ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at:www.ijdsir.com

Volume – 7, Issue – 4, August – 2024, Page No. : 62 - 68

Gingival Crevicular Fluid Galectin-3 Levels in Periodontitis Patients with Coronary Heart Disease

[1]Dr. Ruthvik K S, Post Graduate Student, Department of Periodontology, Ramaiah University of Applied Sciences, Bengaluru-560054

[2]Dr. Kranti Konuganti, Professor & HOD, Department of Periodontology, Ramaiah University of Applied Sciences, Bengaluru-560054

Corresponding Author: Dr. Kranti Konuganti, Professor & HOD, Department of Periodontology, Ramaiah University of Applied Sciences, Bengaluru-560054

Citation of this Article: Dr. Ruthvik K S, Dr. Kranti Konuganti, "Gingival Crevicular Fluid Galectin-3 Levels in Periodontitis Patients with Coronary Heart Disease", IJDSIR- August – 2024, Volume –7, Issue - 4, P. No. 62 – 68.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Periodontitis and coronary heart disease are the most prevalent inflammatory conditions that have common pathogenic mechanisms. Galectin-3 is a β-galactoside-binding lectin which is known to be a prominent factor in various inflammatory conditions. Thus, the aim of this study was to estimate gingival crevicular fluid levels of Galectin-3 concentrations in patients with periodontitis and coronary heart diseases.

Materials &Methodology: A total of 60 patients were enrolled and divided into four groups: Group 1 (Healthy individuals), Group 2(Periodontitis patients), Group 3 (Coronary heart disease patients) and Group 4 (Patients with both periodontitis with coronary heart disease). Gingival crevicular fluid (GCF) samples were obtained to measure the levels of galectin 3. The data was analyzed using Anova followed by Tukey's post hoc test.

Results: The age of the participants ranged between 32 – 72 years. Group 4 participants had the highest galectin-3 levels (0.6869±0.0536) followed by group 3 (0.3695±0.0316), group 2 (0.2267 ±0.0330) and it was the least in group 1 (0.1023±0.0158).

Conclusion: The study showed that patients with CHD and with Periodontitis + CHD had higher level of galectin-3 in GCF as compared to patients with periodontitis and healthy population.

Keywords: Galectin-3, Coronary Heart Disease, Periodontitis

Introduction

Periodontitis and coronary heart disease (CHD) represent two considerable health burdens, contributing substantially to the growing morbidity and mortality rates globally. Periodontitis encompasses a pathogenic pathway that can cause tissue death, bone resorption, and tooth loss[1]. Globally, periodontitis is prevalent among

20-50% of the population and is marked as the eleventh most common condition worldwide[2]. On the other hand, CHD comprises various heart conditions, including angina pectoris, myocardial infarction, and coronary artery disease (CAD). These conditions are frequently characterized by inflammation and atherosclerosis in the coronary arteries. CHD accounts for around 17.8 million deaths and is the third leading cause of mortality globally [3].Despite the fact that CHD and periodontitis are two seemingly disparate conditions, research evidence has suggested a potential link between the two non-communicable diseases. The link is potentially rooted in shared risk factors like smoking, diabetes, hypertension, and obesity and the pathophysiological mechanisms involved, like systemic inflammation, oxidative stress, and immune dysregulation[4].

The pathways proposed to establish a link between periodontitis and CHD have highlighted the fact that periodontal pathogens and their by-products can enter the bloodstream and cause systemic dissemination and activation of inflammatory cascades and contribute to endothelial dysfunction, oxidative stress, immune cell action, and alterations in lipid metabolism, all of which are crucial to the pathogenesis of CHD [1]**.** In addition, continuous exposure to these inflammatory mediators from periodontitis may further aggravate the existing cardiovascular risk factors and result in accelerated plaque formation, and promote a pro-atherogenic environment within the arterial walls [1,5]**.**

Various epidemiological studies have demonstrated an increased risk of CHD among individuals with periodontitis, with some studies reporting a two-fold to three-fold higher risk compared to individuals without periodontal disease[6–8]**.** It has also been shown that in patients with periodontitis, the risk of developing CHD was 14% higher, with a relative risk of 1.14 as compared to the healthy population. In addition, the severity of the periodontal disease was also significantly associated with the incidence of CHD [6]**.**

