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Salivary AST Enzyme Level as a Biomarker for Tracking the Progress of Gum Disease Treatment in Children

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ABSTRACT

Background: Periodontal disease is a persistent inflammatory illness characterized by the destruction of supporting components, which may ultimately result in tooth loss. The most often seen kind of periodontal disease in children and adolescents is gingivitis, which has a significant correlation with certain socioeconomic variables and oral health practices. Numerous salivary biomarkers, such as AST, have been suggested as potential indicators for the timely identification and advancement of periodontal disease.

Aims of the study: The aim is to demonstrate the impact of non-surgical periodontal therapy on children with periodontal disease (PD) and determine whether the AST marker may be used to forecast the advancement of gingival illnesses at various stages in pediatric patients.

Materials and Methods: This case-control research included the observation of a cohort of 30 children, with ages ranging from 6 to 12 years. The individuals were categorized into two cohorts according to their periodontal well-being: a cohort of 17 children diagnosed with gingivitis and another cohort of 13 children diagnosed with chronic periodontitis. All participants' salivary AST enzyme levels, LI, GI, and CAL were assessed at two time points: baseline and one month follow-up.

Conclusion: Children with gingivitis and chronic periodontitis may benefit from non-surgical periodontal therapy and dental hygiene instruction. Tracking therapy progress in children with periodontal disease may be facilitated by monitoring salivary AST enzyme levels. The measurement of salivary AST enzyme levels provides a non-invasive method for evaluating the effectiveness of therapy in the field of pediatric dental care.

Keywords: PD in children, Gingivitis, Chronic periodontitis, AST enzyme

INTRODUCTION

Periodontal disease (PD) is characterized by pathological processes that impact the periodontium, which encompasses the anatomical tissues responsible for tooth support. The architecture included in this kind consist of the gingival tissue, periodontal ligament, cementum, and alveolar bone.[1,2] The onset of Inflammation of the free gingival tissue without accompanying of any clinical attachment loss is a hallmark of the disease's start. In the initial stages of gingival disease, there is a period called gingivitis. Changes in the biofilm microbial ecology are a hallmark of gingivitis.[3]. Nevertheless, it is important to note that not all cases of gingivitis will ultimately progress into periodontitis. The evolution of the problem and the consequent influence it has on the periodontal structures that are related with it may occur if the issue is not precisely detected and then treated adequately. The disease that occurs in this later stage is known as periodontitis, which leads to clinical attachment loss defined by the resorption of

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alveolar bone and destruction of the periodontal ligament.[3,4] If the illness continues to be undiagnosed and untreated, it might ultimately result in dental mobility and consequent tooth loss. Bacterial biofilm formation triggers gingival inflammation. However, the onset and progression of periodontitis are influenced by dysbiotic ecological shifts in the microbiome, which occur in response to nutrients from gingival inflammatory and tissue breakdown products.[5] Additionally, anti-bacterial mechanisms are activated to control the microbial challenge within the gingival sulcus area once inflammation has begun. As a result, many important molecular pathways are triggered, leading to the activation of host-derived proteinases.[6] These proteinases facilitate the breakdown of marginal periodontal ligament fibers, the migration of the junctional epithelium towards the root apex, and the development of the bacterial biofilm along the root surface.[7,8]

Substantial differences exist between the periodontium of the primary dentition and that of the permanent dentition. The periodontium in the primary dentition has a pronounced vascularity, leading to a more intense red coloration. The presence of diastemata causes the gingival tissues to have a larger volume, a rounder form, and less stippling, which helps the teeth to emerge. The radiographic evaluation shows that the periodontal ligament gap is somewhat wider in primary dentition than in permanent teeth. The connective tissue stays unchanged; however, the junctional epithelium of the primary dentition is thicker, whereas the alveolar bone shows less calcification. When it comes to children, gingivitis is more common than periodontitis.[9,10] Dental biofilm-induced gingivitis is the most common kind of gingivitis seen in children. Dental biofilm production initiates the immunological response of the host, resulting in inflammation of the gingiva. If gingivitis, which is marked by inflammation of the gums, is present even when there is not much dental biofilm, it might indicate a connection with systemic diseases like diabetes mellitus. The common periodontal problems often seen in juvenile patients include gingival infections, chronic periodontitis, aggressive periodontitis, and periodontitis as a symptom of systemic illnesses. Necrotizing periodontal infections, periodontal abscess, and endo-perio lesions. Conditions that may either be present from birth or acquired later in life .[11]

