Original Article

Association of Conjunctival Dysplasia (Squamous Intraepithelial Neoplasia) with Melanosis (Microscopic Non-Proliferative Melanin Pigmentation)

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Abstract

Purpose: To determine the association between conjunctival epithelial dysplasia (squamous intraepithelial neoplasia) and its melanosis (microscopic non-proliferative melanin pigmentation) in conjunctival biopsies.

Methods: In this retrospective case series, histopathological slides from all conjunctival biopsies obtained in Khalil Hospital affiliated with Shiraz University of Medical Sciences for a period of 6 years (April 2009–July 2015) were reviewed. After considering the exclusion criteria (non-melanotic pigmentation, melanocytic proliferations, and squamous cell carcinoma), conjunctival biopsies were divided histopathologically into two groups of dysplastic and non-dysplastic. Then, the slides were reviewed by one ophthalmopathologists and one general pathologist. Melanin pigmentation was recorded in both groups as 0, 1+, 2+, and 3+. The data were analyzed, and the groups were compared.

Results: Overall, 685 cases with a mean age of 47.78 (±17.74) years were included in this study. Dysplastic and non-dysplastic groups comprised 135 (19.7%) and 550 (80.3%) specimens, respectively. Seventy-six percent (76%) of the specimens in the dysplastic group versus 40% in the non-dysplastic group had melanosis ($P = 0.001$). However, the degree of dysplasia (1+, 2+, and 3+) was not statistically correlated with the degree of melanosis (1+, 2+, and 3+) ($P = 0.393$).

Conclusion: Our results demonstrated that melanosis is a common finding in conjunctival epithelial dysplasia and might indicate an association with conjunctival epithelial dysplasia.

Keywords: Conjunctival biopsy, Epithelial dysplasia, Melanosis, Premalignant lesion

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Introduction

Epithelial dysplasia, carcinoma *in situ*, and squamous cell carcinoma (SCC) of the cornea and conjunctiva are the diseases categorized as ocular surface epithelial dysplasia.[1] However, some authors do not categorize invasive carcinoma as dysplasia, and different terms have been used at different times for intraepithelial forms of squamous neoplasms such as epithelial plaque, bowenoid epithelioma, and precancerous epithelioma.[2] In another classification, these neoplasms can present as mild (involvement of one-third of the conjunctiva), moderate (involvement of two-thirds of it), and severe (lesions with full-thickness) dysplasia.[3] The term ocular surface squamous neoplasia (OSSN) has been coined for a wide range of dysplastic and carcinomatous ocular lesions,[4] and usually, a subepithelial chronic inflammatory response is

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present.[5] It is histologically defined as "epithelial hyperplasia with loss of goblet cells, nuclear hyperchromasia, and cellular pleomorphism, and often shows surface keratinization, dyskeratosis, and increased mitotic figures." Although OSSN is usually seen in places near the equator with tropical temperature,[6] it has been found in different racial groups as well, Caucasians being the predominant group.[3,7-9]

The association between conjunctival epithelial melanosis and melanocytic tumors, such as malignant melanoma, has been established.[10] However, to the best of our knowledge, the association between epithelial dysplasia and microscopic non-proliferative melanin pigmentation has not been studied previously. Ocular melanosis is the flat melanotic pigmentation or intra- and extra-cellular hyperpigmentation seen on slit-lamp examination with the naked eye.[11] Researchers have also categorized the disease to melanosis and melanocytic proliferation. Since melanosis may refer to any melanocytic pigmentation visible to the naked eye and may be used to encompass both melanin hypersecretion and melanocytic proliferation, in the present study, the term melanosis is used as intraepithelial non-proliferative melanocytic pigmentations. It can be primary (freckles or racial melanosis) or secondary (SCC, inclusion cyst, etc.). Melanocytic proliferation consists of nevus and conjunctival melanocytic intraepithelial neoplasia (C-MIN).[12]

Conjunctival epithelial dysplasia (squamous intraepithelial neoplasia) is considered the most prevalent premalignant lesion of the conjunctiva[13] and is very common in our geographical region (10.2% of all conjunctival biopsies).

The aim of the present study was to determine the association between epithelial dysplasia and melanosis (microscopic non-proliferative melanin pigmentation) in conjunctival biopsies, regardless of the state of gross conjunctival pigmentation in the slit-lamp examination.

Methods

In this retrospective case series, histopathological slides from all conjunctival biopsies that have been obtained in Khalil Hospital and archived in the ophthalmic pathology laboratory affiliated with Shiraz University of Medical Sciences for a period of 6 years (April 2009–July 2015) were reviewed. This hospital is a tertiary referral center for ocular diseases in the south of Iran, where most patients are Caucasian. After considering the exclusion criteria, conjunctival biopsies were divided histopathologically into two groups: dysplastic and non-dysplastic. The tenets of the Declaration of Helsinki were respected, and the study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences.

