



# Assessment of stromal eosinophilia in cutaneous squamous cell carcinoma

Farah Falah Hassan Abu deka\* Alaa Qasim Yahya\*\*  
Mohammed Haider Fadhil\*\*\* Haider latteef mohammed \*\*\*\*



## ABSTRACT

**Background:** Tumor associated tissue eosinophilia (TATE) has been described in a variety of neoplasms. In regard to squamous cell carcinoma, some studies worldwide done to assess stromal eosinophilia in oral and cervical squamous cell carcinoma. The objectives of this study is to evaluate the association of stromal eosinophilic infiltration of cutaneous squamous cell carcinoma and to detect the significance of this association.

### Aim of the study

The aim of our study is to establish the relationship between the degree of stromal eosinophilia and the level of invasion and the histological grade in cutaneous squamous cell carcinoma.

**Methods:** In this retrospective study done at the histopathology department of al Wasitiy teaching hospital for orthopedic and plastic surgery in Baghdad, all paraffin embedded blocks for excisional biopsies diagnosed as primary cutaneous squamous cell carcinoma from December 2015 to January 2017 were retrieved. Incisional biopsies and those with margin positive tumor cells were excluded. After a careful evaluation for histological grading and assessment of level of invasion according to an established histological criteria, the assessment of tissue eosinophils was done by three pathologist through counting eosinophils in tumor stroma in 10 high power field using a light microscope and categorized as none (0), low (1-4), medium (5-19) and high (more than 20).

**Results :** of 56 cases of primary cutaneous squamous cell carcinoma 48(85.7%) cases were conventional. The mean age was  $43.14 \pm 1.76$ . Regarding conventional squamous cell carcinoma, of 48 cases 30(62.5%) were well differentiated, 16(33.3%) had

clarck level III, 18(37.5%) had clarck level IV and 14(29.1%) had Clark level V. There is a significant statistical relationship between histological grading of tissue eosinophilia and level of invasion with p value 0.044 and a non-significant relationship with degree of differentiation.

**Conclusion :** The majority of cutaneous squamous cell carcinomas were conventional well differentiated with a mean age in the fifth decade of life, a male predominance and often had a degree of stromal eosinophilia that is strongly associated with the level of invasion. Thus more Intense tissue eosinophilia is correlated with a higher level of stromal invasion. The significance of such an association should be regarded as either a potential predictive tool for stromal invasion in small incisional biopsy or as a prognostic indicator that could be included in the pathology report for conventional squamous cell carcinoma

**Key words:** Squamous cell carcinoma, tissue eosinophilia, clarck level of invasion.

\*Department of pathology/college of Medicine/Kerbela University/Iraq. (FIBMS) dr.farah.histopathology@gmail.com

\*\* Department of pathology/al Kindy College of medicine/Baghdad University (FIBMS) alaakasim1983@yahoo.com

\*\*\* Department of plastic surgery/(FIBMS) Medical city complex dr\_mohammed.h\_maash@yahoo.com

\*\*\*\* Department of pathology/ (FIBMS) Alwasiti teaching hospital/Bagdad /Iraq. haiderlatteef5@gmail.com

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## INTRODUCTION

Cutaneous Squamous cell carcinoma (cSCC) is the second most common type of skin cancer after basal cell carcinoma that arise from epidermal keratinocytes.<sup>1</sup> Actinic keratosis is regarded as the premalignant precursor for cSCC<sup>2</sup>, it has a strong association with ultraviolet light from sun exposure.<sup>3</sup> Men are more commonly affected, probably due to higher head and neck exposure to sun light (UVR).<sup>4</sup> The most typical presentation is a non-healing ulcer or growth in a sun exposed area.<sup>5</sup> wide varieties of SCC subtypes present, some of

which are associated with a more worse prognosis like basosquamous carcinoma.<sup>6</sup> However cSCC can be divided into three histologic grades, well differentiated, moderately differentiated and poorly differentiated according to degree of nuclear atypia and keratinization.<sup>7</sup> Verrucous carcinoma (VC) is believed to be a relatively indolent form of SCC that has bland, verrucous-like features. It was first described by Ackerman in 1948, and is closely related to both low-risk (types 6 and 11) and high-risk (types 16 and 18) types of

HPV.<sup>8</sup> Tumor stroma is infiltrated with many inflammatory cells like lymphocytes, macrophages, neutrophils, plasma cells, mast cells and eosinophils. The inflammatory cells in tumor stroma are mainly due to host response to tumour.<sup>9</sup> Tumor associated tissue eosinophilia (TATE) means the presence of eosinophils in tumor stroma and has been reported for almost 100 years in many carcinomas located in larynx, pharynx, lung, external genitalia, gastrointestinal tract and oral cavity.<sup>10</sup>

Eosinophils are regarded as destructive effector white blood cells with a cytotoxic activities and are also participate in tissue remodeling and in innate and acquired immune response but the exact cause and mechanism of action of eosinophil infiltration in tumor stroma have remained unknown.<sup>11</sup>

