ORIGINAL ARTICLE

QUANTIFICATION OF CD1A+ LANGERHANS CELLS IN ORAL EPITHELIAL DYSPLASIA AND ORAL SQUAMOUS CELL CARCINOMA

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Background: To quantify the immunohistochemical expression of CD1a positive Langerhans cell population and find their association with tumour progression in oral epithelial dysplasia and oral squamous cell carcinoma. **Methods:** A total of 119 biopsies were collected out of which 61 were oral epithelial dysplasia (20 mild, 20 moderate, 21 severe) and 58 were oral squamous cell carcinoma (well differentiated only). Fresh haematoxylin and eosin slides were prepared followed by application of CD1a immunohistochemical marker to quantify Langerhans cell in epithelium and connective tissue separately. To compare quantitative results One Way ANOVA and Independent Sample T test was used. ≤0.05 was considered a significant *p*-value. **Results:** A statistically significant association between CD1a+ Langerhans cell count and advancing degrees of dysplasia to well differentiated oral squamous cell carcinoma in both epithelium (<0.05) and connective tissue (<0.05) was seen. Significant difference was also seen when oral epithelial dysplasia was compared with well differentiated oral squamous cell carcinoma in epithelium (p<0.05) but, no significant difference (p>0.05) was seen in case of connective tissue. **Conclusion:** The mean CD1a+ Langerhans cell count decreased with increase in degree of dysplasia and an increase in well differentiated oral squamous cell carcinoma was seen which needs further studies. Mean CD1a+ LC count can be used as a tool for highlighting disease progression for epithelial dysplasia and increase in mean CD1a+ Langerhans cell in oral squamous cell carcinoma can be argued with its better prognosis among other histological grades of this carcinoma.

Keywords: CD1a; Langerhans cell; Oral epithelial dysplasia; Oral squamous cell carcinoma; Tumour progression; immune response

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INTRODUCTION

Oral cancer remains a serious public health problem globally as *GLOBOCAN* (2018) data showed 354,864 new cases worldwide of lip and oral cavity cancer with oral squamous cell carcinoma (OSCC) being the most common histological type and even more prevalent in south central Asia.[1] Compared with approximately 4% in developed countries, in Southeast Asia 40% of all cancers are OSCC.[2] Despite widespread advancements in the field of research, significant mortality and morbidity rates are associated with OSCC.[3] For the past 50 years, the five year survival rate remains approximately 50%.[2] The risk factors linked with OSCC include use of tobacco, alcohol consumption, toxic cultural habits, human papillomavirus infection, epstein-barr virus infection and oral potentially malignant disorders.[4] These oral potentially malignant disorders include oral lichen planus and oral leucoplakia which can develop dysplasia, and in one third of cases these lesions may develop into OSCC.[5] This led to finding biomarkers with early diagnostic relevance to predict which oral epithelial dysplasia (OED) lesion has higher potential to develop into malignancy. Immune system plays a key role in defence against epithelial tumours, hence identifying the distribution of tumour infiltrating immune cells help in the management of tumour by predicting prognosis.[6]

Dendritic cells which present antigens play a key role in B and T cell mediated immune response. Distribution of Langerhans cells (LC), dendritic cells of epithelium, in mucous membranes and epidermis are responsible for this immunosurveillance in defence against epithelial tumours, hence of prognostic value.[7] These LC present antigen to T-cells and act as a linkage between innate and adaptive immune responses.[8] Compared to normal lip mucosa, a decrease in LC number is seen in lip squamous cell carcinoma and actinic cheilitis.[9] Also a decrease in LC is seen in OSCC compared to OED.[10] However, some other studies have shown a gradual increase in LC number during progression of oral carcinogenesis.[11]

Hence comparison of distribution of these cells in OSCC and OED need further studies especially in south east Asia, as 90% of global smokeless tobacco users are present in this region according to the report of WHO published in September 2013.[12]

