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ORIGINAL PAPER

Investigation of conjunctivochalasis histopathology with light and electron microscopy in patients with conjunctivochalasis in different locations

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Abstract

Purpose To investigate changes in conjunctival tissue of conjunctivochalasis (CCh) patients and to determine the relationship between pathological findings and localization of loose conjunctiva.

Methods Our study included nineteen eyes of 19 patients who were referred to Cukurova University Ophthalmology Department based on ocular surface symptoms and CCh detected in ocular examination. Amniotic membrane was applied after conjunctival excision as surgical treatment. The control group was formed with five eyes of five patients who are similar in terms of age and gender distribution with our study group. Tissue samples obtained from the study and control groups were investigated with light and electron microscopy.

Results Results of pathological examination of conjunctival tissues revealed increased inflammation in 13 patients (68%), lymphatic ectasia in 12 patients (63%), and loss of goblet cells in 17 patients (89%). Destruction of elastic fibers was detected in all cases by staining with elastic van Gieson. After semiquantitative assessment, varying degrees of light microscopic findings were noted considering the localization of CCh. No statistically significant relationship was observed between light microscopic findings and CCh location ($p > 0.05$ for all). Electron microscopic investigation revealed increase in intercellular spaces, increased cytoplasmic electron density, and the presence of slight vacuolization in cell cytoplasm, and heterochromatin clumping in nuclei of cells in conjunctival samples.

Conclusions Mechanical and inflammatory factors induce development of CCh, and signs associated with these factors can be detected with light and electron microscopy of conjunctival tissue. No relationship was observed between CCh localization and pathological changes in tissues examined in our study, and large-scale case series are required to evaluate the possible effect of CCh localization on pathological findings.

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Introduction

Conjunctivochalasis (CCh) is characterized by loose, non-edematous, and redundant conjunctival tissue. Loose conjunctival tissue may form folds between the globe and inferior eyelid, which may disrupt tear distribution [1–3]. Redundant conjunctival tissue may not cause any symptom or may produce ocular irritation symptoms. This disease is also characterized by subconjunctival hemorrhage, epiphora, dry eye, and corneal ulceration [1, 2, 4]. Tear meniscus disruption, impaired tear distribution, and punctal occlusion play roles in symptom manifestation [1, 4].

Although CCh is frequently detected in the temporal conjunctival tissue near the lower eyelid margin, it may also be observed in central, nasal, and superior localizations. Cases with nasal CCh exhibit some characteristics due to different effects of loose conjunctival tissue on punctum and tear drainage mechanism. In addition to clinical findings, in terms of pathologic findings, nasal CCh may differ from cases with other localizations [3–5].

After excision of loose conjunctival tissue, covering naked sclera with amniotic membrane is an effective surgical treatment for CCh [2, 6, 7].

Although etiopathogenesis of the disease has not been clearly understood, various theories have been proposed. This study aims to evaluate changes in conjunctival tissue in CCh, to determine the relationship between pathologic findings and localization of loose conjunctiva, and to reveal factors involved in etiopathogenesis of the disease.

Materials and methods

Nineteen consecutive patients who were admitted to Cukurova University Medical Faculty Ophthalmology Department due to ocular surface symptoms and who were diagnosed with CCh in ophthalmologic examination were evaluated. The present study included patients whose symptoms persisted despite artificial tear drops and gel treatment. Patients with a history of ocular surface surgery, ocular trauma, contact lens wear, and cases with an additional ocular disease (lacrimal system disorders, eyelid anomaly, ectropion, entropion, and corneal pathology) except meibomianitis and blepharitis were excluded from the study.

Schirmer test was conducted, and tear breakup time was measured in all cases. Corneal and conjunctival staining detected by fluorescein and Rose–Bengal was evaluated [8]. The study also excluded patients with dry eye due to tear deficiency.

When surgical treatment was planned for patients with CCh accompanied by meibomianitis and blepharitis, patients were asked to continue of ocular surface symptoms despite regression of infection after eyelid cleaning and topical antibiotic therapy. Surgical treatment was planned for nineteen eyes of 19 patients after these evaluations.