The hematoxylin- and eosin-stained slides were reviewed by one ophthalmic pathologist and one general pathologist. Rarely, there was a difference between their grades. In such a situation, in the same session, they reached a consensus, and ultimately one grade was recorded for each patient.

In practice, the effect of racial melanosis as bias was omitted because this parameter is supposed to be equally distributed in both groups since both groups are from the same racial population. Other causes of conjunctival pigmentation, such as argyriasis and drug side effects (e.g., calcium channel blockers), were excluded by reviewing the patients' charts. All melanocytic proliferations such as nevus, primary acquired melanosis, C-MIN, and malignant melanoma, as well as invasive SCC, were excluded from this study.

Initially, conjunctival biopsies were assessed for the degree of microscopic melanin pigmentation and also for the grade of epithelial dysplasia. Considering dysplasia, we divided the biopsies into dysplastic and non-dysplastic groups, and then, the grade of dysplasia was determined for each case. The criteria of dysplasia and its grade were determined using a previous review by Surendra Basti and colleagues.[2] We defined epithelial melanosis as increased pigmentation of the basal cell layer without the proliferation of the melanocytes. The epithelial melanosis was classified as: mild (1+) when it was visible only with magnification ×1000; moderate (2+) when it was visible with ×400; and distinct (3+) when it was visible even with ≤×100. The slides were examined under a light microscope (Model BX-53, Olympus, Japan). Finally, the two groups were compared statistically with respect to the association of epithelial dysplasia and melanosis.

All statistical analyses were performed using IBM SPSS Statistics software version 22 (SPSS Inc., Chicago, IL, USA). Chi-square tests and independent *t*-test were used for comparison between groups. A Chi-square test was used to evaluate the association between dysplasia and melanosis. Spearman correlation test was used to evaluate the relationship between age and melanosis. A *P* value less than 0.05 was considered statistically significant.

Results

Of the 830 conjunctival specimens received, 685 were included in the study. The mean (±standard deviation [SD]) age of the patients whose specimens were studied was 47.78 (±17.74) years (range, 2–89 years) [Table 1]. Three hundred and seventy-six (54.9%) specimens were from men and 309 (45.1%) from women. In the dysplastic group, 92 (68.1%) and 43 (31.9%) specimens were from men and

Table 1: Age and sex distribution in dysplastic and non-dysplastic groups

Groups	Dysplastics	Non-dysplastics	Total
n	135	550	685
Mean age (±SD)	55.17 (±18.20)	45.92 (±17.14)	47.78 (±17.74)
Minimum age	12	2	2
Maximum age	89	88	89
Male, *n* (%)	92 (68)	284 (51.6)	376 (54.9)
Female, *n* (%)	43 (31)	266 (48.4)	309 (45.1)

SD: Standard deviation

women, respectively. However, in the non-dysplastic groups, there were 284 (51.6%) specimens from men and 266 (48.4%) from women. One hundred and thirty-five (19.7%) lesions were dysplastic, and 550 (80.3%) were non-dysplastic, including pterygium, epithelium containing tissues with lymphoid hyperplasia, inclusion cyst, lipodermoid or limbal dermoid cyst, and conjunctival inflammation.

We found a statistically significant difference between dysplastic and non-dysplastic groups with respect to sex. Moreover, the mean age (±SD) of the dysplastic group was 55.17 (±18.20) years, and that of the non-dysplastic group was 45.92 (±17.14) years ($P \leq 0.05$) [Table 1].

A comparison of the groups revealed that there was a statistically significant difference between the two groups regarding the presence of melanosis (scores none, 1+, 2+, and 3+), [Table 2]; there was more melanosis in the dysplastic group compared to the non-dysplastic group. However, the degree of dysplasia (1+, 2+, and 3+) was not statistically associated with the degree of melanosis (1+, 2+, and 3+) [Table 3].

There was no statistically significant correlation between age and melanosis in either the dysplastic or non-dysplastic groups. In the dysplastic group, the correlation coefficient (r) was 0.015 ($P = 0.86$). In the non-dysplastic group, the correlation coefficient (r) was 0.062 ($P = 0.152$).

Figure 1 shows different scores of conjunctival melanosis and the associated dysplasia.

Discussion

Conjunctival epithelial dysplasia is more prevalent in the Caucasian race.[2] In one study in Iran, the prevalence of conjunctival epithelial dysplasia comprised 10.2% of all conjunctival specimens; it was considered the most prevalent premalignant lesion.[13] It is important to study the histopathological details of dysplasia because it might help to identify the etiologic factors of dysplasia. It may also provide clues for understanding the pathophysiology of dysplasia.

