The European Research Journal

Original Article

http://www.eurj.org

e-ISSN: 2149-3189

DOI: 10.18621/eurj.2015.1.3.88

Tissue eosinophilia: a histopathological marker associated with stromal invasion but not histopathological grade in cutaneous squamous neoplasia

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ABSTRACT

Objectives. The literature does not include any comparative study on the eosinophil count in premalignant and malignant cutaneous squamous neoplasias. Our aim was to compare the tissue eosinophilic count in actinic keratosis (AK) and cutaneous squamous cell carcinoma (SCC). *Methods.* The study included 20 AK and 20 invasive SCC patients. Age, gender, and lesion location were retrospectively evaluated as clinical parameters. Histopathological parameters included density of inflammation score, lesion grade, and the lesion-associated eosinophil count per 10 high-power (40×) microscopic fields, all of which were compared between groups. *Results.* In all, 65% of the AK group had lesions with moderate inflammation, whereas 85% of the SCC group had lesions with dense inflammation (p=0.001). The mean eosinophil count in the SCC group was significantly higher than that in the AK group, independent of the density of inflammation (p=0.000). In addition, lesion grade was not associated with the eosinophil count in either group (AK group: p=0.601; SCC group: p=0.416). *Conclusions.* Cutaneous SCC lesions had higher eosinophil counts than AK lesions, indicating the role of the eosinophil count as a histopathological marker of stromal invasion.

Eur Res J 2015;1(3):88-93

Keywords: Eosinophilia; actinic keratosis; squamous cell carcinoma; invasion

Introduction

Tumor-associated tissue eosinophilia was first described in 1896 in cervical carcinoma and is defined as eosinophilic infiltration in a tumor that is not associated with necrosis or ulceration. Its functional role remains unclear [1]. Eosinophilic infiltration has been reported in carcinomas located in the oral cavity, larynx, pharynx, gastrointestinal tract, lungs, cervix, and external genitalia; however, the literature includes limited data on eosinophilic infiltration in cutaneous squamous cell carcinoma (SCC) [1, 2].

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Received: 25.02.2015; Accepted: 12.04.2015: Published Online: 04.11.2015

Currently, it is thought that cutaneous SCC manifests as a spectrum, ranging from precursor actinic keratosis (AK) to SCC in situ (SCCIS), invasive SCC, and metastatic SCC [3]. Very few studies have evaluated the role of eosinophils in such premalignancies as oral leukoplakia, and laryngeal, vulvar, and cervical carcinoma in situ, and it has been suggested that an elevated tissue eosinophilia count is a histopathological marker of stromal invasion; however, to the best of our knowledge no study has compared the eosinophil count in AK and invasive cutaneous SCC [1]. As such, the present study aimed to evaluate the eosinophil count in different grades of AK and SCC, and to elucidate the role of the eosinophil count as a histopathological marker of lesion grade and/or stromal invasion in cutaneous squamous neoplasias.

Methods

The study included 20 histopathologically proven AK patients and 20 histopathologically proven invasive cutaneous SCC patients that were diagnosed between January 2013 and March 2014. Clinical data were obtained from pathology reports and included age, gender, and lesion location. Lesion locations were grouped as follows: scalp, forehead, periorbital region, malar region, ear, nose, and other regions (neck, trunk, and extremity). Histopathological parameters were determined via hematoxylin and eosin (H&E) stained sections that were microscopically re-examined. Diagnosis, lesion-associated inflammation, and lesion grade were evaluated as histopathological parameters, and the number of eosinophils was enumerated. As lesion-associated inflammation, in SCC; perilesional stromal inflammation and in AK; dermal inflammation underlying the lesion was evaluated.

The density of inflammation was scored, as follows:

- 0: no inflammation;
- 1: mild inflammation; scattered clusters of inflammatory infiltrate involving < 25% of the lesion;
- 2: moderate inflammation; inflammatory infiltrate involving 25%-75% of the lesion;
- 3: dense inflammation; thick clusters or sheets of inflammatory infiltrate involving > 75% of the lesion.

To minimize interobserver variability standard grading systems were used and lesion grading was based on the agreement of 2 of 3 observers. For dysplasia grading of AK lesions a 3-tiered classification scheme proposed by Cockerell et al. was used, and the lesions were subgrouped as AK-I, AK-II, and AK-III [4]. AK-I was defined as cellular atypia of basal and suprabasal keratinocytes confined to the lower 1/3 of the epidermis. AK-II was defined as atypia involving the lower 2/3 of the epidermis, and AK-III represented carcinoma in situ with full thickness atypia involving the epidermis.

