Original Article

Gingival Crevicular Fluid and Salivary Levels of Galectin-3 in Patient with Gingivitis and Patient with Stage III Periodontitis: An Observational Study

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Abstract

Aim: The study was aimed at assessing the levels of Galectin-3 in the GCF and Saliva in gingivitis and stage III periodontitis patients. **Subjects and methods:** The present cross-sectional study was conducted on 66 participants including periodontal healthy, gingivitis, as well as stage III periodontitis individuals. Periodontal examination was continued at the outpatient clinic, Department of Oral Medicine and Periodontology, Faculty of Dentistry, Cairo University. Pocket depth, clinical attachment level, bleeding on probing, and plaque index were measured using a manual UNC periodontal probe. GCF samples were collected from the deepest site by inserting absorbent paper strips into the gingival sulcus for 30 seconds. Five ml of saliva sample was collected from each subject into Eppendorf tubes which were immediately frozen & stored at -70 °C. GCF and Salivary Galectin-3 levels were quantified by using a commercial Enzyme-Linked Immunosorbent Assay Kit. All these parameters were subjected to statistical analysis. **Results:** There was a statistically significant higher levels of Galectin-3 in the GCF and Saliva of periodontitis than in periodontal healthy. also, gingivitis group had higher levels compared to healthy group, while there was no statistically significant difference between gingivitis and periodontitis. **Conclusion:** Galectin-3 is a proinflammatory protein that might play essential roles in periodontal disease pathogenesis.

Keywords: GCF, Saliva, Gingivitis, Periodontitis and Galectin-3.

I. INTRODUCTION

Periodontal health is defined as the absence of clinically detectable inflammation. It may be seen in intact periodontium without clinical attachment loss and bone loss. Also, it may be seen in non-periodontitis patients with reduced periodontium or in currently periodontally stable patients with a history of periodontitis. It can be restored after gingivitis and periodontitis treatment. Moreover, patients with a history of periodontitis should be continuously monitored because they are still at increased risk of recurrent periodontitis (Chapple et al. 2018).

In intact periodontium, gingival health is characterized by the lack of erythema, attachment loss, edema, bone loss as well as, less than 10 % bleeding on probing. The Physiological bone level range from 1.0 to 3.0 mm apical to the cementoenamel junction (Mariotti and Hefti 2015; Chapple, et al. 2018). Also, on reduced periodontium accompanied with reduced clinical attachment levels and bone levels, it is characterized by an absence of bleeding on probing, erythema, edema, as well as a lack of patient symptoms (Lang and Bartold 2018; Chapple, et al. 2018).

Gingivitis and periodontitis are the most common clinical forms of human periodontal diseases. Gingivitis is generally regarded as a site-specific inflammatory condition initiated by dental biofilm accumulation and characterized by gingival redness and edema and the absence of periodontal attachment loss (Chapple, et al. 2018). It is usually painless and uncommonly leads to spontaneous bleeding. Due to its characterization by precise clinical changes, almost of patients are unaware of the disease or unable to recognize it. It differs between those according to systemic risk factors, known as; modifying factors, and local risk factors, known as: predisposing factors (Holmstrup, Plemons, and Meyle 2018; Chapple, et al. 2018).

Meanwhile, periodontitis is a chronic multifactorial inflammatory disease associated with bacterial plaque biofilms, which leads to progressive destruction of tooth supporting tissues. Microbiota maintains immune homeostasis in oral tissues mucosa. It is a significant public health problem that has high prevalence and potential for tooth loss, (Papapanou et al. 2018). It has a negative effect on aesthetics and chewing function therefore it causes worse quality of life and social inequality (Velickovic et al. 2021). According to Akkaya (2022), Galectin-3 may be involved in the pathogenesis of periodontal disease because its levels are elevated. As a result, these biomarkers may be used in the diagnosis of periodontal diseases.

Galectins are a family of galactoside binding proteins that regulate the interactions for cell-to-cell and cell-to-matrix in different tissues and organs (Fukumori et al. 2003). They were classified into three subgroups according to their carbohydrate recognition domain number and function (Dumic, Dabelic, and Flögel 2006). Galectin-3 that is a 30 kDa molecule that has a single carbohydrate recognition domain and an amino-terminal polypeptide tail region (Johannes, Jacob, and Leffler 2018). Galectin-3 is produced by multiple cell types, which include immune, epithelial, as well as fibroblast cells, affecting the immune cell's function. It has controversial pro-inflammatory and anti-inflammatory activities according to various factors. These factors comprise the target cell implicated in the processes, as well as the intracellular or extracellular localization (Chen et al. 2014; Loimaranta et al. 2018).

