ORIGINAL ARTICLE

Salivary tumour necrosis factor-alpha as a diagnostic marker of desquamative gingivitis

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ABSTRACT

Background

Desquamative gingivitis (DG) is a non-plaque induced gingival disease. Clinical examination coupled with histopathological and immunofluorescence studies diagnose it, however, diagnosis of about one-third of DG cases is still elusive which leads to poor clinical outcomes. Therefore, biomarker profiling of DG will help in predicting better treatment outcomes. The objective of the study was to evaluate the salivary TNF-α as a biomarker of DG and compare it with chronic gingivitis (CG) and clinically healthy (CH) subjects.

Materials and Methods

A case-control study was done with 94 subjects having; DG (n=31), chronic gingivitis CG (n=31), and clinically healthy CH (n=32) were recruited in the study after they fulfilled subject selection criteria and signed the consent form. Detailed history and clinical examination were done. Subjects were told to abstain from eating 2 hours before saliva collection. Three ml of saliva was collected in the saliva collection tubes between 9 AM to 12 PM. The collected samples were stored at -80 centigrade and TNF-α levels were assessed employing an ELISA technique (R&D systems). The data were analyzed using SPSS (Version 20, IBM, USA) statistical software.

Results

Salivary TNF-α levels were significantly increased in DG (33.71±12.15 pg/ml,) as compared to CH (5.99±2.11) and CG (13.48±1.16 pg/ml) subjects. The receiver operating curve (ROC) of DG for salivary TNF-α biomarker showed an area under the curve (AUC) of 0.973.

Conclusion

Salivary TNF-α is a good diagnostic biomarker that is significantly increased in desquamative gingivitis.

Keywords

Gingival disease; Saliva; Diagnosis, Biomarker; Tumour necrosis factor-alpha

INTRODUCTION

Desquamative gingivitis (DG) presents as erythema, ulceration and desqaumation of free and attached gingiva. DG is seen in the 4th to 5th decades of the life with female predilection. Clinically presentation may be asymptomatic or symptomatic with mild burning to intense pain. [1]

DG is not a disease as such but clinical manifestation of variety of diseases and

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conditions; oral lichen planus, mucous membrane pemphigoid, pemphigus and contact allergic reactions to oral hygiene products, food and other chemicals which come in contact with the oral mucosa.[2]

Its diagnosis is done using clinical examination coupled with histopathological and immunofluorescence studies, however around one third of the DG cases still could not be diagnosed. In this scenario clinician resorts to local as well systemic empiric therapy which are effective usually but sometimes becomes refractory.[2,3,4] Therefore, biomarker profiling can serve as a personalised predictive diagnostic aid for better treatment outcomes.

Saliva is a diagnostic fluid used for the diagnosis of the oral diseases and conditions. Saliva collection is inexpensive and causes minimal patient discomfort.[5] Tumour Necrosis factor-alpha (TNF-α); a pro inflammatory cytokine secreted by macrophages, endothelium, fibroblasts and mast cells. It plays central role in inflammation, infection, angiogenesis, tissue modelling, cell proliferation, differentiation and apoptosis. It is a key biomarker in periodontal disease, diabetes mellitus, rheumatoid arthritis and mutagenesis. Interestingly, TNF-α diminishes epithelial barrier function by changing the structure and function of tight junctions.[6] Also, TNF-α is a main cytokine in skin as well as oral lichen planus (OLP) pathogenesis due to increased mRNA expression of TNF-α cytokine. [7,8,9]

In the light of above background, the objective of the study was to assess salivary TNF-α levels as a biomarker of desquamative gingivitis and compare its level in clinically healthy and chronic gingivitis subjects.

MATERIALS AND METHODS

Study Design: Case-Control

The study was done in the Department of Periodontics in collaboration with Department of Biochemistry. The study was approved by Institutional Ethical Committee of our institute. Subjects who agreed to participate and signed the consent form were recruited in the study.