In this regard, Galectin-3, which is known to play a pivotal role in inflammatory pathways, can be implicated in both periodontal diseases and cardiovascular diseases. Galectins belong to a family of evolutionarily conserved proteins characterized by carbohydrate-recognition domains (CRD) comprising approximately 135 amino acids that form a globular structure with a strong affinity for β-galactosides[9]**.** Galectin-3 has been implicated as a primary factor in cardiovascular diseases, which can cause inflammation, endothelial dysfunction, atherosclerosis, myocardial fibrosis, and cardiac remodeling. Adverse outcomes like heart failure, myocardial infarction have been strongly correlated with elevated levels of galectin-3, thus establishing it as an excellent biomarker for disease prognosis[9]**.**

In periodontal disease, Galectin-3 may influence the progression of periodontitis through a signaling pathway that is largely mediated by β1-integrin, particularly in response to lipopolysaccharides from certain periodontal pathogens like Porphyromonas gingivalis [10]**.**Various research have indicated that higher Gal-3 levels correlate with the seriousness of periodontitis, and that undergoing initial periodontal treatment can lower these levels. This suggests that Galectin-3 could serve as a promising biomarker for assessing gum inflammation and the existence of periodontal disease [11]**.**

Several studies have independently assessed the levels of Galectin-3 in patients with periodontitis [12,13]or other systemic condition or estimated the levels of Galectin-3 in serum or salivary fluids [10,11]. However, there is limited research evaluating Galectin-3 levels in gingival crevicular fluid (GCF) among individuals with these coexisting conditions. Thus the present study was

conducted with the aim to estimate gingival crevicular fluid levels of Galectin-3 concentrations in patients with periodontitis and coronary heart disease.

Methodology

The present observational study was conducted in the Department of Periodontology, Faculty of dental sciences, RUAS and M S Ramaiah Memorial Hospital, Bangalore after obtaining ethical clearance from the Institutional ethical committee. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all the participants before initiating the study.Considering the effect size to be measured (dz) at 45% [calculated based on the results of the previous literature[10], power of the study at 80% and the alpha error at 5%, the sample size was estimated at 60.A total of 60 patients in the age group between 30-70 years and fulfilling the inclusion and exclusion criteria were selected and divided into 4 groups of 15 participants each.

In Periodontitis Group Presence of at least 15 teeth 40% sites with a probing depth (PD) ≥4 mm and clinical attachment level (CAL) ≥2 mm ,≥40% of sites with bleeding on probing (BOP) and at least 2 interproximal sites with radiographic alveolar bone loss (ABL) of ≥2 mm verified on periapical X-rays.

CHD diagnosis was made by coronary angiography or percutaneous coronary intervention with the presence of ≥50% of stenosis of at least one coronary artery. Clinical history, previous records, and the pharmacological treatment for CHD were recorded during the first visit. Patients were subjected to an electrocardiographic examination to evaluate the presence of atrial fibrillation or other pathological conditions.

Patient who were on anti-inflammatory, immunosuppressants or antibiotics or drugs six months before the study, pregnant or lactating woman and who have undergone periodontal treatment six months prior to the study were excluded

For GCF collection**,** the areas with deepest probing pocket depth were gently dried and isolated with cotton rolls to avoid saliva contamination. GCF samples were collected and immediately transferred to airtight plastic vials and will be stored at -80-degree celcius until assayed. Samples were assayed using Human GAL-3 (galectin 3) Enzyme Linked Immuno Sorbent Assay (ELISA) kit, according to the manufacturer's instructions.

For analyzing the sample, the standard solution was prepared. The stds/samples/blanks were loaded to their respective wells of the plate. The plate was incubated for 1hour at 37°C Bacteriological Incubator. 100ul of Biotynylated GaLectin-3 antibody working solution was added and the plate was incubated for 1hour at 37°C Bacteriological Incubator. 100ul of Streptavidin: HRP conjugate working solution was added and the plate was incubated for 30mins at 37°C Bacteriological Incubator. 100ul of TMB substrate was added and the plate was incubated for 10mins at 37°C Bacteriological Incubator. 100ul of Stop solution was added and the wells turn from blue to yellow in colour. The absorbance was read at 450nm with microplate reader.

