Original Article

Semilunar sign of cornea: A multimodal analysis of the posterior corneal opacity in non-infectious anterior scleritis

Dhivya Ashok Kumar, Amar Agarwal, Radhika Chandrasekar, Raja M Chinnappan

Purpose: To analyze the morphological outcomes of the posterior corneal opacity or "semilunar sign" in noninfectious anterior scleritis using multimodal imaging. **Methods:** This was a prospective observational case series. Patients with anterior scleritis from January 2018 to January 2019 were included. Clinical and demographic data were collected. Posterior cornea was visualized using the digital slit lamp photography (Elite, mega digital vision), spectral domain optical coherence tomography (MS39), and specular count analyzer (EM-3000). "Semilunar sign" was defined by the (1) presence of posterior corneal opacity, (2) concave semilunar pattern, (3) absence of blood vessels, and (4) normal anterior cornea. Incidence, clinical characteristics and significance, correlation with Mantoux sensitivity, and role of multimodal valuation were assessed. **Results:** Overall 76 eyes of 72 patients were recruited with anterior scleritis. Fifteen eyes of 11 patients (15.3%) presented with semilunar sign. The scleritis was both nonnecrotizing ($n = 8$) and necrotizing ($n = 7$). The semilunar configuration appeared as isolated ($n = 9$) and continuous lesion ($n = 6$). The extent was directly related to the scleral disease extent ($P = 0.002$). The mean thickness measured 212.5 ± 129.3 μm. The mean central endothelial cell density (ECD) was 2540.8 ± 351.7 cells/mm^2, which was significantly higher than the involved peripheral cornea ($P = 0.05$). The mean surface area of the semilunar sign was 7.7 ± 5.2 mm^2. There was no significant correlation between the opacity thickness and the best-corrected visual acuity ($P = 0.895$, r = −0.39), ECD ($P = 0.52$, r = −0.188), and Mantoux ($P = 0.696$, r =− 0.142). **Conclusion:** Corneal semilunar sign of scleritis affected the peripheral cornea and caused no functional abnormality in early presentation. Multimodal analysis can aid in clinical assessment and severity.

Key words: Endothelial opacity of scleritis, posterior corneal opacity, semilunar sign

The sclera is constituted by the interwoven dense collagen, which imparts the unique tensile strength and contour to the globe. The diseases related to sclera are often inflammatory, and rarely infective or degenerative.[1-4] Various systemic associations have been identified with anterior scleral inflammation including collagen vascular diseases and autoimmune syndromes.[4,5] A persistent anterior scleral disease gradually may lead to corneal changes due to its close proximity. The common morphological changes on the cornea induced by anterior scleritis are astigmatism, sclerokeratitis, limbitis, and peripheral ulcerative keratitis (PUK).[6-8] In 1976, Watson *et al.*[9] described about 29% of anterior scleritis causing corneal changes predominantly involving the anterior and the stromal layers of cornea. In 1974, Holt and Watson reported 4 cases of nonsyphilitic deep nummular keratitis with vascularization involving the corneal endothelium in scleritis.[10] However, studies are scarce on the isolated posterior corneal clinical changes in anterior scleritis. In the current study, we report the clinical presentation of posterior corneal endothelial opacity as "semilunar sign" in anterior scleritis, its functional outcome, and the multimodal assessment of the same with easily available methods.

Methods

This was a single center, prospective, observational case series that adhered to the tenets of the Declaration of Helsinki. Institutional review board approval and informed consent was obtained. Clinical information and multimodal imaging of the posterior corneal surface of eyes with anterior scleritis during the time period from January 2018 to January 2019 were acquired. A comprehensive slit lamp examination was performed in all eyes as preliminary step to detect corneal "semilunar sign." Eyes with the presence of semilunar sign was then included in the study and proceeded further.

Definition of semilunar sign

Posterior corneal opacity termed (by D.A.K) as "Semilunar sign" [Fig.1] was defined clinically by the following criteria: (1) presence of posterior corneal opacity, (2) concave semilunar pattern of opacity on diffuse direct illumination, (3) absence of blood vessels, and (4) normal anterior cornea.