The patient was comfortably seated on the dental chair. A comprehensive history of each subject was recorded. Complete intraoral examination was done using mouth mirror, UNC-15 periodontal probe (Hufriedy,Chicago,USA). Plaque Index (PI), Bleeding on probing(BOP),Probing pocket depth(PPD) and Clinical attachment level (CAL) were recorded for diagnosing chronic gingivitis and clinically healthy subjects by the single examiner. Routine haematological blood investigations –complete blood count(CBC) and haemogram was also advised in each subjects.

Subject Selection Criteria:

Desquamative gingivitis (DG):

a. Non plaque induced gingival inflammation.
b. Erythema, erosion, desquamation of free and attached gingiva clinically as fiery red, glazed,atrophic or eroded gingiva.
c. Painful to touch, intolerance to salt & spicy food, burning sensation.

Chronic gingivitis (CG):

a. Dental plaque induced gingivitis.
b. Presence of atleast 20 teeth.
c. Bleeding on probing(BOP) present in more than 30% of sites.
d. Probing Pocket depth(PPD)≤ 4mm.
e. CAL ≤ 1 mm.

Clinically healthy(CH):

a. Periodontal healthy individual with no apparent sign of inflammation.
b. Presence of at least 20 teeth.
c. Probing Pocket depth (PPD)= 2-3 mm.
d. Bleeding on probing (BOP)< 10%.

Exclusion Criteria:

Subjects with following diseases/conditions were excluded due to cofounder effect on salivary TNF-α level.

a. Periodontitis.
b. Tobacco and Alcohol consumption.
c. Systemic diseases like diabetes mellitus, rheumatoid arthritis etc.
d. Patient under any medication for last 3 months
e. Pregnancy and lactation.
f. leukoplakia, Oral Submucous fibrosis, oral cancer.

A total of 1120 subjects were screened from the outpatient clinic using simple random sampling. Out of 1120 subjects recruited, 210 subjects did not meet the subject selection criteria and 816 subjects did not gave consent for the study. The remaining 94 subject recruited were as follows groups viz; Controls; Clinically healthy (CH) (n=32) and Cases; Desquamative gingivitis(DG) (n=31), Chronic gingivitis (CG) (n=31).[**Figure 1**]

Sample size: Taking type 1 or alpha at 5% with the power of study 80% with odds ratio of 5 and ratio of cases to control of 1.The required subjects comes around 28 each in cases and controls. Therefore, the total required subjects in all the three groups (1 control and 2 cases) come around 84.

Saliva Collection: The subjects were abstained from eating or drinking about 2 hours before saliva collection. The subjects were seated comfortably on the dental chair and asked to rinse 3 ml of unstimulated whole expectorated saliva into 5 ml of saliva collecting tubes as per the method described by Navazesh. [10] The saliva collection was done in the outpatient in the morning from 9AM to 12 PM. The collected samples were stored at -80°C. Salivary TNF-α levels in each subjects were determined using the Quantikine Human total TNF-α immunoassay kit employing an ELISA technique (R&D systems).[11]

Statistical analysis**:** The data thus collected were analysed using Statistical Package for Social Sciences (SPSS ,version 20.0, IBM ,USA). The data were presented in Mean± Standard deviation and 95% Confidence Interval (95% CI).The data were tested for normality using Kolmogorov-Smirnov test and found to be normally distributed(p=0.20) thus parametric tests were used for the analysis. The power of study was taken at 80% and significant levels was taken at 5% ($p \leq 0.05$).

Ethical clearance: The study was approved by Institutional Ethical Committee, Faculty of Medicine, Jawaharlal Nehru Medical College and Hospital Aligarh Muslim University, Aligarh, UP, India.

RESULTS:

The mean age in DG group mean age was 45.85± 7.55 years (95% CI; 43.05 to 48.63 years), CG group was 42.29±8.47 years (95% CI; 39.18 to 45.40 years), CG group was 40.5± 8.86 years (95% CI ;37.31 to 43.69 years). In terms of gender distribution DG group had 5 males and 26 females whereas CG group had 16 males and 15 females and CH group had 16 males and 16 females.