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp. was used to perform statistical analyses. Descriptive analysis of all the explanatory and outcome parameters was done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Anova followed by Tukey's post hoc test was used to compare the difference in the galectin-3 levels among various groups. The level of significance was set at $p<0.01$.

Results

The age of the participants ranged from 32 – 72 years. The mean age of the participants in group 1 was 48.52 ± 8.33, in group 2 was 49.80 ±9.69, in group 3 was 52.87 ±8.73 and in group 4 was 56.33 ±9.10. However, there was no statistically significant difference in the age group of the participants between the groups (p=0.14) (Table 1). With respect to gender, majority of the participants in all groups were males as compared to females and this difference was not statistically significant (p=1.000) (Table 2).

The assessment of the Galectin-3 levels in the GCF revealed that Group 4 participants had the highest galectin-3 levels (0.6869±0.0536) followed by group 3 (0.3695±0.0316), group 2 (0.2267 ±0.0330) and it was the least in group 1 (0.1023±0.0158) (Graph-1). This difference in the mean galectin-3 levels was statistically significant (p <0.001). Post hoc analysis revealed that the mean galectin-3 levels between all the 4 groups was significantly different from each other (p <0.001) (Table 3).

Table 1: Mean Age & Gender distribution among different study groups

Mean Age & Gender distribution among different study groups										
Variable	Category	Group 1		Group 2		Group 3		Group 4		p-value
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age	Mean	48.53	8.33	49.80	9.69	52.87	8.73	56.33	9.10	0.14[a]
	Range	32 - 65		35 - 69		40 - 67		42 - 72		
		n	%	n	%	n	%	n	%	
Gender	Males	10	66.7%	12	80.0%	11	73.3%	11	73.3%	1.00[b]
	Females	5	33.3%	3	20.0%	4	26.7%	4	26.7%	
a. Kruskal Wallis Test & b. Chi Square Test										

Table 2: Comparison of mean GCF Galectin-3 levels (in ng/ml) b/w groups

Biomarkers	Groups	N	Mean	SD	Min	Max	p-value
GCF Galectin-3 levels (in ng/ml)	Group 1	15	0.1023	0.0158	0.083	0.130	<0.001*
	Group 2	15	0.2267	0.0330	0.181	0.276	
	Group 3	15	0.3695	0.0316	0.310	0.417	
	Group 4	15	0.6869	0.0536	0.610	0.780	

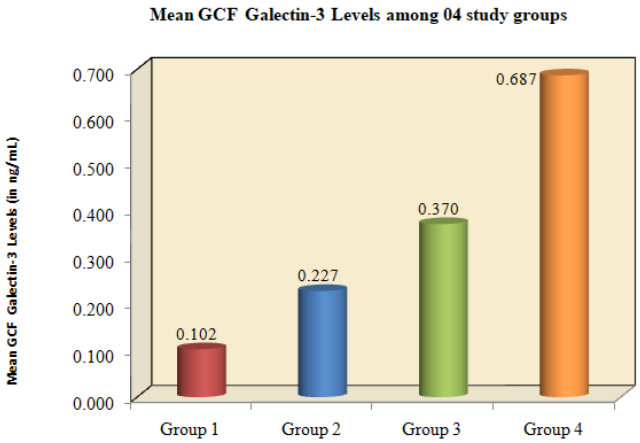
SD- Standard Deviation, Min- Minimum, Max- Maximum, * - Statistically Significant

Table 3: Multiple comparison of mean difference of GCF Galectin-3 levels b/w groups

Parameters	G1 vs G2	G1 vs G3	G1 vs G4	G2 vs. G3	G2 s G4	G3 vs. G4
GCF Galectin-3	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

