in cases of caustic ingestion.[7,14,15] The late complications of caustic ingestion are closely related to the depth and extent of esophageal or gastric injury, which has an effect on inflammation and collagen deposition. The extent and depth of injury depends on the quantity, concentration, and pH of the causative substance, as well as tissue contact time.[1,7,16]

Determining the presence and extent and depth of the injury is critical for predicting the risk of late complications of corrosive ingestion. While clinical findings may provide information concerning esophageal and gastric injury, several studies report that clinical signs and symptoms are not always helpful in predicting the degree of injury and subsequent stricture formation.[16,17] Endoscopy, which is usually performed under anesthesia, remains the best method for evaluating the depth and extent of injury. The present study induced different degrees of esophagitis in rats using different concentrations of NaOH, to investigate if injury and late complications could be predicted based on biochemical analysis, which is less invasive and easier to perform than endoscopy.

FORs are generated as products of cellular metabolism. Under normal conditions, the body's natural defense enzymes, such as superoxide dismutase, catalase, and GPx, render free radicals innocuous by reducing superoxide radicals and peroxides, concurrently oxidizing glutathione. Abnormally high levels of free radicals and the simultaneous decline in antioxidant defense mechanism activity can lead to cellular organelle and enzyme damage, increased lipid peroxidation, and the development of insulin resistance.[18,19] Increased inflammation associated with increased levels of FORs and inflammatory mediators was reported in previous experimental studies of CE.[1,7,8]

Somuncu et al. reported that the level of malondialdehyde (MDA)-a known reactive oxygen radical-increased in tissue after 24 h in an experimental study of CE. They also reported that the level of MDA decreased significantly in response to trapidil treatment, which caused a reduction in the levels of inflammatory cytokines IL-6 and IL-12.[3] Gunel et al. noted high FOR levels in esophageal tissue within 24 h of injury in an experimental study. They observed that this high tissue level of FORs persisted for 72 h after injury.[11] Another experimental study reported that the tissue level of FORs, and collagen deposition in submucosal and muscular layers decreased in animals that were administered antioxidant therapy.[20] Ocakçı et al. reported high levels of MDA in esophageal tissue and low levels of antioxidant enzymes such as GPx, superoxide dismutase, and catalase, in a study of experimental CE.[21]

The present study aimed to determine the blood level of LHP, an indicator of FORs, and GPx level as a member of antioxidant defense systems. There was no significant difference in the blood level of LHP between any of the study subgroups. GPx increased more in the A2 and A3 subgroups than in the A sham subgroup (A1), but the difference between the three subgroups of group A was not significant. Histopathological score in subgroup A1 was significantly lower than in the corrosive esophagitis subgroups (A2 and A3) ($p= 0.001$). Although significant histopathological changes were observed in the present study, FOR levels were not high, which is inconsistent with previous reports.[3,8,11,21]

Tissue damage commonly seen after trauma, burns, and sepsis, induces an inflammatory cascade that releases various mediators, especially pro-inflammatory mediators that arouse reticuloendothelial cells. Anti-inflammatory mediators minimize the extent of tissue damage caused by these pro-inflammatory mediators.[22-24] Experimental burn and pancreatitis studies reported increased TNF-α, IL-1, and IL-6 levels.[25-27] TNF-α is known to be the first PIC to increase. In a liver ischemia model, Neblina et al. blocked selectin, an effective leukocyte adhesion and migration molecule, which led to a significant decrease in TNF-α and a significant increase IL-10, which has tissue protective effects.[28] Li et al. suggested that IL-6 was important in the pathophysiology of reflux esophagitis.[29] Nonetheless, to the best of our knowledge, no study has investigated the levels of PICs and AICs in CE.

Measurement of the pro-inflammatory and anti-inflammatory mediators in the present study showed that, although insignificant, there was a difference in TNF-α levels between the sham subgroups, and the moderate and severe corrosive esophagitis subgroups. IL-10 levels did not differ significantly between the subgroups. We did observe a significant difference in IL-6 levels between the severe and moderate esophagitis subgroups. In addition, the difference in IL-6 levels between the sham and severe esophagitis subgroups was statistically significant.

The stricture index is usually used to evaluate experimental esophageal stricture in experimental studies.[2,21] In the present study, there was no significant difference in the stricture index between any of the subgroups, except the subgroups A2 and A3; on the other hand, there were significant differences in histopathological findings. We think that the sectional line, preservation, and embedding configuration of specimens affected the measurements. Consequently, in accordance with the literature, we think that the barium meal study, which we could not run due to technical inadequacy, is more effective than the stricture index for determining and evaluating esophageal stricture in rats.

CONCLUSION

The statistically non-significant results obtained in the present study may be attributed to the small study population. As such, the present study is considered a preliminary study, but we think that the findings are significant. Larger clinical studies that use less invasive biochemical methods to determine damage severity and the risk associated with late complications are warranted.