Department of Uvea, Oculoplasty and Oncology, Dr Agarwal Eye Hospital and Eye Research Centre, Chennai, Tamil Nadu, India

Correspondence to: Dr. Amar Agarwal, Dr. Agarwal's Eye Hospital and Eye Research Centre, 222, TTK Road, Chennai - 600 018, Tamil Nadu, India. E-mail: dragarwal@vsnl.com

Multimodal analysis

Clinical digital slit lamp photography (10× magnification) (Elite mega digital vision, Germany), spectral domain optical coherence tomography (OCT) (VL, MS39, Italy), and specular count analysis (EM-3000, Tomey, Japan) were performed. High resolution anterior segment OCT using the spectral (3.9 µm axial resolution) domain platform was performed to measure the extent of the posterior corneal opacity in micrometers (µm), Descemet's status, and corneal thickness (µm). Digital images from slit lamp photography were converted to 8-bit greyscale. Using a scale bar on cornea, scaling was set at 1 mm equivalent to 30 pixels on a digital image. The endothelial surface area involved by the opacity was then analyzed [Fig. 2] using the filtered JPEG images from the digital pictures by ImageJ software.[11] Endothelial morphology was determined by the specular analysis method evaluating the mean endothelial cell density (cells/mm²), coefficient of variation (%), polymegathism in 800 to 900 µm² area (%), and apex pleomorphism hexagonality (%).

Ancillary tests

The supplementary ocular examination included the visual assessments, namely, the best-corrected visual acuity (BCVA, by Snellen visual acuity chart in decimal equivalent), uncorrected visual acuity (UCVA), auto-refractometer (diopters), intraocular pressure (IOP) (in mmHg by noncontact tonometry), and dilated fundus examination.
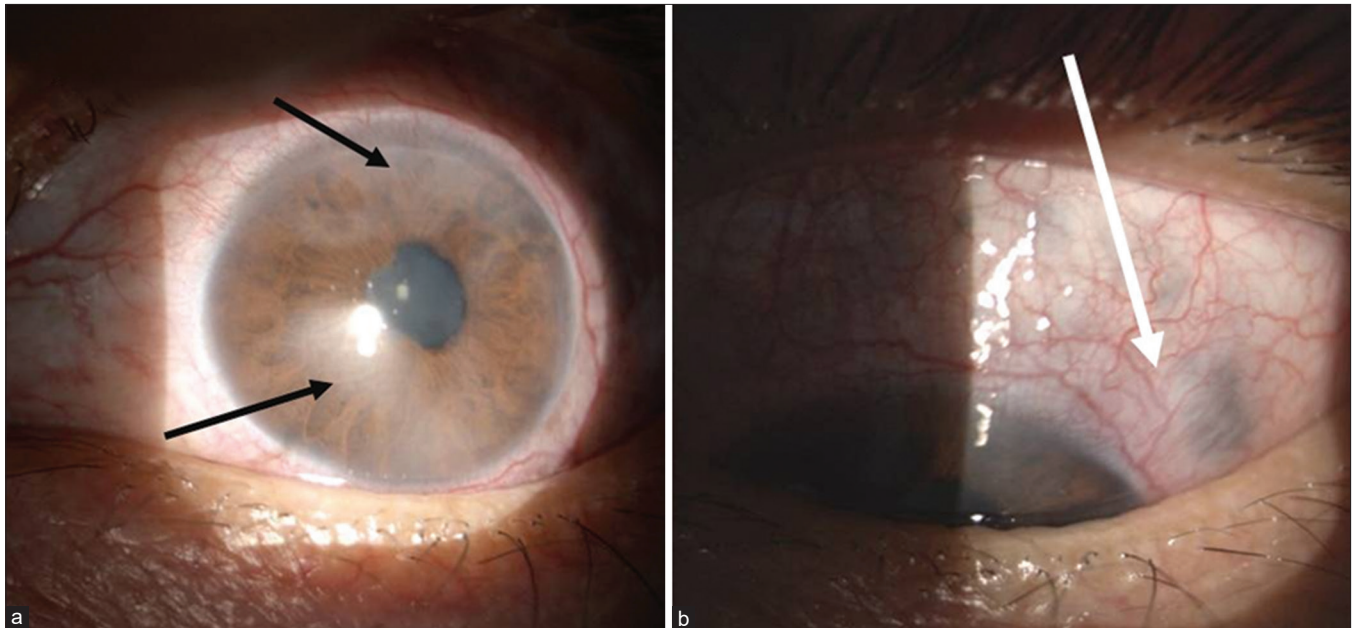
Figure 1: Clinical picture (a) of the semilunar sign visible on the corneal endothelial side (black arrow) and the healed scleritis (white arrow) in same eye (b)
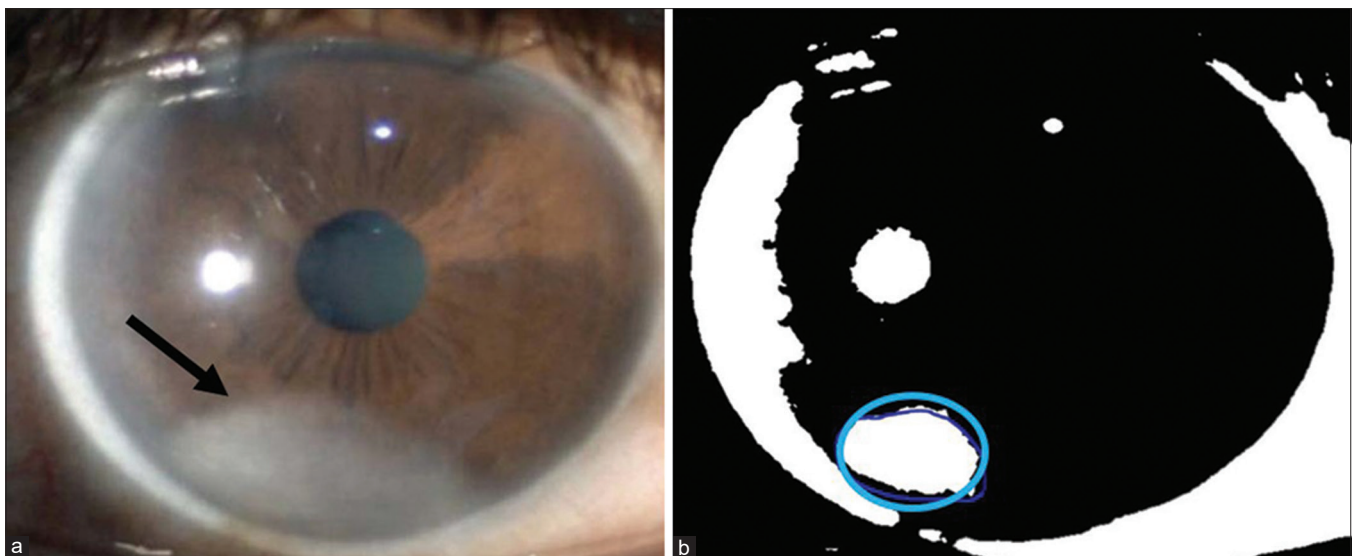
Figure 2: Image analysis showing the assessment of surface area. (a) The slit lamp image showing the semilunar sign (black arrow) and the corresponding 8-bit greyscale image (b) with blue lined area representing the region of interest (ROI) on the filtered image