* - Statistically Significant

Graph 1: Mean GCF Galectin-3 Levels among the 04 study groups



Discussion

Galectin-3 is a β-galactoside-binding lectin that plays an important role in the progression of periodontitis and CHD. In periodontal inflammation, Galectin-3 modulates the inflammatory responses, and due to matrix metalloproteinases, tissue destruction is initiated. It also contributes significantly in the process of immune response dysregulation and is thus responsible for chronic inflammation of periodontal tissues[9].On the contrary, galectin-3 has also been implicated in atherosclerosis, plaque instability, and myocardial fibrosis, which influences the progression of cardiovascular diseases [14].This dual connotation emphasizes the interrelation of periodontitis and CHD, highlighting Galectin-3 as a potential link between oral health and systemic disease outcomes like cardiovascular diseases.

Several studies have linked the direct association of galectin-3 levels and mortality rates in individuals. A study prospective study by Medvedeva et al has shown that with a 26 month follow up period, galectin-3 expression levels especially >21 ng/ml was an independent predictor of mortality in patients with a history of chronic heart failure[15].A study by Dong R et al also proved that in coronary heart disease, serum galectin-3 levels were amplified, and it was an independent predictor of mortality and re-hospitalization[16]. This could possibly be due to the fact that higher galectin-3 levels could augment the possibility of endothelial dysfunction and stroke. These findings demonstrate the fact that Galectin-3 levels were independently associated with augmented cardiovascular events[15,16].

Thus, the present observational study was conducted to recognize the potential association of galectin-3 in periodontitis and CHD by estimating the gingival crevicular fluid levels of Galectin-3 concentrations in patients with periodontitis and coronary heart disease. The observational study was undertaken on a sample of 60 subjects who were divided into 4 groups consisting of healthy individuals, individuals with periodontitis, individuals with coronary heart disease, and individuals with both periodontitis and coronary heart disease, following which thegalectin-3 levels in the GCF and periodontal disease parameters were assessed in all the participants.

The present study revealed that the galectin-3 level in the GCF was higher in subjects who had both periodontitis and CHD, and it was the least in healthy individuals. These findings are similar to epidemiological studies conducted wherein individuals diagnosed with periodontitis and those with periodontitis + CHD cohorts exhibited elevated median levels of Galectin-3 in both serum and saliva when compared to those with only CHD and healthy participants [10].Furthermore, studies assessing the galectin-3 levels in periodontally compromised patients have found that patients with established periodontitis had a higher level of galectin-3 as compared to patients with gingivitis and healthy periodontium[17]. The increased levels of galectin-3 in

patients with a history of periodontitis and CHD could be attributed to the common inflammatory pathway shared by both diseases. The inflammatory pathway involved in periodontitis can cause destruction of tissues, and systemic inflammation contributes to increased levels of galectin-3 in individuals. Likewise, in CHD, the inflammatory process only plays a significant role in atherosclerosis and plaque formation, which further raises Galectin-3 levels as part of the immune response [10].

However, the present study had certain limitations. The sample size considered was small, and the study design was a descriptive study. To overcome the limitations, further studies with a larger sample size, considering a multitude of risk factors, assessing and comparing the levels of the biomarkers in both serum and saliva are recommended. Multi-centric clinical studies, including in-vitro and in-vivo studies comparing various biomarkers and understanding the interplay between the diseases should also be carried out. Longitudinal studies investigating Galectin-3 as a potential biomarker for predicting CHD risk and monitoring disease progression in individuals with periodontitis can be conducted that can help in risk stratification, decision-making, and treatment.

The present observational study showed that patients with CHD and with periodontitis + CHD had higher levels of galectin-3 in GCF as compared to patients with periodontitis and a healthy population. Galectin-3 levels demonstrated to be a valuable early prognostic biomarker for periodontitis and CHD. Further, long-term intervention studies with a larger sample size are required to validate our results.

