ORIGINAL RESEARCH

Estimation and Correlation of Alkaline Phosphatase Enzymatic Activity in Saliva with and without Early Childhood Caries in South Indian Children: A Randomized Clinical Trial

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Abstract

Introduction: Early childhood caries (ECC) is a major common problem seen in children and is the most prevalent chronic disease that leads to discomfort, pain, and poor quality of life, affecting the health of children. Alkaline phosphatase (ALP) is a nonspecific phosphomonoesterase that functions through a phosphoery 1 intermediate to produce free inorganic phosphate. It has different isoenzymes produced by different cell types such as polymorphonuclear leukocytes, osteoblasts, macrophages, and fibroblasts within alveolar bone and/or salivary glands. Various studies show that higher ALP activity is related to periodontal disease and dental caries.

Aim: This study aims to estimate and correlate salivary Alkaline Phosphatase enzyme activity in the saliva of children with and without ECC.

Materials and methods: A total of 50 children were included in the study, divided into two groups—caries-active and caries-free, each consisting of 25 participants. Unstimulated saliva samples were collected and subjected to a spectrophotometer for analysis. ALP enzyme activity levels were estimated and correlated between caries-active and caries-free children.

Results: The correlation between caries score and ALP activity was statistically significant, with a moderate correlation. The comparison of mean ALP activity between caries-active and caries-free groups was statistically significant. However, the comparison of ALP based on different age-groups and gender was not statistically significant. There was a statistically significant correlation between caries scores and the caries-active group.

Conclusion: In conclusion, there is a substantial correlation between ALP enzyme levels and the severity of dental caries. An increase in ALP enzyme level is linked to a considerable rise in caries severity. Therefore, prevention may be possible with early detection.

Keywords: Alkaline phosphatase enzyme, Early childhood caries, Saliva.

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Introduction

Early childhood caries (ECC) is the most common chronic condition that affects children's health, causing discomfort, suffering, and a low quality of life. It is a severe problem frequently observed in children.[1,2] Early detection of disease is critical for prognosis. The need for accessible and noninvasive early detection instruments makes salivary diagnostics a viable substitute for blood testing. Alkaline phosphatases (ALPs) have received significant attention as salivary biomarkers, particularly in dental contexts.[3]

The membrane-bound enzyme known as ALP (AP, EC 3.1. 3.1 orthophosphoric-monoesterase, alkaline optimum) is present in nearly all living organisms, including bacteria and humans. Its primary function is to catalyze the transphosphorylation process in the presence of high phosphate acceptor concentrations and to hydrolyze phosphoric acid monoesters. Gingival crevicular fluid (GCF), serum, and saliva all contain increasing amounts of these intracellular enzymes released from injured cells. Salivary ALP levels increase in response to inflammation and the breakdown of healthy tissues, indicating its role as a biomarker.[4] Adults typically have between 20 and 140 IU/L of ALP. Pregnant women and children have much higher ALP levels.[5] It contains many isoenzymes made by various cell types found in the salivary glands and/or alveolar bone, including fibroblasts, osteoblasts, macrophages, and polymorphonuclear leukocytes. Higher ALP activity has been linked to periodontal disease and tooth decay, according to several studies.[6]

A spectrophotometer measures the amount of light and its wavelength absorbed by the sample. It identifies components in the sample solution with high specificity. Additionally, it supports customer workflows with a fast, easy-to-use, and trustworthy analytical instrument.[7]

Since there is a deficiency in the literature regarding ALP activity and its correlation with ECC in the Indian population, the study

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aims to assess ALP activity and its correlation with ECC using the spectrophotometer method.

Materials and Methods

A total of 50 children between the ages of three and six who visited the Department of Pediatric and Preventive Dentistry participated in the current study. To reduce bias, children residing in and around South Bengaluru were considered. The patients were selected using random sampling, and allocation was determined using a computer-generated random assignment sequence. Ethical clearance number: RRDCH/IEC/23/20.

A study proforma was designed that included demographic details, chief complaint, and recording of all subjective and objective symptoms, as well as clinical and radiographic findings. The experimental group (caries-active) comprised 25 patients with severe ECC, while the control group (caries-free) consisted of 25 patients without caries aged 3–6 years. Informed consent was obtained from the parents or guardians of the children participating in this study.

Inclusion/Exclusion Criteria

The study included children between the ages of 3 and 6, specifically those with four or more active carious lesions and those without caries, whose parents provided informed consent. Excluded from participation were children with genetic disorders or syndromes, pulpally involved teeth, requiring special medical attention, having received topical fluoride application within the previous month, or currently taking any medication.

