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Is it possible to diagnose infectious oesophagitis without seeing the causative organism? A histopathological study

ESOPHAGUS

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Original Article

ABSTRACT

Background/Aims: We investigated the utility of using histological changes to diagnose infectious oesophagitis when causative organisms cannot be seen.

Materials and Methods: Sixty-seven endoscopic biopsy specimens (51 Candida, 9 herpes simplex virus, 4 tuberculosis, and 3 cytomegalovirus oesophagitis) collected from 2000-2010 that matched the investigative criteria were included in the study. Cases were re-evaluated for histological changes observed in oesophagitis, and the findings were statistically compared using nonparametric tests.

Results: Thirty-nine cases occurred in male patients, and 28 occurred in female patients; the mean age of the patients was 51±20.1 years (range, 5-94 years). All cases showed lymphocytic and neutrophilic infiltration; while 27 (40.3%) showed eosinophilic infiltration. The density of lymphocytes and eosinophils were 8.43±6 and 1.07±1.62 per high power field, respectively, and these rates were higher in tuberculosis oesophagitis cases. Lamina propria infiltration was present in herpes simplex virus and Candida oesophagitis. Dense neutrophilic infiltration (>50/high power field) was noted in herpes simplex virus oesophagitis. Candida colonization was observed in 82% of cases with eosinophilic infiltration, and 80% of cases with erosion. Ulceration was present in all tuberculosis oesophagitis cases ($p<0.001$). Basal cell hyperplasia, papillary elongation, and dilated intercellular spaces were seen in all cases except for 2 Candida oesophagitis cases. Lamina propria fibrosis was especially noted in cytomegalovirus oesophagitis cases.

Conclusion: It is not possible to distinguish infectious oesophagitis from other subtypes, especially reflux oesophagitis, if the causative organism is not detected. Clinicopathological correlation and control with repeat targeted biopsies are essential for diagnosis.

Keywords: Infectious, oesophagitis, cytomegalovirus, herpes simplex virus, candida

INTRODUCTION

Oesophagitis is the damage and inflammation of the oesophageal mucosa caused by various physical, chemical, and biological factors (1). Approximately 5% of the adult population suffers from oesophagitis in the United States and other Western countries; this frequency is much higher in certain regions including Northern Iran and some areas of China (2).

The most frequent aetiological factors are gastro-oesophageal reflux disease (GERD), followed by infectious agents and intake of corrosive substances and drugs. An increase in the frequency of eosinophilic oesophagitis (EO) has been observed in recent years. Other causes of oesophagitis include radiation therapy; graft-versus-host disease; autoimmune diseases; and systemic diseases such as pemphigus, epidermolysis bullosa, and Crohn's disease (3). Infectious oesophagitis (IO) is generally seen in immunosuppressive conditions (including transplantation, aggressive cancer treatments, old age, debilitating diseases, chronic alcoholism, diabetes mellitus, hematologic malignancies, and acquired immune deficiency syndrome) (1,3). While bacterial, viral, fungal, and parasitic infections play a role in disease aetiology, the most frequent causative agents are Candida species, herpes simplex virus (HSV), and cyto-

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megalovirus (CMV). However, bacterial and parasitic infections are rare (3). A histological diagnosis is made by observing the causative organism and confirming the diagnosis by immunohistochemistry.

