*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 8 Ver. XI (Aug. 2017), PP 36-42*
*www.iosrjournals.org*

# Comparative Quantification of Mast Cells in Desquamative Gingivitis And Chronic Periodontitis: A Histopathologic Study

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***Abstract***
***Objectives:*** *Mast cells play an important role in the pathogenesis of allergic and inflammatory diseases. This study aims to quantify mast cells in the gingival specimens of patients with desquamative gingivitis (DG) and chronic periodontitis (CP), and to compare it with clinically healthy gingiva (CHG).*
***Materials and methods:*** *15 DG, and 15 CP and 15 CHG were included in the study. Histopathologic examination was done with toluidine blue staining method to assess mast cell counts in DG subjects and also in CP and CHG.*
***Results:*** *Mast cells were significantly increased in DG as compared to CP and CHG. DG showed a nine fold increase in the mast cell count as compared to clinically healthy gingiva and a fourfold increase as compared to chronic periodontitis. CP showed a two fold increase in mast cell count when compared to CHG.*
***Conclusion:*** *Mast cells counts were elevated in DG and CP when compared to CHG. Mast cells may have a role in the pathogenesis of DG and CP. The difference in the mast cell count in DG and CP observed in the present study may be due to the difference in the stimulus for activation of immuno inflammatory pathway. Further studies are essential to clarify the underlying pathophysiological mechanisms in these diseases.*
***Keywords:*** *chronic periodontitis, desquamative gingivitis, mast cells*

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Date of Submission: 01 -08-2017 Date of acceptance: 14-08-2017

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## I. Introduction

Chronic desquamative gingivitis (DG) is a peculiar condition characterized by the presence of intense erythema, desquamation, and ulceration of the free and attached gingiva.[1] Desquamative gingivitis is not a specific disease entity, but a gingival response to a variety of conditions like lichen planus, cicatricial pemphigoid, pemphigus vulgaris, bacterial, fungal and viral infections and as allergic manifestations to a variety of allergens. Major part of desquamative gingivitis is accounted by lichen planus or lichenoid reactions. Mast cells play an important role in the pathogenesis of allergic and inflammatory diseases. Mast cells on degranulation release a variety of mediators like trypsin and chymotrypsin like enzymes that have an effect on the vasculature and on the surrounding cells.[2] Lichen planus may simplistically represent a type IV hypersensitivity response to an exogenous agent[3] or an autoimmune reaction.[4,5] It has been suggested that mast cells present in lichen planus might play a role in the pathogenesis of the disease.[6,7] A characteristic feature of oral lichen planus is the presence of subepithelial band of T cells. [8,9] The bidirectional T lymphocyte- mast cell interaction in lichen planus has regulatory and modulatory effects on immune response. Mast cells may affect the recruitment of T cell to the site of inflammation by several mechanisms like direct release of chemotactic molecules, regulation of expression of adhesion molecules and induction of cytokine release by endothelial cells.[8, 9] Mast cell activation and degranulation may be an essential prerequisite in the induction and elicitation of hypersensitivity reactions.

Lipopolysaccharides (LPS) present in the cell walls of gram negative bacteria play a prominent role in the pathogenesis of periodontitis. LPS from dental plaque may trigger the migration and degranulation of mast cells and release of histamine from the mast cells in periodontal tissues in susceptible individuals.[10] Based on all this, we hypothesized that mast cells have a role in the pathogenesis of DG and chronic periodontitis. This study aimed to quantify the distribution of mast cells in the gingival specimens of patients with desquamative gingivitis and chronic periodontitis and to compare the number of mast cells in desquamative gingivitis and chronic periodontitis with clinically healthy gingiva.