It is of immense importance to find immune responses in OED so that strategies to enhance immunity in these can be strengthened.[13] Antigen presenting cells induce primary immune response and among them dendritic cells are most potent and a potential target for immunotherapy. Even in cancer immunotherapy dendritic cells are best suitable for delivery of tumour specific antigens.[7] Dexosomes secreted by dendritic cells have been used as cell-free anticancer vaccine vehicle in several clinical trials.[14] Immunopathogenesis of lesions such as gingivitis and periodontitis, recurrent aphthous stomatitis, contact hypersensitivity, oral lichen planus and even reasonably oral cancers are linked with LC of oral cavity.[15] For detecting LC available immunohistochemistry markers include CD1a, CD83, CD207 etc.[5] To identify these LC in inflammatory, dysplastic or neoplastic disorders, CD1a is considered the most reliable immunohistochemistry marker.[16] CD1a is a human protein which mediates the presentation of self or microbial origin primarily lipid and glycolipid antigens to T cells.[17] In OSCC, a strong independent prognostic factor is the depletion of CD1a+ cells.[18] Therefore, our study is intended to determine the quantification of CD1a+ LC and their association with tumour progression which may help in predicting prognosis in OED and OSCC. This may also help in the management of tumours as immunotherapeutic agents.

MATERIAL AND METHODS

The study was conducted in Department of Histopathology of Armed Forces Institute of Pathology, Combined Military Hospital, Rawalpindi, Pakistan. All instruments, machinery and reagents needed for conducting this research project were available in their histopathology and immunohistochemistry labs.

It was a cross-sectional study done on recently biopsied samples of one year. The total sample size was 119 which included 61 biopsies of OED and 58 biopsies of OSCC. This sample size was calculated by using WHO sample size calculator using their respective global prevalence. The confidence level of 1.96, margin of error at 0.05, design effect 1 and expected response rate of 0.8 were used to calculate this sample size. The sampling technique was non-probability convenience sampling and among 61 biopsies of OED, 20 were mild, 20 moderate and 21 biopsies were of severe OED but all 58 biopsies of OSCC were well differentiated.

The inclusion criteria included patients from both genders, all age groups, all recent biopsies of mild, moderate and severe OED and well differentiated OSCC diagnosed on routine haematoxylin and eosin staining. The exclusion criteria however included poorly fixed or autolyzed tissue specimens and very scanty tissue specimens. Biopsies of post-chemotherapy and radiotherapy patients were also excluded from this study.

An ethical approval was taken beforehand from the Institutional Review Board (IRB) of Armed Forces Institute of Pathology. Already diagnosed biopsy specimens of OED and OSCC from the histopathology department of Armed Forces Institute of Pathology were selected through non-probability convenience sampling method till we reached the required sample size.

For the selected cases, patients were called and informed consent forms were signed for the use of their biopsy samples and clinical records. These were recorded from the histories presented onto the data collection questionnaire. Confounding factors were excluded by firmly following the exclusion and inclusion criteria.

Haematoxylin and eosin slides were prepared freshly for already diagnosed cases, but to eliminate bias the slides were viewed first by trainee and then independently confirmed by two investigators. After confirmation of diagnosis on freshly prepared haematoxylin and eosin slides, CD1a immunohistochemical markers by Leica Microsystem (Germany) were applied according to the company's guidelines along with control of CD1a for each batch.

The sections with an inter observer difference of more than 10% were re-examined by means of a multi-headed light microscope to reach consensus. Results were then statistically analysed.

Skin biopsy was used as a control as in Figure 1. CD1a shows predominantly membranous staining in mucosal tissues as in Figure 2.

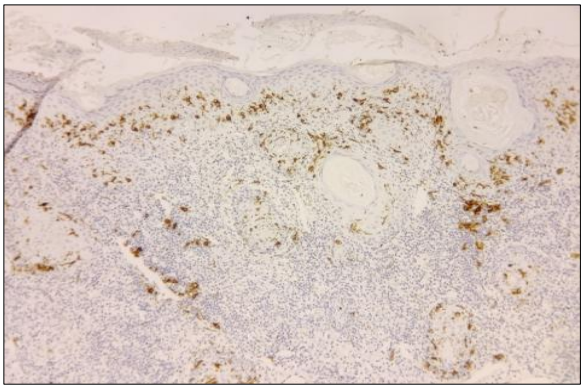

Figure-1: Skin biopsy as control (40X)

