Estimation of Salivary Sialic Acid Levels in Chronic Periodontitis Patients

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Abstract:

Background: Gingivitis and periodontitis stand as the most common oral ailments, impactingapproximately 50% of the adult human population. SA serves as a significant source of bacterial pathogens and plays a crucial role in the colonization of periodontal pathogens. It facilitates bacterial aggregation and contributes to the formation of pellicle and dental plaque. **Aim:** Toestimate and comparethesalivarySialicacidlevelsinhealthycontrols,gingivitis&chronic periodontitis patients. **Material and Methods:** Unstimulated whole saliva was collected from 60 participants Group I, (healthy controls, n=20), Group II (gingivitis, n=20), Group III (Individuals with periodontitis, n=20). Evaluation of periodontal health was done by calculating plaque index, measuring probing depth, and Clinical Attachment Loss (CAL). The saliva samples were collected, and centrifuged at8000 rpm for 12mins and biochemical analysis was done using the Acidic Ninhydrin method. **Results:** The mean salivary sialic acid levels in Group 3, Group 2, and Group 1 were 1.4785µg/ml, 1.115µg/ml, and 0.43µg/ml. These differences were found to be statistically significant ($p<0.001$). It is clear that as the severity of periodontal disease progresses from Group I to III, the average levels of SA in saliva also noticeably rise from Group I to Group III. **Conclusion:** Our study has shown significant results of increased salivary Sialic acid levels in periodontitis compared to healthy controls. Thereby estimation of salivary sialic acid levels is noninvasive, reliable, and cost-effective can be used as an adjunctto determine the current periodontal disease status, and monitor response to therapy, and assess the treatment outcomes.

Keywords: Sialic Acid, Periodontitis, Gingivitis, Saliva

Introduction:

Human saliva is a biological fluid, that comprises molecules recognized as valuable biomarkers in contemporary research, offering insights to the diagnosis and prognosis of both oral and systemic ailments. Due to its easy collection and storage, saliva stands out as an ideal tool for the early detection of diseases through the detection of

biological markers (1). One such marker is sialic acid or N-acetylneuraminic acid (Neu5Ac),which is a monosaccharide with nine-carbon atoms and present as terminal residues of glycoproteins and glycolipids on the cellsurface. It is important for cell-to-cellinteractionsignaling and regulates innate immunity (2, 3). Certain bacterial species cansynthesize sialic acid, while others can obtain sialic acid from the host. This holds significance in understanding the host-parasitic interactions, shedding light on evolutionary relationships between them (4, 5).Sialic acid (SA) demonstrates diverse biological roles, such as increasing the viscosity of glycoproteins, and aiding in the binding and transportation of certain molecules, thereby maintaining the structural stability of proteins and enzymes (6. 7). SA has been proposed as a marker for the acute phase responses, with increased blood levelsin inflammatory processes as a result of elevatedsialylated glycoproteins (2, 8). Gingivitis and periodontitis stands as the most common oral diseases, affecting approximately 50% of the adult human population. Periodontitis, a prevalent dental condition, results in the deterioration of the supporting structures of the teeth. Originating from the gingival tissue, untreated periodontitis progresses deeper disrupting bone homeostasis, leading to tooth loss. Bacterial biofilm growth on the tooth surface however emerges as the primary contributor to periodontitis (9).Research has shown that SA is linked to various inflammatory conditions, including periodontal disease (10). Previous studies havereported that oral diseases can influence levels of SA in saliva, and individuals with periodontal disease may exhibit increased levels of inflammatory markers in circulation. SA serves as a significant source for bacterial pathogens and plays a crucial role in the colonization of periodontal pathogensfacilitates bacterial aggregation and contributes to the formation of pellicle and dental plaque (11). Research reports have suggested that SA should be regarded as a biomarker for early disease detection, precise diagnosis, assessment of disease progression, and evaluation of therapy effectiveness in managing periodontitis. Hence, the current study attempted to estimate the salivary sialic acid levels in chronic periodontitis cases.