Periodontal status: The CG group had significantly increased Plaque index (PI) and Bleeding on Probing(BOP) as compared to CH and DG groups ($P<0.001$).[**Table 1**]

Salivary TNF-α levels: The mean and 95% CI (confidence interval) values of salivary TNF-α level were DG group 33.71±12.15 pg/ml (95% CI; 29.25 to 38.17pg/ml), CG group 13.48±1.16 pg/ml (95% CI;13.05 to 13.91pg/ml) where as in CH group 5.99±2.11pg/ml (95% CI;5.23 to 6.76 pg/ml). **[Table 2, Figure 2]**

On comparing the salivary TNF-α levels in all the three groups using analysis of variances analysis (ANOVA) with 0.05% significant level and post hoc bonferroni analysis. There was highly significant difference in salivary TNF-α among all the three groups with the highest value in DG group ($p<0.001$).

Further, on plotting receiver operating curve (ROC) of salivary TNF-α level for DG the area under curve was 0.973 (95% CI;0.936 to1) showing it as a good biomarker of DG **[Figure 3]**

The *screening cut off* value of salivary TNF-α level in DG cases was 11.5 pg/ml with sensitivity of 100% and specificity of 52.4% having likelihood ratio (LR) of 2.10. However, the *diagnostic cut off* value of salivary TNF-α level for the DG cases was 15.56 pg/ml with sensitivity of 93.5% and specificity of 84% and likelihood ratio of 5.84 **[Table 3]**.

DISCUSSION

Our study show that salivary TNF-α is significantly elevated in DG as compared to chronic gingivitis and clinical health subjects. The receiver operator characteristic (ROC) curve of salivary TNF-α for DG had an area under curve (AUC) of 0.973.The *diagnostic cut off* value of salivary TNF-α for the DG was 15.56 pg/ml with sensitivity of 93.5% and specificity of 84% and likelihood ratio of 5.84 making it a good diagnostic marker**.**

Our findings are in agreement with study of Rhodus et al., [12] where salivary TNF-α levels in erosive oral lichen planus (35.63 ±19.87 pg/ml) were significantly increased than controls (2.24 ± 0.78 pg/ml). Similarly, Zhang et al., [13] showed that the salivary TNF-α values

in OLP patients (29.92 ± 9.99pg/ml) were significantly elevated than healthy controls(6.16 ± 1.93 pg/ml) which may be attributed due to local production of TNF-α.[14]

Sonja pezeli- Ribaric et al.,[14] compared salivary TNF-α levels between atrophic/erosive form of OLP and clinically healthy volunteer controls. Salivary TNF-α levels were significantly elevated and also correlated with disease. They found that salivary TNF-α levels were more increased in erosive or atrophic OLP which manifest as DG rather than reticular OLP. Similarly, Ghallab et al., [15] observed increased salivary TNF-α levels in erosive lichen planus than controls.

Furthermore, Robati et al., [16] demonstrated increased salivary IL-6 and TNF-α levels in OLP as compared to healthy controls and also found higher TNF-α and IL-6 levels in erosive OLP patients than its reticular variant. Also, Taghvi Zenouz et al.,[18] showed increased serum TNF-α and transforming growth factor –beta (TGF-β) levels in lichen planus patients as compared to age and sex matched controls suggesting critical role of TNF-α in pathogenesis of the disease. Interestingly, Rhodus et al [12] observed that salivary IL-6 and TNF-α levels were significantly increased in squamous cell carcinoma in comparision to precancerous lesions and healthy control.