## METHODS

This is a retrospective study which done at the histopathology department of al Wasity teaching hospital for orthopedic and plastic surgery in Bagdad , where all paraffin embedded blocks for excisional biopsies diagnosed as primary cutaneous squamous cell carcinoma from December 2015 to January 2017 were retrieved from patient archives together with the associated clinical information such as age, gender, and tumor location . Incisional biopsies and those with positive margins were excluded. From each block 4  $\mu$ m thickness sections were cut and stained with routine hematoxylin and eosin (H and E ) and carefully evaluated for histological grades and assessment of level of invasion according to an established

histological criteria <sup>12</sup>.Then assessment of tissue eosinophils was done by counting eosinophils in 10 high power field in the stroma at advancing edge of tumor using a light microscope and categorized as none (0), low (1-4), medium (5-19) and high (more than 20)<sup>13</sup> .Statistical analysis done using Freeman-Halton extension of the Fisher exact probability test

## RESULTS

This study enrolled 56 cases of primary cutaneous squamous cell carcinoma ,age range from 24 to 80 years with a mean age of  $43.14 \pm 1.76$  , of total sample 42 were male and 14 were female with a male to female ratio 3/1 , 28 (50%) were located in sun exposed areas ,20(35.7%) were in unexposed areas and 8(14.2%) in an area of previous burn .Regarding histological types, 48(85.7%) cases were conventional squamous cell carcinoma ,4(7.1%) were basosquamous carcinoma and 4(7.1%) were verrucous carcinoma .

Regarding conventional squamous cell carcinoma, of 48 cases 2(4.1%) were poorly differentiated, 16(33.3%) were moderately differentiated and 30(62.5) were well differentiated. By assessment of level of invasion according to certain histological criteria developed by Wallace H. Clarck <sup>12</sup> of 48 cases, 16(33.3%) had clarck level III, 18(37.5%) were clarck level IV and 14(29.1%) had Clark level V.

By assessment of stromal eosinophilia in squamous cell carcinoma, the results listed in table 1.

**Table 1: Assessment of tissue eosinophilia in cutaneous squamous cell carcinoma**

Eosinophil's count	Conventional squamous cell carcinoma	Basosquamous cell carcinoma	Verrucous carcinoma	Total
Low	14	1	0	15
Medium	20	1	0	20
High	14	2	4	21
Total	48	4	4	56

By correlation of stromal eosinophilia in conventional squamous cell carcinoma with Clark level of invasion, there is a significant statistical relationship with p value 0.044 (<0.05) as shown in table 2

**Table 2: Correlation of stromal eosinophilia and level of invasion in conventional squamous cell carcinoma**

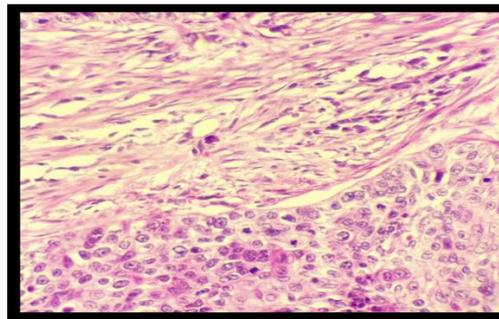
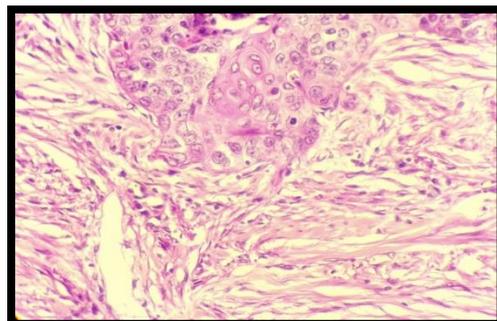
Eosinophil's count	Clarck level III <sup>(a)</sup>	Clarck level IV <sup>(b)</sup>	Clarck level V <sup>(c)</sup>	Total
Low	8	4	2	14
medium	6	10	4	20
High	2	5	8	14
Total	16	18	14	48

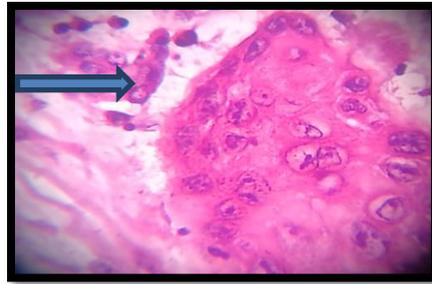
a, Invasion reach the junction between papillary and reticular dermis. b, Invasion of reticular dermis. c, Invasion of subcutaneous tissue

By correlation of stromal eosinophilia in conventional squamous cell carcinoma with histological grade, there is a non-significant statistical relationship with p value 0.044 (<0.05) as shown in table 3