Although the causative agents and endoscopic appearances of various forms of oesophagitis differ, the histological findings seen in oesophagitis are very similar and nonspecific. The most frequent findings are inflammatory infiltration and epithelial hyperplasia characterized by basal cell hyperplasia and papillary elongation (1). Depending on the severity of the disease, erosions and ulcerations are also seen. Basal cell hyperplasia is defined as the basal cell layer exceeding 20% of the squamous epithelium thickness (3). Papillary elongation is the elongation of the papillae to cover 75% of the epithelium thickness towards the surface (4). In addition, the enlargement of papillary vessels and the presence of vascular pools; haemorrhage; dilated intercellular space (intercellular oedema); lymphocyte, eosinophil, and polymorphonuclear leukocyte (PNL) infiltration within the epithelium; and ballooning of the squamous cells can also be seen (5-8). The density of lymphocytes and eosinophils in reflux oesophagitis varies but does not usually exceed 20 lymphocytes and 7 eosinophils within 1 high power field (HPF). The presence of acute inflammatory cell infiltration, erosion, and ulceration are more prominent features in serious injuries (1). In GERD, all of the above-mentioned histological changes can be seen. EO is diagnosed when the number of eosinophils exceeds 25 in 1 HPF, or exceeds 15 in at least 2 HPFs (9,10). The diagnosis of IO generally requires identification of the causative organism. In contrast with reflux oesophagitis, EO can be treated with steroids, whereas IO requires specific treatments for the index agent (3). Therefore, subtyping of oesophagitis is pivotal in directing treatment and follow-up. Patient history can be of help in diagnosis of corrosive, drug-induced, radiation-related oesophagitis or other oesophagitis forms accompanying systemic diseases. However, there is no consensus on which findings should bring forth the suspicion of IO in cases where the causative organism cannot be seen.

In the present study, we evaluated the histological features of IO, other than the causative organisms themselves, and discussed their diagnostic value.

MATERIALS AND METHODS

A total of 67 cases with endoscopic biopsy specimens suggestive of IO were enrolled in the study and re-evaluated retrospectively. Cases were selected from a 10-year cohort, excluding biopsy specimens displaying only superficial characteristics, those without immunohistochemical staining for viral oesophagitis, and those without available clinical information. All biopsy specimens were fixed in 10% neutral formalin for 6-8 hours, cut into 4-5-micron thick sections, and stained with haematoxylin-eosin, following routine tissue processing procedures.

Histological evaluation was performed with a standard light microscope (Olympus BX50; Olympus corporation, Tokyo, Japan; ×40 magnification, 0.54-mm diameter, ocular magnification: ×10), without using a special ocular grid. Slides were reviewed for the presence of erosion/ulceration, basal cell hyperplasia, papillary elongation, dilated intercellular space, vascular pooling/congestion, existence of fibrosis and eosinophils in lamina propria, eosinophilic microabscess (>4 eosinophils), superficial eosinophil 'layering' (eosinophil aggregates becoming more intense in the epithelial surface), and 'surface sloughing' (desquamated epithelial cells intermingled with eosinophils). Number of lymphocytes, neutrophils, and eosinophils were measured in hot spots and recorded as number per high power field.

For the diagnosis of Candida oesophagitis, tissue invasion of the agent was sought, since Candida can be found commensally in oral mucosa and the gastrointestinal tract. Pseudohyphae located perpendicular to the surface were accepted as an indicator of invasion (Figure 1). Tuberculosis was diagnosed from the presence of typical granuloma formation and confirmed by either basal stain or clinical findings (Figure 2). HSV oesophagitis and CMV oesophagitis were diagnosed from their intranuclear inclusions and confirmed immunohistochemically (Figure 3, 4). The monoclonal antibodies used for diagnosis were HSV1 (clone OVTL 12-30, SC-52322, Santa Cruz, CA, USA; 1:100), HSV2 (clone OVTL 12-30, SC-52322, Santa Cruz, CA, USA; 1:100) and CMV (clone OVTL 12-30, SC-52322, Santa Cruz, CA, USA; 1:100)

According to the above-mentioned criteria, 51 cases of Candida oesophagitis, 9 of HSV oesophagitis, 4 of tuberculosis, and 3 of CMV oesophagitis were included in the study. Cases were re-evaluated with regards to the findings seen in reflux oesophagitis and EO, and were statistically compared using nonparametric tests (chi-square and Kruskal-Wallis) (IBM Corporation, New York, USA).

