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Phagocytic Activity of Salivary Neutrophils in Recurrent Aphthous Ulcer

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KEYWORDS

Recurrant aphthous ulcer, salivary neutrophil, phagocytic function, ingestion capacity.

ABSTRACT:

Objectives: Recurrent Aphthous Ulceration (RAU) is an inflammatory condition of unknown etiology characterized by painful, recurrent, single or multiple ulceration of oral mucosa. The etiology has not been completely understood, although, many local and systemic predisposing factors are known. Patients with RAU show increased Antibody Dependent Cell Mediated Cytotoxicity in the early stage of disease. Concentration of neutrophils at the periphery of the ulcer suggests the contribution of neutrophils in the pathogenesis of the ulcer. Hence, we aimed to compare the Phagocytic function of salivary PMN in patients with active RAU and during remission phase.

Method: Saline rinse was collected from patients suffering with RAU during ulcerative stage and during remission stage. Neutrophils were isolated from this and tested for their phagocytic function and ingestion capacity.

Result and Conclusion: Reduced neutrophils phagocytic function was seen in patients with ulcerative lesion when compared to remission phase. This suggests that neutrophils have an active role in pathogenesis and / or healing of RAU.

1. Introduction

Recurrent aphthous ulceration (RAU) is an inflammatory condition of unknown etiology characterized by painful, recurrent, single or multiple ulcerations of the oral mucosa (Fig 1). According to Stanley (1972) recurrent aphthous ulceration has three different variants: Minor aphthous, Major aphthous, Herpetiform ulcers. Recurrence is the hallmark of RAU, and patient generally present with only one variant of the disease, but two forms may coexist, or a change in clinical expression may be seen with time [1].

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The etiology of this disease has not been completely understood, although many local and systemic predisposing factors are known. Systemic predisposing factors which give rise to ulceration are hormonal changes, food hypersensitivity, drugs, hematinic deficiency (Iron, vitamin B12, Folic acid), Zinc deficiency and environmental factors like stress, local trauma and tobacco [2,3].

It has been documented that patient with RAU show increased Antibody Dependent Cell Mediated Cytotoxicity (ADCC) in the early stage of disease [4]. Neutrophils constitute a fundamental component of the innate immunity and play a role not only in the inflammation via phagocytosis but also in the promotion of adaptive response and in the development of autoimmune process [5]. Marked concentration of neutrophils at the ulcer area suggests that they may play an active role in the pathogenesis and / or healing of RAU [1]. However the exact role of neutrophils in the pathogenesis or healing of recurrent aphthae is still unknown and remains to be identified. Most of the studies on role of neutrophils in RAU have been done on the peripheral blood neutrophils. So we have attempted to study the phagocytic function of salivary neutrophils in patients with RAU.

Fig 1: Clinical photographs of the patient showing recurrent aphthous ulcer on the lower lip.

2. Objectives

The objective of the present study was to compare the phagocytic function of salivary neutrophils in ulcerative phase and remission phase of RAU

3. Methods

A total number of 30 patients with the history of RAU were included in the study {following the criteria of Natah et al. [6]}. Age of patients ranged from 12 – 54 yrs, 12 female and 18 male patients were included.

Unstimulated whole saliva was collected after taking the consent of the patient, by rinsing method. Saliva was collected during active ulcerative and during remission phases. Patient was asked to rinse with 5ml of normal saline for 3 min and expectorate into a sterile container. The procedure was repeated thrice to collect around 15 ml of sloution.

Neutrophils were separated from this rinse by centrifugation at 450 x g for 10 min and the supernatant was discarded. Pellet was washed with phosphate - buffered saline (PBS) thrice and suspended in 0.25 ml of Hanks balanced salt solution (HBSS, Hi Media). The pellet was refrigerated until use. Phagocytic function and ingestion capacity of the neutrophils were tested with yeast cells as targets.

‘`Determination of Ingestion ability: 0.25µl minimum essential medium (MEM), 0.25µl pooled sera and 0.25µl of leukocyte suspension was added and left aside for 30min at room temperature. Smears were made from this and stained with Giemsa. 100 neutrophils and ingested candida were counted (Fig 2).

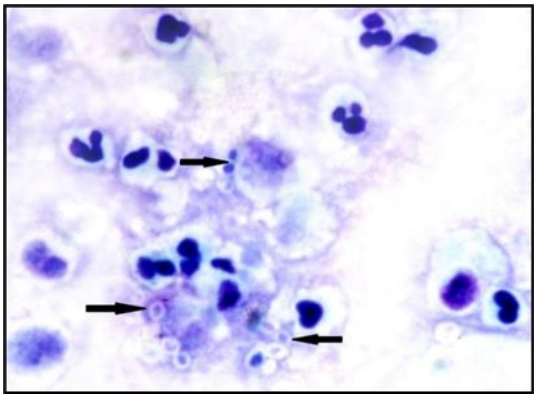

Fig 2: Photomicrograph showing neutrophils with ingested candidal cells, stained with Giemsa stain (under oil immersion).