Materials and Methods:

Informed consent formsand ethical approval from the institution's ethical committee (2022/IRB-APRIL-OP01/APDCH)were obtained before starting the procedure.Patients of age18 to 60 years were included in the study.Patients undergone oral prophylaxis, periodontal surgery, or periodontal debridement for six months andpatients under medication for any other systemic illnesses were excluded.A total of 60 subjects were grouped as Group I - Control (healthy), comprising 20 subjects exhibiting clinically healthy periodontium, devoid of any clinical attachment loss or indications of gingival inflammation. Group II – gingivitis comprising 20 subjects exhibited clinical manifestations such as pronounced redness, swelling, ulceration, and a propensity for spontaneous bleeding, yet lacked any signs of clinical attachment loss. GroupIII–periodontitiscomprising 30subjects evaluating

the periodontal health by calculating plaque index, measuring probing depth, and Clinical Attachment Loss (CAL) (Table 1).

Sample Collection:

Unstimulated whole saliva was collected from 9 am to 11 am, in a sterile container. The patient was instructed to abstain from consuming food or water for at least two hours preceding saliva collection. Subsequently, they were asked to expel saliva for five minutes by a standardized spitting technique. After the collection of saliva samples, the samples were centrifuged at8000 rpm for 12mins and the supernatant was stored at -20^{O}ctill proceeding with biochemical analysis.

Biochemical Analysis:

The salivary sialic acid levels in patients with periodontitis were determined using spectrophotometry at a wavelength of 490nm, employing the Acidic Ninhydrin method. The Acidic Ninhydrin reagent was prepared by dissolving 250mg Ninhydrin in 6 ml of glacial acetic acid and 4 ml of concentrated sulfuric acid, followed by thorough vortexing for 30 minutes. For the N-acetyl neuraminic acid (NANA) standard, 10mg NANA was dissolved in 100 ml of distilled water. 0.9% NaCl solution was prepared. In the assay procedure, 0.1ml of saliva was added to 0.9ml of normal saline, resulting in a total volume of 1ml, and then centrifuged at 3000 rpm for 30 minutes. To this, 1 ml of glacial acetic acid and 1 ml of freshly prepared acidic Ninhydrin reagent were added. Subsequently, the test tubes were immersed in a boiling water bath for 10 minutes, then cooled under tap water, and the absorbance was assessed at 470nm (12). (Fig 1, 2, 3, 4)

The data was entered in an excel sheet and descriptive analysis was done, inter-groupingcomparisonwasanalyzedusingthe ANOVAtestbySPSSsoftwareversion2.0.Statistically significant results were obtained with a p-value of<0.001.

Results:

A total of 60 patients were selected and grouped satisfying all the inclusion criteria.The meansalivary sialic acid levels in periodontitis (Group 3), gingivitis (Group 2), and healthy controls(Group 1) were 1.4785µg/ml, 1.115µg/ml, and 0.43µg/ml (Graph 1).It is clear that as the severity of periodontal disease progresses from Group I to III, the average levels of SA in saliva also noticeably rise from Group I to Group II. The levels of sialic acid peaked in Group III. A notable correlation has been identified between periodontal conditions and saliva sialic acid levels, indicating an association between periodontal diseases and salivary sialic acid concentrations.

Analysis shows differences in salivary sialic acid levels in Group I and GroupII, Group I and Group III, and Group II and Group III. These differences were found to bestatistically significant (p<0.001) (Table 2, Graph 1).

Discussion:

In our present study, we estimated sialic acid levels in saliva. The mean salivary sialic acid levels were significantly higher in Group III compared toGroup I and II. Thus, we found apositive correlation between chronic periodontitis cases and salivary sialic acid levels. The results of our studyweresimilar to various previous studies.

Our findings revealed elevated SA levels in saliva samples collected from individuals with both periodontitis and gingivitis. This suggests that the immune system responds to the presence of periodontal pathogens in the oral cavity. Inflammation serves as the principal immune response mechanism aimed at eradicating harmful stimuli and pathogens while restoring damaged host cells to their original state (9). The inhibitory effect exerted by pathogens might heighten the propensity for gingival bleeding upon gentle probing. Overproduction of reactive oxygen species by inflammatory cells results in tissue damage during active inflammatory responses (10). SA, serving as an inflammation marker, could potentially regulate immunological processes and contribute to mitigating oxidative stress by removing reactive oxygen species (11).