Desquamative gingivitis is a clinical entity which is routinely diagnosed using specific clinical features along with adjunct histopathological and immunoflouresence studies with diagnosis of about one third of DG cases not certain. As, erosive OLP which manifests clinically as DG is more prone to transform into cancer, therefore, biomarker profiling has potential to contribute as an adjunct in diagnosis and prognosis of these lesions.[18,19]

Saliva is an easily collectable diagnostic fluid. The collection mode is non-invasive which requires minimal time and resources. Saliva is a rich source of biological molecules which can be used to decipher undergoing clinical phenomenon.[4]

Tumour necrosis factor-alpha (TNF-α) comes as a pro inflammatory cytokine with critical role in inflammation, immunity and apotosis. TNF-α decreases epithelial barrier function via altering structure and function of tight junction and diminishes trans-epithelial resistance by 81% and thereby increases epithelial permeability which is mediated by by Nuclear factor kappa B(NF-KB) dependent pathway.[5,9,20]

In addition, expression of both TNF-α and Intercellular adhesion molecule-1 (ICAM-1) are significantly increased in lichen planus as compared to controls and TNF-α plays a key role in the development of lichen planus.[21] Also, serum TNF-α levels in lichen planus were found to be significantly elevated than healthy controls.[22]

Moreover, TNF-α antagonists; Infliximab, Etanercept, Adalimunab have promising role in the treatment of oral mucosal disorder like OLP, Psoriasis thus delineating role of TNF-α in the pathogenesis of impeded epithelial barrier function.[23,24,25] Furthermore, decrease in baseline TNF-α levels in biopsy specimens of oral lichen planus was observed after treatment with local steroid.[26,27] Othmon et al., [27] showed that topical steroid reduced serum-TNF-α levels more than laser treatment in oral lichen planus.

In the our study the age distribution among all groups was similar however in sex distribution there was skewed female frequency in DG as compared to CH and CG groups, which is in conformity with previous studies showing female predominance in DG lesions.[1,2]

The limitations of the study is the recruitment of DG subject solely on clinical characteristics as our aim was biomarker profiling of the clinical condition. The desquamation, erythema and ulceration denotes a discontinuity in the mucosal epithelium barrier which is underpinned by increased expression of mRNA for TNF-α expression; inhibits cell-cell epithelial cohesion mediated by tight junctions/gap junctions.[8,13,14,15, 26 ,27,28,29]

Several studies show that salivary TNF-α is a diagnostic as well as prognostic marker as its increased levels are indicative of local environment milieu and depicts underlying epithelial aberrations and mutagenic changes in oral mucosal diseases.[16,17,18,30,31,32]

In conclusion, salivary TNF-α levels is a potential diagnostic marker of desqumative gingivitis as it depicts local oral environment milieu and defective epithelial barrier function.

Conflicts of interest:

All the authors declare that they have no conflicts of interest pertaining to this study.

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Authors's contribution:

Data gathering and idea owner of this study: Saif Khan, Afshan Bey Pankaj Bansal

Study design: Saif Khan

Data gathering: Saif Khan, Afshan Bey Pankaj Bansal, Shagufta Moin

Data analysis: Saif khan, Syed Ziaur Rahman

Writing and submitting manuscript: Saif Khan, Syed Ziaur Rahman

Editing and approval of final draft: Saif Khan, Afshan Bey Pankaj Bansal, Shagufta Moin , Syed Ziaur Rahman

Table 1: Showing periodontal parameters in desquamative gingivitis (DG), chronic gingivitis (CG) and clinically healthy (CH) subjects. Plaque index (PI), Bleeding on probing (BOP) in percentage, Probing pocket depth (PPD) and Clinical attachment level (CAL) in millimeters.

		DG	CG	CH
1	PI	0.52±0.24	2.06±0.56	0.12±0.45
2	BOP(%)	8.06±2.11	41.47±16.10	3.03±5.06
3	PPD (mm)	2.71±0.70	3.5±0.57	2.44±0.29
4	CAL (mm)	1.03±0.10	0.92±0.10	0.90±0.10

Table 2: Showing the mean and 95% confidence interval salivary TNF-α levels(pg/ml) in all the three study groups viz; Desquamative gingivitis (DG), Chronic gingivitis (CG) and Clinically healthy (CH).