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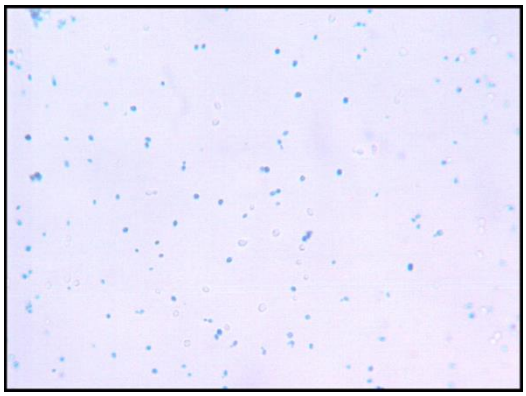

Fig 3: Photomicrograph showing wet mount preparation of killed candidal yeast cells stained with 0.1% Methylelne blue (magnification X 400).

Determination of Phagocytic activity: The test sample was left as such for another 30 min at room temperature and 0.25µl of 2.5% Sodium deoxycholate was added which caused lysis of neutrophils. After 5min 0.1% Methylene blue (vital staining) was added and mixed well and centrifuged. A wet mount preparation was done with the pellet and mounted with cover slip. The killed candida that escaped after neutrophil lysis stained blue with methylene blue. The readings were determined by the ratio of candida killed to the total number of candida ingested and expressed as percentage (Fig 3). The results were tabulated and stastical analysis was done using Student T test.

4. Results

30 patients with the history of RAU were included in the study with age ranging from 12 – 54 yrs, 12 female and 18 male patients were included (Table 1). 53.3 % of the cases were in the age group of 20 – 40 yrs, 33.3% were under 20 yrs and 13.3% were above 40 yrs.

Table 1: Age & Sex distribution of patients with RAU

Age distribution	Sex distribution		Percentage (%)
	Male (9 cases)	Female (6 cases)	
Below 20 yrs	6	4	33.3
20 – 40 yrs	10	6	53.3
Above 40 yrs	2	2	13.3

The salivary neutrophils were assayed for their phagocytic function and ingestion capacity.

- The Phagocytic function (proportion of ingesting neutrophils) of the salivary neutrophils ranged for 16 – 35% during ulcerative phase and 20 – 38% during remission phase (Table 2).
- The Ingestion capacity (number of ingested targets) of neutrophils ranged from 2 – 4 ingested candidal cells during ulcerative phase and 3 – 4 ingested candidal cells during remission phase (Table 2).

The phagocytic function of the salivary neutrophils RAU showed a reduction in the ulcerative phase when compared to the remission phase, the calculated value of t was (12.25) > Table value at 5% l.o.s (level of significance). Hence the null hypothesis was rejected with probability 0.0001. The difference was highly significant.

Table 2: Range of Phagocytic functions and Ingestion capacity of salivary neutrophils in both Ulcerative phase and Remission phase.

	Ulcerative phase	Remission phase	t value	Probability
Phagocytic function	16– 35 %	20 – 38 %	12.25	< 0.0001
Ingestion capacity	2 - 4	3 - 4	1.35	0.18

The ingestion capacity of the salivary neutrophils in RAU showed a reduction in the ulcerative phase when compared to the remission phase, the calculated value of t was (1.35) > Table value at 5% l.o.s. Hence the null hypothesis is rejected with probability 0.18. The difference was not significant.

5. Discussion

RAU is an inflammatory condition of an unknown etiology with the incidence of 20%. Aphthous ulcers have been reported in association with inflammatory bowel disease, peptic ulcer, primary and secondary immunodeficiency, and severe stress, suggestive of an immune-pathologic disorder [7]. Several workers have now detected circulating immune complexes in patients who have the disorder. In addition, aphthous ulcers have histopathologic features suggestive of an immune complex – mediated vasculitis [8].

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Innate immune system plays an important role in defense against pathogens and controls the induction and modulation of adaptive immune response. Neutrophils constitute a fundamental component of innate immunity and play a role not only in inflammation via phagocytosis, but also in the promotion of adaptive immune response and in the development of autoimmune process [4].

The study comprised of 30 cases with 18 males and 12 female patients (Table 1). Sun et al., reported that RAU presents more commonly in females with a female to male ratio of 2: 1 [9]. 53.3 % of the patients were in the age group 20 – 40 yrs. Scully et al. quoted that in about 80% the condition develops before 30 yrs of age [10].

The phagocytic function in the ulcerative phase of RAU ranged from 16 - 35% and in the remission phase it ranged from 20 - 38%. There was a definite reduction in the phagocytic function in the ulcerative phase when compared to the remission phase, which was highly significant, with the t value of 12.25 (p - > 0.0001). This was consistent with the only available reports of Lukac et al. [11] and Kumar et al., [7] who noted that there was a significant reduction in the phagocytic function and intracellular microbicidity of salivary neutrophils in RAU when compared to that of healthy controls.