In periodontitis, the secretion of proinflammatory cytokines escalates due to pathogenic microorganisms, leading to oxidative stress buildup. Consequently, this escalation fosters radical formation and prompts the loss of terminal SA residues from glycoproteins (12). Furthermore, the release of proinflammatory mediators stimulates the liver and extrahepatic tissues, causing an elevation in acute phase reactants within the circulatory system (13).

Rathod et al (2014) studied the concentration of total sialic acid (TSA) levels in saliva and serum andits association with periodontal health and disease andconcluded that elevated TSA levels in both saliva and serum could indicate its involvement in the development of periodontal disease (2).Jawan Ibrahim et al (2017) conducted a study aimed at identifying diagnostic sialic acid fractions and their scavenger effect for periodontal diseases in 62 smokers and 62 individuals with varying periodontal health status and revealed a significant correlation between elevated levels of free sialic acid in young smokers with medium and deep pocket depth. The study concluded that salivary-free sialic acid could serve as a diagnostic biomarker for oxidative stress associated with periodontal diseases among young smokers (14).Sudhir Rama Varma et al (2019) conducted a study involving 75 patients to evaluate the importance of sialic acid and IL10 in both early and moderate stages of periodontitis. IL10 levels showed insignificant changes, suggesting that both IL10 and sialic acid levels increased progressively as patients transitioned from a healthy state to periodontitis(15).Shiny Inasu et al (2016) undertook a study to assess and establish a correlation between serum sialic acid and nitric oxide levels in individuals with healthy periodontium and chronic periodontitis. Increased levels of salivary free sialic acid were observed in chronic periodontitis compared to the healthy controlsandconcluded that this

disparity might stem from the release of various lysosomalexo-glycosidases during the advancement of periodontal disease, suggesting a connection between the severity of the disease and the concentration of salivary and serum sialic acid (16).Sujeeth Muthukumar et al (2021)evaluated and compared the effects of nonsurgical periodontal therapy on the levels of sialic acid and nitric oxide in both serum and saliva of 100 patients diagnosed with generalized chronic periodontitis and found that nonsurgical periodontal therapy led to a decrease in both serum and salivary levels of sialic acid and nitric oxide among individuals with generalized chronic periodontitis. This reduction in biochemical markers correlated with a decrease in probing pocket depth and an increase in attachment level (17).
The current data suggests a significant correlation between salivary and serum levels of TSA (total sialic acid) and the state of periodontal health and disease. Likewise, numerous studies documented in the literature have highlighted fluctuations in salivary sialic acid levels across various oral diseases(16, 17).

Conclusion:
This study was conducted to detect the salivary sialic acid levels in gingivitis and chronic periodontitis cases for early detection, accurate diagnosis,and evaluation ofthe treatment outcome.Our study has shown significantlyelevated levels of salivary sialic acid in periodontitis compared to gingivitis and healthy controls, which was similar to many published studies. Hence,the estimation of salivary sialic acid seems to bea reliable,cost-effective, and noninvasiveprocedure. It can be used as an alternativeto determineperiodontitis progression monitor therapeutic response and assess the treatmentoutcome.