Human saliva is a readily available bodily fluid that carries a diverse range of indicators associated with diseases, making it a promising candidate for diagnostic purposes. Whole saliva is produced by three sets of primary salivary glands—the parotid, submandibular, and sublingual glands—and other secondary salivary glands from non-glandular origins, such as gingival crevicular fluid (GCF).[12,13] In normal settings, the daily production of saliva ranges from 0.5 to 1.5 L. Saliva primarily consists of 98% water and the other 2% is composed of electrolytes, mucus, antimicrobial chemicals, and different enzymes.[14,15] The oral fluid possesses various functions, including rinsing, dissolving food substances, removing food and bacteria, lubricating soft tissues, forming a bolus, diluting debris, facilitating swallowing and speech, and aiding in chewing. These functions are all attributed to the fluid's specific components and fluid-like properties. additionally, the constituents of saliva contribute in the protection of mucous membranes, aiding in the process of digestion, and the defense against microorganisms in oral cavity. The collection of salivary samples is straightforward, noninvasive, and simple secure technique, with undemanding and cost-effective storage. The enzyme Aspartate Aminotransferase is also referred to glutamic oxalotransferase. The AST is an enzyme that relies and depend on pyridoxal phosphate (PLP) to functions as a transaminase. This enzyme has a crucial role in the metabolic process of amino acids metabolism. Traditionally, the AST enzyme is often termed as a hepatic enzyme. The AST is often released from necrotic cells into the extracellular fluid and assessed in various body fluid (blood, saliva, tears, and gingival crevicular fluid). Any simple alteration in the activity of enzyme in the saliva may provide insights into metabolic changes in the gingiva and indicate inflammation of the periodontal tissues.[16] By testing the enzyme levels, the severity of periodontitis can be evaluated. Tatjana et al., 2005 and Mojgan and Praveen et al., 2014 manifestoed a significant increase

in the activity of this enzyme in saliva among patients with periodontal diseases compared to the control group. Moreover, the activity of this enzyme statistically significant decrease after a traditional periodontal therapy.[17,18] The objective of the research is to evaluate the effect of non-surgical treatment (scaling, polishing and oral hygiene instruction) and if there is an association between the concentration of salivary AST enzyme (an indicator of inflammation) and the condition of periodontal health in children. This is accomplished by assessing the clinical periodontal parameters and comparing the salivary AST levels to these parameters at various stages of therapy. The objective is to see whether this marker may be used to predict the progression of gingival diseases at different stages in pediatric.

Materials and Methods

The samples of this case- control study include the investigation of 30 children ranged from 6 to 12 years old. The participants chosen for this research were gathered from the Department of Pedodontics at the Teaching Hospitals of the College of Dentistry, University of Tikrit and Baghdad, as well as from a specialized clinic in Tikrit city, Iraq. Gingivitis and chronic periodontitis patients in excellent medical condition, aged 6–12, are included in the study. On the other hand, youngsters with any systemic disorder, those who have undergone periodontal treatment, or those who have used anti-inflammatory, anti-microbial, or other medication in the 2 months prior to the study are excluded from the measurement. The participants were provided with detailed information on the aims and intentions of the research, with the approval of their parents, and were granted complete liberty to either accept or refuse the participation. The permission form of the patient's parents is documented by the act of signing on a specifically developed informed consent. Each participant completed a questionnaire in the manner of a case sheet, which included diverse details or information such as their name, age, medication use, dental history, and medical history.

After the participants were selected, samples of unstimulated whole saliva were obtained, and all of these procedures would be performed prior to the analysis of clinical periodontal parameters. The subjects should be instructed to abstain from consuming or ingested any food or beverages, with the exception of water, for a duration of one hour before to the saliva sample collection. In addition, they should instruct to consistently rinse their mouth with water in order for removing any food particles debris and potentially contaminated substances. Afterward, it is advisable to wait for a period of 1-2 minutes for the water to clear away. Once the participants were selected, un stimulated salivary samples were obtained, and all of these procedures would be performed prior to the examination of clinical periodontal characteristics.

Before conducting the collection procedures, the participants were instructed to adopt a sitting posture with their head positioned downwards, their arms supported, and their elbows resting on their knees. It is important to instruct the patients to refrain expectorating from coughing up mucus and to prevent any motion of their jaw, cheeks, lips, and tongue. Passive saliva drooling and collection was performed by loosely and partially opening the lips, allowing the saliva to flow over the lower lip naturally run into the test tube. Moreover, it is essential that participants must clear request to avoid from swallowing throughout the procedures and from spitting into the test tube to ensure their safety.[22] It is recommended to dispose of salivary samples that include blood. Once collected, all samples are placed in a chilling box to prevent bacterial growth and the breakdown of nucleic acids and proteins. Each tube was assigned a unique number, which corresponded to the corresponding number on the case file for the same patient. The clinical periodontal parameters examination for all subjects was conducted after the completion of salivary sample collection. This examination involved the use of a periodontal probe on the four surfaces of all teeth, namely the mesial, distal, lingual/palatal, and buccal/labial surfaces. The collected data included the plaque index (PLI) of,[22] gingival index (GI) of,[19] and clinical attachment level (CAL) of 22. Subsequently, scaling and polishing procedures were performed, accompanied by comprehensive instructions provided to both the children and their parents regarding the nature of diet and brushing technique. The process of saliva

collection and periodontal index examination would do two times for each patient:

1. The first visit includes saliva collection, examination of the periodontal index, motivational analysis, scaling and polishing procedures, as well as education on the use of mechanical plaque control techniques at home (home care technique).
2. The second visit, which occurs one month after the first appointment, incorporates many clinical procedures such as collection of saliva, periodontal index assessment, motivation, scaling and polishing if necessary, and training on oral and dental hygiene and home care techniques.

Groups:

- (19)
- The Chronic Periodontitis Group (CP) included 13 children, was characterized by the presence of at least four sites with periodontal disease (PD) measuring 4mm or more, together with clinical attachment loss of 1-2mm or more. The classification was determined according to the worldwide criteria for characterizing the periodontal disease (PD).[20]

We confirm that our research, which include human subjects, complies with the Helsinki Declaration of 1975, as revised in 201.[21] Moreover, the research has obtained acceptance from the relevant institutional Ethics Committee (approved by the committee of ethical approval collage of dentistry university of Tikrit, reference no. 2023-53-UOT, date 2023-8-1).

Statistical Analysis

I used the SPSS V. 26 software application to do the statistical analysis on my results. A descriptive statistical analysis was performed in the research, using metrics such as mean, mean percentage, standard deviation (SD), and paired t test. Significant levels (S) were used in statistical analysis, where non-significant (NS) was indicated when $P > 0.05$, highly significant (HS) was indicated when $P \geq 0.01$, and very significant (HS) was indicated when $0.05 \geq P \geq 0.01$.

Results

A cohort of 30 youngsters diagnosed with gingivitis and chronic periodontitis participated in this case-control research. All the patients from the department of pedodontics. The gingivitis group had a mean age of 7 years (SD = 1.04), whereas the chronic periodontitis group had a mean age of 12 years (SD = 1.73). The proportion of boys in the gingivitis group is around 52.94% (n=9), while the proportion of girls is 47.05% (n=8) out of 17 patients. In contrast, the proportion of boys in the CP group is 61.53% (n=8) and the proportion of girls is 38.56% (n=5) out of 13 patients. All 30 patients included in the study were inhabitants of Tikrit, all of these mention on table 1.

Table 2 displays the mean and standard deviation of the clinical periodontal parameters, namely plaque index (PLI), gingival index (GI), and AST enzyme, for the gingivitis group at both the baseline and one-month follow-up. The findings also demonstrated statistically significant disparities across all parameters by the use of a paired t-test.

Table 3 displays the average and variability of the clinical periodontal parameters, such as plaque index (PLI), gingival index (GI), clinical attachment level (CAL), and AST enzyme, during both the initial assessment and the one-month follow-up for the CP group. The findings also

Table 1: Characteristics of study subjects

Characteristic	Ging.			CP		
	Mean ± SD					
Age	7 ± 1.045			12 ± 1.731		
Gender		No.	%		No.	%
	Boy	9	52.94	Boy	8	61.53
	Girl	8	47.05	Girl	5	38.46
Residency	Tikrit city 17			Tikrit city 13		

indicated statistically significant changes for all variables in terms of PLI and GI, as well as very significant differences for the CAL parameter. However, non-significant variations were seen in.

Table 2: Statistical analysis and comparison to evaluate clinical periodontal parameters and AST enzyme(U/L) across Gingivitis group

		Ging. Mean ±SD	p-value
PLI	Baseline	1.74±0.24	0.04*
	One month follow up	1.43±0.15	
GI	Baseline	1.65±0.35	0.05*
	One month follow up	1.45±0.26	
CAL	Baseline	-	-
	One month follow up	-	
AST	Baseline	25±2.35	0.02*
	One month follow up	20±2.06	

*Significant difference<0.05 (Paired t- test)

Table 3: Statistical analysis and comparison to evaluate clinical periodontal parameters and AST enzyme(U/L) across CP group

		CP Mean ±SD	p-value
PLI	Baseline	1.97±0.31	0.07**
	One month follow up	1.74±0.27	
GI	Baseline	1.83±0.24	0.002**
	One month follow up	1.36±0.31	
CAL	Baseline	4.15±2.14	0.000**
	One month follow up	n/a	
AST	Baseline	32±2.93	0.13***
	One month follow up	30±2.54	

* Significant difference<0.05 (Paired t- test)

** Highly significant difference ≤ 0.000

*** Non-significant difference > 0.05

Table (4) demonstrate non-significant association in CP group between salivary AST enzyme level and (PLI and CAL parameters) while significant correlation showed between AST and clinical periodontal parameters in gingivitis group and GI in CP group.