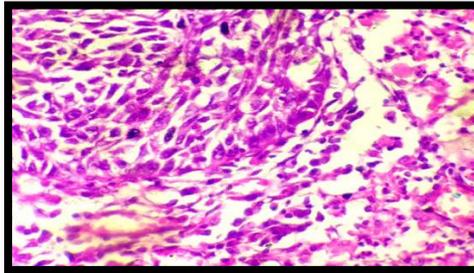
**Table 3: Correlation of stromal eosinophilia in conventional squamous cell carcinoma with histological grade (degree of differentiation)**

Eosinophil's count	Well differentiated	Moderately differentiated	Poorly differentiated	Total
Low	8	5	1	14
medium	12	7	1	20
High	10	4	0	14
Total	30	16	2	48

**Figure 1 (x100):** Histological feature of well differentiated squamous cell carcinoma with stromal inflammation**Figure 2: (x200):** Histological section of well differentiated squamous cell carcinoma with low stromal eosinophilia



**Figure 3:** (x400): Histological section of well differentiated squamous cell carcinoma with low stromal eosinophilia showed only one eosinophil (arrow) in this high power field



**Figure 4:** (x200): Histological section showed intense stromal eosinophilia in conventional squamous cell carcinoma.

## DISCUSSION

Development of invasive carcinoma is not simply a result of genetic mutations within the tumor cell alone but also associated with numerous changes in host endothelial, stromal, and inflammatory or immune cells<sup>(14)</sup>. The intratumoral and peritumoral inflammatory infiltrates when found in tumors have been considered as the host's immune reactions to the neoplasia<sup>(14, 15)</sup>. Eosinophils are commonly found in human cancer, however; their role in malignancies remains not clear. The literature described a tendency to consider TATE as favorable prognostic factor in head and neck squamous cell carcinoma (HNSCC), but TATE has also been described to be a poor prognostic sign or having no effect on patients' outcome showing that this issue remains a matter of controversy<sup>(14,15)</sup>.

Tumor associated tissue eosinophilia (TATE) has been studied in a variety of neoplasms<sup>10</sup>. In regard to squamous cell carcinoma some studies worldwide done to assess stromal eosinophils in oral squamous cell carcinoma and only limited studies done to evaluate the association of

stromal eosinophilic infiltration of cutaneous squamous cell carcinoma .

The study showed that the mean age of patient with cutaneous squamous cell carcinoma was  $43.14 \pm 1.76$  which is lower than the mean age in one Iraqi study done by Khalil H. Al-Jeboori et tal.<sup>16</sup> which found that the mean age was in the sixth decade of life ,the difference may be due to a smaller sample size used by the latter or may indicate a recent decrease in the mean age for cutaneous squamous cell carcinoma in Iraq and necessitate early application of a protective measure ,but our results agrees with a worldwide study done by Christenesonn LJ et tal.<sup>17</sup> which also found the incidence of cutaneous SCC is increasing significantly in younger individuals. This study showed men were more commonly affected which agree with two studies.<sup>18, 19.</sup>

The present study revealed that the majority of squamous cell carcinoma were well differentiated which agree with an Iraqi done by Khalil H. Al-Jeboori et tal.<sup>16</sup> ,the study also showed that 50% of squamous cell carcinoma were located in sun exposed areas which agree with a study of Pedro Andrade et tal.<sup>19</sup>

By correlation of stromal eosinophilia in conventional squamous cell carcinoma with Clark level of invasion in our study, there is a significant statistical relationship with p value 0.044 (<0.05)

This result agree with one study done by Reyhane et al.<sup>20</sup> In which there was significant relationship between clinical stage and mean eosinophil count in both oral squamous cell carcinoma and cutaneous squamous cell carcinoma groups with (P<0.0001) supporting the fact that eosinophils produce proteases which stimulate invasion in carcinomas .No such Iraqi study done to compare with.

By assessment of stromal eosinophilia with histological grade there is a non-significant relationship, a result disagrees with a study done by S. Rahrotaban et al.<sup>13</sup> in which, the mean eosinophil count in squamous cell carcinoma group was found to be significantly higher than dysplastic group, suggesting that they might have a role in stromal invasion and that well differentiated squamous cell carcinoma had significantly higher counts compared to moderately differentiated squamous cell carcinoma probably because the latter study focused on both cutaneous and oral squamous cell carcinoma as a total sample. Thus stromal eosinophilia in cutaneous squamous cell carcinoma unlike oral squamous cell carcinoma doesn't correlate with histological degree of differentiation.

## CONCLUSION

The majority of cutaneous squamous cell carcinomas were conventional, well differentiated, with a mean age in the fourth decade of life ,a male predominance and often had a degree of stromal eosinophilia that is statistically associated with the level of invasion .Thus more intense tissue eosinophilia is correlated with a higher level of stromal invasion. The significance of such an association should be regarded as either a potential predictive tool for stromal invasion in small incisional biopsy or as a prognostic indicator that could be included in the pathology report for conventional squamous cell carcinoma.

## Recommendation

A prospective study using a larger sample size is recommended to detect any clinical association of stromal eosinophilia in

conventional squamous cell carcinoma and if there is an association with blood eosinophilia in order to investigate the role of eosinophils in tumor growth , an issue of great importance in cancer therapy in future .

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