Broder's classification was used to grade SCC and lesions were graded on the basis of the degree of differentiation and keratinization [5]. SCCs were graded, as follows:

- Grade I: Well-differentiated lesion; 75%-100% of cells are differentiated.
- Grade II: Moderately differentiated lesion; 50%-75% of cells are differentiated.
- Grade III: Poorly differentiated lesion; 25%-50% of cells are differentiated.
- Grade IV: Anaplastic lesion; 0%-25% of cells are differentiated.

Eosinophils were enumerated under high-power (40×) microscopic fields in regions with lesion-associated inflammation. A high-power field (HPF) with the maximum number of eosinophils was identified first, and then eosinophils in that field and in 9 adjacent contiguous HPFs were enumerated and recorded as eosinophils10 HPFs-1. Areas of necrosis or ulceration were not included. Only intact nucleated cells with intense red granules in cytoplasm were accepted as eosinophils-eosinophils in lymphovascular spaces were not included. In addition, the eosinophil counts in the AK and SCC groups were compared, and the effect of density of inflammation on differences in the count was determined. Furthermore, the association between lesion grade and the eosinophil count was evaluated in both groups. The study has been carried out in accordance with Declaration of Helsinki and research protocol was approved by Institutional Review Board.

Statistical Analysis

Statistical analysis was performed using SPSS v.18.0 for Windows (SPSS Inc., Chicago, IL, USA). Continuous variables are presented as mean ± SD, and categorical variables as frequency and percentage. The chi-square test was used to determine associations between categorical variables. The normality of the distribution of numeric variables was evaluated using the Kolmogorov-Smirnov test. For normally distributed variables between-group differences were determined using the independent samples t-test, and the Mann-Whitney U test was used for variables not distributed normally. Eosinophil counts were categorized as < 10 eosinophils 10 HPFs-1 and > 10 eosinophils 10 HPFs-1. To compare eosinophil counts according to lesion grade AK lesions were grouped as AK-I and AK-II + AK-III, and SCC lesions were grouped as SCC-I and SCC-II + SCC-III due to the small number of samples. In addition, the eosinophil counts in AK-II/III lesions and SCC grade I-IV lesions were compared. The association between the eosinophil count and the density of inflammation in each group was evaluated using Spearman's correlation coefficient. Linear regression was performed to identify the effect of the density of inflammation on the difference in the eosinophil count between the AK and SCC groups. The level of statistical significance was set at p=0.05.

Table 1. Comparison of the clinical and histopathological features in the AK and SCC groups.

Feature	**AK (n=20)**	**SCC (n=20)**	*p*
Mean±SD age (years)	59.5±15.3	68.4±10.5	**0.038**
Gender (female/male) (%)	10/10 (50/50)	2/18 (10/90)	**0.014**
Lesion Location (%)			
Malar	4 (20)	5 (25)	
Periorbital	3 (15)	3 (15)	
Ear	3 (15)	4 (20)	
Nose	2 (10)	5 (25)	0.756
Forehead	3 (10)	1 (5)	
Scalp	2 (10)	1 (5)	
The other	3 (15)	1 (5)	
Density of Inflammation (%)			
Mild	0 (0)	1 (5)	
Moderate	13 (65)	2 (10)	**0.001**
Dense	7 (35)	17 (85)	
Mean±SD Eosinophil Count	2±1.6	45±26.3	**<0.001**
Eosinophils 10 HPFs^{-1} (%)			
< 10 eosinophils 10 HPFs^{-1}	20 (100)	0 (0)	**<0.001**
≤ 10 eosinophils 10 HPFs^{-1}	0 (0)	20 (100)	

Bold *p* value denotes significant difference (p<0.05).

Table 2. Comparison of the eosinophil count, according to group and histological grade.

Group (n)	Mean±SD Eosinophil Count	*p*
AK (n=20)	2±1.6	**<0.001**
SCC (n=20)	45±26.3	
AK-II/III (n=11)	2.18±1.8	**<0.001**
SCC (n=20)	45±26.3	
AK-I (n=9)	1.78±1.48	0.601
AK-II/III (n=11)	2.18±1.8	
SCC-I (n=8)	39±27.5	0.416
SCC-II/III (n=12)	49.1±25.9	

Bold *p* values denote significant difference ($p<0.05$).