## II. Materials And Methods

**Study design**

This study was conducted in the Department of Periodontics, Govt. Dental College, Calicut, Kerala, India. The study involved 45 subjects who attended the Out Patient Division of Dept of Periodontics, Govt. Dental College, Calicut, Kerala, India. Among them 15 subjects were diagnosed clinically as having desquamative gingivitis (DG), 15 subjects were diagnosed with chronic periodontitis (CP) and 15 subjects with clinically healthy gingiva (CHG) were selected. Patients were evaluated using a detailed questionnaire including the patients name, age, sex, socioeconomic status, oral hygiene practices, and duration of the present illness. The study was independently reviewed and approved by the Institutional Ethics Committee, Govt. Dental College, Calicut, Kerala, India with regard to the ethical principles, of World Medical Association Declaration of Helsinki. The experiments were undertaken with the understanding and informed written consent was obtained from all subjects.

The subjects were included in the study based on the following criteria: the desquamative gingivitis (DG) group included male and female patients with desquamative gingivitis, patients with at least 20 teeth remaining in the oral cavity and no history of previous periodontal therapy (fig1). The chronic periodontitis group was identified based on the Centers for Disease Control and Prevention criteria put forward by the American Academy of Periodontology.[11] Subjects with severe chronic periodontitis were considered for inclusion into the study. Severe periodontitis was defined as ≥2 interproximal sites with clinical attachment loss ≥6mm, not on the same tooth, and ≥1 interproximal site with PD ≥5 mm. Clinically healthy gingiva (CHG) group included patients with Silness and Loe Plaque index score of 0 and Loe and Silness gingival index score of 0 who were posted for orthodontic treatment. Subjects with known systemic diseases and conditions, such as cardiovascular diseases, renal diseases, rheumatoid arthritis, diabetes mellitus, nutritional deficiencies, and pregnant and lactating females, patients with previous history of periodontal therapy in the past six months, or history of antibiotic therapy in the past 6 months were excluded from all the three groups.

## III. Histopathologic Examination

In DG group, incision biopsy was performed from the most representative site of the lesion, under local anesthesia. In CP group, the biopsy was obtained from the site with clinical attachment loss of ≥6 mm and indicated for extraction due to periodontal reasons. In the CHG group, the specimen was obtained from the first or second premolar area at the time of extraction for orthodontic correction. The obtained specimens were fixed in 10% formalin, processed and routine histopathologic examination was carried out to arrive at a diagnosis. A second section was stained with toluidine blue to assess the mast cell count.For the assessment of mast cell count, prepared sections were deparafined in xylene and passed through descending grades of alcohol and then hydrated. The sections were then stained with toluidine blue for 1-2 minutes, washed rapidly first in absolute alcohol and then in xylene. The sections were then cleared in xylene and mounted in DPX medium. The mast cell counting was done using a 10x eyepiece fitted with $1cm^2$ ocular grid and a 40 x objective. The area encompassed by the graticule under 40 x objectives was taken as one microscopic field. Mast cell counts were taken from 3 areas per section and 2 zones were assessed in each area – superficial (subepithelial) zone [zone 1] and deep zone [zone 2]. The superficial zone was the connective tissue zone that was immediately below the epithelium. For the deep zone, one microscopic field immediately below the superficial zone was left and the next microscopic field was taken. The number and distribution of mast cells in DG group were then compared with that of CP and CHG subjects in both zones.

## IV. Statistical Analysis

All data were analyzed using a statistical software package (SPSS 13.0 for Windows). The mean number of mast cells in desquamative gingivitis, chronic periodontitis and clinically healthy gingiva was statistically evaluated using the Students t test. The results were considered to be statistically significant if the p value was less than 0.05.

## V. Results

Of the 45 patients in the study, 15 subjects were diagnosed clinically as having desquamative gingivitis (DG), 15 subjects with chronic periodontitis (CP) and 15 subjects with clinically healthy gingiva (CHG). 80% of the cases who belonged to the DG group were females suggesting a female predilection for DG. 73% of the cases who belonged to the DG group had gingival lesions and lesions on some other areas of the oral cavity. 27% had lesions restricted to the gingiva. The age of the patients in DG group ranged from 25 -55 years and the mean age of the study group was 39.4 years. The mean age for CP group was 43.7 years and that of CHG group was 25.6 years (Table 1). There was no significant difference in the plaque score between DG and CP group. However, there was a

significant difference in the calculus index between these groups (Table 1). The scores of the plaque index and the calculus index for the CHG group was 0.