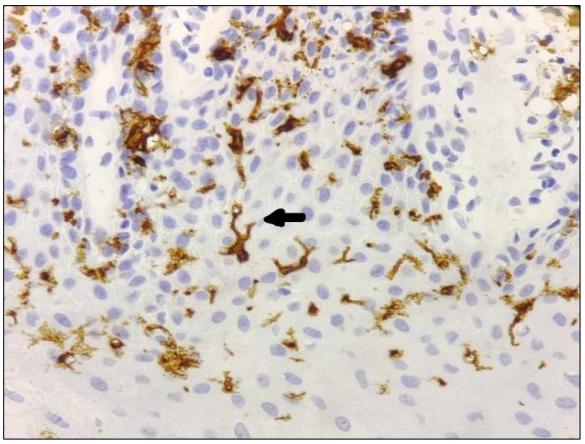

Figure-2: LCs in oral mucosa showing membranous staining (400X)

The tissue sections were scanned at low power initially. Areas showing positive staining with prominent heterogeneous pattern were considered for counting of LC. LC were counted positive when:

1. A prominent brown staining was seen.
2. Cell body with at least one dendritic process was seen.

CD1a+ cells were counted manually in three high power fields (400X) of epithelium and connective tissue each which were selected haphazardly. LC count was the mean number of positive cells in three high power fields. In sections with more than 10% inter observer variation; a consensus was achieved using a multi-headed light microscope.

SPSS version 23.0 was used for statistical analysis and Microsoft Excel 2010 was used for diagrammatic representations. Quantitative variables (age, quantification of CD1a IHC marker in epithelium and connective tissue) were presented as mean and standard deviation and qualitative variables (gender, site, degrees of dysplasia and well differentiated OSCC) were presented as frequency and percentages. To compare results and assess the significance of data Independent Sample T test and One Way ANOVA were applied. ≤0.05 was considered a significant *p*-value.

RESULTS

Out of 119 patients the gender distribution was almost same, 60 (50.4%) were men and 59 (49.6%) were female. For OED, 31 (50.8%) were males and 30 (49.2%) were females. Male and female patients of OSCC were equal, 29 (50.0%) each. These are shown as in Figure-3.

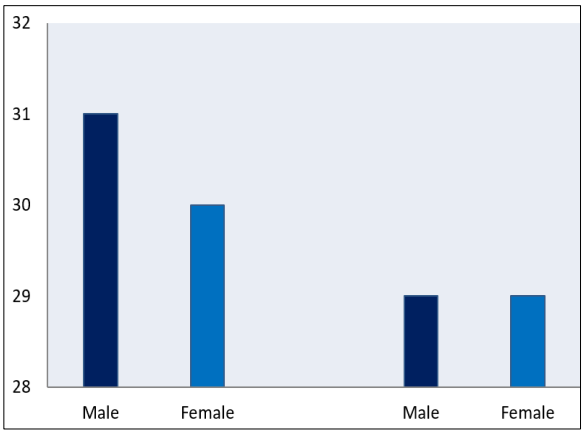

Figure-3: Gender distribution of patients in OED and OSCC

The overall mean age of participants who participated in the study was 57.04±12.21 (mean±SD) with overall mean age of males being 56.75±12.48 and overall mean age of females being 57.34±12.03. The oldest patient was of 90 years old and the youngest of 24 years. Among the patients of OED, the mean age of males was 57.13±13.21 and 60.77±10.45 for females. Similarly for patients of well differentiated OSCC the mean age of males and females were 56.34±11.88 and 53.79±12.70, respectively. These are represented as in Table-1.

Table-1: Mean age distribution in OED and OSCC of both genders

Gender:	Mean Overall Age±SD	Mean OED Age±SD	Mean OSCC Age±SD
Male	56.75±12.48	57.13±13.21	56.34±11.88
Female	57.34±12.03	60.77±10.45	53.79±12.70

The overall age distribution of participants according to age groups of <50 years and ≥50 years included 31 (26.1%) and 88 (73.9%) participants, in respective groups. These age groups distribution in OED and OSCC is shown as in Figure 4.