Discussion

An endeavor was made to establish a connection between oral and overall health by utilizing biological indicators found in saliva. Saliva is regarded as a straightforward and non-intrusive diagnostic tool for assessing the extent of periodontal damage. Salivary enzymes, hormones, and immunoglobulins are employed to monitor various bodily functions, including bone metabolism and immune response. The inflammatory response in periodontal connective tissue involves the production of several enzymes by inflammatory cells, resulting in the destruction of collagen and alveolar bone inside the connective tissue. During this physiological process, certain enzymes undergo migration towards the gingival sulcus or periodontal pocket, subsequently reaching the gingival calcific fluid (GCF). Once in the GCF, these enzymes are released and contribute to the composition of saliva. The alterations in enzymatic activity observed in the GCF serve as indicators of metabolic changes occurring in the gingiva and periodontium, leading to inflammation.[22–24]

The findings of our research indicate that a significant prevalence of gingivitis and CP is seen among males in both study groups. However, it is important to note that this is not a definitive factor, since children's oral health is impacted by factors such as toothbrush type, age at which brushing begins, and other self-performed methods to manage plaque (25,26) . In their study,

Table 4: Correlation between AST (U/L) level with clinical periodontal parameters in gingivitis CP groups after one month follow up.

Groups	PLI			GI			CAL		
	r	P	Sig.	r	p	Sig.	r	p	Sig.
Gingivitis group	0.372	0.02	S	0.654	0.04	S	0.517	0.01	S
CP group	0.532	0.07	NS	0.456	0.03	S	0.725	0.06	NS

P=probability

r= Simple person's correlation coefficients

Francisca Varas et al. (2011) determined that there was no statistically significant difference (P=0.838) between genders. Consequently, they concluded that sex does not serve as a predictor of gingivitis risk in this particular cohort.[27] However, another research concurs with our findings, which indicate a notably reduced occurrence of periodontal disorders in women compared to males throughout their early years (28,29).

Multiple studies have examined the efficacy of non-surgical periodontal therapy on periodontal health in adults, using various biomarkers. However, there is currently no research specifically focusing on this topic in children. The findings of the study revealed significant disparities in plaque-like index (PLI) and gingival index (GI) between the gingivitis and CP groups at the beginning and one-month post-scaling and polishing, accompanied by instruction on home care equipment. This observation aligns with previous research.[30] Inadequate oral hygiene has been identified as a recognized causative element for several oral ailments, such as dental caries and periodontal disease. One straightforward method for evaluating oral hygiene is assessing periodontal health via the examination of dental plaque accumulation. Dental plaque is a bacterial film that forms a bond with the surfaces of teeth, making mechanical hygienic treatments the most efficient approach for its removal.[31] Therefore, toothbrushes, floss, and non-surgical periodontal therapy are considered crucial instruments for maintaining oral health, and their use may be regarded as the most straightforward self-care practice in the realm of oral health.[30–32]

A substantial statistical change was seen between the baseline and one month following scaling and teaching, which is consistent with previous findings.[33,34] Therefore, it was observed that patients with periodontal disease (CP) and gingivitis exhibited elevated levels of clinical periodontal parameters (PLI, GI, BOP, PPD, and CAL) in conjunction with increased levels of salivary enzymes. This finding establishes a significant correlation between the severity of CP and salivary AST enzyme levels, suggesting that salivary AST enzyme can serve as a biomarker for assessing periodontal tissue damage. Consequently, this biomarker holds potential utility in the diagnosis, prognosis, and evaluation of post-therapy effects in periodontal disease,[34,35] also in a study conducted by Hiromasa Yoshie et al. (2007), it was shown that salivary levels of AST, ALT, and LDH may serve as indicators of inflammation and degradation of periodontal tissue. These findings imply that these markers may have clinical significance in the context of periodontal treatment.[36]

Conclusion

The present research provided evidence of the beneficial effects of non-surgical periodontal therapy, oral hygiene education, and consistent dental care on pediatric patients diagnosed with gingivitis and chronic periodontitis. Furthermore, it has been proposed that the evaluation of salivary AST enzyme levels holds potential as a non-invasive method for monitoring the progress of periodontal disease treatment in children. By offering valuable insights into the effectiveness of treatment and the management of the disease, this biomarker presents a valuable contribution to the field of pediatric dental care. Nevertheless, further investigation is necessary to authenticate its dependability and effectiveness across various groups of patients and treatment approaches. However, the findings of this study have significant consequences, as they have the potential to contribute to the development of more effective monitoring systems and better oral health outcomes in the field of pediatric dentistry.

Conflict of Interest: No conflict of Interest

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