Table 2: Distribution of dysplasia with melanosis

Diagnosis	Melanosis	Non-melanosis	Total	*P*
Dysplasia	106	29	135	<0.05
Non-dysplasia	215	335	550	
Total	321	364	685	

Table 3: Association between the degree of dysplasia and the degree of melanosis

Dysplasia	Melanosis			Total	*P*
	1.00	2.00	3.00		
1.00	28	8	6	42	>0.05
2.00	24	10	2	36	
3.00	22	3	3	28	
Total	74	21	11	106	

P-value for all subgroups is ≥0.05

The results of our study on 685 specimens of conjunctival dysplasia showed that male gender and an increase in age were related to conjunctival dysplasia. These results are in the same line with a major review done by Lee and Hirst in 1995, concluding that OSSN is more prevalent among older men with a mean age at the occurrence of 56 years.[4]

We found that the presence of microscopic melanosis in the conjunctival epithelium was statistically correlated with epithelial dysplasia. To the best of our knowledge, this is the first study on the association between epithelial dysplasia and melanosis (microscopic non-proliferative melanin pigmentation). In the present study, this prevalence was 76% in the dysplastic group and 40% in the non-dysplastic group. There was an attempt to eliminate the racial effect (primary melanosis) by comparing groups from the same race. In a study on Africans using 234 conjunctival biopsies, the researchers found that melanocytic proliferation mostly occurred in lesions that had severe dysplasia.[14] As mentioned before, we excluded all conjunctival specimens with obvious melanocytic proliferation from our study. Furthermore, our results showed that the degree of melanosis (1+, 2+, and 3+) was not statistically correlated with the degree of dysplasia (1+, 2+, and 3+), so it can be concluded that in our dysplastic cases, overproduction of melanin pigment occurred that did not necessarily have a direct correlation with the level of dysplasia. One of the most important factors contributing to OSSN is ultraviolet (UV) radiation.[1,3,7-9,14-17] Ultraviolet-B rays damage the human epithelial cell deoxyribonucleic acid,[18,19] and prolonged or frequent exposure also increases the melanocyte size and functional activity.[20,21] We evaluated melanosis and dysplasia at the same time in the histopathological slides; therefore, we cannot comment on the temporal order of their occurrence in the patients. We may conclude that UV light is a shared risk factor for both dysplasia and melanosis. UV light that is a major risk factor for dysplasia[22-24] causes overproduction of melanin as a defense mechanism,[14] and both dysplasia and melanosis are the results of the cumulative effects of prolonged and excessive sun exposure. Although this deduction may be correct, as the results indicate, almost 91% of the specimens from the non-dysplastic group were pterygia, a lesion which shares some risk factors with dysplasia, in particular exposure to UV radiation. Therefore, as both groups share exposure to UV radiation as an important risk factor, exposure to UV light cannot exclusively explain why in the dysplastic group, the amount of microscopic pigmentation was greater than that in the pterygia group. Another explanation is that melanosis could also be secondary to the response to the localized trauma induced by the tumor into the ocular surface. This trauma may cause the resident melanocytes to release their granules into the surrounding keratinocytes. The association of melanosis with neoplasms of other organs may justify this explanation. Although the conjunctiva is the only mucous membrane in the human body that is exposed to a high level of UV light, there has been an

