

# Leukocyte Esterase and Protein Levels in Saliva, as Indicators of Gingival and Periodontal Diseases in Children

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## Abstract

**Purpose:** This study was to determine if dip stick assays of children's saliva for leukocyte esterase or protein (Serim Research Corporation, Elkhart, Ind) reflect the presence or severity of gingival or periodontal diseases in children.

**Methods:** The study included 13 children with periodontitis [study group] and a control group of 17 children without periodontitis. The saliva leukocyte esterase and protein values (scales from 1 to 4) were tested with dip stick analyses. The gingival (GI) and plaque indices (PI) presence and number of sites with periodontitis, demographic data, systemic condition, caries prevalence, and the presence of dental restorations were recorded and their relationship to leukocyte esterase or protein values were analyzed.

**Results:** Most children had a protein value of 2 or 3 or a leukocyte esterase value of  $\geq 3$ . Significant differences in the distribution of protein values by the presence/absence of periodontitis (chi-square,  $P < 0.001$ ), or the number of sites with periodontitis by protein value (chi-square,  $P = .005$ ; ANOVA,  $P = .03$ ) were evident; No. 4 protein values were found only in children with periodontitis, and No. 2 and No. 3 protein values were mostly found in children without it.

**Conclusions:** Dip stick protein analysis of saliva of children has the potential to differentiate children with periodontitis or with fewer periodontal lesions. (*Pediatr Dent.* 2004;26:310-315)

**KEYWORDS:** LEUKOCYTE ESTERASE, PROTEIN, SALIVA, PERIODONTITIS

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The fact that children and adolescents are not immune to periodontal diseases has been clearly evidenced in the literature.<sup>1-6</sup> Furthermore, it has been indicated that:

1. individuals, families, and populations may have an increased susceptibility to periodontal diseases;<sup>1-6</sup>
2. there may be a relationship between the presence and severity of periodontal diseases in childhood and older ages;<sup>7-9</sup>
3. periodontopathogenic bacteria may be transmitted among family members.<sup>10-11</sup>

These facts, in addition to the fact that the incipient periodontal diseases require relatively simple and less costly treatments than those required for more severe periodontitis, emphasize the need for diagnostic techniques that allow for the early diagnosis of gingival and periodontal diseases. In fact, several methods may be utilized to iden-

tify etiologic factors for periodontal diseases, including the examination of the microbial composition of the subgingival dental plaque.<sup>12-15</sup> However, these methods require the availability of suitable materials and equipment, are expensive, and may take several days.

Dry reagent technology with test strips that include an indicator pad saturated with a dry solution of 1 or more chemicals has recently been proven to be a simple and inexpensive diagnostic tool for the diagnosis of destructive inflammatory diseases.<sup>16-22</sup> The technique involves dipping a test strip with an indicator pad into the fluid sample that is being tested. The chemicals in the pad react with the substance being detected, and the color of the pad changes proportionally to the concentration of the substance under analysis.<sup>16</sup>

Gingivitis and periodontitis are inflammatory diseases that involve leukocyte and protein changes in reaction to the

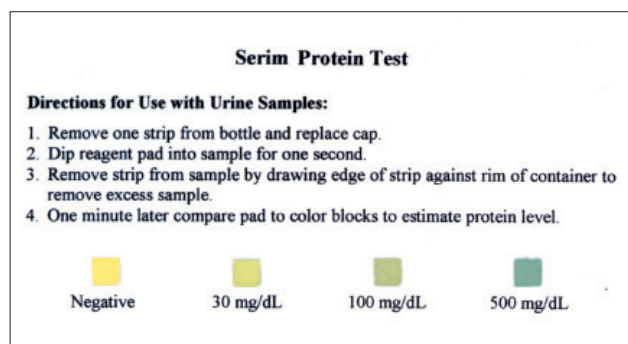


Figure 1. Serim protein test levels color scale.

infection with periodontal pathogens.<sup>15,23,24</sup> In addition, there is evidence that human salivary glands may respond to inflammatory diseases in the oral cavity, such as periodontitis, by enhanced synthesis of acinar proteins.<sup>25</sup> Furthermore, gingival crevicular fluid collagenase levels have been found to be related to periodontitis.<sup>26</sup> Therefore, the hypothesis of the present study was that increased values of leukocyte esterase and protein in saliva would reflect the presence and severity of gingival or periodontal diseases in children.

Consequently, the purpose of the present study was to determine if dip stick assays of saliva for leukocyte esterase or protein reflect the presence and severity of gingival or periodontal diseases in children.

## Methods

After Institutional Review Board approval, the study was conducted in the Pediatric Dentistry Clinic of the College of Dentistry of the University of Florida. Over a period of 6 months, children with radiographic evidence of periodontitis (study group) and their parents were invited to participate in the study. It should be noted that the children were approached only after informed consent was given by their parents. In addition, after informed consent was received from parents and their children, a matched control sample of children with no radiographic evidence of periodontal diseases was included in the study.

The small size of the indicator pad in the dip sticks requires only a few drops of saliva for each analysis. Therefore, the children were requested to spit an unspecified amount of unstimulated saliva into a plastic cup. In every clinical session, >1 sample was examined simultaneously within 5 minutes after saliva collection, without the examiner being aware if a specific sample was from a child with periodontitis or a control. The saliva samples were analyzed with a dip stick for leukocyte esterase and a dip stick for protein, as indicated by the manufacturer (Serim Research Corporation, Elkhart, Ind). The results were determined at 1 minute by comparing the color of the dip stick indicator with a 4-color key provided by the manufacturer:

1. leukocyte esterase:
  - a. negative;
  - b. trace;
  - c. small;
  - d. large.