REFERENCES

1. Huang YC, Ni YH, Lai HS, Chang MH. Corrosive esophagitis in children. Pediatr Surg Int 2004;20(3):207-10.
2. Yukselen V, Karaoglu AO, Ozutemiz O, Yenisey C, Tuncyurek M. Ketotifen ameliorates development of fibrosis in alkali burns of the esophagus. Pediatr Surg Int 2004;20(6): 429-33.
3. Somuncu S, Cakmak M, Erdogan S, Caglayan O, Akman H, Kaya M. Trapidil, an inhibitor for phosphodiesterase and platelet-derived-growth factor, ameliorates corrosive esophageal burn in rats. Tohoku J Exp Med 2005;207(3):203-8.
4. Doğan Y, Erkan T, Cokuğraş FC, Kutlu T. Caustic gastroesophageal lesions in childhood: an analysis of 473 cases. Clin Pediatr (Phila) 2006;45(5):435-8.
5. Kamijo Y, Kondo I, Kokuto M, Kataoka Y, Soma K. Miniprobe ultrasonography for determining prognosis in corrosive esophagitis. Am J Gastroenterol 2004;99(5):851-4.
6. Millar AJ, Numanoglu A, Mann M, Marven S, Rode H. Detection of caustic oesophageal injury with technetium 99m-labelled sucralfate. J Pediatr Surg 2001;36(2):262-5.
7. de Jong AL, Macdonald R, Ein S, Forte V, Turner A. Corrosive esophagitis in children: a 30-year review. Int J Pediatr Otorhinolaryngol 2001;57(3):203-11.
8. Ozel SK, Dagli TE, Yuksel M, Kiyan G, Kotiloglu E. The roles of free oxygen radicals, nitric oxide, and endothelin in caustic injury of rat esophagus. J Pediatr Surg 2004;39(9): 1381-5.
9. Bakan V, Demirtas I, Dülger H. The antioxidant paradox or antioxidant damage. Pediatr Surg Int 2004;20(11-12):903.
10. Keane MP, Strieter RM. The importance of balanced pro-inflammatory and anti-inflammatory mechanisms in diffuse lung disease. Respir Res 2002;3:5. doi:10.1186/rr177
11. Günel E, Cağlayan F, Cağlayan O, Akillioğlu I. Reactive oxygen radical levels in caustic esophageal burns. J Pediatr Surg 1999; 34(3):405-7.
12. Li CQ, He LC, Jin JQ. Atractylenolide I and atractylenolide III inhibit Lipopolysaccharide-induced TNF-alpha and NO production in macrophages. Phytother Res 2007;21(4):347-53.
13. Arbell D, Udassin R, Koplewitz BZ, Ohana M, Genina O, Pines M, et al. Prevention of esophageal strictures in a caustic burn model using halofuginone, an inhibitor of collagen type I synthesis. Laryngoscope 2005;115(9): 1632-5.
14. Baskin D, Urganci N, Abbasoğlu L, Alkim C, Yalçin M, Karadağ C, et al. A standardised protocol for the acute management of corrosive ingestion in children. Pediatr Surg Int 2004;20(11-12):824-8.
15. Temiz A, Oguzkurt P, Ezer SS, Ince E, Hicsonmez A. Long-term management of corrosive esophageal stricture with balloon dilation in children. Surg Endosc 2010;24(9):2287-92.
16. Lamireau T, Rebouissoux L, Denis D, Lancelin F, Vergnes P, Fayon M. Accidental caustic ingestion in children: is endoscopy always mandatory? J Pediatr Gastroenterol Nutr 2001;33(1):81-4.
17. Gaudreault P, Parent M, McGuigan MA, Chicoine L, Lovejoy FH Jr. Predictability of esophageal injury from signs and symptoms: a study of caustic ingestion in 378 children. Pediatrics 1983;71(5):767-70.
18. Maritim A, Dene BA, Sanders RA, Watkins JB 3rd. Effects of beta-carotene on oxidative stress in normal and diabetic rats. J Biochem Mol Toxicol 2002;16(4):203-8.
19. Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 2000;279(6):L1005-28.
20. Günel E, Cağlayan F, Cağlayan O, Canbilen A, Tosun M. Effect of antioxidant therapy on collagen synthesis in corrosive esophageal burns. Pediatr Surg Int 200218(1): 24-7.

21. Ocakci A, Coskun O, Tumkaya L, Kanter M, Gurel A, Hosnuter M, et al. Beneficial effects of Ebselen on corrosive esophageal burns of rats. Int J Pediatr Otorhinolaryngol 2006; 70(1):45-52.
22. Mullington JM, Hinze-Selch D, Pollmächer T. Mediators of inflammation and their interaction with sleep: relevance for chronic fatigue syndrome and related conditions. Ann N Y Acad Sci 2001;933:201-10.
23. Sies H. Strategies of antioxidant defense. Eur J Biochem 1993;215(2):213-9.
24. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. N Engl J Med 2001; 345(8):588-95.
25. Plackett TP, Colantoni A, Heinrich SA, Messingham KA, Gamelli RL, Kovacs EJ. The early acute phase response after burn injury in mice. J Burn Care Res 2007;28(1):167-72.
26. Jo YJ, Choi HS, Jun DW, Lee OY, Kang JS, Park IG, et al. The effects of a new human leukocyte elastase inhibitor (recombinant guamerin) on cerulein-induced pancreatitis in rats. Int Immunopharmacol 2008;8(7):959-66.
27. Zhang XP, Ye Q, Jiang XG, Ma ML, Zhu FB, Zhang RP, et al. Preparation method of an ideal model of multiple organ injury of rat with severe acute pancreatitis. World J Gastroenterol 2007;13(34):4566-73.
28. López-Neblina F, Toledo-Pereyra LH. Anti-ischemic effect of selectin blocker through modulation of tumor necrosis factor-alpha and interleukin-10. J Surg Res 2007;138(2):275-83.
29. Li FY, Li Y. Interleukin-6, desmosome and tight junction protein expression levels in reflux esophagitis-affected mucosa. World J Gastroenterol 2009;15(29):3621-30.