Results

The study included 20 AK patients and 20 invasive cutaneous SCC patients. In the AK group the female-male ratio was 10:10, whereas in the SCC group, 18 (90%) of the patients were male ($p=0.014$). Mean age in the AK group was 59.5±15.3 years, versus 68.4±10.5 years in SCC group ($p=0.038$).

Lesions in the AK group were located, as follows: malar region (n=4, 20%), periorbital region (n=3, 15%), forehead (n=3, 15%), ear (n=3, 15%), nose (n=2, 10%), scalp (n=2, 10%), and other regions (n=3, 15%). Lesions in the SCC group were located, as follows: malar region (n=5, 25%), nose (n=5, 25%), ear (n=4, 20%), periorbital region (n=3, 15%), forehead (n=1, 5%), scalp (n=1, 5%), and other regions (n=1, 5%). The distribution of lesion location did not differ significantly between the 2 groups ($p=0.756$).

In 13 (65%) of the AK group lesions exhibited moderate inflammation, whereas in 17 (85%) of the SCC group lesions exhibited dense inflammation ($p=0.001$).The eosinophil count in all the lesions in the AK group was < 10 eosinophils10 HPFs-1, versus ≥ 10 eosinophils 10 HPFs-1 in the SCC group ($p<0.001$). Furthermore, the mean eosinophil count 10 HPFs-1 was 2±1.6 in the AK group, versus 45±26.3 in the SCC group, and the difference was significant ($p<0.001$); when the effect of the density of inflammation was adjusted for, the difference remained significant ($p<0.001$). In the AK group the density of inflammation and the eosinophil count were not correlated, whereas in the SCC group there was a moderate positive correlation between the density of inflammation and the eosinophil count (Spearman's correlation coefficient: 0.58; $p= 0.007$). In the AK group histopathological grade of the lesions was, as follows: AK-I in 9 (45%); AK-II in 10 (50%); AK-III/SSCI in 1 (5%) lesions. In the SCC group histopathological grade of the lesions was, as follows: well differentiated/grade I in 8 (40%); moderately differentiated/grade II in 11 (55%): poorly differentiated/grade III in 1 (5%) lesions.

The mean eosinophil count 10 HPFs-1 in AK-I, II and III lesions was 1.78±1.48, 1.8±1.4, and 6, respectively. The mean eosinophil count 10 HPFs-1 in AK-I and AK-II/AKIII lesions did not differ significantly (1.78±1.48 vs. 2.18±1.8, $p=0.601$).The mean eosinophil count 10 HPFs-1 in SCC-I, II and III lesions was 39±27.5, 42.8±14.8, and 118, respectively. The mean eosinophil count 10 HPFs- 1 in SCC-I and SCC-II/III lesions did not differ significantly (39±27.5 vs. 49.1±25.9, $p= 0.416$). Additionally, the mean eosinophil count 10 HPFs-1 in AK-II/AK-III and SCC lesions differed significantly (2.18±1.8 vs. 45±26.3, $p<0.001$).

A detailed comparison between the AK and SCC groups is shown in Table 1, and a comparison of the eosinophil count between the various lesion grades and the 2 groups is shown in Table 2.

Discussion

The literature includes limited data on eosinophilic infiltration in cutaneous SCC. In 1984, Lowe *et al.* published a study on tissue eosinophilia in keratoacanthoma (benign category), and early and late stage cutaneous SCC [6]. They suggested that lesions are more likely to be malignant when there is tissue eosinophilia, and recommended that eosinophilic infiltration be added to the criteria for differentiating the 2 types of lesions.

The role of eosinophils in premalignancies has been examined in a few studies; however, none included cutaneous pre-invasive neoplasias. In 2002 Spiegel *et al.* reported that eosinophils may be used as a marker of invasion in cervical squamous neoplasias and recommended that ≥ 5 eosinophils HPF-1 and/or ≥ 10 eosinophils10 HPFs-1 in biopsy specimens should prompt a search for focal invasion in patients with high-grade intraepithelial neoplasia [7]. Agarwal *et al*. [8] reported findings similar to those of Spiegel et al. and suggested that the presence of stromal eosinophils should result in a search for invasion in deeper sections in lesions of cervical intraepithelial neoplasia II and III Alrawi *et al.* [2] reported higher eosinophilic counts in laryngeal SCC lesions than in SCCIS, concluding that tissue eosinophilia might be a pathological feature associated with stromal invasion. Similarly, Said *et al.* [9] reported that an elevated eosinophil count in squamous neoplasia of the larynx is associated with stromal invasion, and recommended a thorough search for invasion when the number of infiltrating eosinophils is > 10 eosinophils HPF-1 and/or 20 eosinophils 10 HPFs-1. More recently, Jain *et al.* [1] observed higher eosinophil counts in oral SCC than those in oral dysplasia, suggesting they play a role in stromal invasion. In addition, they recommended that determination of the eosinophil count should be included in the routine histopathological evaluation of oral precancer and SCC.