II. SUBJECTS AND METHODS

A. Study Design and Participants:

The present observational cross-sectional study with the Identifier: ID: NCT05281796 at ClinicalTrials.gov, was accepted by the Ethics Committee for Scientific Research at Cairo University January 2022. The purpose of the study was described to all subjects who signed a written consent and committed to participate in this work prior to filling out the questionnaire. The current study included 66 participants of gingivitis and stage III periodontitis of Egyptian patients seeking dental treatment at Cairo University's Diagnostic Center, Faculty of Dentistry. This study was conducted from January 2022 till September 2022 and screening of patients was continued until the target sample was achieved. Identifying and recruiting potential subjects was achieved through patients' database. The study included 3 groups, gingivitis, stage III periodontitis, and healthy subjects. Patients were diagnosed by proper history and periodontal clinical examination.

Inclusion criteria included for healthy subjects with Bleeding on probing was less than 10%, Probing depth less than 3mm and No loss of attachment. For gingivitis subjects with Bleeding on probing was 10% or more, Probing depth less than 3mm and No loss of attachment. For stage III periodontitis subjects with at least two non-adjacent teeth sites in each jaw having clinical attachment level (CAL) ≥ 5 mm and pocket depth (PD) > 5 mm in one or more sites, bleeding on probing (BOP) ≥ 30%, tooth loss due to periodontitis ≤ 4 teeth.

Exclusion criteria were those individuals with a history of systemic diseases or immunological disorders, pregnant and lactating women, individuals that received periodontal treatment within the last 6 months, individuals with a history of systemic antibiotics and anti-inflammatory drugs within the last 3 months and smokers subjects.

B. Clinical periodontal examination:

Clinical examinations were performed by a trained examiner (MS) to reach a case identification and diagnose the periodontal condition. For case identification, to diagnose and define the stage of periodontitis, PD, clinical attachment level, BoP and plaque index (PI) were measured. These parameters were measured using a manual UNC periodontal probe at the site of interproximal defect and were rounded up to the nearest millimeters. All permanent fully erupted teeth, excluding third molars, were evaluated at six different sites for each tooth; mesio-buccal, mid-buccal, distobuccal, disto-lingual, mid-lingual, and mesiolingual. Diagnosis and case identification of the periodontal disease was performed based on the new classification of periodontal disease (Papapanou, et al. 2018).

C. Samples collection:

1. GCF sampling

GCF samples were collected from the deepest site (Akkaya, et al. 2022). A single examiner was performing GCF sampling. Before sampling, the teeth were isolated with cotton rolls, supragingival plaque were removed with a sterile curette and the surfaces will be gently air-dried. GCF was sampled by inserting absorbent paper strips into the gingival sulcus or periodontal pocket for 30 seconds. Paper strips contaminated with blood and saliva were rejected.

The GCF volume absorbed into each strip was determined by an electronic gingival fluid measuring device, which was calibrated based on a protocol described before (calibration of the electronic device by polynomial regression). Samples of the same category (healthy or diseased sites) in each subject were immediately pooled into a dry sterile polypropylene micro centrifuge tube (five paper strips per tube) and kept at -80∘C until analysis. The readings from the electronic device were converted to an actual volume (µL) by reference to a standard curve. GCF Galectin-3 levels were determined using ELISA kit provided by Bioassay Technology Laboratory with Cat. No E3449Hu (Zhejiang. China)

2. Saliva sampling

All subjects who took part in this investigation provided unstimulated whole expectorated saliva samples were collected from all subjects participating in this study. Five ml of saliva sample were collected from each subject between 09:00 and 11:00 hours to avoid circadian rhythm (Navazesh 1993). Saliva samples were obtained in the morning following an overnight fast, patient was requested not to drink (except water) or chew gum.

Subjects rinsed their mouth with tape water. The saliva sample was obtained by expectoration of patient into an Eppendorf tube while seated in an upright position. The supernatant phase was transferred to Eppendorf tubes and stored at −80°C until analysis. Salivary Galactin-3 levels were determined using ELISA kit provided by Bioassay Technology Laboratory with Cat. No E3449Hu (Zhejiang. China).