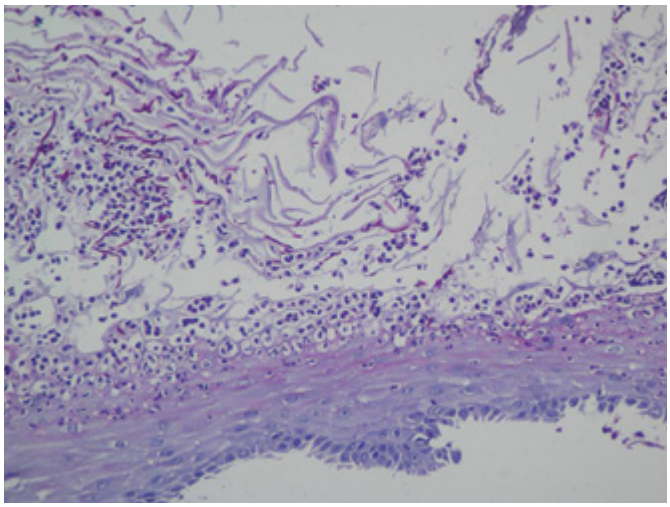

Figure 1. Candida oesophagitis (Hematoxylin and eosin stain, ×200).

RESULTS

Fifty-one of the 67 (76.1%) cases analysed were Candida oesophagitis, 9 (13.4%) were HSV oesophagitis, 4 (6.0%) were tuberculosis oesophagitis and 3 (4.5%) were CMV oesophagitis. Thirty-nine of these cases occurred in male patients and 28 occurred in female patients; the mean age of the patients was 51±20.1 years (5-94 years). The age and gender distribution of the cases according to oesophagitis subtypes are given in Table 1. The patients with CMV infections were younger than those with HSV infections (40.6 years vs 59.4 years); however, no significant relationships were found between oesophagitis subtype and age.

All of the cases exhibited lymphocyte infiltration, and 27 (40.3%) showed eosinophilic infiltration. While lymphocytic infiltration was observed particularly in tuberculosis oesophagitis cases, 22 (82%) cases of eosinophilic infiltration occurred in Candida oesophagitis cases. When all the aetiological factors were considered, the lymphocyte count per HPF ranged from 0 to 23 (mean, 8.43±6), and the eosinophil count ranged from 0 to 7 per HPF (mean, 1.07±1.62). Lymphocyte and eosinophil density in tuberculosis oesophagitis cases was greater than

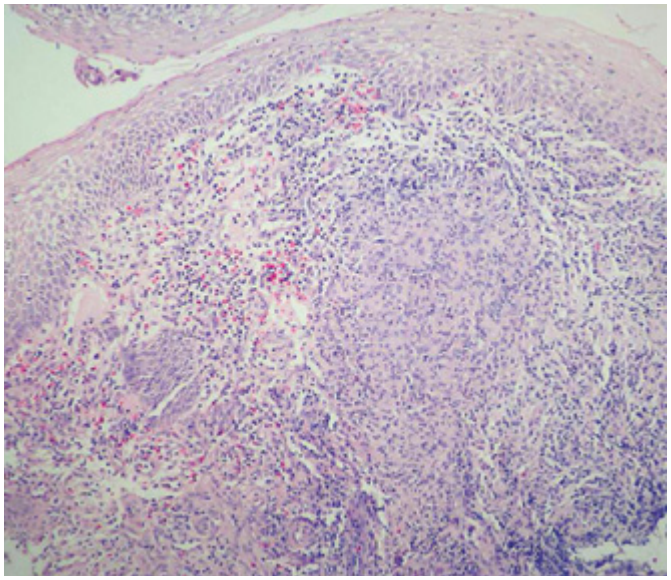

Figure 2. Tuberculosis-induced granuloma formation (Hematoxylin and eosin stain, ×100).

