Analysis of Salivary Amylase Level in Patients with Pain of Endodontic Origin: A Case-Control Study.

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ABSTRACT

Background
Adequate management of pain of endodontic origin requires an understanding of the severity and the need for emergency treatment. Salivary alpha-amylase (sAA) is a major saliva biomarker linked to pain from oral diseases but not established with pain from endodontic/pulpal diseases which form a major proportion of oral pathologies.
Objective: To assess the relationship between salivary alpha-amylase level and pain of endodontic origin.
Methods: A case-control study on patients attending the Dental Centre of the University College Hospital, Ibadan, diagnosed with pain of Symptomatic irreversible pulpitis (SIP) and Symptomatic apical periodontitis (SAP). The pain was assessed with a visual analogue scale (VAS) subjectively. Saliva (stimulated and unstimulated) was collected and the sAA level was analysed using Elisa Kit. IBM SPSS version 25.0 was used for data analysis. Pearson's correlation was used to test the relationship between pain and sAA level with a P-value set at ≤ 0.05.
Results: Participants (43, 30) were included as test and control respectively. The mean unstimulated and stimulated saliva sAA was 123.1 ng/ml ±22.59 and 119.6ng/ml ±27.98 respectively, for the test group and 47.55± 9.54 ng/ml and 48.24± 10.85 ng/m for the control. The sAA level in both saliva samples was significantly higher in the test group compared to the control group. However, no significant correlation was observed between pain and sAA in unstimulated (r = 0.04, p = 0.82) and stimulated (r = 0.01, p = 0.96) saliva.
Conclusion: sAA showed a significantly elevated level in patients with pain of SIP and SAP but did not show a correlation with pain perception.

Keywords: irreversible pulpitis, pulpal pain, apical periodontitis, salivary alpha-amylase, visual analogue scale (VAS).

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INTRODUCTION

Pain by definition is "an unpleasant sensory and emotional experience associated with or resembling that which is associated with actual or potential tissue damage". It is a complex sensation, and the expression of the feeling is subjective.[1]

The pain of dental origin is the major reason most people present at the dental clinic[2-4] and this pain may be more understood and well appreciated for adequate management if the bias of subjectivity of expression of the pain is eliminated by using an objective method of assessment.

Pulpitis is one of the major causes of oral pain and it is primarily caused by an opportunistic infection of the pulp space from the oral microorganisms. Different treatments are advocated based on the state of inflammation of the pulp at presentation. Management of teeth with pulpal pathology is based on the accurate determination of the status of the pulp that will allow repair to occur.[5] Irreversible pulpitis is characterized by constant, spontaneous pain with exaggerated and lingering response to cold stimuli. However, some teeth with irreversible pulpitis can be painless[6] and referred to as asymptomatic irreversible pulpitis.[7] Further progression of the infection, may lead to symptomatic apical periodontitis, a condition that is characterized by acute painful reaction to biting, or percussion of the tooth with or without response to vitality test.[7]

Presently, adequate assessment of pulpal inflammation involves taking a good case history with adequate clinical and radiographic examination. Adequate clinical assessment involves procedures such as inspection of the affected tooth, assessing the pulp sensitivity of the tooth to thermal or electric stimuli, and assessment of pain on palpation or percussion.[5,7] However, the correlation between the various clinical tests being used to detect the histopathological status of the pulp and the level of pain being experienced/ reported by an individual remains controversial.[8]

Current pain assessment tools include the visual analogue scale (VAS), Numerical pain scale, Wong-Baker Facial pain scale (WBFPS), colour analogue scale (CAS) et cetera. All these tools rely on self-reporting, requiring an individual to process both external information and to communicate this personal experience.[9] However, there are situations when this expression is not possible and may be unreliable. Hence, there is a need to develop or make use of an objective method(s) of pain assessment that is sensitive and specific to pain such as the use of markers/parameters that are associated with such pain or changes in human physiology.