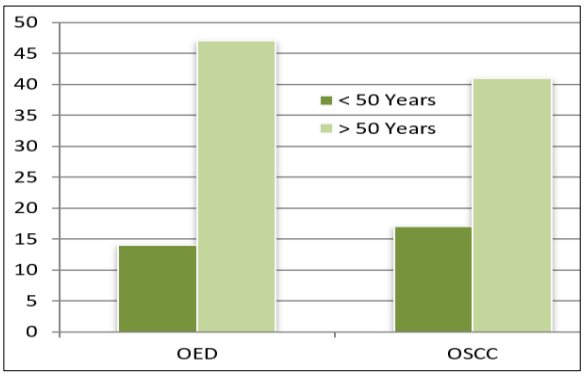

Figure-4: Age distribution according to age groups in OED and OSCC

Figure 5 shows the site distribution of patients with OED and OSCC. Majority of the biopsies were from buccal mucosa 53 (44.5%) as opposed to other sites. This included tongue 33 (27.7%) followed by floor of mouth, alveolus and sites not specified grouped together as others 33 (27.7%).

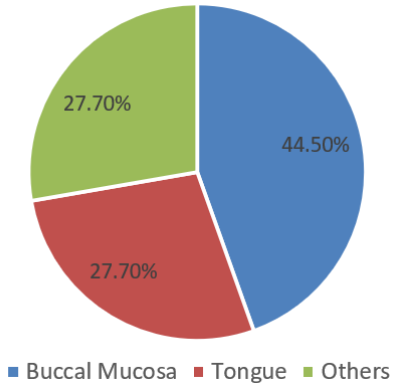

Figure-5: Site distribution of patients with OED and OSCC

The mean CD1a+ LC count within the epithelial compartment of OED and OSCC was significant (p <0.05, Independent Samples T test), whereas within the connective tissue no significant difference (p >0.05) was observed in OED and OSCC as shown in Table-2.

Table-2: Mean CD1a+ LC count in epithelium and connective tissue of OED and OSCC

Groups:	Mean CD1a+ LC count±SD (cells / high power field)	
	Epithelium	Connective Tissue
OED (n=61)	2.57±1.43	0.98±0.76
Well differentiated OSCC (n=58)	2.06±1.31	1.23±0.92
t	2.02	-1.57
p-value	**0.04**	0.11

There was a significant difference in the mean CD1a+ LC count of both the epithelium and connective tissue of mild, moderate, and severe OED to OSCC groups (p<0.05, One Way ANOVA) as shown in Table-3.

Table-3: Mean CD1a+ LC count within grades of OED to well differentiated OSCC

Lesions:	Mean CD1a+ LC count±SD (cells / high power field)	
	Epithelium:	Connective Tissue:
Mild OED (n=20)	3.28±1.57	1.51±0.76
Moderate OED (n=20)	2.76±1.13	1.04±0.68
Severe OED (n=21)	1.72±1.16	0.42±0.38
Well differentiated OSCC (n=58)	2.06±1.31	1.23±0.92
F	6.52	7.49
p-Value	**0.00**	**0.00**

Mean CD1a+ LC count decreases as the grade of dysplasia increases in both the epithelium and connective tissue but a slight increase is seen when we reach OSCC which in epithelium is between the mean LC count of moderate and severe OED but in connective tissue lies between mild and moderate OED as shown in Figures 6 and 7 respectively.

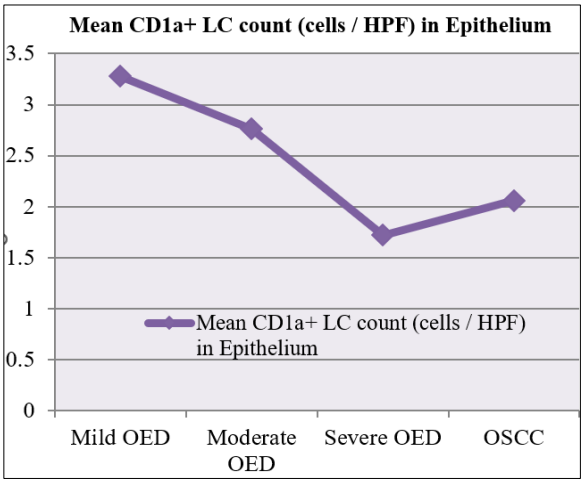

Figure-6: Mean CD1a+ LC count within grades of OED to well differentiated OSCC in Epithelium