Laboratory tests

Preliminary laboratory work up included a complete hemogram (total count, differential count, hemoglobin, and erythrocyte sedimentation rate), C-reactive protein, rheumatoid arthritis factor (RA), venereal disease research laboratory (VDRL), anti-nuclear antibody (ANA), anti-neutrophilic cytoplasmic antibody (ANCA) P and C, and Mantoux skin allergy test. The Mantoux or the tuberculin sensitivity skin test was performed in all patients and the maximum induration (in millimeters) measured at 48 hrs was documented. Induration ≥ 15 mm in otherwise healthy person was taken as positive. Conjunctival swab was obtained in eyes with active scleral disease for Gram and Ziehl–Neelson staining.

Eyes with preexisting corneal endothelial decompensation, viral endothelial disease, traumatic cornea, and infectious anterior scleritis (abscess/positive microbial culture in active cases) were excluded. Active cases were defined when the endothelial opacity presented with pain, redness with clinically active scleritis and nonactive were those with healed scleritis (scleral thinning/ surface change) with no symptoms. Patients with active noninfectious anterior scleritis were started on oral steroids 1 mg/kg body weight for 2 weeks and then tapered 10 mg per week accordingly. Specialty consultation with rheumatologist or chest physician was obtained in patients with specific systemic conditions associated with them. Patients who were refractory to oral steroids required immunomodulators (Methotrexate 5 mg weekly after evaluating the liver function tests and hemogram).

Statistical analysis

Data was entered using excel software (Microsoft corp.) and analyzed by SPSS (version 21; IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). The mean and standard deviation of the evaluated variables were reported. Data normality was tested by Shapiro–Wilk normality test and nonparametric tests were used for intergroup analysis. Correlation coefficient was determined for correlation of parameters. A P value less than or equal to 0.05 was considered statistically significant.

Results

A total of 76 eyes of 72 patients with anterior scleritis were evaluated in the out-patient clinic in the given time period. Only 15 eyes of 11 patients (15.3%) presented with posterior corneal endothelial opacity or semilunar sign. Demographic characteristics of the fifteen eyes were summarized in Table 1. There were 9 females (81.8%) and 2 males (18.1%) with mean age of 40.5 ± 11.8 years (22–56 years). Anterior scleritis was noted in all 15 eyes in either active (n = 3) or inactive (n = 12) state. Four out of 11 (36.3%) patients had bilateral presentation. The referring symptoms were pain (n = 3, 27.2%) and blurred vision (n = 4, 36.3%).