Biological biomarkers are measurable and quantifiable critical indicators of diseases and physiological states.[5,10] Several studies [11-13] have been done to assess objectively, pain from different areas in the body using pain biomarkers. A biomarker should be non-invasively accessible, highly specific, sensitive, easy to interpret, and of low cost.[10] However, due to the complexity of the pain system, a uni-dimensional reliable biomarker for pain is yet to be identified to date.[10]

Although the dental pulp is encased, it is a reactive tissue that spreads its biological products into the immediate environment.[14] Many studies [15-18] have shown that pulpal conditions can be reflected by measuring the levels of some protein markers that correlate with pulpal symptoms in pulpal blood,[15] the dentinal fluid,[16] the periapical fluid,[17] the gingival crevicular fluid (GCF),[18] and the saliva.[19] These protein molecules which are expressed in the cascade of tissue inflammation may be diagnostic biomarkers to objectively determine the presence of inflammation in the pulp.[5]

Saliva proteins, such as substance P and alpha-amylase have been reported to have a significant relationship with dental pain.[19-22] Alpha-amylase makes up 50-60% of salivary protein.[23] It is an essential salivary protein and has been proposed as a possible biomarker of the sympathetic reaction to psychosocial stress and parasympathetic responses.[23-25]

For the adequate management of the pain of endodontic origin presenting as symptomatic irreversible pulpitis (SIP) or apical periodontitis (SAP), it is important to understand the severity of the disease. Identifying those in need of emergency management, may need to be assessed objectively, especially in situations such as a cracked tooth in which there may not be any physical cause or visible injury found for pain. Objective assessment may also be necessary in a situation where the severity of the pain being reported by the patient is not in accordance with the visible injuries. The assessment of salivary alpha-amylase in such situations may, therefore, help to recognize the severity of the condition in order to provide appropriate and adequate management.

Saliva may be an attractive medium for laboratory testing due to the ease of collection compared to blood and other body fluids. More so, there are

clinical studies[26-31] supporting the use of salivary markers as a diagnostic means for the detection and/or confirmation of some pathologic conditions such as head and neck carcinomas,[26] diabetes mellitus,[27] chronic kidney disease,[28] and odontogenic pathologies such as caries[29] and periodontal disease; periodontitis.[30]

Hence, saliva biomarkers may serve as a better and more reliable means of diagnosing endodontic pain objectively. Also, the sample collection is non-invasive, fast, and numerous samples can be taken in a stress-free atmosphere without limitation.

Therefore, this study assessed the level of salivary alpha-amylase (sAA) in patients with pain of pulpal/endodontic origin in the forms of irreversible pulpitis (SIP) and apical periodontitis (SAP) and compared it with healthy individuals. Also, the study evaluated the relationship between salivary alpha-amylase and pain severity as scored using the Visual Analogue Scale (VAS), in patients with pain of pulpal/endodontic origin in the forms of irreversible pulpitis and apical periodontitis. The study hypothesised that there would be no difference between the sAA level in patients with pain of pulpal/endodontic origin and control as well as no relationship between VAS and sAA level in patients with pain of pulpal/endodontic origin.

METHODOLOGY

A case-control study was carried out at the Dental Centre, University College Hospital Ibadan. All patients diagnosed with pain of endodontic origin in the forms of symptomatic irreversible pulpitis and apical periodontitis, attending the Dental Centre of the University College Hospital, Ibadan, during the period of the study and signed the informed consent were included. Gender-matched controls were people without the pain of irreversible pulpitis, apical periodontitis, or any other dental pain. Clinical and Biodata of the participants were obtained through a self-administered data collection form. Ethical approval was given by the institution's ethical review board (UI/EC/21/0075).

Participants were patients aged 16 years and above with severe acute tooth pain of pulpal/endodontic origin, who were mentally stable and could express themselves, while patients with other forms of dental pain, who smoke, who have had medication for the pain within 48 hours before the presentation or had any systemic disease were excluded. Also, patients whose last meal was less than an hour were excluded.