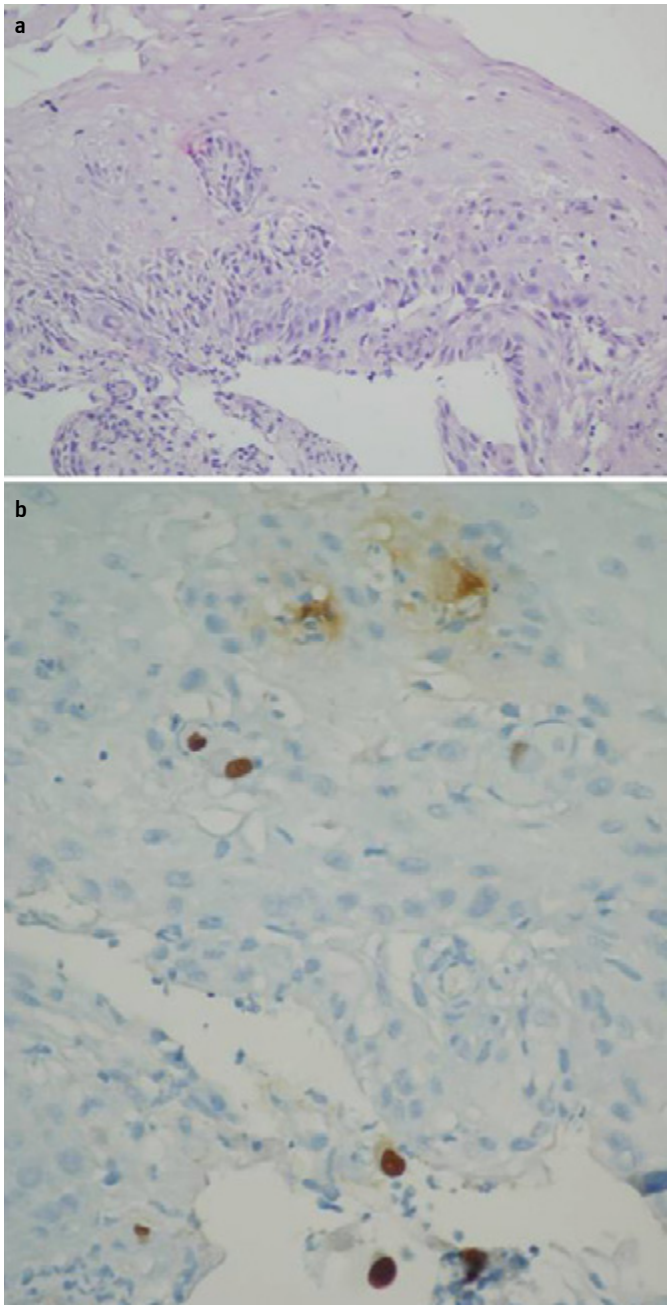

Figure 4. a, b. Cytomegalovirus (CMV) oesophagitis (Hematoxylin and eosin stain, ×200) **(a)**. Inset: Positive CMV immunohistochemistry (Anti-CMV, ×200) **(b)**.