The ingestion capacity of salivary neutrophils in the ulcerative phase of RAU ranged from 2-4 and in the remission phase it ranged from 3 - 4. But this reduction in the ingestion capacity in the ulcerative phase of RAU was not statistically significant with the t value of 1.35 (p - 0.18). Lukac et al. demonstrated significant reduction in the ingestion ability in RAU as compared to controls [11] where as Kumar et al., demonstrated similar reduction which was not significant [7].

Kumar et al. did not find any significant difference in the phagocytic function of the peripheral blood and salivary neutrophils in RAU, suggesting that saliva can be a sole diagnostic tool in determination of phagocytic activity of neutrophils in RAU patients [7].

There is ample evidence to suggest that neutrophils, by exteriorization of free oxygen radicals can cause tissue damage, particularly in autoimmune conditions [8]. Neutrophils may be attracted to the RAU area by chemotactic split products of complement, which have been activated by immune complex deposited in the oral mucosa. They are important for phagocytosing and eliminating antigenic material or products of tissue damage [12]. Modulation of activation status of neutrophils is important in determining the balance between defense and injury [4].

Ueta et al. in their study observed the suppression of neutrophil respiratory burst in RAU [13]. Dagalis et al. could not find any statistically significant difference in chemotaxis, spontaneous migration and chemotactic index between patients with RAU and controls [8]. Wary et al. showed a significantly increase adherence to glass compared to controls and the difference in adherence was quoted as due to serum factors. Increased adherence of neutrophils may increase the duration of ulceration by prolonging sequestration of exogenous antigen with the lesion and indeed, debridement of biopsy tends to promote healing [12].

Natalia Lewkowicz et al. confirmed the increase Reactive oxygen intermediate production in resting and formyl-methionyl-leucyl-phenylalanine stimulated neutrophils [4]. Results of the above studies were based on the neutrophils obtained from peripheral blood. Previous unsuccessful attempts to isolate lesional neutrophils by washing the ulcer thoroughly indicated a potentially increased adherence of neutrophils in recurrent aphthous stomatitis [12].

6. Conclusion

The results of findings presented in the study suggest that the pathophysiology of RAU may be associated with neutrophil suppression. However the exact role of neutrophils in the pathogenesis and / or healing of RAU remain to be identified. Hence a large-scale study is required to know the exact role of neutrophils in the pathogenesis and / or healing of RAU.

Refrences

1. Natah S S, Konttinen Y T, Enattah N S, Ashammakhi, Sharkey K A, Hayrinen-Immonen R. Recurrent aphthous ulcers today: a review of growing knowledge, Int. J. Oral Maxillofac. Surg 2004;33:221-234.
2. Porter S R, Scully C. Recurrent Aphthous Stomatitis, Crit Rev Oral Biol Med, 1993;9(3):306-321.

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3. Mills P M, Mackler F B, Nelms D C, Peavy L D. Quantitative Distribution of Inflammatory Cells in Recurrent Aphthous Stomatitis, J Dent Res, 1980;53(3):562-566.
4. Lewkowicz N, Lewkowicz P, Kurnatowska A et al. Innate immune system is implicated in recurrent aphthous ulcer pathogenesis, J Oral Pathol Med, 2003;32:475-481.
5. Sistig S, Cekic-Arambasin A, Rabatic S, Vucicevic-Boras V, Kleinheinz, Piffko. Natural immunity in recurrent aphthous ulceration, J Oral Pathol Med, 2001;30:275-280.
6. Natah SS, Konttinen YT, Enattah NS, Ashammakhi N, Sharkey KA, Hayrinen-Immonen R. Recurrent aphthous ulcers today: a review of growing knowledge. Int J Oral Maxillofac Surg, 2004;33,221-234.
7. Kumar B P, Keluskar V, Bagewadi S B, Shetti A. Evaluating and comparing phagocytic functions of salivary and blood neutrophils in Patients with recurrent aphthous ulcers and controls. Quintessence Intl, 2010;41(5):411 – 416.
8. Dagalis P, Bagg J, Walker D M. Spontaneous migration and chemotactic activity of neutrophils polymorphonuclear leukocytes in recurrent aphthous ulceration, Oral Surg. Oral Med. Oral Pathol, 1987;64:298-301.
9. Sun A, Chiang CP, Chiou PS, Wang JT, Liu BY, Wu YC. Immunomodulation by Levamisole in patients with Recurrent aphthous ulcers and oral lichen planus. J Oral Pathol Med 1994;23:172-177.
10. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis. J Am Dent Assoc, 2003;134:200-207.
11. Lukac J, Mravak-Stipetic M, Knezevic MA. Phagocytic functions of salivary neutrophils in oral mucous membrane diseases, J Oral Pathol Med. 2003;32:271-4.
12. Wary D, Charon J. Polymorphonuclear neutrophils function in recurrent aphthous stomatitis, J Oral Pathol Med. 1991;20:392-394.
13. Ueta E, Umazume M, Yamamoto T, Osaki T. Leukocyte dysfunction in oral mucous membrane diseases, J Oral Pathol Med. 1993;22:120-125.