Histopathologic examination showed that 63.34% of the DG patients had lichen planus, 23.33% Lichenoid reaction, 13.33% inflammatory hyperplasia. The mean number of mast cells in zone 1 (DG = 24.4667, chronic periodontitis=6.63, clinically healthy gingiva = 3.125), in zone 2 (DG=23.2, clinically healthy gingiva = 2.375, chronic periodontitis=5.63) was analyzed (Table 2). When comparing the mast cell distribution between zone 1 and zone 2 of DG, CP and CHG no statistically significant difference was observed (Table 2). The total number of mast cells in zone 1 and zone 2 together (DG = 45.6667, clinically healthy gingiva = 5.5, chronic periodontitis= 12.25) was analyzed (Table 2). DG showed a nine fold increase in the total number of mast cells as compared to clinically healthy gingiva and a fourfold increase as compared to chronic periodontitis (Table 3), (fig 2,3,4). CP showed a 2 fold increase in mast cell count when compared to CHG (Table 3).

## VI. Discussion

Mast cells are normal components of the gingival connective tissue and are important constituents of the immune system.[12-14] They stain metachromaticaly; their granules contain various substances like histamine, heparin, tryptase, chymase, cytokines like TNF-α, IL-1β, IL-3,4,5,6 and IL-10.[2] When mast cells are activated they degranulate and thereby release this panel of powerful preformed inflammatory mediators which play an important role in immune and allergic reactions.[12-14] Mast cell activation and degranulation might have a regulatory and modulatory effect on various aspects of immune response. Mast cells may be an essential pre requisite in the induction and elicitation of gingival manifestation in these immune and allergic reactions.Mast cells are one of the first few cells which get involved in periodontal inflammation. They play a critical role in host immune defense against gram-negative bacteria.[15] Some investigators have proposed a role for mast cell constituents in periodontal destruction.[16-18] Mast cells strongly express matrix metalloproteinase (MMP)-1, -2 and -8,[19] which are key enzymes in degradation of periodontal soft tissue. However, the contribution of mast cell mediators to periodontal disease progression is not clearly known.

Of the 45 subjects in this study, 15 subjects had DG. Among these 15 patients 80% were females and 20% were males. This shows that there is a sex predilection in the involvement of DG (Table 1). This was in accordance with results of previous reports.[20-22] The age of the patients in this study ranged from 25 -55 years and the mean age of the study group was 39.4 years (Table 1).This is in accordance to reports suggesting that DG appear in the third and forth decades of life. Among the DG subjects studied, 73.3% had associated lesions on the buccal mucosa. Yet another interesting observation in our study was that the lingual gingiva was free of desquamative lesions in all the cases irrespective of the amount of plaque and calculus. Holmstrup P in 1990 had also reported of gingival lesions of lichen planus that involved exclusively the facial aspect of the gingiva.[23]

Histopathologic examination of the cases showed that there was statistically significant increase in the number of mast cells in DG as compared to CHG (Table 3). No statistically significant difference in the number of mast cells between zones 1 and 2 was seen in DG (Table 2). DG showed a nine fold increase in the total number of mast cells as compared to clinically healthy gingiva (Table 3). Mast cells strongly express matrix metalloproteinase (MMP)-1, -2 and -8,[19] which are key enzymes in degradation of soft tissue. DG (oral lichen planus, oral Lichenoid reaction, inflammatory hyperplasia) may simplistically represent a type IV hypersensitivity reaction to an exogenous agent[3] or an autoimmune reaction.[4,5] Apart from the direct action of mast cells in the pathogenesis of DG by releasing enzymes that degrade the basement membrane, they also interact with other cells like keratinocytes and T cell directly or indirectly to amplify the magnitude of the immune responses elicited in the sensitized host.