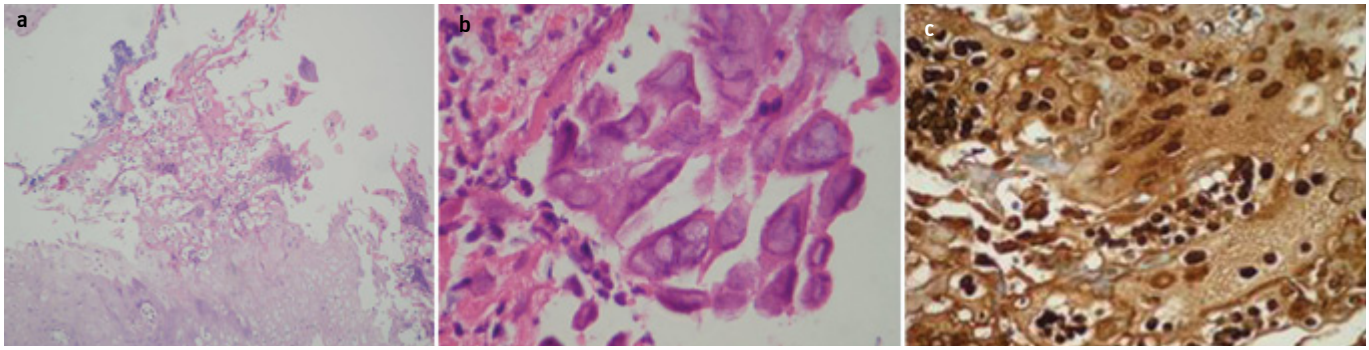

Figure 3. a-c. Herpes simplex virus HSV oesophagitis (Hematoxylin and eosin stain, ×200) **(a)** and HSV intranuclear inclusions (Hematoxylin and eosin stain, ×400) **(b)** and positive HSV immunohistochemistry (Anti-HSV, ×200) **(c)**.

Table 1. Age and gender distribution of the cases according to oesophagitis subtypes

	Age	Gender		Total number of cases
Oesophagitis subtype	Mean±SD (range; years)	Male	Female	(n)
CMV	40.6±8 (32-48)	3	0	3
Tuberculosis	46±17.3 (31-61)	2	2	4
HSV	59.4±24.7 (10-94)	6	3	9
Candida	50.4±19.8 (1-83)	28	23	51
Total	50.9±20.1 (1-94)	39	28	67

CMV: cytomegalovirus; HSV: herpes simplex virus

Table 2. Distribution of inflammatory infiltration according to infectious oesophagitis subtype

	Lymphocyte number/HPF	Eosinophil number/HPF	PNL number/HPF		
Oesophagitis subtype	Mean±SD (range)	Mean±SD (range)	0-25 n (%)	26-50 n (%)	>5 n (%)
CMV	3.7±3.5 (0-7)	0	2 (66.7)	0	1 (33.3)
Tuberculosis	9.7±4.9 (4-16)	2.2±1.5 (0-3)	1 (25)	2 (50)(25)	1
HSV	6.3±6.7 (0-22)	0.3±0.7 (0-2)	2 (22.2)	3 (33.3)	4 (44.4)
Candida	8.9±5.9 (0-23)	1.1±1.7 (0-7)	34 (66.7)	5 (9.8)	12 (23.5)
Total	8.4±6 (0-23)	1±1.6 (0-7)	39 (58.2)	10 (14.9)	18 (26.9)

CMV: cytomegalovirus; HSV: herpes simplex virus; PNL: polymorphonuclear leukocyte; HPF: high power field

Table 3. Distribution of histological findings according to the infectious oesophagitis subtype

	Erosion n (%)	Ulceration n (%)	Basal cell hyperplasia n (%)		Papillary elongation n (%)	
			>50	<50	>50	<50
CMV	2 (66.7)	0	2 (66.7)	1 (33.3)	3 (100)	0
Tuberculosis	0	4 (100)	4 (100)	0	4 (100)	0
HSV	6 (66.7)	3 (33.3)	7 (100)	0	6 (100)	0
Candida	32 (62.7)	2 (3.9)	41 (82)	8 (16)	47 (94)	3 (6)
Total	40 (59.7)	9 (13.4%)	54 (84.4)	9 (14.1)	60 (95.2)	3 (4.8)

CMV: cytomegalovirus; HSV: herpes simplex virus

that observed in other oesophagitis subtypes (mean lymphocytes per HPF, 9.75±4.9 [range: 4-16]; mean eosinophils per HPF 2.25±1.5 [range: 0-3]). Although PNL was observed in all cases, the number of PNLs per HPF did not exceed 25/HPF in 58.2% of the cases; however, it was notable that PNL density in 44% of the cases with HSV infections exceeded 50/HPF (Table 2).