Caries were recorded using the dmft index [decayed, missing, and filled primary teeth (dmft)]. The children were classified based on the presence (dmft ≥4) or absence (dmft = 0) of the disease. Unstimulated saliva was collected using the spit method (Fig. 1).

Following collection, the samples were transferred to the laboratory and stored at −80°C in a hermetically sealed case filled with ice for 1 hour. Each sample was assigned a number, and this process continued until the laboratory study was completed. To prevent bias, the sample investigator was blinded. The activity of the ALP enzyme in all 50 samples was measured using a spectrophotometer (Fig. 2).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) for Windows version 22.0 Released 2013. Armonk, New York: IBM Corp., was utilized for statistical analyses. Descriptive analysis of all explanatory and outcome parameters was conducted using frequency and proportions for categorical variables, and mean and standard deviation for continuous variables using the Mann–Whitney *U* test. Spearman's correlation test was employed to assess the relationship between caries scores and ALP activity levels in the caries-active group. Mann–Whitney *U* test was used to compare gender and age distribution between the two groups. The level of significance was set at $p < 0.05$.

Results

The results indicate that there was a statistically significant difference between the caries-active and caries-free groups, with a mean dmft of 46.35 ± 35.08. ALP levels showed significantly higher activity in the caries-active group compared to the caries-free group (Table 1). Spearman's correlation analysis revealed a statistically significant moderate correlation ($\rho = 0.59$, $p < 0.002$) between caries score and ALP activity (Table 2). In the study, comparisons of ALP activity based on gender and different age groups did not show statistically significant differences

Table 1: Comparison of ALP activity between caries-active and caries-free subjects

Comparison of mean ALP Activity levels between caries-active and caries-free using the Mann–Whitney U test

Parameter	*Groups*	*N*	*Mean*	*Standard deviation*	*Mean difference*	*p-value*
ALP activity	Caries-active	25	46.35	36.08	33.16	<0.001*
	Caries-free	25	13.20	13.09		

*, Statistically significant

Table 2: Spearman's correlation between caries score and ALP activity between caries-active subjects

Spearman's correlation test to assess the relationship between caries scores ALP activity levels in caries-active group

Groups	*Variable*	*Values*	*ALP levels*
Caries-active	Caries scores	*ρ*	0.59
		p-value	0.002*

*, Statistically significant

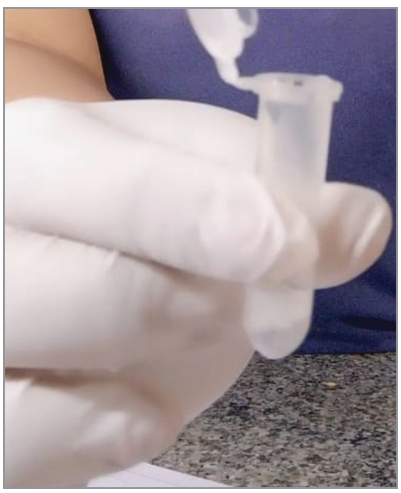

Fig. 1: Collected saliva into Eppendorf tubes by spit method

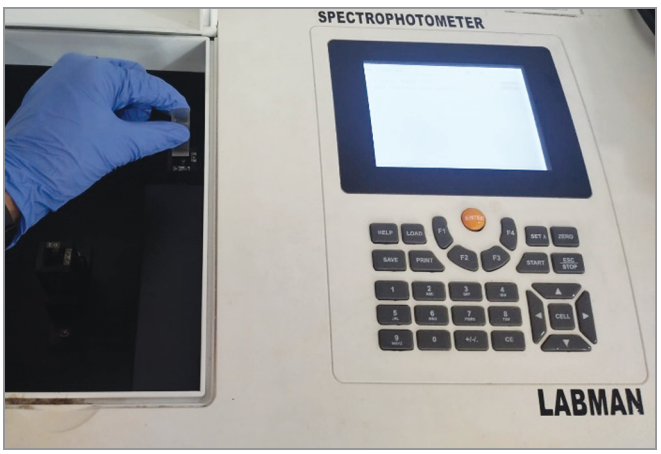

Fig. 2: Spectrophotometer for estimation of ALP activity in saliva

(Tables 3 and 4). Additionally, a comparison of mean ALP activity levels based on caries scores in the caries-active group showed statistically significant results, with a mean of 68.49 ± 35.33 for caries scores greater than 6 and a mean of 28.95 ± 26.51 for scores ≤6 ($p < 0.005$) (Table 5). So as caries increases, ALP activity also increases.

