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Original Article

Langerhans Cells Distribution in Oral Mucosa of Immunosuppressive Mice Associated with and without Oral Candidiasis: A Comparative Study

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Abstract

Context: Langerhans cells (LCs) are dendritic cells residents in epithelial tissue, which motivate the immune system against to pathogen entry, self-antigen, commensal microbes and protect the mucosa. Langerhans cells in oral mucosal are responsive to wide microbes such as oral Candida species that play immunological activity in immunosuppressed cases. *Aims*: The study was aimed at compering the distribution of LCs in in immunodeficiency mice associated with/without oral candidiasis. *Settings and Design:* Thirty female mice were distributed in three groups; control group (C group), immunosuppressed group without candida infection (IM group) and immunosuppressed group with candida infection (IM+*C. albicans* group). *Methods and Material:* The dorsal tongue surface stained with CD1a marker and the number of LCs was calculated. *Statistical analysis used:* was performed using analysis to determine the difference in the number of LCs between groups by analysis of variance or Student t test with unequal variances. A P value of <0.05 was regarded to be statistically significant. *Results:* The statistically examination of lingual sections showed high significantly different in LCs cells number between each group ($p< 0.05$). The greatest number of LCs was observed in (IM+ *C. albicans* group) with the Mean±SD: 102±31.30, and the lowest number was observed in (C group) with the Mean±SD: 17±14.29, while (IM group) recorded Mean±SD: 44.2±9.95. *Conclusions:* The increase in (IM+*C. albicans* group) might return to the role of antigenic exposure that leading to cell mediated immunity in oral candidiasis.

Keywords: Langerhans Cells (LCs); CD1a; Oral Mucosa; Immunosuppressed; *Candida Albicans*.

Introduction

Stratified squamous epithelium of oral mucosa contain Langerhans' cells (LC) which are dendritic, antigen-presenting cells. Langerhans' cells (LC) have a role function the immune systems the peripheral arm [1]. LCs is driving immune response against antigenic pathogens in the epithelial surface for secure of body's integrity and homeostasis , most of them peripheral organ, As sentinels of immune system [2]. The mucosal LCs is imperative mediators of mucosal immunity, it have immune reaction to antigen of microbial and tumor, yet additionally of resilience to self-antigen and commensal microbes. oral mucosal LCs have been discovered receptive to several other diseases such as nickel in patients with nickel allergies, oral Candida species, oral lichen planus, immunodeficiency virus (HIV) infection and oral squamous cell carcinoma [3]. Oropharyngeal candidiasis and gastrointestinal *C. albicans* infection are more wide demonstrated in, immunocompromised patient populations (e.g. AIDS and transplant patients, and patients undergoing steroid therapy) [4]. The defenses mechanisms of innate and adaptive immunity in host against *C. albicans* incorporate mechanical barriers to fungal entrance epithelial surfaces and soluble antimicrobial factors [5].

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Cytokines and chemokines are regulating responses proteins to infection secreted by epithelial cells in response to *C. albicans* hypha attack and activate immune cells. The innate and adaptive immune response mechanisms ultimately clear the fungus or reduce fungal burdens below the activation threshold, there by re-establishing the commensal phenotype [5]. The adaptive arm of the anti-*C. albicans* response is initiated through recruitment of dendritic cells (or langerhans cells) to the site of mucosal infection, Dendritic cells will recognise *C. albicans* through established pattern recognition receptors and traffic to the local lymph node where processed fungal antigen will be presented to T cells to initiate adaptive immunity [4] the current study goals are to quantitatively the number of oral mucosal LCs in immunodeficiency mice associated with/without oral candidiasis with a view to elucidate their role in pathogenesis. Subjects and Methods:

Animal Model

Reduce immunity thirty female mice, 6 week old, weighing 25–30 gm each, were gotten from the Animal House, KFMR and kept under controlled temperature (22±2°C), humidity (55±10%), and 12/12 hours cycle of light and dark with an access to nourishment and drinking water ad libitum. The animals were immunosuppressed and treated with Takakura et al., 2004 method . [6] and arranged into 3 groups; first group was control group (C group) which was resaved physiological solution. Second group was immunosuppressed group with no candaidal infection (IM group). Third group was immunosuppressed group infected with Candida albicans ATCC66027 was obtained from Microbiology laboratory, KAU Hospital (IM+*C. albicans* group).