Staging of CCh was performed in accordance with staging of Meller et al. [9] by assessing the number of conjunctival folds due to loose conjunctiva and the relationship between folds and tear meniscus. All cases included in the study were in stage III as identified by numerous conjunctival folds which exceed the edge of the inferior eyelid and disrupt the tear meniscus. Eyes with severe ocular surface symptoms were included in the study of bilateral cases. All cases were classified according to CCh location preoperatively. Location of CCh was noted in central inferior, nasal, and temporal regions.

A written informed consent was obtained from all patients before surgery. All operations were performed by the same surgeon. (MY) The same surgical steps were applied in all cases for conjunctival excision. The globe was tilted upwards with 8-0 polyglactin sutures passing through near the limbus at three and nine o′clock positions. Limbus and 2-mm adjacent conjunctiva were preserved, arc-shaped conjunctival peritomy was performed in inferior quadrant, and excess conjunctiva was excised. Location of loose conjunctival folds in preoperative examination was considered during determination of the amount of excised conjunctival tissue. Conjunctival tissue adjacent to fornices was fixed to the sclera with 8-0 polyglactin sutures to prevent recession of conjunctiva located distal to the excised conjunctival tissue. Bare sclera was covered with amniotic membrane by using sutures or fibrin glue. A control group consisting of five cases with similar distribution with patient group in terms of gender and age was formed to compare

findings obtained from conjunctival samples collected from cases with CCh. Conjunctival samples were obtained during peritomy from patients who underwent pars plana vitrectomy due to various indications and who presented no history of ocular surgery involving the conjunctiva. All samples collected from the patient and control groups were examined by light and electron microscopy.

Conjunctival tissue samples for light microscopic examination were fixed in 10% formaldehyde solution. Fixation procedure was completed in an autotechnicon device. Sections, 4–5 μm in thickness, were obtained from the paraffin-embedded tissues and stained with hematoxylin–eosin (H&E) and elastic van Gieson (ElVG) and examined under light microscope. Inflammation, lymphatic ectasia, and goblet cell loss were evaluated semiquantitatively in the conjunctiva as +, ++, +++ in H&E-stained preparations. Elastic fiber injury was graded similarly in ElVG staining technics applied specimens.

Conjunctival tissue samples for the electron microscopic examination were fixed for 4 h with 5% glutaraldehyde in Millonig phosphate buffer at pH 7.4 and then post-fixed with 1% osmium tetroxide in the same phosphate buffer for 2 h at 4 °C. The samples were dehydrated in a graded series of ethanol and embedded in araldite. Semi-thin sections, which were prepared with a Reichert Ultracut S ultramicrotome, were stained with toluidine blue, and appropriate areas for electron microscopic evaluation were determined. The thin sections were taken from selected areas, stained with uranyl acetate and lead citrate, and examined using Jeol JEM-1400 transmission electron microscope.

Statistical analysis

SPSS 19.0 package program was used for statistical analysis of data. Categorical measures were summarized as number and percentage. Numerical measurements were summarized as mean and standard deviation (when necessary median and minimum–maximum). Chi-square test was used to compare categorical measurements among groups. In comparison with numerical measurements between groups, t test (Student's t test) was used when assumptions were made. In comparison with numerical measurements between groups, Mann–Whitney U test was used when assumptions were not made. Statistical significance level was $P < 0.05$ in all tests.

Results

Nineteen eyes of 19 patients were surgically treated. Light microscopic examination revealed subepithelial inflammatory cell infiltration in 13 patients (68%) that demonstrated increase in inflammation (Fig. 1a). Lymphatic ectasia in different grades was observed in 12 patients (63%), and different degrees of goblet cell loss were detected in 17 patients (89%) (Fig. 1b–d). ElVG staining showed elastic fiber damage in all cases (Table 1) (Fig. 1e).

Cases were classified according to CCh location: CCh was located in temporal region in seven cases; in central inferior and temporal regions in six cases; in nasal, central inferior, and temporal regions in four cases; in nasal and temporal region in one case; and in central inferior region in one case (Fig. 2a, b). Light microscopic findings, which were evaluated semiquantitatively and noted at different grades, were assessed by considering CCh location. A total of 11 cases with central inferior component and 8 cases without central component; 5 patients with nasal component and 14 patients without nasal component; seven cases with CCh with only temporal localization; and the remaining 12 cases were compared with each other. No statistically significant relationship was observed between light microscopic findings and CCh location (Tables 2, 3, 4).