Table 1 : Demographic Details of Patients

Age/ sex	Eye	Endothelial opacity	Scleritis	Blood vessels	IOP (mmhg)	ECD (cells/ sq mm)	CCT (micro meters)	MANTOUX
26/F	OD	8 clk hrs	HEALED	absent	18	2051	667	23 mm
43/F	OD	12 clk hrs	HEALED	absent	14	2844	483	25 mm
	OS	12 clk hrs	HEALED	absent	12	2850	560	
32/M	OD	1 clk hrs	HEALED	absent	12	2850	560	0
	OS	6 clk hrs	ACTIVE	absent	16	2924	558	
44/F	OD	5 clk hrs	HEALED	absent	10	2427	436	15 mm
	OS	3 clk hrs	HEALED	absent	12	1652	438	
56/F	OD	2 Clk hrs	HEALED	absent	11	2644	499	25 mm
27/F	OD	3 clk hrs	ACTIVE	absent	10	2602	524	5 mm
45/F	OS	3 clk hrs	HEALED	absent	18	2718	549	30 mm
47/F	OD	2 Clk hrs	HEALED	absent	18	2534	532	15 mm
22/F	OD	2 Clk hrs	HEALED	absent	15	2542	520	13 mm
53/F	OD	2 Clk hrs	HEALED	absent	15	2131	539	0
51/M	OD	4 Clk hrs	ACTIVE	absent	16	2748	495	10 mm
	OS	8 Clk hrs	HEALED	absent	13	2595	536	

Clk hrs: Clock hours, ECD: Endothelial cell density, F: Female, M: Male

Table 2: Comparison of interstitial keratitis and semilunar sign of anterior scleritis

	Interstitial keratitis*	Semilunar sign of scleritis
Location	Adjacent to scleritis	Can be anywhere
Corneal layer	Any part of corneal stroma involved	Starts with endothelium and only Posterior stroma involved
Vacularity	Blood vessels present	Blood vessels absent
Role of ATT/steroids	May resolve with steroids/ATT	Do not resolve with steroids
Pattern	May not follow concave semilunar pattern	Follows semilunar pattern on start

ATT: Anti tuberculous treatment, *includes both syphilitic and nonsyphilitic etiology

Six out of 11 patients (54.5%) showed positive Mantoux test (Induration ≥ 15 mm). Three out of 11 patients (27.2%) had taken antituberculous treatment (ATT) already (10 years before) for pulmonary tuberculosis (n = 2) and positive lymph node (n = 1). One patient each had positive RA factor and ANA, respectively. None of them were ANCA (P/C) and VDRL positive. Conjunctival swab was negative for microbial growth in all 3 eyes with active scleritis. The mean uncorrected and best corrected visual acuity was 0.4 ± 0.3 and 0.63 ± 0.4 decimal equivalent, respectively. A BCVA

better than 20/40 was recorded in 73.3% of eyes. The mean astigmatism was − 1.4 D ± 0.7 with a mean myopic (n = 5) and hyperopic (n = 4) refractive error of − 1.8D ± 2.3 and 0.8 ± 0.3D respectively. Four eyes had cataractous lens and 2 patients had posterior chamber intraocular lens, while nine eyes had clear lens. The mean IOP was 14 ± 3 mmHg in the study group.