While erosion was observed in 40 cases (59.7%) and ulceration was observed in 9 (13.4%), the epithelium was sound in 18 cases (27%). It was notable that 80% of the cases with erosion had Candida colonization, and ulceration was present in all the cases with tuberculosis oesophagitis ($p<0.001$). In those cases, it was quite difficult to discern whether the Candida colonization was secondary to ulceration. Endoscopic findings favoured Candida oesophagitis when the Candida plaques were disseminated throughout the oesophagus.

Basal cell hyperplasia and papillary elongation were seen in all cases except for 1 Candida oesophagitis case. Notably, however, basal cell hyperplasia exceeding 50% of the epithelial thickness was seen in all tuberculosis oesophagitis and HSV oesophagitis cases, and 82% and 67% of Candida oesophagitis and CMV oesophagitis cases, respectively, but without statistically significant differences between subtypes. Papillary elongation was observed in all cases of CMV oesophagitis, tuberculosis oesophagitis, and HSV oesophagitis, and in 94% of Candida oesophagitis cases with basal cell hyperplasia exceeding one-half of the epithelial thickness (Table 3).

When the cases were reviewed in terms of vasodilatation--vascular pooling and dilated intercellular space (both regarded as early findings of non-erosive reflux disease)--dilated intercellular space was observed in all oesophagitis cases except for 2 Candida oesophagitis cases. When the lowest epithelial level at which such changes are reflected in these cases was investigated, basal cell hyperplasia and papillary elongation exceeding 50% of the epithelial thickness was found in all of the tuberculosis and HSV oesophagitis cases, and in 76% and 67% of Candida oesophagitis and CMV oesophagitis cases, respectively. While vascular pooling was not observed in CMV and tuberculosis oesophagitis cases, it was observed in 11 cases each of HSV and Candida oesophagitis; however, the differences between subtype was not statistically significant.

All cases were examined for eosinophilic microabscess, superficial layering of eosinophils, and surface sloughing; these are characteristics considered typical of EO. These findings were observed only in 1 Candida oesophagitis case; neither the clinical history of the case nor the findings in the existing biopsy specimens indicated EO. When the presence of fibrosis and eosinophilic infiltration in the lamina propria, features frequently observed in EO, were investigated, fibrosis was present in 82% of all cases and eosinophil infiltration was present in 60% of all cases. Notably, these findings were present in all

of the tuberculosis oesophagitis cases. Lamina propria eosinophilic infiltration was present in 83.5% and 54.5% of Candida and HSV oesophagitis cases, respectively. In addition, all of the CMV oesophagitis cases displayed fibrosis. The rate of fibrosis in HSV and Candida oesophagitis cases was 83.3% and 78.8%, respectively.

DISCUSSION

The histological findings of oesophagitis are similar despite the diverse underlying causes. However, the diagnosis of IO is important, since it allows for specific treatment to target the different causative agents.

Infectious oesophagitis can occur at any age and is frequently associated with immunosuppression. In our study, the youngest patient was 5 years old, while the oldest was 94 years old; the mean age of patients with IO was 51 years. Although the age range was wide, no significant relationship was found between the IO subtype and age. With respect to gender distribution, consistent with previous studies, a male predominance was identified in our study (11). However, no correlation was found between gender and oesophagitis subtype. The reason for the male predominance needs further evaluation.