Multilayered prismatic shaped surface epithelium and lamina propria with fibroblasts, collagen fibers, and capillaries in it were appeared normal in the control group using transmission electron microscopy (Fig. 3a, b). Histopathologic changes were observed in conjunctivochalasis group. Intercellular spaces were enlarged in the epithelial layer. Heterochromatin clumping in the nucleus, increase in cytoplasmic densities, and vacuoles in the cytoplasm were observed. Increased electron-dense elastic fibers organized into clusters between the collagen fibers in the lamina propria were striking (Fig. 3c, d).

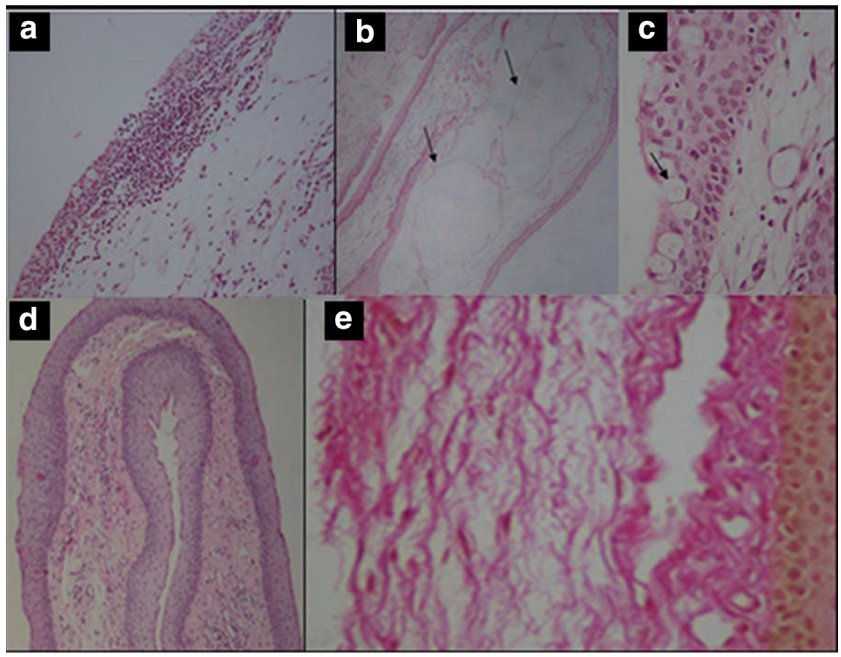

Fig. 1 **a** In the subepithelial area, intense inflammatory cell accumulation progressing in the epithelium in CCh (×200, H&E); **b** lymphatic ectasia (+++) in subepithelial area in CCh (×100, H&E); **c** goblet cells in normal conjunctival tissue (×200, H&E); **d** goblet cell loss (+++) in CCh. (×100, H&E); **e** significant conjunctival elastic fiber damage in Cch. (×200, ElVG)

Table 1 Results of pathological examination with light microscope

Pathologic findings	Number of cases (%)
Inflammation	
None	6 (31)
+	9 (47)
++	3 (15)
+++	1 (5)
Lymphatic ectasia	
None	7 (36)
+	8 (42)
++	2 (10)
+++	2 (10)
Loss of goblet cells	
None	2 (10)
+	5 (26)
++	10 (52)
+++	2 (10)
Elastic fiber damage	
+	12 (63)
++	7 (37)

Discussion

Studies involving histopathologic examination for CCh can be divided into those that suggest inflammatory changes are detected in conjunctiva and those that claim the absence of inflammation [10, 11]. Researchers who suggest that inflammatory changes are not observed in conjunctiva have reported stromal lymphangiectasis and edema in the conjunctiva [10]. ElVG staining revealed pathological changes, such as loss of usual bond pattern, fragmented elastic fibers, and sparseness, in collagen bonds [10]. On the other hand, chronic non-granulamatous plasma and lymphocyte cell infiltration were detected in the conjunctiva in pathologic studies, suggesting that ocular surface inflammation is involved in CCh histopathology [11].