We also observed that there was a statistically significant increase in the number of mast cells in CP as compared to CHG ($p < 0.05$) (Table 3). CP showed a 2 fold increase in mast cell count when compared to CHG. Substantial evidence has implicated certain immune and inflammatory responses in the periodontal disease process.[24,25] One possible host reaction in periodontal breakdown may be mediated by mast cell release and increased mast cell counts have been reported in the diseased gingiva as compared to other healthy tissues. The results of the present study seem to indicate that mast cells have higher counts in chronic periodontitis compared to clinically healthy gingiva, which is consistent with the results of previous studies.[26,28] However, contradictory results are also available in the literature.[29,30] Gemmel in his study observed that the number of mast cells were decreased in chronic periodontititis. When triggered by bacterial products like lipopolysaccharides, mast cells can release large quantities of pre-stored mediators such as histamine, leukotrienes, platelet-activating factor (PAF), proteases and tumor necrosis factor-α (TNF-α).[31,32] The expression of Matrix Metalloproteinases (MMPs) 1, 2, and 8 are strongest in mast cells. MMPs are crucial in the degradation of the main components in extracellular matrices.[33] Furthermore, tryptase activity confined to mast cells can cleave collagen and activate latent collagenases

that participate in tissue destruction in periodontitis. Though plaque index was similar between the DG and CP group, there was a statistically significant difference between the groups for the calculus index scores, CP subjects having a higher calculus index score (Table 1). The plaque observed in DG subjects could either be a cause or a consequence of the distressing condition. Though the calculus index scores and tissue destruction were higher in CP group the mast cell counts were significantly lower in this group as compared to DG.

In this study, DG subjects showed a fourfold increase in mast cells count as compared to chronic periodontitis (Table 3). More than 75% of our DG subjects were diagnosed as lichen planus or lichenoid reaction. It has been well established that mast cells play an important role in the pathogenesis of lichen planus.[6] The increased mast cell count in the DG group of this study may be a manifestation of the underlying diseases like lichen planus/lichenoid reactions. In the "unifying hypothesis" proposed for lichen planus,[34] a lichen planus antigen is expressed in association with MHC class I molecules on basal keratinocytes at the OLP lesion site. Antigen-specific CD8+ cytotoxic T-lymphocytes (CTLs) are activated in the OLP epithelium and trigger keratinocyte apoptosis. Activated T-cells undergo intra-lesional clonal expansion and release RANTES and other cytokines that stimulate intra-lesional mast cell migration and degranulation. This mechanism could have contributed to an increase in mast cell count in DG subjects.

In chronic periodontitis mast cells play important role as the first line of defense against infection. Mast cells are also able to present antigens to T cells in either an MHC-I or MHC-II restricted or costimulatory molecule dependent fashion. Both T cell derived cytokines, and direct contact between mast cell and T cells can cause mast cell activation and release. Mast cell signals memory T cells to differentiate preferentially to the Th 2 subset. T cell derived cytokines IL-3 and chemokines (RANTES) could also serve as growth factors and chemotactic factors for mast cells.[13,29] Thus T cell - mast cell interaction is bidirectional in CP. Increased mast cell counts in the chronic periodontitis may indicate the importance of these cells in the progression of the disease.The difference in the mast cell count in DG and CP observed in the present study may be due to the difference in the stimulus for activation of immuno inflammatory pathway. In CP, the immunoinflammatory mechanism may be triggered by microbial plaque deposits, whereas, in DG the immunoinflammatory mechanism could have been triggered by antigens expressed on the basal keratinocytes. Further studies are essential to clarify the underlying pathophysiological mechanisms in these diseases. The limitation of the present study includes the use of toluidine blue stain for histopathological analysis and a relatively small sample size. The toluidine blue has a low sensitivity in detecting partially degranulated mast cells.