Posterior corneal opacity: A semilunar sign

Corneal endothelial opacity appeared in the form of semilunar configuration. The opacity involved the endothelium and the
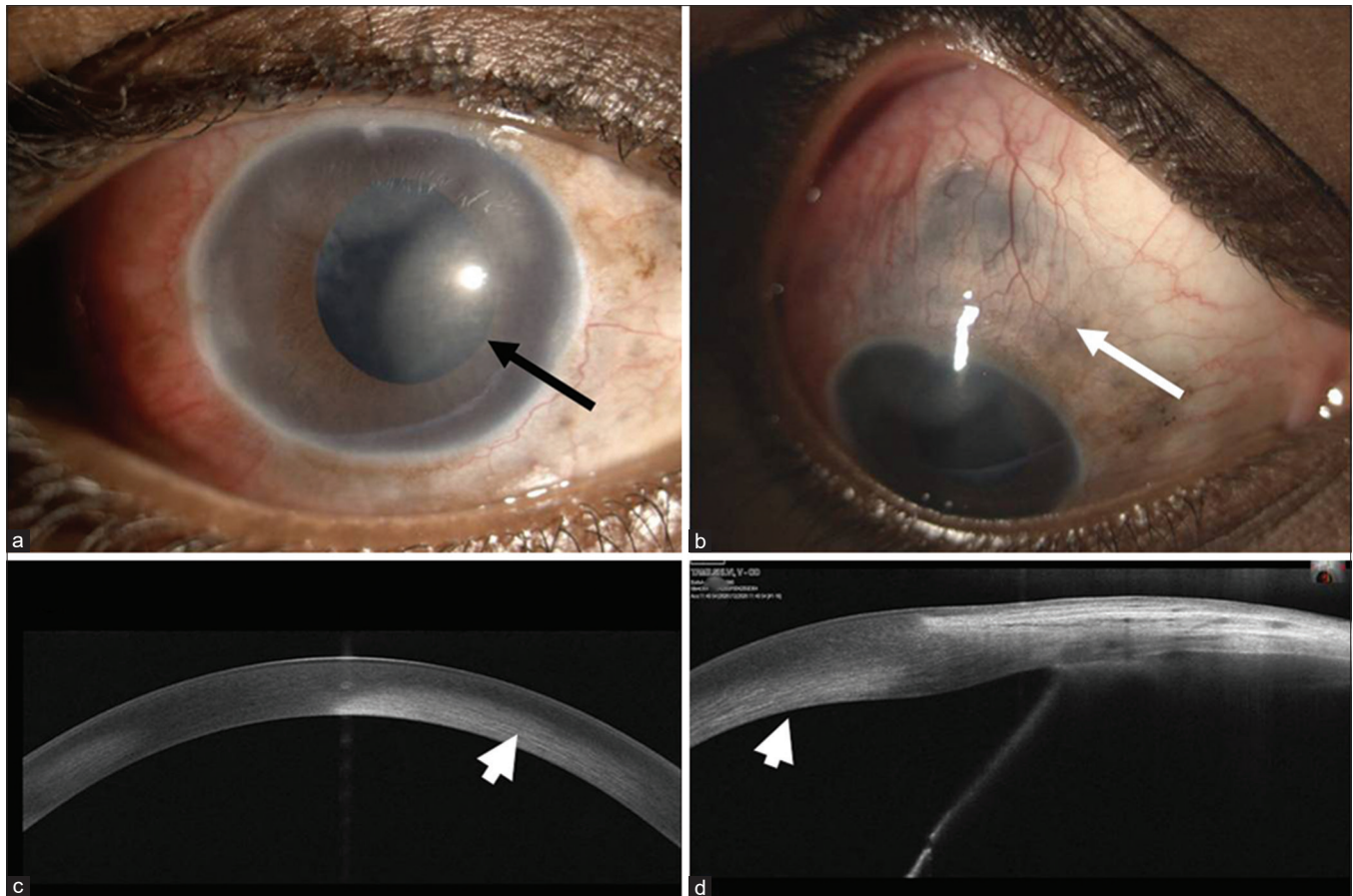
Figure 3: (a) Clinical picture of semilunar sign (black arrow) and adjacent (b) healed thinned sclera (white long arrow) with the (c and d) corresponding spectral domain optical coherence tomography showing endothelial hyperreflective opacity (white arrow head)
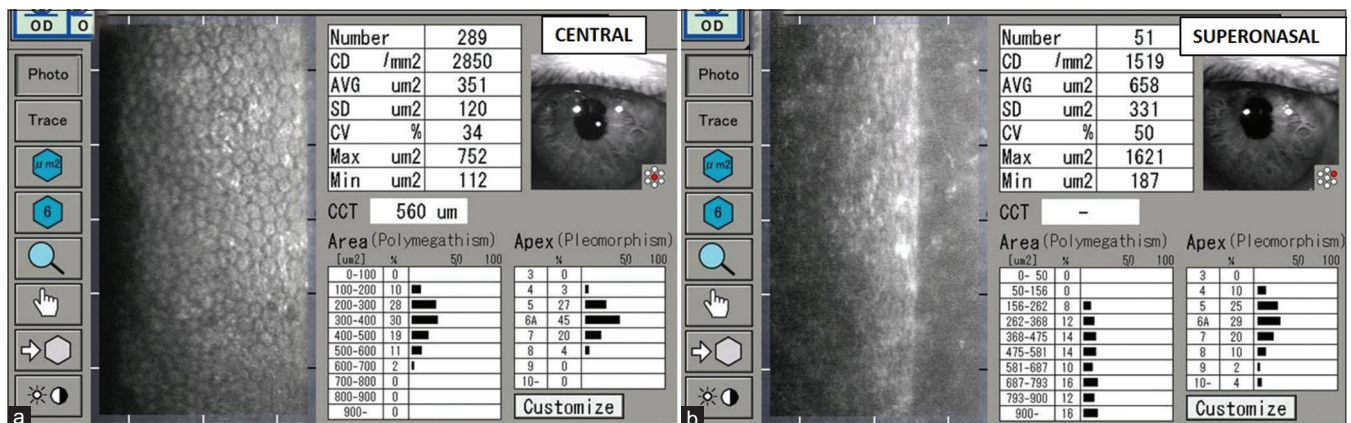
Figure 4: Endothelial cell morphology comparing the central (a) clear cornea and the periphery with supero-nasal (b) semilunar sign of the same eye