Immunohistochemistry

The tongue were fixed in 10% formalin, after embedding in paraffin were sectioned of 4µm thickness Narayanan and Narasimhan 2015 method using CD1a expression [7].

Evaluation

The stained slides were viewed and analyzed under light microscope. Under the microscope brown surface stained cell was taken as positive for CD1a as CD1a is a surface marker. The quantity of LCs per high power field (400x) were calculated from 6 fields in the varying layers of epithelium and the average LCs in a high power field was calculated for each of the sections.

Statistical Analysis

Statistical analysis was performed using analysis to determine the difference in the number of LCs between groups by analysis of variance or Student t test with unequal variances. A P value of <0.05 was regarded to be statistically significant. Computations were done by SPSS software [8].

Results

Immunohistochemistry of lingual sections stained with CD1a revealed amount of LCs population with distributed length of oral basement membrane in each group study and morphological appearance of LCs cells was shown with highly or fewer dendrites.

Histologically examination of lingual sections showed the normal view of tongue in the control group with stratified squamous keratinized epithelium with a few numbers of LCs in basement membrane figure 1, while the IM group specimens

Fig. 1: Control group with a few numbers of LCs in basement membrane CD1a magnification × 400

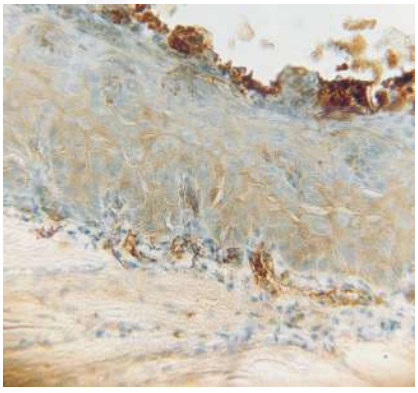

Fig. 2: Immunosuppressive with oral candidiasis showed a large distribution of LCsCD1a magnification × 400

Table 1: Statistical comparison of Langerhans cell counts in control C ,immunosuppressed with no *candida* infection IM, immunosuppressed with *candida* infection IM+*C. albicans* (unpaired student's t test)

	Mean ±SD	C	IM	IM+*C.albicans*
C	5.8±23		*p*= 0.0001	*p*= 0.0001
IM+*C.albicans*	102±31.30	*p*= 0.0001		*p*= 0.0001
IM	45.2±9.07	*p*= 0.0001	*p*= 0.0001	

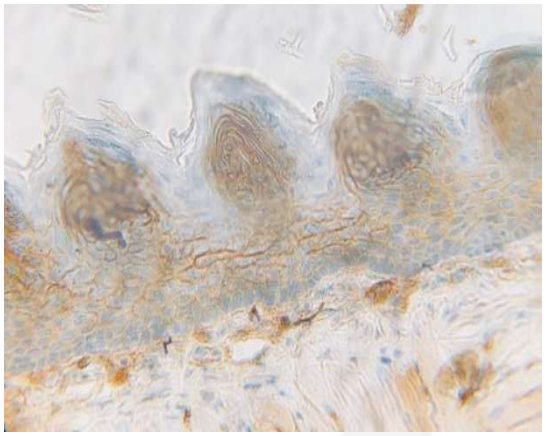

Fig. 3: Immunosuppressive without oral candidiasis showed numbers of LCs in basement membrane CD1amagnification × 400

showed a slight change in filiform papillae distributed and largest number of LCs in basement membrane figure 2, on the other side highly Candidal colonization showed in IM+ *C. albicans* group specimens lead to candida pseudohyphae penetrating the superficial keratinous epithelial layers and number of LCs in basement , connective tissue and few number of LCs cells reach to keratinized layer figure 3.

The Mean number of LCs per unit area in groups is presented in table 1. The greatest number of LCs per unit area was observed in (IM+*C. albicans* group) with the Mean±SD: 102±31.30, and the lowest number of LCs per unit area was observed in (C group) with the Mean ±SD: 17±14.29, while (IM group) recorded Mean ±SD: 44.2±9.95.