## VII. Conclusion

Within the limitations of the present study it could be concluded mast cells counts were elevated in desquamative gingivitis and chronic periodontitis as compared to clinically healthy gingiva. There was a fourfold increase in mast cell count in desquamative gingivitis subjects when compared to chronic periodontitis subjects. Mast cells may have a role in the pathogenesis of desquamative gingivitis and chronic periodontitis. Large prospective multicenter clinical trials with larger sample size, newer staining techniques like immuno histochemical analysis for the detection of mast cells are needed to better elucidate the immunoinflammatory mechanisms regulating mast cell release in these clinically distinct diseases. Interventional studies are necessary to elucidate the influence of periodontal therapy in reducing mast cell levels in desquamative gingivitis and chronic periodontitis.

## Acknowledgements

We are thankful to Dr Shameena P M and Dr Resmi G Nair of the Department Of Oral Pathology And Microbiology, Government Dental College Kozhikode, for their help and support in carrying out the study.

## References

Scully C, el-Kom M. Lichen planus: Review and update on pathogenesis. J Oral Pathol 1985; 14: 431-58.

Barnett ML. The nonkeratinocyte intraepithelial cell population in lichen planus. An ultrastructural characterization of cells in gingival lesions. Oral Surg Oral Med Oral Pathol 1976; 41: 338-53.

Porter SR, Kirby A, Olsen I, Barrett W. Immunologic aspects of dermal and oral lichen planus: A review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997; 83: 358-66.

Sugerman PB, Satterwhite K, Bigby M. Autocytotoxic t-cell clones in lichen planus. Br J Dermatol 2000; 142: 449-56.

Sugerman PB, Savage NW, Zhou X, Walsh LJ, Bigby M. Oral lichen planus. Clin Dermatol 2000; 18: 533-9.

Zhao ZZ, Savage NW, Pujic Z, Walsh LJ. Immunohistochemical localization of mast cells and mast cell-nerve interactions in oral lichen planus. Oral Dis 1997; 3: 71-6.

Lodi G, Scully C, Carrozzo M, Griffiths M, Sugerman PB, Thongprasom K. Current controversies in oral lichen planus: Report of an international consensus meeting. Part 2. Clinical management and malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 100: 164-78.