posterior stroma clinically [Fig. 1]. There was neither superficial nor deep vascularization. The clock hours of endothelial opacity followed the scleral involvement [Table 1]. No corneal edema or Descemet's fold noted. Pupil zone was crossed partially in 3 eyes. Pupil was not covered totally in any of the eyes. There was a 0.2–0.5 mm clear zone noted between the opacity and the limbus in 11 eyes. No clear zone was seen in 4 eyes. Full thickness corneal involvement was not seen in any of the eyes. The corneal endothelial opacity does not reduce with steroid treatment. The clinical characteristics comparing the semilunar sign and the conventional interstitial keratitis have been mentioned in Table 2.

Surface area

The mean surface area of the endothelial opacity and the standard deviation was 7.7 mm^2 and 5.2 mm^2, respectively. The overall surface area involved ranged from 2 mm^2 to 17 mm^2. The percentage of the involved corneal endothelial surface contributed 5.8% of the overall endothelium.

Anterior segment optical coherence tomography

On the anterior segment OCT, the opacity involved the endothelium and the posterior stroma. The opacity represented a well delineated hyperreflective zone on OCT [Fig. 3]. Endothelial surface was normal and smooth without any Descemet's irregularities. The mean OCT thickness of the endothelial opacity was 212.5 ± 129.3 μm. The corneal epithelium remained uninvolved. There was no statistically significant correlation between the posterior corneal opacity thickness and the BCVA ($P = 0.895$, r = −0.39). However, 3 eyes in which the opacity was partially crossing the pupil had reduced BCVA. Mantoux sensitivity (mm) also showed no significant correlation with the posterior corneal opacity (μm) ($P = 0.696$, r =−0.142).

Endothelium

There were no keratic precipitates or pigments on the endothelium in these eyes at presentation. The mean central corneal thickness was 526.4 ± 55.7 μm (range: 436–666 μm). The central endothelial cell density (ECD) ranged from1652 to 2924 cells/mm^2 with a mean of 2540.8 ± 351.7 cells/mm^2. The mean coefficient of variation, hexagonality, and polymegathism were $33.4 \pm 19.6\%$, $32.2 \pm 18\%$, and $1.4 \pm 3.3\%$, respectively. There was no correlation between the ECD and the thickness of opacity ($P = 0.52$, r = −0.188). The morphology of endothelium differed between the involved and the uninvolved region of cornea [Fig. 4]. There was statistical difference in ECD between the central and the involved peripheral cornea ($P = 0.05$).

Scleritis

All the 15 eyes had scleral lesion either in healed (*n* = 12) or active (*n* = 3) state. Scleral thinning following necrotizing scleritis was noted in 7 eyes. The active scleritis was diffuse in 2 eyes and nodular in 1 eye. The scleral inflammation resolved in all patients with systemic steroids. Recurrence was noted in 2 patients and the same has been managed by maintenance therapy by low dose steroids. Two patients underwent phacoemulsification with foldable intraocular lens implantation under local anesthesia after control of inflammation, and the subsequent postoperative period was uneventful.

Discussion

Embryologically, the corneal endothelium and the predominant part of sclera are derived from the neural crest cells.[8] The additional factors for the combined pathologies between the cornea and sclera are the anatomical close proximity, the closely related collagen structures, and the immunological response.[3,9,10] The frequent corneal pathologies following the anterior scleritis were noted as a sequel of a common immune response in both the corneal and scleral tissue in noninfectious anterior scleritis.[8-12]

The acute stromal keratitis of necrotizing anterior scleritis presents as corneal edema with dense white stromal infiltrates. The lesion may be central or peripheral with keratic precipitates or "precipitin ring."[9] However, the configuration and the level of lesion differed from our cases of posterior corneal involvement, uniform semilunar pattern, and absence of corneal edema. Sclerosing keratitis with adjacent scleral disease is seen with cornea edema with upgrowing conjunctival vessels into the cornea. A candy floss opacity is often seen in the anterior or mid stroma unlike the "semilunar sign," which is posterior.[9] They can circumferentially coalesce with central clear cornea, forming the so called "scleralized cornea."[1] Keratolysis is a severe form of corneal response seen in necrotizing scleral inflammation, characterized by severe loss of corneal tissue. Limbal guttering or PUK with scleritis has also been noted in association with anterior scleritis in collagen diseases.