D. Statistical and power analysis:

This power analysis Galectin-3 levels in the GCF and saliva of patient with periodontal disease as primary outcome. According to (Akkaya, et al. 2022), Galectin-3 levels in gingivitis group 76.17 ±15.96 and Galectin-3 levels in periodontitis group, 88.36 ± 11.4. the effect size of the difference between patients with gingivitis and patient with periodontitis stage III in GCF levels of Galectin-3 was 0.878. Using power 80% and 5% significance level, patients in each group will be 22 per group and total patients in study are 66 patients. The sample size was calculated by G-power program. Data was analyzed using MedCalc software, version 19 for windows (MedCalc Software Ltd, Ostend, Belgium). Data was explored for normality using Kolmogorov Smirnov test and Shapiro Wilk test. The mean and standard deviation were used to describe continuous data with a normal distribution. Intergroup comparison between continuous data was performed using one-way ANOVA followed by tukey post-hoc test. Non-parametric data were described as median and range and were compared using the Kruskal-Wallis test followed by Dunn post-hoc test. A value of 0.05 or less was considered statistically significant and all tests were two tailed.

III.   RESULTS

A. Results of clinical Parameters:

Table 1 shows periodontal parameters for the groups. The highest PI scores were found in periodontitis and gingivitis groups, with no statistically significant difference between them. While, the lowest PI scores were found in healthy controls. Additionally, the highest BoP scores were found in periodontitis groups. While, the lowest BoP scores were found in healthy controls. Also, a statistically significant difference in BoP scores was reported between healthy and gingivitis groups as well as gingivitis when compared to the periodontitis group. Moreover, the results obtained from the current study showed a statistically significant difference between PPD and CAL within the healthy and gingivitis groups compared to periodontitis group. However, there was no statistically significant difference between the healthy and gingivitis groups.

B. Levels of Galectin-3 in the GCF and saliva in the different study groups:

Table 2 shows Levels of Galectin-3 in the GCF and saliva for the groups. The highest GCF and saliva levels of Galectin-3 were found in periodontitis and gingivitis groups, with no statistically significant difference between them. While the lowest GCF and saliva levels of Galectin-3 were found in healthy controls.

Table (1): Clinical periodontal parameters throughout the study period

Group/Outcome	Healthy	Gingivitis	Periodontitis	P value
	Median (range)	Median (range)	Median (range)	
PI score	0[a] (0-0)	3[b] (1-3)	3[b] (3-3)	P < 0.001*
BoP %	0[a] (0-7.1)	40.8[b] (17.9-94.6)	100[c] (61.5-100)	P < 0.001*
	Mean±SD	Mean±SD	Mean±SD	
Pocket depth	1.34[a] ± 0.23	1.38[a] ± 0.21	4.22[b] ± 0.47	P < 0.001*
Clinical attachment level	0.0[a] ± 0.0	0.0[a] ± 0.0	5.7[b] ± 0.61	P < 0.001*

Means that do not share a letter are significantly different, * corresponds to statistically significant difference.

Table (2): Mean and standard deviation of GCF and Galectin-3 (ng/ml) for all groups

Group/Outcome	Healthy	Gingivitis	Periodontitis	P value
	Mean±SD	Mean±SD	Mean±SD	
Galectin GCF (ng/ml)	163.35[a] ± 10.77	679.21[b] ± 114.66	619.21[b] ± 148.02	P < 0.001*
Galectin Saliva (ng/ml)	384.47[a] ± 80.5	589.78[b] ± 131.3	521.46[b] ± 126.49	P < 0.001*

Means that do not share a letter are significantly different, * corresponds to statistically significant difference.

IV. DISCUSSION

Periodontal diseases are a group of inflammatory conditions that affect all tooth-supporting structures such as gingiva, periodontal ligament, cementum, and alveolar bone (**Lang and Bartold 2018**). Researchers find it challenging to perform full-mouth clinical assessments due to the time involved. As a result, there is an increased demand for newer reproducible diagnostic tools that accurately represent the current disease state and estimate future disease risk (**Fatima et al. 2021**). Galectin-3 is a proinflammatory protein that might be involved in the progression of inflammation by inducing the expression of proinflammatory mediators during the periodontal inflammatory process. Previous studies found an increase in levels of Galectin-3 in GCF of periodontitis patients, as it plays a key role in immune cell homeostasis and also acts as a modulator for cell signaling and cell surface functioning in a variety of inflammatory conditions (**Karsiyaka Hendek, Olgun, and Kisa 2021; Akkaya, et al. 2022; Afacan et al. 2023**). Based on the data mentioned above, this observational cross-sectional study aimed to assess levels of Galectin-3 in saliva and gingival crevicular fluid of patients with gingivitis and patients with stage III periodontitis compared to healthy individuals.