In this study, 76% of the cases were found to be Candida oesophagitis. Candida oesophagitis presents with odynophagia and dysphagia. Plaques resembling cottage cheese can be seen in endoscopy together with erosion, ulceration, strictures, and/or pseudodiverticles. In the case of Candida oesophagitis, the main diagnostic challenges include ruling out swallowed commensal oral fungal colonization or secondary colonization due to erosion and ulcerations. Its presence in the superficial exudate is not considered disease-specific, and invasion of the tissue with the infectious agent has to be confirmed by histological examination. Pseudohyphae located perpendicular to the surface are generally accepted as indicators of invasion. To accurately confirm Candida oesophagitis, biopsy must be taken from the plaques located in the mid-oesophagus (3). The histological findings in our study correlated with those from endoscopic evaluation; however, erosion was observed in 80% of our Candida oesophagitis cases, indicating that it could reflect the development of a secondary fungal colonization on erosion areas, rather than a change directly related to fungal infection. In addition, the presence of basal cell hyperplasia and papillary elongation in both Candida oesophagitis and reflux oesophagitis cases, although nonspecific, also support this hypothesis.

Herpes simplex virus was the second most common causative agent in our study. The onset of HSV oesophagitis is very noisy. It initially manifests as acute odynophagia; general pain and retrosternal pain may also occur simultaneously. HSV is seen in neonates as a primary infection (HSV2) and as a reactivation of the primary infection in immunosuppressed adults (HSV1). Endoscopically, it is characterized by multiple well-defined small ulcers and vesicles. Pseudomembranes can also be present (2). HSV involvement is widespread; however, it ends at the gastro-oesophageal border (3). Histologically, it is characterized by hollow ulcers. In contrast with CMV and varicella zoster virus, it infects the squamous epithelium. Nuclear inclusion bodies are typically located at the periphery of the ulcers. Cowdry type A can be observed as more typical, small, pink inclusions with a transparent zone between the inclusions and the nuclear membrane. Cowdry type B is seen more frequently and is characterized by large, greyish-blue, frosty glass-like inclusions that fill the entire nucleus. We also observed that Cowdry type B inclusions were much more recognizable than Cowdry type A inclusions. In addition, multinuclear giant cells, balloon degeneration, desquamation, nuclear moulding, and macrophage aggregations can also be found. Biopsy must be taken from the distal oesophagus and borders of the ulcers (1). In our study, in addition to those well-defined histological changes, we observed prominent basal cell hyperplasia exceeding 50% of the epithelial thickness accompanied by papillary elongation. Although both findings are nonspecific and are also observed in GERD, the level of basal hyperplasia was comparable to that seen in EO. The reason for this epithelial regeneration activity and its relationship with the disease progress warrants further research.

Clinically, CMV oesophagitis generally manifests as nausea, vomiting, and diarrhoea. Odynophagia and retrosternal pain is rarer compared with HSV oesophagitis. In the endoscopic examination, there are well-defined, single (rarely multiple) distal ulcers. There were 3 cases of CMV oesophagitis in our study group, which involved patients who were younger than those with HSV oesophagitis. Contrary to HSV oesophagitis, intracytoplasmic 'owl's eye appearance' inclusions were seen in subepithelial tissue, together with cytomegalic changes in the endothelium, fibroblasts, and stromal cells or granular basophils. It is noteworthy that among the cases excluded from the study, there were cases with superficial biopsies, emphasizing the importance of deeper, granulation tissue biopsies to accurately diagnose CMV oesophagitis (1,3).

Recent studies have shown that macrophage aggregates are found in HSV and CMV oesophagitis. The macrophage aggregates found in CMV oesophagitis are smaller in size and number compared with those in HSV oesophagitis. In addition, their location is different from those in HSV oesophagitis. They are located in the perivascular area in CMV oesophagitis and in granulation tissue, while they are located in the squamous epithelium in HSV oesophagitis (12). However, in our study, we did not observe any macrophage aggregates in either HSV or CMV oesophagitis cases, probably due to the limited number of cases.