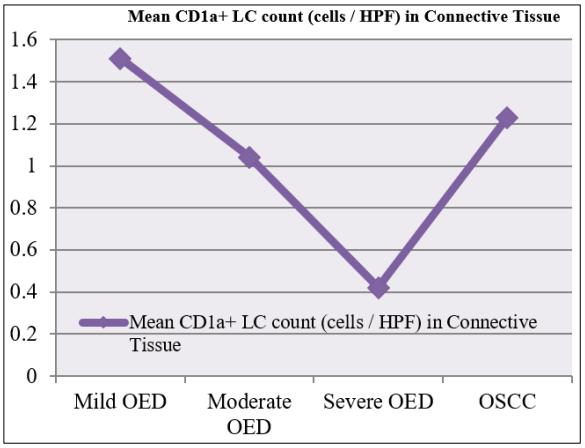

Figure-7: Mean CD1a+ LC count within grades of OED to well differentiated OSCC in Connective Tissue

Associations between mild OED versus severe OED, mild OED versus OSCC, moderate OED versus severe OED and moderate OED versus OSCC were statistically significant (p <0.05) for the mean CD1a+ LC count in epithelium. Similarly, associations between mild OED versus moderate OED, mild OED versus severe OED, moderate OED versus severe OED and severe OED versus OSCC were statistically significant (p<0.05) for the mean CD1a+ LC count in connective tissue. All other possible comparisons done are insignificant (p>0.05) as shown in Table-4.

Table-4: Comparison of mean CD1a+ LC count between all four lesions

Lesions:	Epitheliu m:		Connective Tissue:	
	t	*p*-value	t	*p*-value
Mild OED vs Moderate OED	1.19	0.24	2.04	**0.04**
Mild OED vs Severe OED	3.58	**0.001**	5.84	**0.00**
Mild OED vs OSCC	3.10	**0.001**	1.34	0.18
Moderate OED vs Severe OED	2.89	**0.006**	3.60	**0.001**
Moderate OED vs OSCC	2.28	**0.02**	-0.82	0.41
Severe OED vs OSCC	-1.10	0.27	-3.85	**0.00**

Independent Sample T test showed that there was no significant difference ($p<0.05$) between the gender and age of patients in relation to mean CD1a+ LC count in epithelium and connective tissue of patients with OED and OSCC. One Way ANOVA also showed similar insignificance ($p<0.05$) between the site of lesion and mean CD1a+ LC count in epithelium and connective tissue of OED and OSCC patients. These are as shown in Table-5.

Table-5: Clinicopathological correlation of mean CD1a+ LC count in epithelium and connective tissue of OED and OSCC

Clinicopathological Parameter	Quantification of Mean CD1a+ LC count±SD (cells / high power field)	
	Epithelium	Connective Tissue
Gender of Patients		
Male (Mean±SD)	2.32±1.32	1.07±0.87
Female (Mean±SD)	2.33±1.48	1.14±0.84
t	-0.02	-0.47
p-value	0.98	0.63
Age of Patients:		
<50 Years (Mean±SD)	2.18±1.24	1.10±0.89
≥50 Years (Mean±SD)	2.38±1.45	1.10±0.84
t	0.73	0.01
p-value	0.46	0.99
Site of Lesion in Patients:		
Buccal Mucosa (Mean±SD)	2.03±1.36	1.08±0.93
Tongue (Mean±SD)	2.59±1.43	1.11±0.82
Others (Mean±SD)	2.53±1.36	1.15±0.76
F	2.15	0.06
p-value	0.12	0.93

DISCUSSION

OSCC is a serious global health issue especially in Southeast Asia and it comprises majority of the oral cancer burden.[2] Among other risk factors, OED has the highest chance to develop into a malignancy and sometimes on routine H&E it is not easy to state which OED has a higher chance to develop into a malignancy.[5] Early and accurate diagnosis remains the key to effectively tackle this disease; hence biomarkers with early diagnostic and prognostic relevance are of paramount importance. Currently no treatment is available to prevent OED to transform into OSCC and no definite prognostic modality present to predict which OED has a higher potential to develop into a malignancy.[13]

Identifying the dispersal of tumour infiltrating immune cells and their association with tumour progression help in predicting prognosis.[6] One such immune cell is the LC which is responsible for this immunosurveillance and CD1a is considered the highest reliable immunohistochemical marker to detect these cells.[16] In our study we assessed the quantification of mean CD1a+ LCs and their association with tumour progression in mild, moderate, severe OED to well differentiated OSCC.