With regards to PI scores, stage III periodontitis group showed significantly greater PI scores than the periodontally healthy group. Also, the PI scores of the gingivitis

group were significantly higher than that of the healthy group. However, PI scores were statistically insignificant between gingivitis and stage III periodontitis. These results were consistent with **Karsiyaka Hendek et al. (2021)** who stated that the values of the PI scores in periodontally healthy individuals were significantly lower than the other groups. The present results were further supported by **Isola et al. (2021)** who reported that the PI were significantly high in periodontitis as well as a combination of periodontitis with coronary heart disease groups compared to healthy and coronary heart disease groups.

Interestingly, though **Akkaya et al. (2022)** stated that stage III periodontitis group had higher PI value than that of the healthy group, however, it was claimed that the gingivitis group had significantly greater PI value than healthy group as well. Meanwhile, stage III periodontitis group had higher PI value than gingivitis group. This result may be due to the fact that PI scores reflect the patient oral hygiene measures, but it is not the indicator which differentiate between gingivitis and periodontitis (**Tonetti and Sanz 2019; Carvalho et al. 2023**). The results of this current investigation were in line with **Afacan et al. (2023)** who stated that both stage III grade B and C periodontitis had significantly higher PI scores than periodontally healthy controls as well as gingivitis had significantly higher PI scores than periodontally healthy controls. Furthermore, as mentioned by **Rajaram et al. (2023)**, the mean PI were significantly high in periodontitis compared to that of the healthy group which supported the results of the current study.

Regarding BoP results, the present study exhibited a higher BoP score in the periodontitis group than the healthy and gingivitis groups. Likewise, there was statistically significant difference between the healthy and gingivitis groups. These findings were comparable with the results of a study conducted by **Isola et al. (2021)** who stated that the BoP was significantly high in periodontitis as well as a combination of periodontitis with coronary heart disease groups compared to healthy and coronary heart disease groups. Also, these results were consistent with **Akkaya et al. (2022)** found that BoP values were significantly high in gingivitis and stage III periodontitis groups with no statistical significance between them. These results were further supported by **Afacan et al. (2023)** who found that periodontitis and gingivitis groups had significantly higher BoP scores than periodontally healthy controls, and periodontitis had significantly higher BoP than gingivitis group. These results could be attributed to the fact that BoP values represent the severity of periodontal inflammation, which commonly indicate the difference between periodontally healthy individuals and others with periodontal diseases **(Tonetti and Sanz 2019)**. Moreover, periodontitis patients have a high prevalence of Porphyromonous gingivalis which affects the values of BoP. As mentioned by **Rodrigues et al. (2020),** the presence of P. gingivalis was linked to stage III grade C periodontitis along with increased periodontal destruction. While the P. gingivalis fim-A genotype was linked to increased BoP, lending support to the hypothesis that P. gingivalis fim-A II is an important virulence factor for pathogenesis of periodontal diseases.

The PPD and CAL results of this cross-sectional study stated that periodontitis values were significantly greater than healthy and gingivitis groups. However, healthy and gingivitis groups were the same with significantly insignificant difference in PPD and CAL values. These results were consistent with **Karsiyaka Hendek et al. (2021)** who concluded that PPD and CAL values were significantly higher in periodontitis groups than gingivitis and health groups. The present findings were also consistent with a cross-sectional study performed by **Isola et al. (2021)** who stated that the PPD and CAL were significantly high in periodontitis as well as a combination of periodontitis with coronary heart disease groups compared to healthy and coronary heart disease groups.

The results of the current investigation were further in line with **Akkaya et al. (2022)** who stated that Group stage III periodontitis had the highest CAL values compared with groups gingivitis and healthy groups, while, healthy and gingivitis groups had similar CAL values. Similarly, **Afacan et al. (2023)** concluded that the mean CAL scores in gingivitis and periodontally healthy groups were significantly lower than in periodontitis group. These results may be attributed to the fact that CAL is considered the mainly clinical parameter to differentiate periodontitis from gingivitis, so the increase in those parameters is expected with periodontitis groups **(Tonetti and Sanz 2019; Carvalho, et al. 2023)**.

On the contrary with this present findings, concerning the PPD, **Akkaya et al. (2022)** and **Afacan et al. (2023)** found that the PPD value in the healthy group was significantly lower than in the gingivitis group. These current findings could be attributed to the fact that the PPD value alone should not be used as evidence for periodontal healthy or gingivitis, since there might be pseudo-pockets present due to the inflamed tissues which may mask the PPD values. It must be considered in conjunction with BoP, as well as modifying and predisposing factors. Furthermore, PPD should be less than 3 mm in case of periodontal healthy or gingivitis, without any clinical attachment loss **(Chapple, et al. 2018; Lang and Bartold 2018; Murakami et al. 2018)**.