Groups	Mean±SD*	95% Confidence Interval values		Minimum value	Maximum value
		Lower bound	Upper bound		
DG	33.71±12.15	29.25	38.17	11.64	60.18
CG	13.48±1.16	13.05	13.91	11.36	15.65
CH	5.99± 2.11	5.23	6.76	2.60	10.54

*Standard deviation

Table 3: Showing the screening, optimal and diagnostic cut-off values of salivary TNF-α in the DG group. ($p<0.05$,significant)

	Salivary TNF-α levels (pg/ml)	Sensitivity	Specificity	Likelihood Ratio(LR)
Screening Cut off	11.5	100%	52.4%	2.1
Optimal Cut off	13.65	93%	78%	4.2
Diagnostic Cut off	15.56	93.5%	84%	5.8

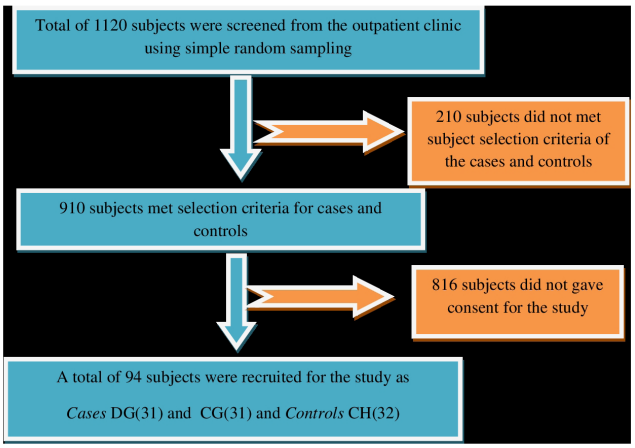

Figure 1: Showing the subject recruitment flow of the study.

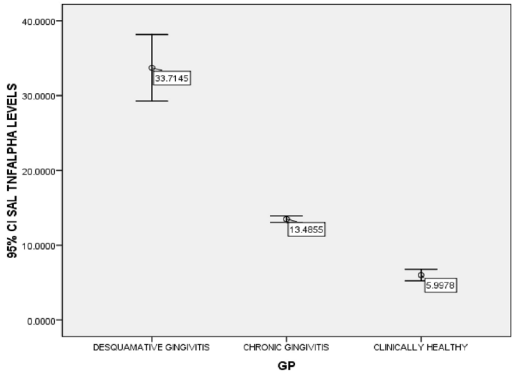

Figure 2: Showing the mean and 95% confidence interval (95% CI) values of salivary TNF-α (pg/ml) among all the three study groups.

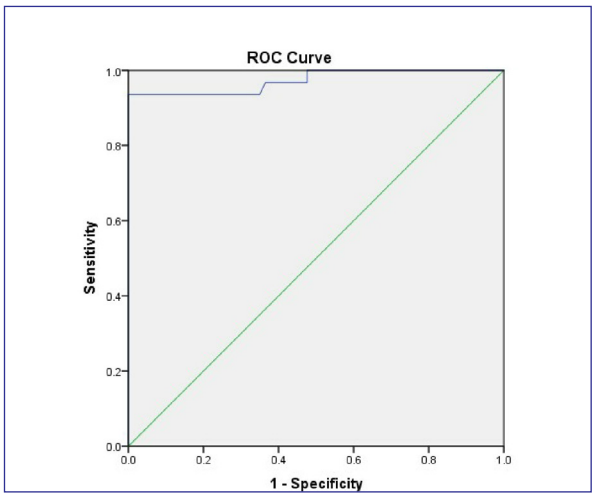

Figure 3: Showing the receiver operating characteristic (ROC) curve of salivary TNF-α in desquamative gingivitis (DG) having area under curve of 0.973.

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