In our current study, a total of 119 biopsies were selected which comprised 58 biopsies of well differentiated OSCC and 61 biopsies of OED from department of Histopathology, AFIP, Rawalpindi. Male to female ration was almost 1:1 as overall 50.4% were males and 49.6% were females. This conflict with a recent local study carried on nearly the same sample size in which males constituted 77.7% of the cases and 22.3% were females showing a huge male predominance.[19] As this study was conducted in Karachi where use of tobacco related products is a main cause of oral cancer and its predominance among males may be attributed to their social freedom to use these products. Another study from our same setting also showed a male predominance of 59.4% males compared to 40.6% females. This corresponds with international and local studies.[20] Male predominance is seen in majority of researches all over world.[21] These contradictory results with our study might be due to sampling technique and sample selection bias where we kept almost equal male to female ratio.

The mean age of participants was 57.04±12.21 (mean±SD) with the oldest patient of 90 years and youngest of 24 years and majority of the patients presented with lesions on buccal mucosa followed by tongue. This corresponds with the previous studies conducted in AFIP Rawalpindi, where mean age was 60.63±13.814 and buccal mucosa was the commonest site.[20] Other studies from Pakistan also showed buccal mucosa being the commonest site followed by tongue which conflicts with the international studies where tongue is the first common site and buccal mucosa being the second.[19] This is due to oral habits of our population with regards to use to tobacco products kept in the buccal pouch. In a study conducted in India where oral habits are similar to our population, we noticed that buccal mucosa was again the most common site.[22]

The frequency of patients with OED and OSCC below 50 years of age were 23% and 29.3%, respectively and the ones 50 and above were 77% and 70.7%, respectively. These relate with research conducted in America with a huge sample size of 454, where participants below the ages of 50 years for OED

and OSCC were 17.7% and 19.4%, respectively and those 50 and above were 82.3% and 80.6%, respectively.[23]

The key finding of our study was a reduction in mean CD1a+ LC count in the epithelial and connective tissue compartments from mild to moderate to severe dysplasia. This finding portrays the association of tumour progression with respect to distribution of tumour infiltrating immune cells such as LCs. Interaction of dysplastic and immune cells characterizes the concept of cancer immunoediting in which immune cells during initial stage of dysplasia can eradicate damaged cells that have resulted in neoplastic change.[13]

Another important finding was decrease in mean CD1a+ LC count in epithelial compartment of well differentiated OSCC compared with all OED lesions combined but in connective tissue an increase was seen. However, when degrees of dysplasia and well differentiated OSCC were compared, it was observed that mean CD1a+ LC count in epithelial compartment of well differentiated OSCC increased than severe dysplasia but was less than mild and moderate dysplasia. Similarly, an increase was seen in the connective tissue compartment where well differentiated OSCC was more than severe and moderate both but was still less than mild dysplasia.

Possible reasoning for the increase of CD1a+ LC count in epithelial compartment of well differentiated OSCC compared to severe OED could be ulceration, secondary infection or even trauma. Whereas increase of CD1a+ LC count in connective tissue of well differentiated OSCC more than moderate and severe OED and also overall OED could be due to organized recruitment of these cells by tumour microenvironment as suggested by substantial literature evidence.[24]

There have been many conflicting results in published studies with regard to LC counts in OED and OSCC and with different possible reasoning. According to a study conducted in Taiwan in 2017, mean CD1a+ LCs in OED lesions with malignant transformation was found to be less than the OED lesions without malignant transformation, suggesting LCs immunosurveillance ability decreases with increase in degree of dysplasia to ultimately invasive carcinoma. However, this same study also showed that as the degree of dysplasia increased from mild to moderate to severe the mean CD1a+ LC count in both epithelial and connective tissue compartments increased.[11] Girod *et al* displayed similar results where the mean S100+ LCs in OSCC was found to be less than oral benign lesions.[25] Both these studies contradict with our results especially in connective tissue compartments where a rise in LC count was seen in well differentiated OSCC from OED. Also, in our study a decrease in mean CD1a+ LC count was observed as the degree of dysplasia increased from mild to severe dysplasia which does not correlate with the results shown in first study. But a decrease in LC count in epithelial compartment of well differentiated OSCC with combined OED lesions is similar to the results shown in second study, though those results included epithelium and connective tissue means collectively and S100 IHC was used to quantify LCs.