The chief findings of the present study were levels of Galectin-3 in the GCF and saliva. Concerning the levels of Galectin-3 in GCF, there was a statistically significant differences between healthy and gingivitis groups, also, healthy compared to periodontitis groups.

However, Galectin-3 levels in the GCF were similar in periodontitis and gingivitis groups. These findings were supported by **Karsiyaka Hendek et al. (2021)** who stated that the levels of GCF galectin-3 was significantly lower in periodontal healthy group than the other two groups, and there was no significant difference between gingivitis and periodontitis groups.

One of the tenable explanations of the present results was the potential role of Galectin-3 as a pattern recognition receptor has become an area of increased attention. It is essential in the progression from prediabetic state to diabetic state, since it is considered a proinflammatory molecule that causes beta-cell dysfunction and insulin resistance because it acts as a receptor for advanced glycation end products **(Vasta 2012)**. It was examined whether Galectin-3 had a possible effect on periodontitis with or without DM as a factor in DM and bone metabolism. It was concluded that osteocyte-derived exosomes carrying miR-124-3p might regulate Galectin-3 expression of osteoblasts. This occurs specially under high-glucose conditions, suggesting a possible mechanism for DM-related alveolar bone pathologies **(Pugliese et al. 2014; Yilmaz et al. 2015)**.

Additionally, the higher Galectin-3 levels in periodontitis may also be linked to the association between Galectin-3 and P. gingivalis. According to **Miyauchi et al. (2018)**, P. gingivalis Lipopolysaccharide stimulation raised Galectin-3 expression in placental cells, consequently, in this investigation, the presence of P. gingivalis in periodontitis lesions may increase Galectin-3 production. Moreover, Galectin-3 play an active role in the progression of inflammation by inducing the expression of proinflammatory mediators in the periodontal inflammatory process **(Karsiyaka Hendek, Olgun, and Kisa 2021)**.

On the other hand to this present findings, **Akkaya et al. (2022)** reported that stage III grade C periodontitis group had highest GCF volume compared with gingivitis and healthy groups. Gingivitis group had also significantly greater GCF volume than healthy group.

Additionally, **Afacan et al. (2023)** found that stage III grade C periodontitis had significantly higher GCF Galectin-3 levels than gingivitis and healthy. However, there was no statistically significant difference between gingivitis and periodontitis in stage III grade B periodontitis.

These current findings could be attributed to the facts claimed in the study conducted by **Hong et al. (2020)** who reported that gingivitis is a prerequisite for periodontitis, as well as it would seem natural to believe that gingivitis and periodontitis can share the same diagnostic biomarkers. However, there are differences in the severity and stage of inflammation. Also, these contradictory results could be explained by the ethnic differences between the subjects enrolled in the studies that resulted in heterogenicity of their features. Furthermore, all of these studies were observational with small sample sizes and a lack of representativity, making it was impossible to interpret of this finding **(Nagarajan et al. 2015; Barros et al. 2016; Afacan, et al. 2023)**.

Regarding the levels of Galectin-3 in saliva, the current statistical analysis showed significant differences samples between healthy and gingivitis as well as in healthy compared to periodontitis groups, while gingivitis and periodontitis had comparable results regarding salivary Galectin-3 levels. These findings were consistent with **Isola et al. (2021)** and **Rajaram et al. (2023)** who stated that the values of salivary Galectin-3 levels were significantly high in periodontitis compared to healthy.

Limitation with this current study could be the cross-sectional design of the study and relatively small sample size, where a causal association between the biomarkers assessed and periodontal state could not be established. Strong evidence can be obtained from well-designed studies with larger sample sizes and matching results after a longer follow-up period following periodontal treatment.

V.   CONCLUSION

1. Within the limits of this study, increased GCF levels of Galectin-3 in periodontitis compared to healthy controls imply that these molecules play essential roles in periodontal disease pathogenesis.
2. Galectin-3 biomarkers may be beneficial in the detection of periodontal disorders.
3. Furthermore, additional studies are needed to research will aid in clarifying the specific mechanisms, time-dependent evaluation, and its role in chronic inflammation and systemic-periodontal disease interactions.

Conflict of Interest:

The authors declare no conflict of interest.

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Ethics:

This study protocol was approved by the ethical committee of the faculty of dentistry-Cairo university on: 25-01-2022, approval number: 15122.

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