Even though the stromal and the anterior corneal changes were reported extensively in literature, posterior corneal changes in scleritis were neither studied nor analyzed widely.[8-12] Watson *et al*.[9] in their review reported seeing patients with posterior corneal opacity, which they mentioned as indistinguishable from interstitial keratitis of congenital syphilis. However, there are few clinical features in the semilunar sign which differentiated it from the interstitial keratitis as tabulated in Table 2. Our cases differed from the case series of Holt and Watson by the absence of anterior chamber inflammation, absence of vascularization, absence of nummular coin shape, and the peripheral corneal semilunar location.[10]

There are few noncorneal conditions like chronic anterior uveitis, uncontrolled glaucoma, complicated intraocular surgeries, and immune-mediated endotheliopathy, which can cause endothelial dysfunction.[12-18] Nonsyphilitic deep interstitial keratitis shown by Holt and Watson had nummular features with vascularization along with inflammatory signs in anterior chamber.[10] All the above conditions are often symptomatic and are caused by the anterior stromal involvement with vascularization unlike nonvascular posterior opacity or semilunar sign of anterior scleritis.

In our case series, the noninfectious scleritis was diagnosed clinically and was observed to respond exceptionally well to oral steroids and no antibiotic was required in any of the eye during the active scleral disease. Though three patients had taken ATT for pulmonary and lymph adenitis, it has been long (10 years) before the presentation of scleritis. Moreover, Mantoux positivity alone does not indicate an active disease, as it correlates to cell-mediated immune response in the patient. Strong Mantoux positivity seen in 54% of patients showed good delayed (type IV) immune response in these patients. The rationale behind the correlation of Mantoux severity and the corneal opacity was that the endothelial opacity was suspected as an associated immune response similar to Mantoux. If we consider it to be a form of immune-mediated response from the cornea, which has triggered the endothelial change, it should have shown response to systemic steroids. However, it does not disappear after steroid course. Thus, the pathophysiology remains unclear in such eyes as it does not

fit into the conventional interstitial keratitis or acute stromal keratitis. With no vascularization and asymptomatic corneal involvement, it may be entitled as additional clinical sequel of anterior scleritis. Since extensive scleral involvement was noted to have more endothelial involvement, early treatment may prevent the severity. This was in terms with Cocho *et al.*[19] who reported that necrotization has been known to have more corneal involvement. Though tuberculous keratoscleritis has been known as a common differential in endemic countries, it has been often noted as scleral abscess and necrosis and not been observed as isolated corneal endothelial opacity.[7,20,21]

Though there have been reports of endothelial changes in scleritis, to the best of our knowledge, no studies reported on the multimodal evaluation of posterior corneal opacity in anterior scleritis.[3,9,10] Even though there have been studies on scleral assessment using OCT, no reports are available on the corneal endothelial OCT assessment in scleral disease.[22] The imaging using OCT can aid in diagnosis and provide long-term documentation. Swept source OCT and in-vivo confocal microscopy can also be utilized for further assessment.[23,24] The main objective of highlighting the endothelial sign in scleritis was to create an awareness of a dormant or indolent condition, which still on prolonged course can affect the endothelial function. We also insist that the presence of semilunar sign will mandate a comprehensive scleral examination in routine checkup. Additionally, we would like to give a warning alarm to the operating surgeon to take additional intraoperative precautions during cataract extraction like proper planning of surgical incision, use of limited ultrasound power, and viscoelastic guided endothelial protection, thereby preventing further endothelial loss.

The presence of healthy concomitant uninvolved endothelium, peripheral location, and younger age group may be the probable reasons for the asymptomatic course. Smaller population group and absence of serial progression analysis were the limitations of our current study. We therefore recommend a long-term follow-up study with serial imaging to identify the role of recurrence and progression of semilunar sign.

Conclusion

To conclude, the study showed that the semilunar sign of anterior scleritis was not uncommon and it was often seen in dormant phase. We believe our study will place extra light on the clinical entity and thereby recommend further observational and histopathological exploration in future.

Conflicts of interest
There are no conflicts of interest.

References

1. Goldstein DA, Tesler HH. Episcleritis, scleritis and other scleral disorders. In: Yanoff M, Duker JS, editors. Ophthalmology. Missouri: Mosby, Inc; 2004. p. 511-9.