In our study, conjunctival samples were examined by light microscopy using ElVG and H&E dyes. Increased inflammation was detected in 13 cases, lymphatic ectasia in 12 cases, goblet cell loss in 17 cases, and elastic fiber damage in all cases. Considering previous studies on CCh histopathology, intensive inflammatory cell deposits identified in the subepithelial area in the majority of tissue specimens and goblet cell loss are considered evidence of inflammatory factors involved in etiopathogenesis. Lymphatic ectasia and elastic fiber damage detected in conjunctiva were considered in favor of mechanical factors to which the tissue was exposed.

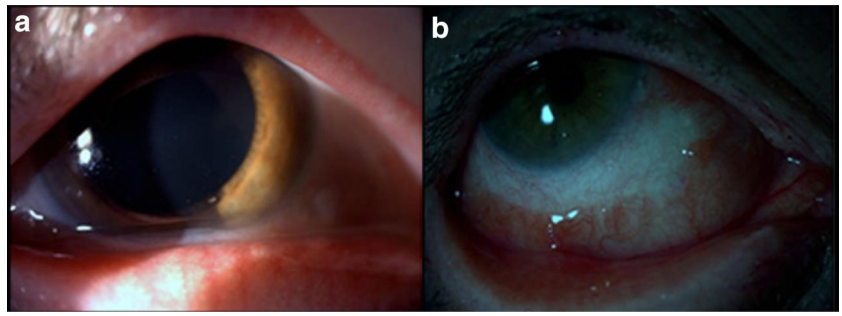

Fig. 2 Clinical images of cases with Stage 3 CCh. **a** CCh with central inferior settlement, **b** first month after conjunctival excision and amniotic membrane transplantation

Table 2 Localization of CCh–pathologic findings of cases with central inferior component and others

Pathologic findings	Cases with CI component ($N = 11$)	Others ($N = 8$)	P
Inflammation			0.642
None	4 (36%)	2 (25%)	
+	5 (45%)	4 (50%)	
++	2 (18%)	1 (12%)	
+++	0 (0%)	1 (12%)	
Lymphatic ectasia			0.842
None	5 (45%)	2 (25%)	
+	4 (36%)	4 (50%)	
++	1 (9%)	1 (12%)	
+++	1 (9%)	1 (12%)	
Loss of goblet cells			0.715
None	1 (9%)	1 (12%)	
+	2 (18%)	3 (38%)	
++	7 (64%)	3 (38%)	
+++	1 (9%)	1 (12%)	
Elastic fiber damage			0.999
+	7 (64%)	5 (62%)	
++	4 (36%)	3 (38%)	

CI central inferior

Loose conjunctival tissue can be detected in the upper or lower bulbar conjunctiva in patients with CCh. Excess conjunctival tissue at the lower eyelid margin may localize on the nasal or temporal quadrant. Mimura et al. [12] observed CCh at higher stages in the temporal localization in the study of 1416 patients with CCh. In two different studies, loose conjunctival tissue was most commonly detected in temporal localization and at least in an inferior location with six o′clock alignment in patients with CCh in the lower half of the conjunctiva [8, 13]. In our studied cases, loose conjunctiva was most common in temporal localization. CCh with temporal component was present in 18 of the 19 cases. This finding, which was determined in accordance with previous studies, can be explained by the fact that conjunctival area assessed along the lower eyelid margin in temporal region is wider than the central inferior and nasal regions.

Through electron microscopic investigation, Ward et al. [14] have detected weakness of the relationship between epithelial cells, diminished intercellular connections, aggregation of nuclear material of epithelial cells, pyknotic nucleus, and accumulation of elastic fibers in the conjunctival stroma in patients with CCh.