References

1. Hajishengallis G. Periodontitis: from microbial immune subversion to systemic inflammation. Nat Rev Immunol. 2015 Jan;15(1):30–44.
2. Nazir M, Al-Ansari A, Al-Khalifa K, Alhareky M, Gaffar B, Almas K. Global Prevalence of Periodontal Disease and Lack of Its Surveillance. The Scientific World Journal. 2020 May 28;2020.
3. Brown JC, Gerhardt TE, Kwon E. Risk Factors for Coronary Artery Disease. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Jul 26]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK554410/
4. Donders HCM, Veth EO, van ‘t Hof AWJ, de Lange J, Loos BG. The association between periodontitis and cardiovascular risks in asymptomatic healthy patients. International Journal of Cardiology Cardiovascular Risk and Prevention. 2021 Dec 1;11:200110.
5. Janakiram C, Mehta A, Venkitachalam R. Prevalence of periodontal disease among adults in India: A systematic review and meta-analysis. Journal of Oral Biology and Craniofacial Research. 2020 Oct 1;10(4):800–6.
6. Alwithanani N. Periodontal Diseases and Heart Diseases: A Systemic Review. J Pharm Bioallied Sci. 2023 Jul;15(Suppl 1):S72–8.
7. Beck JD, Eke P, Heiss G, Madianos P, Couper D, Lin D, et al. Periodontal Disease and Coronary Heart Disease. Circulation. 2005 Jul 5;112(1):19–24.
8. Nikolaos Andreas Chrysanthakopoulos, Aggelos Antonios Oikonomou, Panagiotis Andreas Chrysanthakopoulos, Rajiv Saini. Associations between Periodontal Disease Parameters and Coronary Heart Disease in Greek Adults: A Cross-

sectional Study. International Journal of Experimental Dental Science. 2015 Jun;4(1):4–10.

9. Velickovic M, Arsenijevic A, Acovic A, Arsenijevic D, Milovanovic J, Dimitrijevic J, et al. Galectin-3, Possible Role in Pathogenesis of Periodontal Diseases and Potential Therapeutic Target. Front Pharmacol. 2021 Mar 19;12:638258.
10. Isola G, Polizzi A, Alibrandi A, Williams RC, Lo Giudice A. Analysis of galectin-3 levels as a source of coronary heart disease risk during periodontitis. J Periodontal Res. 2021 Jun;56(3):597–605.
11. Abdulmajeed S, Sh Mahmood M. Evaluation of the Salivary Levels of Interleukin-17 and Galectin-3 in Patients with Periodontitis and Type 2 Diabetes Mellitus. Medical Journal of Babylon. 2023 May 5;Volume 20 ¦ January-March 2023:175–80.
12. Barut Z, Nalbantoğlu AM, Korkmaz H, Demir Z, Hatipoğlu M, Özkan A, et al. The role of salivary galectin-3 and galectin-9 levels in plaque-induced gingivitis and periodontitis. Heliyon. 2023 Sep 1;9(9):e19979.
13. Rajaram V, Sudhakar U, Ravindran N, Mithradas N, Ram V, Warrier V, et al. Evaluation of Salivary Galectin-3 Level and its Potential Role in Increasing the Severity of COVID-19 Infection in Patients with Periodontitis. World Journal of Dentistry. 2023 Mar 27;14:3–8.
14. Cao ZQ, Yu X, Leng P. Research progress on the role of gal-3 in cardio/cerebrovascular diseases. Biomedicine & Pharmacotherapy. 2021 Jan 1;133:111066.
15. Medvedeva EA, Berezin II, Surkova EA, Yaranov DM, Shchukin YV. Galectin-3 in patients with chronic heart failure: association with oxidative stress, inflammation, renal dysfunction and prognosis. Minerva Cardioangiol. 2016 Dec;64(6):595–602.
16. Dong R, Zhang M, Hu Q, Zheng S, Soh A, Zheng Y, et al. Galectin-3 as a novel biomarker for disease diagnosis and a target for therapy. International Journal of Molecular Medicine. 2018 Feb 1;41(2):599–614.
17. Afacan B, Ilhan HA, Köse T, Emingil G. Gingival crevicular fluid galectin-3 and interleukin-1 beta levels in stage 3 periodontitis with grade B and C. Clin Oral Invest. 2023 Jul 1;27(7):3749–58.