2. Watson PG, Young RD. Scleral structure, organisation and disease. A review. Exp Eye Res 2004;78:609-23.

3. Okhravi N, Odufuwa B, McCluskey P, Lightman S. Scleritis. Surv Ophthalmol 2005;50:351-63.

4. Wakefield D, Di Girolamo N, Thurau S, Wildner G, McCluskey P. Scleritis: Immunopathogenesis and molecular basis for therapy. Prog Retin Eye Res 2013;35:44-62.

5. Yoshida A, Watanabe M, Okubo A, Kawashima H. Clinical characteristics of scleritis patients with emphasized comparison of associated systemic diseases (anti-neutrophil cytoplasmic antibody-associated vasculitis and rheumatoid arthritis). Jpn J Ophthalmol 2019;63:417-24.

6. Gupta N, Sachdev R, Sinha R, Titiyal JS, Tandon R. Herpes zoster ophthalmicus: Disease spectrum in young adults. Middle East Afr J Ophthalmol 2011;18:178-82.

7. Shoughy SS, Jaroudi MO, Tabbara KF. Clinical manifestations and outcome of tuberculous sclerokeratitis. Br J Ophthalmol 2016;100:1301-3.

8. Azar NF, Davis EA. Embryology of eye. In: Yanoff M, Duker JS, editors. Ophthalmology. Missouri: Mosby, Inc; 2004. p. 22-7.

9. Watson PG, Hayreh SS. Scleritis and episcleritis. Br J Ophthalmol 1976;60:163-91.

10. Holt-Wilson AD. Watson PG. Non syphilitic deep interstitial keratitis associated with scleritis. Trans Ophthamol Soc UK 1974;94:52-7.

11. Rasband W. Image J. Image Processing and Analysis in Java. Bethesda, Maryland, Research Services Branch, National Institutes of Health, Bethesda. Available from: http://rsb.info.nih.gov/ij/. [Last accessed on 2018 Feb 27].

12. Sainz de la Maza M, Jabbur NS, Foster CS. Severity of scleritis and episcleritis. Ophthalmology 1994;101:389-96.

13. Alfawaz AM, Holland GN, Yu F, Margolis MS, Giaconi JA, Aldave AJ. Corneal endothelium in patients with anterior uveitis. Ophthalmology 2016;123:1637-45.

14. Hillenaar T, Weenen C, Wubbels RJ, Remeijer L. Endothelial involvement in herpes simplex virus keratitis: An *in vivo* confocal microscopy study. Ophthalmology 2009;116:2077-86.

15. Moshirfar M, Murri MS, Shah TJ, Skanchy DF, Tuckfield JQ, Ronquillo YC, *et al*. A review of corneal endotheliitis and endotheliopathy: Differential diagnosis, evaluation, and treatment. Ophthalmol Ther 2019;8:195-213.

16. Paul RH. Presumed autoimmune corneal endotheliopathy. Am J Ophthalmol 1988;105:519-22.

17. Chen LG, Finger PT, Dhrami-Gavazi E. Peripheral pigmented placoid corneal endotheliopathy. Cornea 2013;32:1483-7.

18. Thomas AS, Ali A, Arepalli S, Suhler EB. Inferior corneal haze and inflammatory endotheliopathy related to pars planitis. Ocul Immunol Inflamm 2020;28:798-801.

19. Cocho L, Gonzalez-Gonzalez LA, Molina-Prat N, Doctor P, Sainz-de-la-Maza M, Foster CS. Scleritis in patients with granulomatosis with polyangiitis (Wegener) Br J Ophthalmol 2016;100:1062-5.

20. Ramenaden ER, Raiji VR. Clinical characteristics and visual outcomes in infectious scleritis: A review. Clin Ophthalmol 2013;7:2113-22.

21. Dutta Majumder P. Presumed tuberculous sclerokeratitis presenting with hypopyon. Ocul Immunol Inflamm 2019;27:1121-3.

22. Axmann S, Ebneter A, Zinkernagel MS. Imaging of the sclera in patients with scleritis and episcleritis using anterior segment optical coherence tomography. Ocul Immunol Inflamm 2015;24:29-34.

23. Kuroda Y, Uji A, Morooka S, Nishijima K, Yoshimura N. Morphological features in anterior scleral inflammation using swept-source optical coherence tomography with multiple B-scan averaging. Br J Ophthalmol 2016;101:411-7.

24. Wang EF, Misra SL, Patel DV. *In vivo* confocal microscopy of the human cornea in the assessment of peripheral neuropathy and systemic diseases. Biomed Res Int 2015;2015:951081.