We detected by electron microscopic investigation marked enlargement of intercellular spaces as a result of reduced connections between epithelial cells,

Table 3 Localization of CCh–pathologic findings of cases with nasal component and others

Pathologic findings	Cases with nasal component ($N = 5$)	Others ($N = 14$)	P
Inflammation			0.356
None	1 (20%)	5 (36%)	
+	4 (80%)	5 (36%)	
++	0 (0%)	3 (21%)	
+++	0 (0%)	1 (7%)	
Lymphatic ectasia			0.507
None	1 (20%)	6 (43%)	
+	3 (60%)	5 (36%)	
++	0 (0%)	2 (14%)	
+++	1 (20%)	1 (7%)	
Loss of goblet cells			0.576
None	0 (0%)	2 (14%)	
+	2 (40%)	3 (21%)	
++	3 (60%)	7 (50%)	
+++	0 (0%)	2 (14%)	
Elastic fiber damage			0.999
+	3 (60%)	9 (64%)	
++	2 (40%)	5 (36%)	

Table 4 Localization of CCh–pathologic findings of cases with temporal CCh only and others

Pathologic findings	Only temporal ($N = 7$)	Others ($N = 12$)	P
Inflammation			0.311
None	2 (28%)	4 (33%)	
+	4 (57%)	5 (41%)	
++	0 (0%)	3 (25%)	
+++	1 (14%)	0 (0%)	
Lymphatic ectasia			0.631
None	1 (14%)	6 (50%)	
+	4 (57%)	4 (33%)	
++	1 (14%)	1 (8%)	
+++	1 (14%)	1 (8%)	
Loss of goblet cells			0.703
None	1 (14%)	1 (8%)	
+	2 (28%)	3 (25%)	
++	4 (57%)	6 (50%)	
+++	0 (0%)	2 (16%)	
Elastic fiber damage			0.333
+	6 (85%)	6 (50%)	
++	1 (14%)	6 (50%)	

heterochromatin clumping in cell nuclei, and collagen fibers arranged in different directions in lamina propria and elastic fibers arranged in groups in conjunctiva. Especially, the expansion of intercellular distances and observation of elastic fibers collectively were concluded to result from mechanical effects. However, we assumed that increase in inflammatory cells, which can be considered as evidence of