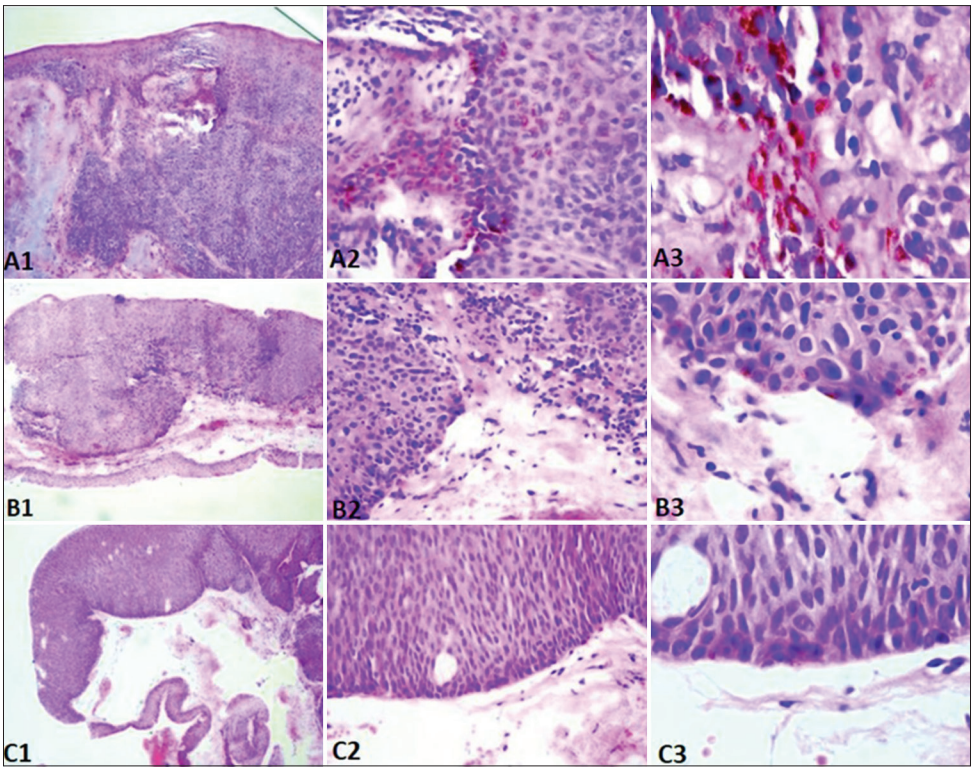

Figure 1: Conjunctival epithelium with severe dysplasia and 3+ melanosis. Melanin pigment is obviously seen at low magnification. H&E stain, magnification 100, 400, and 1000, respectively. B1, B2, B3: The same as A1, A2, A3, in another patient (conjunctival epithelium with severe dysplasia and 3+ melanosis seen at magnification 100, 400, and 1000, respectively). C1, C2, C3: Conjunctival epithelium with severe dysplasia and 2+ melanosis. Melanin pigment is visible at power 400. H&E stain, magnification 100, 400, and 1000, respectively

association between melanosis and neoplasms of other mucosa, such as respiratory, laryngeal, and gastroesophageal mucosa. There are some studies that have evaluated the association of melanosis with epithelial dysplasia and SCC in other mucosal membranes. Cordes *et al*. in a study on African-American smokers concluded that laryngeal mucosal melanosis signals the injury or chronic inflammation of the mucosa and could be a possible sign of the head-and-neck malignancy.[25] Gonzalez-vela *et al*. showed that an irritant stimulus transforms the respiratory mucosa, leading to either melanogenic metaplasia or increased melanin pigment production.[26] Another study by Yokoyama *et al*. showed that the prevalence of melanosis was higher in alcoholic Japanese men with esophageal dysplasia, esophageal SCC, and oropharyngolaryngeal SCC. The researchers concluded that melanosis in the upper aerodigestive tract should be considered one of the high-risk biomarkers for neoplasm.[27] Moreover, trauma or chronic irritation might also be responsible for uterine-cervical mucosal melanosis.[28]

The present research is a basic study and might open a window in the molecular chemistry of OSSN. In addition, it has clinical application as the detection of melanosis during clinical examination, and slit-lamp biomicroscopy might indicate a higher likelihood of conjunctival dysplasia and OSSN. Therefore, the detection of melanosis might provide a simple new sign for the identification of patients who are at high risk for conjunctival dysplasia and OSSN. Another importance of this finding is that it could be added to the diagnostic clues of histopathological examination in patients with conjunctival epithelial dysplasia. If it is also confirmed in future studies, melanosis could be considered a biologic marker of conjunctival dysplasia and OSSN.

There are some limitations in our study. Since it was a retrospective study, we could not match the age and sex between the two groups; the mean age of the dysplastic group was 55.17 years, and that of the non-dysplastic group was 45.9 years. It occurred because, according to previous studies, OSSN is more prevalent among older men with a mean age of occurrence of 56 years that is in line with our results. As to sex, in the non-dysplastic groups, 51.6% were specimens from men and 48.4% from women ($P > 0.05$). However, in the dysplastic group, 68.1% and 31.9% of the specimens were from men and women, respectively. It is in line with previous studies as OSSN is more prevalent among older men. Another limitation was that some details of history, such as the exact prior history of receiving local chemotherapy such as mitomycine or interferone were not recorded in some patients. This is, in fact, the drawback of many retrospective studies. However, it is unlikely that it has affected our results. Conducting prospective studies to assess this subject seems necessary.

In conclusion, based on the results of our study, there is an association between conjunctival epithelial dysplasia and its melanosis. Increased melanosis in patients with dysplasia

might be the result of both UV exposure and the localized trauma induced by the tumor, and melanosis may be considered a biomarker of the conjunctival dysplasia. Thus, it is important to consider and report the presence of melanosis in the histopathological examination of conjunctival specimens.

As our study was retrospective in nature, further investigations are required to clarify whether there is a causal relationship between conjunctival melanosis and conjunctival dysplasia. The results of such studies help to have a better understanding of the pathophysiology of conjunctival dysplasia and OSSN that subsequently might result in better therapeutic strategies.

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Conflicts of interest

There are no conflicts of interest.

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