Jungell P, Konttinen YT, Malmstrom M. Basement membrane changes in oral lichen planus. Proc Finn Dent Soc 1989; 85: 119-24.
Jungell P, Konttinen YT, Nortamo P, Malmstrom M. Immunoelectron microscopic study of distribution of t cell subsets in oral lichen planus. Scand J Dent Res 1989; 97: 361-7.
Madianos PN, Bobetsis YA, Kinane DF. Generation of inflammatory stimuli: How bacteria set up inflammatory responses in the gingiva. J Clin Periodontol 2005; 32 Suppl 6: 57-71.
Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol 2007; 78 Suppl 7 : 1387-99.
Chi DS, Fitzgerald SM, Krishnaswamy G. Mast cell histamine and cytokine assays. Methods Mol Biol 2006; 315: 203-15.
Chi DS, Walker ES, Hossler FE, Krishnaswamy G. Bacterial activation of mast cells. Methods Mol Biol 2006; 315: 383-92.
Krishnaswamy G, Ajitawi O, Chi DS. The human mast cell: An overview. Methods Mol Biol 2006; 315: 13-34.
Batista AC, Rodini CO, Lara VS. Quantification of mast cells in different stages of human periodontal disease. Oral Dis 2005; 11: 249-54.
Walsh LJ. Mast cells and oral inflammation. Crit Rev Oral Biol Med 2003; 14: 188-98.
Steinsvoll S, Helgeland K, Schenck K. Mast cells--a role in periodontal diseases. J Clin Periodontol 2004; 31: 413-9.
Walsh LJ, Savage NW, Ishii T, Seymour GJ. Immunopathogenesis of oral lichen planus. J Oral Pathol Med 1990; 19: 389-96.
Naesse EP, Schreurs O, Helgeland K, Schenck K, Steinsvoll S. Matrix metalloproteinases and their inhibitors in gingival mast cells in persons with and without human immunodeficiency virus infection. J Periodontal Res 2003; 38: 575-82.
Simpson HE. The age and sex incidence and anatomical distribution of oral leucoplakia and lichen planus. Br J Dermatol 1957; 69: 178-80.
Nisengard RJ. Periodontal implications: Mucocutaneous disorders. Ann Periodontol 1996; 1: 401-38.
Nisengard RJ, Rogers RS, 3rd. The treatment of desquamative gingival lesions. J Periodontol 1987; 58: 167-72.
Holmstrup P, Schiotz AW, Westergaard J. Effect of dental plaque control on gingival lichen planus. Oral Surg Oral Med Oral Pathol 1990; 69: 585-90.
Doty SL, Lopatin DE, Syed SA, Smith FN. Humoral immune response to oral microorganisms in periodontitis. Infect Immun 1982; 37: 499-505.
Ishikawa I. Host responses in periodontal diseases: A preview. Periodontol 2000 2007; 43: 9-13.
Kennett CN, Cox SW, Eley BM, Osman IA. Comparative histochemical and biochemical studies of mast cell tryptase in human gingiva. J Periodontol 1993; 64: 870-7.
Kabashima H, Nagata K, Maeda K, Iijima T. Involvement of substance p, mast cells, tnf-alpha and icam-1 in the infiltration of inflammatory cells in human periapical granulomas. J Oral Pathol Med 2002; 31: 175-80.
Jeffcoat MK, Williams RC, Johnson HG, Wechter WJ, Goldhaber P. Treatment of periodontal disease in beagles with lodoxamide ethyl, an inhibitor of mast cell release. J Periodontal Res 1985; 20: 532-41.
Gemmell E, Carter CL, Seymour GJ. Mast cells in human periodontal disease. J Dent Res 2004; 83: 384-7.
Carranza FA, Jr., Cabrini RL. Mast cells in human gingiva. Oral Surg Oral Med Oral Pathol 1955; 8: 1093-9.
Befus AD, Dyck N, Goodacre R, Bienenstock J. Mast cells from the human intestinal lamina propria. Isolation, histochemical subtypes, and functional characterization. J Immunol 1987; 138: 2604-10.
Befus D. The role of the mast cell in allergic bronchospasm. Can J Physiol Pharmacol 1987; 65: 435-41.
Zhao ZZ, Sugerman PB, Zhou XJ, Walsh LJ, Savage NW. Mast cell degranulation and the role of T cell RANTES in oral lichen planus. Oral Dis 2001; 7: 246-51.
Sugerman PB, Savage NW, Walsh LJ, Zhao ZZ, Zhou XJ, Khan A, et al. The pathogenesis of oral lichen planus. Crit Rev Oral Biol Med 2002; 13: 350-65.

**Legend**

Fig 1: Figure showing a case of desquamative gingivitis.

Fig 2: Toluidine blue stained histopathologic specimen under a magnification of 40x showing the presence of mast cells in a clinically healthy gingiva.

Fig 3: Toluidine blue stained histopathologic specimen under a magnification of 40x showing the presence of mast cells in a case of chronic periodontitis.

Fig 4: Toluidine blue stained histopathologic specimen under a magnification of 40x showing the presence of mast cells in a case of desquamative gingivitis.

**Tables**

**Table 1:** Demographic and Periodontal Parameters in desquamative gingivitis (DG), chronic periodontitis (CP) and clinically healthy gingiva (CHG) Subjects

| Parameters | Dg | Cp | Chg |
|---|---|---|---|
| Age | 39.4 | 43.7 | 25.6 |
| Calculus Index∗ | 1.822 | 2.93 | 0 |
| Plaque Index | 2.15 | 2.45 | 0 |

∗ $p < 0.05$ statistically significant

**Table 2:** Mast Cell Counts in desquamative gingivitis (DG), chronic periodontitis (CP) and clinically healthy gingiva (CHG) subjects

| Groups (N=15) | ZONE 1 | ZONE 2 | TOTAL |
|---|---|---|---|
| DG | 22.47 | 23.2† | 45.67 |
| CP | 6.63 | 5.63† | 12.25 |
| CHG | 3.13 | 2.38† | 5.5 |