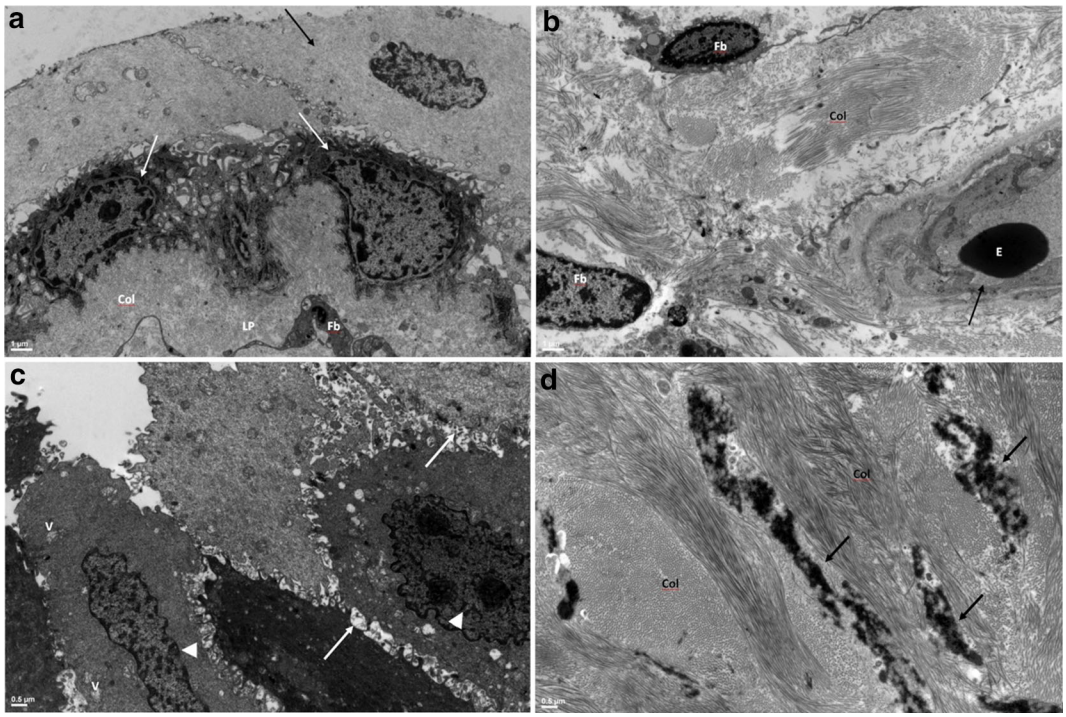

Fig. 3 Electron microscopic examination: **a** control group. Multilayered prismatic shaped surface epithelium is seen. Although apical epithelial cells have an electron-lucent appearance (black arrow), the basal epithelial cells are seen to have a more electron-dense appearance (white arrow). Lamina propria (LP), with fibroblasts (Fb) and collagen fibers (Col), is observed normally under the epithelium. Bar 1 µm. **b** Control group. Fibroblasts (Fb), collagen fibers (Col), and a capillary (arrow) with erythrocyte (E) in it are seen normally in the lamina propria. Bar 1 µm. **c** Conjunctivochalasis group. Intercellular spaces are enlarged (arrow) in the epithelial layer. Heterochromatin clumping in the nuclei (arrow head), increase in cytoplasmic electron density, and vacuoles (V) in the cytoplasm are observed. Bar 0.5 µm. **d** Conjunctivochalasis group. Increased electron-dense elastic fibers organized into clusters (arrows) between the collagen fibers (Col) are striking. Bar 0.5 µm

inflammatory factors, cannot be detected in studied specimens possibly due to the small area examined by electron microscopy.

Tear clearance is delayed due to punctal occlusion caused by loose conjunctiva in patients with nasal CCh. In these cases, ocular surface inflammation resulting from reduced tear clearance has been shown in previous studies [3–5]. Erdoğan-Poyraz et al. [4] evaluated 75 patients with CCh and discovered the correlation between the size of loose conjunctiva near the lower eyelid margin and its proximity to punctum and secondary punctal occlusion findings, such as epifora and delayed fluorescein clearance. In another study, an increase in tear interleukin levels and ocular surface disease index scores were observed in CCh cases with central or inferior nasal component in addition to the temporal component [3]. In an another study, Wang et al. [5] classified 29 patients according to the presence or absence of nasal CCh, and they reported increased ocular surface staining with Rose–Bengal, increased visual symptom scores, and decreased conjunctival goblet cell density in the nasal CCh group.

Nasal components of CCh were observed in 5 of the 19 patients. We observed that no relationship existed between localization of CCh, including the nasal region, and pathologic changes in the conjunctiva. We assumed that the effect of location of CCh on clinical signs cannot be observed due to the small number of patients and low proportion of patients with nasal component.

In another study in which the localization of the CCh was taken into consideration, Kheirkhah et al. [6] reported that the main feature of superior CCh is poor

adhesion, but not redundancy or excess, of the conjunctiva to the sclera. The studies mentioned above about both nasal and superior CCh suggested that localization may be an important factor in the pathophysiology of CCh. To the best of our knowledge, our study is the first case series and report in the literature evaluating changes in conjunctival tissue in CCh, to determine the relationship between pathologic findings and localization of loose conjunctiva including temporal and inferior localizations. Although we could not demonstrate a relationship between CCh localization and pathological changes, localization-specific histopathologic features could be detected in the temporal and inferior CCh similar to the nasal and superior CCh in larger case series. Thus, the physiopathological mechanism may be clarified more clearly for these cases.

In previous studies, the decrease in secretory mucins, such as MUC2 and MUC5AC, was demonstrated immunohistochemically or by polymerase chain reaction method. These methods allow observation of loss of conjunctival goblet cells [5, 15]. Some studies have also demonstrated increased levels of interleukin and metalloproteinases on the ocular surface [3, 14]. The fact that these markers were not evaluated in conjunctival tissues and ocular surface can be considered as the limiting aspect of our study.

In our study, pathologic findings detected in the majority of specimens evaluated by light microscopy suggest that inflammatory and mechanical factors participate in etiopathogenesis of CCh. Examination by electron microscopy revealed findings that can prove mechanical effects to which conjunctival tissues were exposed to. No relationship was observed between CCh localization and pathological changes in tissues examined in our study. Large-scale case series are required to establish possible roles of inflammatory and mechanical factors in etiopathogenesis of CCh and evaluation of the effect of CCh localization on pathological findings.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee (Ethics Committee of the Cukurova University, Medicine Faculty, Adana, Turkey) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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