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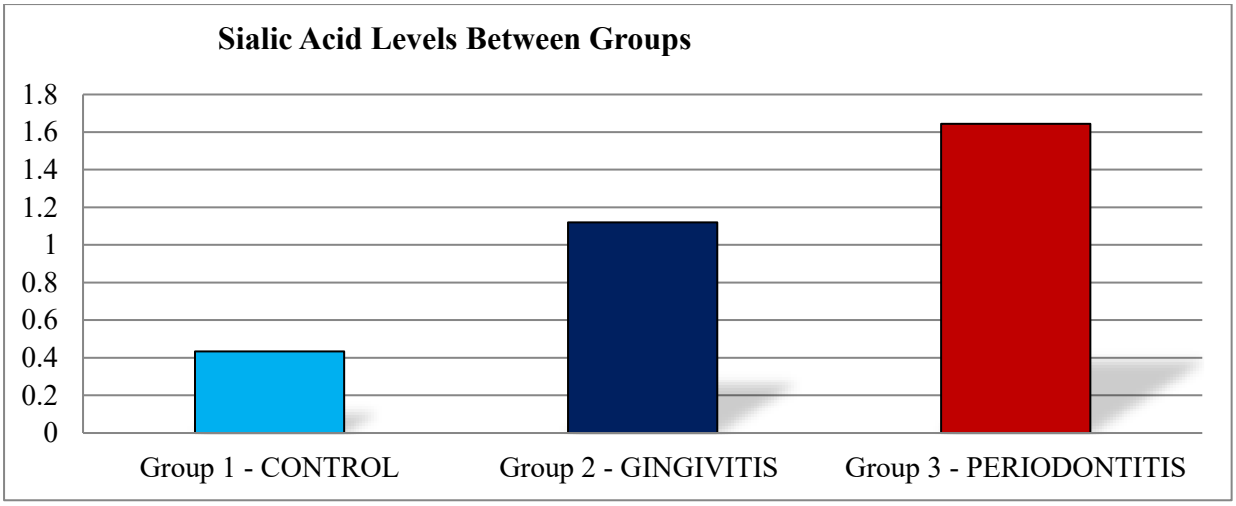
GROUP	STUDY GROUP	SAMPLE
GROUP 1	Control(healthy)	20
GROUP 2	Individuals with gingivitis	20
GROUP 3	Individuals with chronic periodontitis	20

Table 1: study sample grouping

n	Inter grouping (A-B)		DF	T	p value
	A series	B series			
20	1 – Healthy control	2 –Gingivitis	38	8.6	<0.001*
20	1 – Healthy control	3 – Chronic periodontitis	38	17.23	<0.001*
20	2 –Gingivitis	3 – Chronic periodontitis	38	6.9	<0.001*

Table 2: inter-grouping values between groups with P value (<0.01 considered as statistically significant)



GRAPH 1: graphical representation between groups showing gradual elevation in SA levels

Figures:

Fig 1: Centrifugation of salivary sample

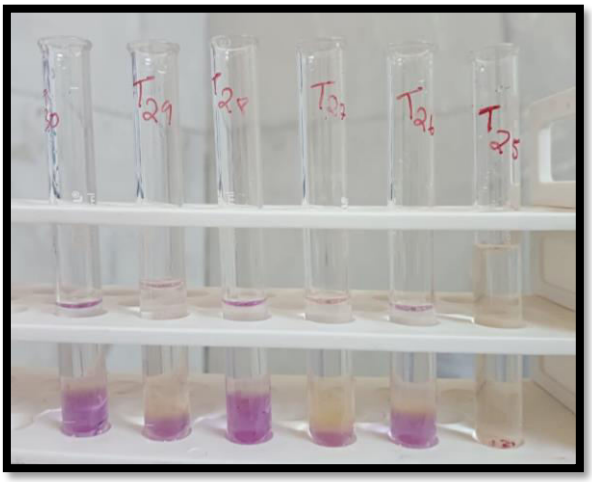


Fig 2: Pre heated sample mixture

Fig 3: Sample mixture heated in water bath

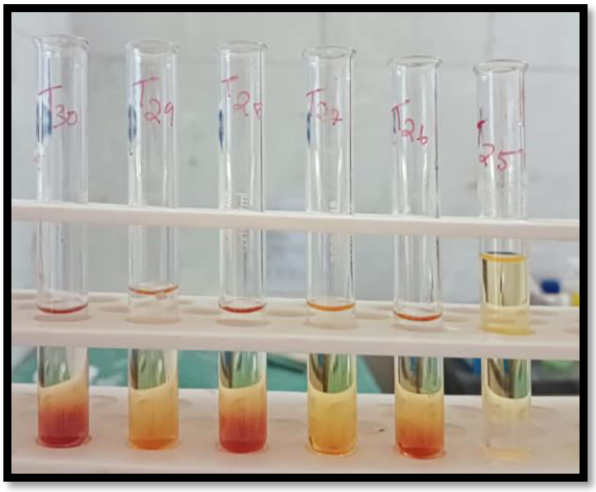


Fig 4: Change in colour noticed after heating the sample mixture