Discussion

Saliva consists of complex substances originating from the salivary glands and other sources including the oropharynx, gastric reflux, GCF, and blood. Analyzing the composition of salivary components has long been recognized as a highly useful method for assessing health status. Variations in salivary compounds serve as a "window" reflecting the physiological and pathological conditions of the body. Therefore, analyzing chemicals found in saliva can aid in monitoring the onset, progression, recurrence, and treatment of systemic and oral diseases. Using saliva as a diagnostic tool instead of serum or tissues offers several benefits, including its noninvasive collection approach, reduced sample fraction, high patient compliance, cost-effectiveness, ease of storage and transportation, increased sensitivity, and correlation with blood levels.[4] Salivary biomarkers have been discovered for a variety of medical conditions, including infections, autoimmune disorders, cancers, and metabolic diseases, thanks to advancements in technology. ALP has received significant interest as a salivary biomarker, particularly in dental contexts.

Table 3: ALP activity levels based on different ages in both caries-active and caries-free subjects

Comparison of mean ALP activity levels based on different age groups in each group using the Mann–Whitney U test						
Groups	*Age*	*N*	*Mean*	*Standard deviation*	*Mean difference*	*p-value*
Caries-active	4–5 years	9	40.19	39.36	–9.62	0.40
	6 years	16	49.81	34.94		
Caries-free	4–5 years	11	13.51	15.28	0.56	1.00
	6 years	14	12.95	11.69		

Table 4: ALP activity levels based on gender in both caries-active and caries-free subjects

Comparison of mean ALP activity levels based on gender in each group using the Mann–Whitney U test						
Groups	*Gender*	*N*	*Mean*	*Standard deviation*	*Mean difference*	*p-value*
Caries-active	Males	14	48.62	37.71	5.16	0.83
	Females	11	43.46	35.49		
Caries-free	Males	9	16.92	11.53	5.82	0.14
	Females	16	11.10	13.80		

Table 5: ALP activity levels based on caries scores in caries-active subjects

Comparison of mean ALP activity levels based on caries scores in caries-active using the Mann–Whitney U test						
Groups	*Caries*	*N*	*Mean*	*Standard deviation*	*Mean difference*	*p-value*
Caries-active	≤6 nos.	14	28.95	26.51	–39.54	0.005*
	>6 nos.	11	68.49	35.33		

*, Statistically significant

Alkaline phosphatase is an enzyme found in various organisms, including bacteria and humans. Its primary function involves catalyzing the hydrolysis of phosphoric acid monoesters. Additionally, in the presence of high phosphate acceptor concentrations, it catalyzes a trans-phosphorylation process.[3] Our results indicate that ALP activity levels in saliva samples from subjects with caries were higher compared to those in subjects without caries, indicating a higher level of caries severity. The correlation between caries score and ALP activity was statistically significant, showing a moderate correlation, suggesting that as caries severity increases, ALP enzyme activity also increases.

In comparison, a study involving 75 children divided into three groups—nonrampant caries, rampant caries, and a control group—found that the rampant caries group exhibited higher ALP activity compared to the control group.[8] In another study involving 374 children aged between 4 and 6, it was observed that the study group had significantly higher concentrations of ALP compared to the control group. Additionally, there was a positive correlation between the study group's age- and gender-specific decayed, missing, and filled surfaces (dmfs) index and ALP levels.[9] The observed positive correlation between ALP and pathogenic flora may be attributed to the release of intracellular enzymes into the GCF and saliva. This release occurs when periodontal tissues become diseased or when cellular membranes are damaged due to edema or bacterial infections. These enzymes can be measured to assess their activity in diagnosing and monitoring oral health conditions.[10] Alterations in ALP levels can affect calcium and phosphate ion concentrations, potentially influencing the balance between demineralization and remineralization processes in dental health. However, contrary to expectations, a study found no significant evidence of a correlation between the progression of caries and salivary ALP activity or ratio.[11]

In our study, the comparison of ALP activity based on different age and gender groups did not show statistically significant differences. Similarly, another study concluded that there was no significant difference between age and gender groups in terms of ALP activity.[12]

The comparison of caries scores within the caries-active group was found to be statistically significant in our study. Specifically, the higher the number of caries, the higher the concentration of ALP enzyme activity observed. Currently, there are no similar studies specifically focused on the correlation between caries scores and ALP enzyme activity.

Saliva is increasingly recognized as a crucial diagnostic fluid, offering a noninvasive method to evaluate numerous biomarkers and providing new insights into preventive dentistry. Estimating ALP enzyme activity levels could be a valuable approach to identifying individuals at high-risk of dental caries, as these levels often increase in patients with active caries.

Conclusion

In conclusion, there is a significant moderate correlation between ALP enzyme activity levels and the severity of dental caries. An increase in ALP enzyme levels is associated with a notable increase in caries severity. Therefore, early detection may enable preventive measures against dental caries.

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