Clinical examination was done to assess the test patients who were diagnosed with pain of endodontic origin in the forms of irreversible pulpitis and symptomatic apical periodontitis. The diagnosis of symptomatic irreversible pulpitis was based on symptoms of spontaneous, severe, and sharp pain that was prolonged after the removal of the stimulus. They were assessed for the presence of tenderness on apical percussion in addition to apical periodontitis.[32] Standardized periapical dental radiographs, taken with a film holder, were used to confirm the pathology.

The participants were asked to use the Visual analogue scale (VAS) to assess the severity of the pain being experienced. The VAS is a 10 cm horizontal line, where the patient marks between distances of 0 cm indicating no pain to 10 cm which indicates the most severe pain ever.[33]

Saliva Sample collection: saliva samples were taken at least 1 hour after meal from both the test patients and the control. After rinsing the mouth with clean water, whole unstimulated and stimulated (using paraffin gum) saliva was collected by asking participants to spit into a graduated universal bottle for 5 minutes.[34] This was immediately stored at -80°C before laboratory analysis.

Sialochemical analysis: The saliva samples were centrifuged (KD2-TDSA, Nantong Hailun Bio-medical Apparatus Manufacturing C., Ltd., Haimen City, Jiangsu, China) for 3 - 5 minutes to acquire pure saliva. The level of alpha-amylase activity in the saliva was determined using ELISA (Melsin Medical Co. Limited). The Kit makes use of a double-antibody sandwich enzyme-linked immunosorbent one-step process to assay α-Amylase. Standard, test sample and HRP-labelled α-Amylase (sAA) antibodies were added to the pre-coated wells. The α-Amylase (sAA) antibody was used for the pre-coating. Incubation and washing of samples were done to remove the uncombined enzyme, Chromogen Solution A and B were added and samples were incubated for 15 minutes at 37°C. The colour change was measured spectrophotometrically (6300, Jenway, Staffordshire, UK) at a wavelength of 450 nm.

Data analysis. The data were entered on a spreadsheet and analysed with SPSS IBM software version 23.0. Descriptive statistics were done, frequency and mean of normally distributed data were reported. Shapiro-wilk test of normalcy was done for the sAA and VAS data. Median and Interquartile range were reported for sAA data as it was not normally distributed. Mann-Whitney U non-

parametric test was used to test the association between salivary alpha-amylase and gender while Chi-square was used to test the association between normally distributed VAS score, the diagnosis and gender. The level of significance was set at $p<0.05$.

RESULTS

Forty–three participants that presented with pain of endodontic origin during the study period, and were recruited for meeting the criteria for inclusion, while 30 gender-matched participants (without pain) were included as control.

In the test group, there were more females (27; 62.8%) than males. This was also reflected in the control group where there were 17 (56.7%) females against 13 males. The mean age for the test group was 47.95 ± 13.95 years while that of the control was 32.17± 6.52. (Table 1). Majority of the participants had tertiary education (33; 76.7%), and 31 (72.1%) were married. Pain was the reason for presentation in all the cases in the test group and duration of presenting complaints ranged from one day to one year. Pain from symptomatic irreversible pulpitis (SIP) was diagnosed in 11 cases while all the remaining (32; 74.4%) were pain from symptomatic apical periodontitis (SAP). (Table 1).