† p>0.05 (when zone 1 and zone 2 were compared

**Table 3:** Comparison of the Mast Cell Counts in desquamative gingivitis (DG), chronic periodontitis (CP) and clinically healthy gingiva (CHG) subjects

| Groups | N | Mast cell count | p value |
|---|---|---|---|
| DG<br>CHG | 15<br>15 | 45.67<br>5.5 | 0.000∗ |
| DG<br>CP | 15<br>15 | 45.67<br>12.25 | 0.001∗ |
| CHG<br>CP | 15<br>15 | 12.25<br>5.5 | 0.039∗ |

∗ p <0.05 statistically significant

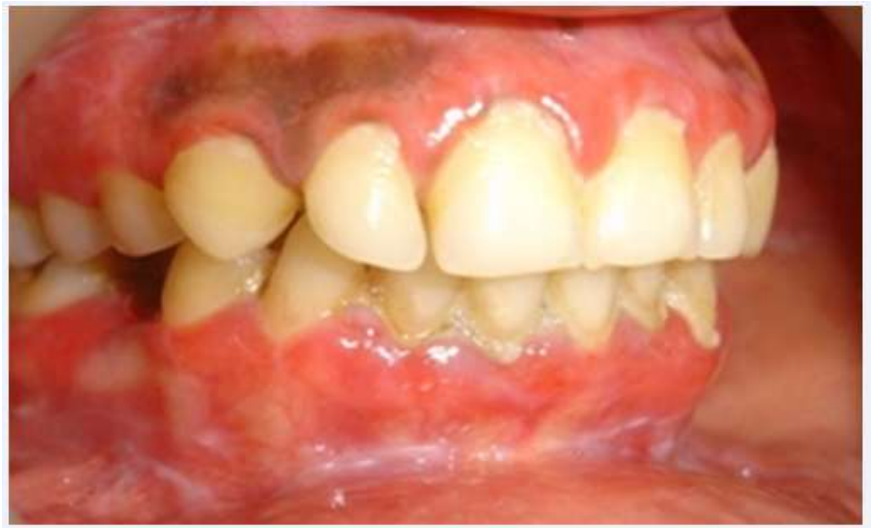

Fig 1

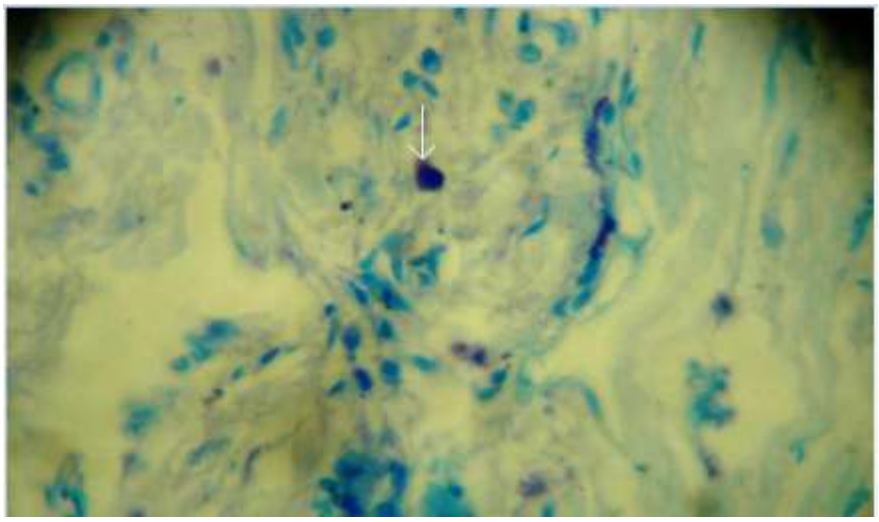

Fig 2

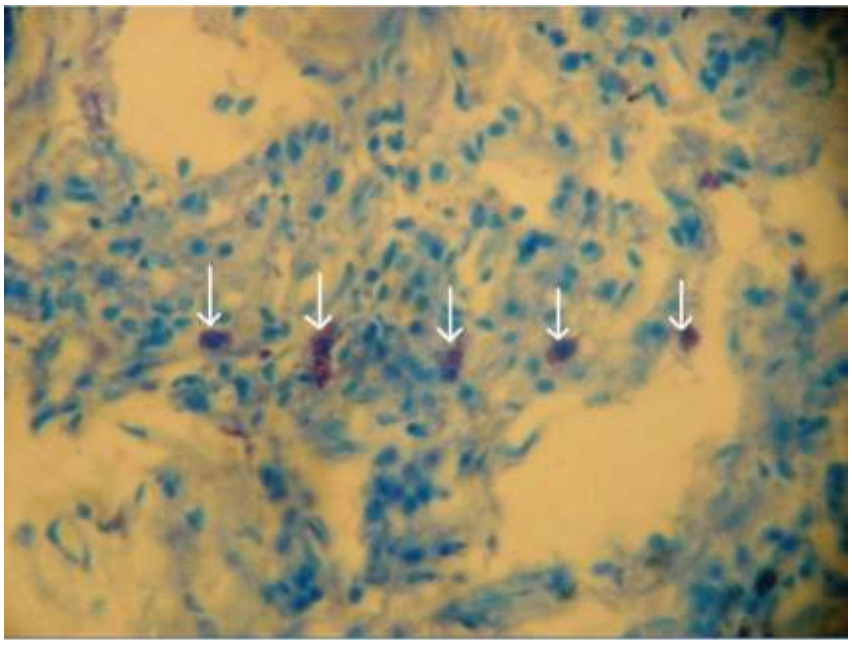

Fig 3

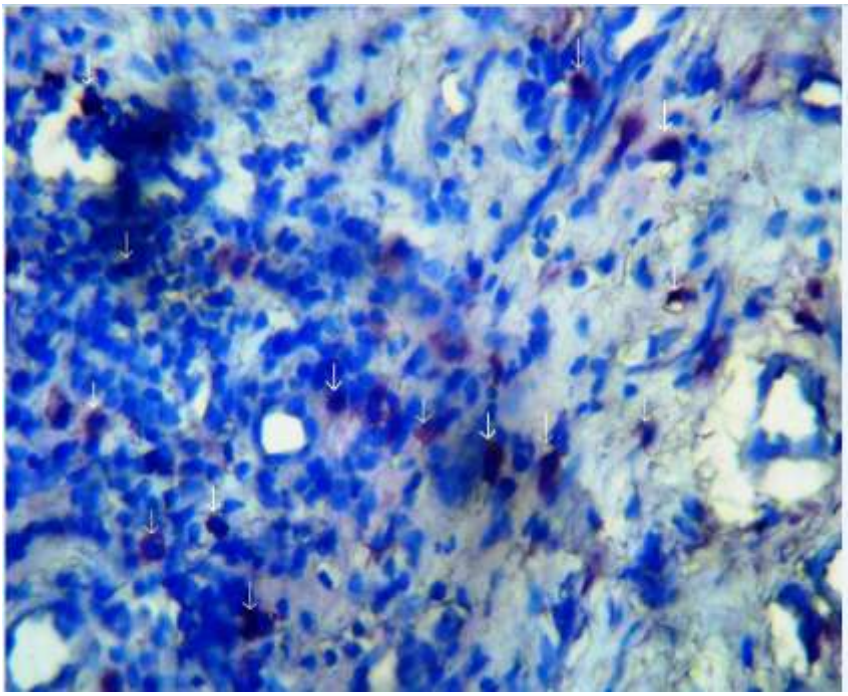

Fig 4

*Shiny Joseph. "Comparative Quantification of Mast Cells in Desquamative Gingivitis And Chronic Periodontitis: A Histopathologic Study." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.8 (2017): 36-42