It has also been reported in the literature that marked mononuclear cell infiltration is characteristic of HSV oesophagitis. Greenson et al. (13) used Candida and bacterial oesophagi-

tis cases as the control group in their study and saw marked mononuclear cell infiltration in HSV oesophagitis, with statistically significant results. Although we did not observe any increase of infiltration in HSV or CMV oesophagitis cases in our study, it was notable that lymphocytic and eosinophilic intensity in tuberculosis oesophagitis cases was greater than that observed in the other oesophagitis subtypes. Regarding acute inflammatory cells, although PNLs were present in all of the cases, the number of PNLs per HPF did not exceed 25 in 58.2% of the cases; however, PNL density in HSV oesophagitis cases exceeded 50 PNLs per HPF.

Tuberculosis has an important place among the granulomatous oesophagitis cases. It has been reported that when the oesophagus is infected with with *Mycobacterium tuberculosis*, gross appearance of the lesions are either ulcerative and/or hyperplastic (pseudotumoral) (14,15). In our study, ulceration was present in all of the tuberculosis oesophagitis cases.

Basal cell hyperplasia and papillary elongation are the 2 basic lesion types seen in oesophagitis cases. In our study, while basal cell hyperplasia and papillary elongation were seen in all of the cases except for 1 Candida oesophagitis case, basal cell hyperplasia exceeding 50% of the epithelial thickness was seen particularly in tuberculosis oesophagitis and HSV oesophagitis cases, and in 82% and 67% of Candida oesophagitis and CMV oesophagitis cases, respectively; although the difference was statistically insignificant between each subtype. Papillary elongation was observed in all cases of CMV oesophagitis, tuberculosis oesophagitis and HSV oesophagitis, and in 94% of Candida oesophagitis cases that exceeded 50% of the epithelial thickness.

Vasodilatation--vascular pooling and dilated intercellular space--are regarded as early findings of non-erosive reflux disease. In our study, dilated intercellular space was observed in all of the oesophagitis subtypes, with the exception of 2 Candida oesophagitis cases. However, when the lowest epithelial level at which such changes are reflected in these cases was investigated, basal cell hyperplasia and papillary elongation exceeding 50% of the epithelial thickness was observed in all the tuberculosis and HSV oesophagitis cases, and in 76% and 67% of the Candida oesophagitis and CMV oesophagitis, respectively. While vascular pooling was not seen in CMV and tuberculosis oesophagitis cases, it was observed in 11 (16.4%) cases each of HSV and Candida oesophagitis; however, this difference was not statistically significant between each subtype. In this respect, we can conclude that histological findings in IO are no different than in reflux oesophagitis.

Eosinophilic microabscess, superficial layering, and surface slime, features considered typical of EO, are rare occurrences in other oesophagitis subtypes (3). In the present study, these findings were observed in only 1 Candida oesophagitis case; however, the clinical history of the case and the findings of the existing biopsy did not indicate EO. The presence of fibrosis in the lamina propria or eosinophils in the superficial epithelium or in the lamina propria is a strong indicator of EO. These findings are seen in the early stages of the disease and are more frequent in children than in adults (3). In our study, fibrosis was observed in 82% of cases and eosinophil infiltration was observed in 60%. Notably, both of these findings were observed in all tuberculosis oesophagitis cases. Eosinophils in the lamina propria were seen in 83.5% and 54.5% of Candida and HSV oesophagitis cases, respectively, but were not seen in any of the CMV oesophagitis cases. In addition, all of the CMV oesophagitis cases displayed fibrosis, with the rates of fibrosis in HSV and Candida oesophagitis being 83.3% and 78.8%, respectively. Therefore, one can speculate that although eosinophils and/or fibrosis can occur in IO, the density of eosinophilic infiltration and accompanying epithelial changes is helpful in its differentiation from EO.

We concluded that there were no significant histological differences between the IO subtypes with regards to the characteristics of inflammatory infiltration or accompanying epithelial changes. Although the distinction from EO may be easier, it is not possible to differentiate between oesophagitis subtypes, especially reflux oesophagitis, using only histological findings when the causative organism is not detected. Clinicopathological correlation and control with repeat targeted biopsies are therefore essential for diagnosis.

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