Table 1: Demographics of participants

	Test N (43)	%	Control N (30)	%
Gender				
Male	16	37.2	13	43.3
Female	27	62.8	17	56.7
Marital status				
Single	8	18.6	15	50
Married	31	72.1	15	50
Widow	4	9.3	0	0
Education				
Primary	2	4.7	0	0
Secondary	5	11.6	1	3.3
Post-secondary	3	7.0	1	3.3
Tertiary	33	76.7	28	93.3
Diagnosis				
Symptomatic Irreversible pulpitis	11	25.6	-	-
Symptomatic apical periodontitis	32	74.4	-	-

The overall mean sAA levels in stimulated and unstimulated saliva for both control and test groups are as shown in table 2. The table 2 also shows the mean sAA levels according to gender. The mean sAA in the unstimulated and stimulated saliva for the test group were 123.1 ng/ml ±22.6 and 119.6ng/ml ±27.98 respectively, while that of the control were 47.55± 9.54 ng/ml and 48.24± 10.85 ng/ml respectively.

Table 2: Mean salivary alpha-amylase according to gender.

	Gender	Mean Unstimulated (ng/l)	OPD	Mean Stimulated (ng/l)	OPD
Control					
	Male	45.5038	0.599	50.5769	0.604
	Female	49.11±8.75	0.602	46.45±10.4	0.598
Total mean		47.55± 9.54	0.600	48.24± 10.0	0.601
Median		46		46.25	
Interquartile Range		8		10	
Symptomatic pulpal disease (Test group)	Male	126.09± 31.57	0.649	119.17± 28.4	0.621
	Female	121.32± 51.52	0.603	119.85± 28.2	0.591
Total Mean		123.09± 22.6	0.620	119.59± 27.9	0.603
Median		122.25		121.00	
Interquartile Range		22.25		22.25	

Furthermore, a non-parametric Mann-Whitney U statistics test was used to conduct a pairwise comparison between the sAA of the test and control groups. This showed statistically significant higher values in the test (patients with pain of endodontic origin in the form of symptomatic irreversible and apical periodontitis) compared to control in both the stimulated and unstimulated saliva. ($p<0.001$) Table 3.

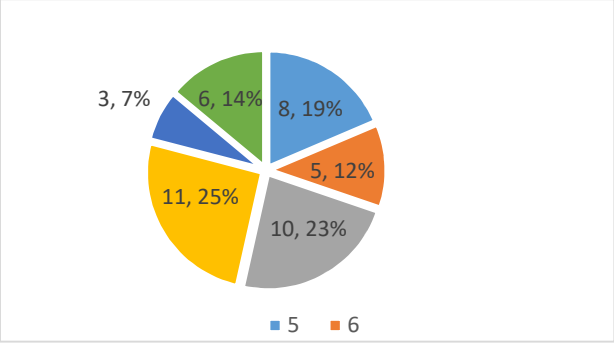
Table 3: The mean rank values of the test and control groups

	Mean rank Values	
	Unstimulated	Stimulated
Test Group (symptomatic pulpal disease)	51.84	51.14
Control	15.73	16.73
Mann-Whitney U value	7.0003	37.000
P-value	<0.001	<0.001

The VAS score of the pain of symptomatic irreversible and apical periodontitis is shown in figure 1. The mean VAS score of pain was 7.33 ± 1.6. Moderate pain (scores 5-6) was noted in 13 (30.2%), while severe/worst pain (7-10) was in 30 (69.8%) participants. Significant higher scores of VAS pain rating were seen in apical periodontitis than in irreversible pulpitis ($p= 0.05$). This also correlated significantly (spearman correlation $r= 0.5$, $p=0.001$). However, no significance was observed when the values were compared among gender ($p = 0.89$).

There was, however, no correlation between the VAS for the test group and the alpha-amylase level in both unstimulated and stimulated saliva ($r = 0.04$, $p = 0.82$; $r = 0.01$, $p = 0.96$ respectively).

Figure 1: Figure 1: Distribution of the VAS scores in symptomatic endodontic disease.



There was, however, no correlation between the VAS for the test group and the alpha-amylase level in both unstimulated and stimulated saliva ($r = 0.04$, $p = 0.82$; $r = 0.01$, $p = 0.96$ respectively).