The Statistically compared of Langerhans cell counts in control, immunosuppressed, candidal infected immunosuppressed groups table1, showed highly significantly different between C and IM+ C.albicans group (p<05) as was different between IM and C+IM group (p<05). The LCs cells counts for C and IM+ *C. albicans* groups was also significantly different (p< 0.05).

Discussion

This study has shown the normal distribution of LCs cells in the basement membrane of control specimens C, while LCs cells reach to connective tissue in IM group specimens, and the largest distribution of LCs cells appeared in IM+C.albicans group specimens. The authors viewed lowest quantities of LCs in the floor of mouth and highest quantities in the buccal mucosa and dorsum of the tongue.

LCs cells arranged as a line along the length of epithelium and were found in basal and supra-basal layers of lateral tongue border and floor of mouth [9]. The recent result recorded two types of LCs have been defined based on their dendritic to highly dendritic LCs and fewer dendritic LCs in common with Breathnach 1977 the electron microscopic appearance of LCs noted two types: Type 1: described by highly dendritic with an electronlucent cytoplasm, numerous granules and is usually found in the suprabasal layers; Type 2: described by fewer dendrites, a more electron-dense cytoplasm with fewer Birbeck granules and is usually located in the basal layer [10].

The result shown significantly different between C and IM+ *C. albicans* group (p<05), many studies have highlighted LCs are capable of engaging with a wide kind of pathogens, and, upon endocytosis, they can process antigens, prime naive T-cells, and initiate adaptive immune responses. Also an increase of LCs number associated with the development of the oral micro flora has been observed [11].

LCs mobilize and mature in response to inflammatory cytokines and pathogen associated molecular patterns from oral mucosal Pathogens. Oral mucosal LCs appear to be oriented in a manner to efficiently sample the oral fluids and bacteria, with their dendrites extending toward the surface, and often represent a hetrogenous population [12]. The LCs counts in immunosuppressed with no candida infection group IM and, immunosuppressed with candida infection group IM+ *C. albicans* was p<05 that result reflect to LCs response for candida

infection. Newman and Holl report the LCs have defense role against candidiasis with phagocytic capacity of candidal yeasts and hyphae as well as processing their antigens [13].

Our histological recorded data similar to Romagnoli et al., 1997 in heavily candida-infected sections the CD1a positive LCs were particularly intense and the localization was quite variable. The dynamic nature of LCs and its involvement in local disease mechanisms such as chronic hyperplastic candidiasis lead to highly LCs variation in its number or localization. Furthermore, LCs were significantly numerous and richer in dendrites and Birbeck granules in erythematous areas than in areas of pseudomembranous candidiasis. [14] This finding is consistent with the prevailing view that a high density of Langerhans cells CD1a in the epithelium of Oral lichen planus has an important role in the antigen presentation and immunity cells activation [15].

Some reports, however, Confirmed LCs in oral mucosa have been response to some oral disease; Candida species, oral lichen planus (OLP), rhomboid median glossitis, human immunodeficiency virus (HIV) infection, hairy leukoplakia of the tongue, oral squamous cell carcinoma, and several other diseases [3-16-17].

This study shown highly significant between LCs cells counting in IM and IM+ C.albicans groups with $p< 0.05$ this difference is refers to candida in IM+ *C. albicans* sections. This reason agrees with Romagnoli et al., 1997 reported this difference is appears because the clinical appearance of a reaction to Candida antigens as a result of defense mechanism activation in HIV seropositive subjects where oral tissues influenced by candidiasis showed decrease of LCs number, than oral tissues without candidiasis; this reduction being more significant in oral pseudomembranous than erythematous candidiasis [14].

Conclusion

From the above results it was obvious Langerhans cells (LCs) played an important role in the immune system against pathogens attack especially in immunosuppressed cases, finally, it recommended that studies investigating the factors effected the numbers and activation of oral LCs found in immunosuppressed candidiasis cases, and immune interaction between epithelial host and pathogens.

Key Messages

The quantities of Langerhans' cells (LC) in oral mucosa are clear evidence on the efficacy of the peripheral immune system. The number of Langerhans' cells (LC) in oral mucosa influenced in immunosuppressed cases and increased in microbial infections such as white *Candida albicans* attacked.

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