Another study conducted in Japan opposes our results as according to their results, LC count in epithelium and connective tissue both increases from mild to severe dysplasia, but one similarity among the results was that LC count was more in epithelium compared to connective tissue.[26] The difference in outcomes might be due to difference of type of assessment methods and site from where specimens were taken intraorally.

Outcomes of a study in 2017 showed some similarity to ours in results where mean CD1a+ LC count reduced from OED to OSCC in epithelium with a possible reasoning that these antigen presenting LCs might have travelled to lymph nodes to stimulate the immune system by presenting tumour antigens.[10] Other reasoning could be that under the effect of tumour derived factors there was a lack of CD1a expression as it was the sole IHC marker used to detect these cells.[27]

A very detailed study conducted to compare epithelial and connective tissue compartments of all grades of OED and OSCC revealed that mean CD1a+ LC count decreased from mild to severe OED in epithelial compartment similar to our results. A similar decrease was seen from mild to severe OED in connective tissue. For well differentiated OSCC, huge increase was seen in mean CD1a+ LC count in connective tissue compartment which was even more than mild dysplasia but in epithelium the increase was less than mild dysplasia yet still more than moderate and severe dysplasia. Overall, a decrease in mean LC count in epithelium and an increase in mean LC count of connective tissue was seen which was exactly similar to our results.[24]

A study done in Belgium to assess LC count as a prognostic factor for head and neck squamous cell carcinoma (HNSCC) proved that increase in quantity of these cells, especially in stromal compartment, is an independent prognostic factor for HNSCC and allied with longer recurrence free survival.[28] This correlated with our results as we only included well differentiated OSCC cases which have better prognosis then moderate and poor OSCC cases and the mean LC count of these well differentiated OSCC was higher than even severe to moderate dysplasia in epithelium and connective tissue, respectively, possibly indicating better prognosis. The study also showed that

LC count was higher in HNSCC than oral dysplastic lesions in both epithelium and connective tissue and this too correlates with our results for connective tissue.

Araujo *et al* in one of the studies assessed the variation in mean CD1a+ LC count in actinic chelitis with degrees of epithelial dysplasia. This showed that mean CD1a+ LC count was highest in severe dysplasia and a decrease was seen from mild to moderate dysplasia.[29] This decrease correlated with our results but the increase in severe dysplasia might have been due to disproportionate number of the three lesions as severe dysplasia was only 14.29% of the whole sample size. Also, in this study there was no statistical difference in mean LC count amongst degrees of dysplasia and actinic chelitis.

According to the conclusion of a study by Da Silva *et al* in 2020, a reduction in mean CD1a+ LC count maybe linked to the development of OSCC and in OPMDs this might be a display of malignant transformation.[5] These observations strengthen the hypothesis of our study that there is an association of CD1a+ LC count with tumour progression in OED and OSCC. Our results also support this to some extent as a decrease in CD1a+ LC count is seen from mild to severe dysplasia indicating a tumour progression and also a decrease is seen in the epithelial compartment when LC count in combined OED lesions is compared with well differentiated OSCC though this differs from the results of connective tissue and also when severe dysplasia and well differentiated OSCC is compared. Difference in sample size between severe dysplasia and well differentiated OSCC could be one of the reasons.

Another study by Vargas *et al* in 2017, with equal cases of epithelial dysplasia and OSCC discovered an increased CD1a+ LC count in epithelial dysplasia matched with OSCC. This study also strengthened our hypothesis and concluded that this increase showed that there is an association of CD1a+ cell count with tumour progression.[30] Possible justification for this is that an increase in CD1a+ LC count indicates an increase in immune response to dysplastic cells and hence, higher count means longer recurrence free survival as mentioned in one of the previous studies.[24]

We performed Independent Sample T test, on any two lesions independently in both epithelium and connective tissue, to compare mean CD1a+ LC count among all four lesions and all results were significant except when we compared mild OED with moderate OED in epithelium and severe OED with well differentiated OSCC in epithelium. Possible reasoning for these both could be that these comparisons are among lesions which are close to each other in tumour progression where presence of difference in LC counts might not be significant. However, according to the study by Wang *et al*, all similar comparisons in epithelium and connective tissue were done and they were all significant.[11] Contrary to this, results of another study showed no statistical difference in LC count with respect to degrees of dysplasia.[29] Insignificant results were also seen when we compared mean CD1a+ LC count in mild OED and moderate OED with well differentiated OSCC in connective tissue individually.