DISCUSSION

The use of saliva biomarkers in the diagnosis of oral diseases is becoming more established recently. Biomarkers such as IgA, salivary alpha-amylase, cortisol, and other proteins have been used in the assessment of oral diseases such as caries, pain, periodontal disease, and apical periodontitis from pulpal disease. This study looked at the relationship between sAA level and pain of endodontic origin and related it with controlled healthy individuals.

Similar to some studies, this study found more females presenting with pain of pulpal disease.[20,35] The gender presentation of the participants in the present study also corroborated the report by Cardoso et al.[36] where a higher female-to-male ratio was seen in both the test and control groups for salivary alpha-amylase level in patients with aphthous ulcer. This presentation may be because females present more at dental clinics and give more preference to their oral health than males.[37] However, the difference in the sAA of the genders was not statistically significant in agreement with the report of Surin et al.[35]

The sAA levels in the individuals with pain of endodontic origin from irreversible pulpitis and apical periodontitis were significantly higher than that of the control in this study. This finding is similar to that of Surin et al.,[35] and Cardoso et al.,[36] where sAA values were higher than that of asymptomatic cases and healthy control respectively. However, Cardoso et al.,[36] considered pain from recurrent aphthous ulcer while Surin et al.,[35] looked at sAA level in symptomatic and asymptomatic cases in relation to third molar surgery. Thus, these variations in study participants may limit the comparison with our findings.

Contrary to the report of Ahmadi-Motamayel et al.,[20] where a significant positive correlation of sAA in irreversible pulpitis with age, gender, and visual analogue scale was reported, this study observed no relationship with the mentioned variables. With the effect of sAA on microbes and the pathology of apical periodontitis, Harian et al.,[38] observed positive correlations between the level of sAA and the level of periodontitis while the elevation of sAA in chronic periodontitis is reported to serve a protective role

during the inflammatory stage.[39] There is, therefore, the need for more research in this field to have a reference for comparison such as the use of case-control in this study

The VAS scale is a well-known scale that is used to assess pain and many other variables. In this study, the pain value was significantly higher in apical periodontitis than in symptomatic irreversible pulpitis. This report agrees with that of Erdogan et al.,[40] who reported significantly higher pain in adult patients with apical periodontitis. Contrarily, Rechenberg et al,[41] reported the same level of pain irrespective of the diagnosis. Apical periodontitis is a more advanced pathology of pulpal origin than irreversible pulpitis with the involvement of the periodontium. Thus, this may be the reason for higher pain than in irreversible pulpitis where the pathology is expected to be confined within the pulp canal. This observation may, however, be further explored to be able to distinguish more between the pain of symptomatic irreversible pulpitis and symptomatic apical periodontitis especially in relation to demographic factors such as gender. It may also, serve as a clinical but subjective assessment of the pathology.

Furthermore, no significant correlation was observed between VAS and the levels of sAA in this study contrary to other reports.[20,35] This difference may be due to the sample size, study population, and study design. This present study, however, had more cases, and looked at both symptomatic apical and symptomatic irreversible pulpitis, unlike the study of Ahmadi-Motamayel et al,[20] where only irreversible pulpitis was considered. Also, worthy of note is the difference in the study population. While other studies [35,36,20] were among subsets of Caucasians and Arabs, the present study was among a subset of Africans. This, therefore, calls for further research on the subject with larger sample size considered.

To the best of the authors' knowledge, this study is the first to be done in this clime relating level of salivary alpha-amylase with endodontic pain from symptomatic irreversible pulpitis and apical periodontitis. It may be necessary, however, to compare the sAA of same patients pre- and post-treatment for more inferences on the use of this biomarker in the monitoring of treatment of oral pain specifically in relation to those of endodontic origin.

CONCLUSION

The study observed a higher level of salivary amylase in individuals with pain of endodontic origin (symptomatic irreversible pulpitis and apical periodontitis) compared to control. This suggests that assessing the salivary alpha-amylase level may be an alternative method of endodontic pain evaluation.

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Nil
Conflict of interest
None declared

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