As previously reported, in the present study the eosinophil count was higher in patients with invasive cutaneous SCC than in those with AK. Furthermore, the difference in the eosinophil count remained significant when it was compared between the SCC group and AKII/III lesions. In all the patients in the AK group-including 1 with SCCIS-the eosinophil count in all the lesions was < 10 eosinophils 10 HPFs-1, whereas in the SCC group the eosinophil count was > 10 eosinophils 10 HPFs-1 in all the lesions. As such, we recommend-as did Spiegel et al.-that an eosinophil count > 10 eosinophils 10 HPFs-1 should prompt a search for invasion, especially in patients in which differentiating between pre-invasive neoplasia and invasive SCC is difficult [7].

In the present study, an evaluation of the relationship between the eosinophil count and AK lesion grade showed that they weren't strongly correlated. To the best of our knowledge, there is only one study that examined the relationship between dysplasia grade and the eosinophil count in patients with premalignancies [1]. In the present study there were not any significant differences in the eosinophil count according to dysplasia grade confirming that study.

The present study also evaluated the relationship between SCC grade and the eosinophil count, but the eosinophil count did not differ significantly according to SCC grade. The relation between SCC grade and the eosinophil count remains contentious; in some earlier studies an elevated eosinophil count was associated with non-keratinizing carcinomas [10, 11], whereas in others there wasn't a significant difference between keratinizing and non-keratinizing carcinomas [7]. In a more recent study Joshi *et al.* [12] reported that there was not a correlation between different grades of oral SCC and eosinophilic infiltration, which is in agreement with the present findings.

Eosinophil counts might have been higher in the present study's SCC group because the density of inflammation was higher; most of the AK lesions had moderate inflammation, whereas inflammation was dense in most of the SCC lesions. In the AK group there wasn't a correlation between the density of inflammation and the eosinophil count, whereas in the SCC group there was a moderate positive correlation; however, when the effect of the density

of inflammation on the eosinophil count was adjusted for via regression analysis the difference in the eosinophil count between the AK and SCC groups remained significant. In most of the earlier published studies the effect of the density of inflammation on the eosinophil count was not analyzed. Joshi *et al.* [12] suggested that there isn't an association between overall inflammatory response and the eosinophil count in their study on oral SCC, as all their patients had dense inflammation; however, they did not score the density of inflammation as in the present study, making a comparison of the findings difficult.

The mechanisms underlying eosinophilic accumulation in invasive SCC are not fully known [9]. It was suggested that lesion-derived eosinophil chemotactic factors induce eosinophilic infiltration.

Furthermore, lesion-associated eosinophils release eotaxin, which induces further eosinophil chemotaxis to the lesion. Similarly, mast cells in lesional infiltration release histamine and eosinophil chemoattractant factor, causing further eosinophil accumulation [1].

The functional role of eosinophils in stromal invasion is not known. Eosinophils produce several angiogenic factors and may promote lesion angiogenesis [1, 13]. Furthermore, they can break down basement membrane and extracellular matrix via the release of several matrix metalloproteinases and their inhibitors [1]. The exact mechanisms by which eosinophils induce stromal invasion need to be elucidated via additional research.

The limitations of the present study are its retrospective design and the small number patients included.

In conclusion, the eosinophil count was higher in the patients with invasive cutaneous SCC than in those with AK. Additionally, lesion histological grade and the eosinophil count in the AK group and SCC group were not correlated. The present findings show that an elevated eosinophil count (especially ≥10 eosinophils 10 HPF-1) might be indicative of stromal invasion in cutaneous squamous neoplasias. As such, we think that quantitative evaluation of eosinophils should be included in routine histopathological examination of such lesions, and that additional sections should be obtained and examined thoroughly for focal invasion in pre-invasive cases with high eosinophilic infiltration.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

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