We also compared mean CD1a+ LC count in degrees of dysplasia and well differentiated OSCC in both epithelium and connective tissue by One Way ANOVA and a significant difference was seen among them ($p<0.05$). Comparable results were witnessed in three studies, where significant difference were found in mean CD1a+ LC counts in epithelium and connective tissue between degrees of dysplasia and OSCC.[10,28,31]

Another comparison between mean CD1a+ LC count in OED combined was compared with well differentiated OSCC in epithelial compartment and a significant difference was found in our results ($p<0.05$). This compares with a study done in 2017 where significant results were seen but, in this study, the epithelial and connective tissue were combined.[30] In one more similar study comparing OED combined with well differentiated OSCC only like ours, significant difference was seen as well.[22]

Results of clinicopathological correlation of mean CD1a+ LC counts with gender, age of patients and site of lesions were insignificant in our study. Yet comparing LC count to another study, it was noticed that tongue had the highest number of LC counts.[31]. Similar results were also observed in one more study in which females and patients 50 years or above had highest number of mean CD1a+ LC count.[11] These both results were similar to ours.

There may be many reasons due to these overall conflicting results among available literature and also our study. These differences might be due to different types of IHC markers used to quantify LCs, although CD1a is still considered the most widely used and reliable one.[16] Also, the assessment methods to quantify these cells vary from one study to another resulting in inter observer variations, to the extent that some quantified epithelium and connective tissue separately and others even collectively. Even the sites from where these dysplastic and malignant lesions were taken vary, as in our population these lesions were common in buccal mucosa whereas in other international studies tongue was the commonest site and this could result in difference in the presence of these immune cells.[19]

These reasons present possible limitations for comparison among several studies, yet this can be

agreed upon that an imbalance of immune system, especially in patients who are immuno-compromised, can result in the development of cancer. Hence, an ample response of mucosal immune cells is required to guard the oral mucosa against malignant transformation.[10]

One of the limitations of our study was difference of sample size between each degree of dysplasia compared with well differentiated OSCC. This was due to the limited time and resources for collection of OED samples. Also, ideally equal numbers of moderate and poorly differentiated OSCC should have been added but as very few cases of these could have been recovered, they were not included in our study. Another limitation of this study was being carried out in one centre which cannot be generalized. The immune status and other risk factors of the participants were not taken into account which could have altered the results but this study offers a platform for future analysis of these versatile LCs.

Further recommendations include a larger sample size and equal number of mild to severe OED and well to poor OSCC. Also, the immune status and other risk factors should be considered and large-scale multi-centre studies are recommended to validate the results. Lastly to confirm the prognostic value of LCs, longitudinal studies are encouraged to assess association with recurrence free survival in OED and OSCC which may help in classifying individuals as low or high risk.

CONCLUSION

LCs are antigen presenting cells of mucosal epithelium and first in the line of defence against any foreign antigen. Our study showed that their number varies in different oral mucosal disease conditions. This change in LC count shows the activity of immune system in protecting against epithelial changes. The changes range from normal to hyperplastic to dysplastic (degrees) and ultimately invasive carcinoma with different grades. Hence, this can be indicated that quantifying the LC count is an important prognostic marker and this can be utilized in cancer immunotherapies.

We may conclude that our study showed a statistically significant association between CD1a+ LC count and advancing degrees of dysplasia to well differentiated OSCC in both epithelium and connective tissue. Mean CD1a+ LC count decreased with increase in degree of dysplasia showing they can be used as a tool for highlighting disease progression. However, an increase in well differentiated OSCC was seen which can be argued with its better prognosis among other histological grades of OSCC for which further studies are needed. Significant difference was also seen when OED was compared with well differentiated OSCC in epithelium and an increase in mean CD1a+ LC count in OED advocated the protective role of LCs against epithelial tumours. Larger and clinically correlated longitudinal studies are needed to further validate this.

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AUTHORS' CONTRIBUTION

SWB: Methodology, investigation, writing, original draft. MA: Supervision, funding acquisition, resources. FZ: Writing, review & editing, formal analysis, project administration. AA: Visualization, validation. AH: Data curation. SM: Conceptualization.

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