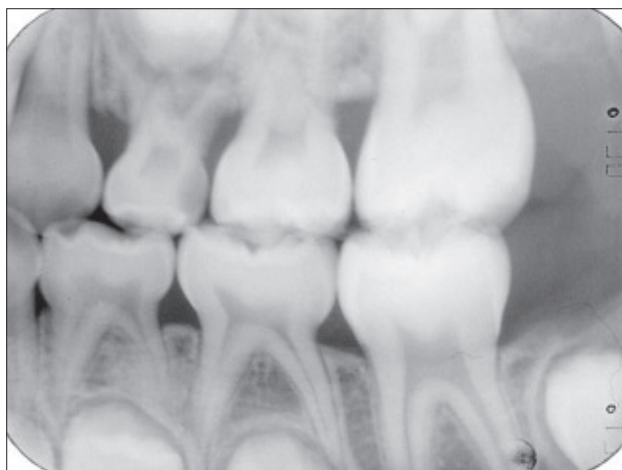


Figure 2A. Bite-wing radiographs in which chronic periodontitis related to a proximal caries lesion, contact loss, and food impaction between the maxillary primary molars is evident.

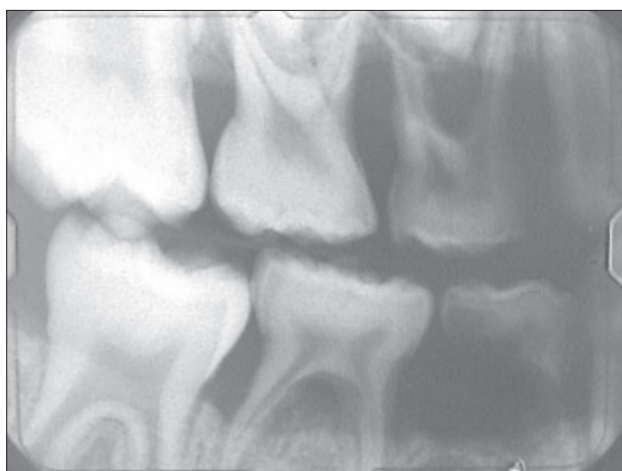


Figure 2B. Bite-wing radiographs in which aggressive periodontitis affecting all primary molars is evident.

2. protein (Figure 1):
  - a. negative;
  - b. 30 mg/dL;
  - c. 100 mg/dL;
  - d. 500 mg/dL.

Reproducibility of the dip stick exams was not evaluated, since they can be used only once and the reading is made only once. Repeated measures of the same sample with several dip sticks or for longer periods of time could be affected by evaporation of the water content of the saliva and/or contamination of the saliva by the reagents in the dip stick.

In addition to the saliva analysis, the following data were recorded:

1. sex;
2. age in years;
3. systemic condition: healthy or with a systemic disease or syndrome, and type of disease or syndrome when present;
4. ethnic origin: white, Hispanic, African American, Asian;

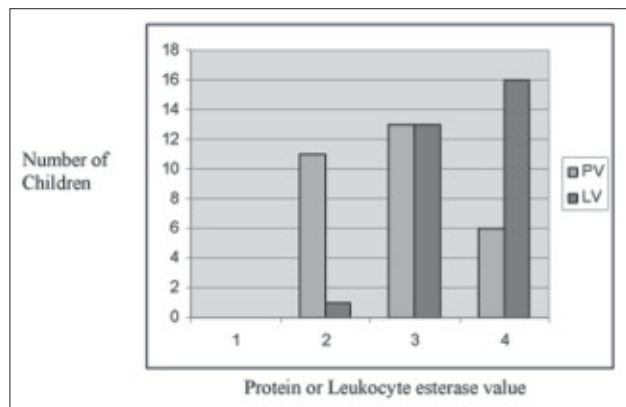


Figure 3. Graphic presentation of the number of children by their protein (PV) or leukocyte esterase (LV) values.

5. dentition: primary, mixed, or permanent;
6. type of dental treatments present: no treatment; composite and sealants; amalgam; stainless steel crowns;
7. number of dental restorations when present;
8. number of tooth surfaces with caries, missing, or filled: DMFS+dmfs;
9. number of teeth with severe caries: crown destroyed or residual roots;
10. evidence of swelling or fistula associated to a pulp pathologic process: yes/no;
11. plaque index (PI)<sup>27</sup>:
  - a. no plaque;
  - b. plaque invisible, found with probe;
  - c. visible small amount of plaque;
  - d. severe accumulation of plaque.
12. gingival index (GI)<sup>28</sup>:
  - a. no inflammation;
  - b. inflamed gingiva, no bleeding on probing;
  - c. inflamed gingiva, bleeding on probing;
  - d. severe inflammation, spontaneous bleeding.
13. radiographic evidence of periodontitis, based on an increased distance from the alveolar bone to the cemento-enamel junction, and the complete loss of lamina dura above the alveolar bone crest<sup>29</sup>:
  - a. present or absent;
  - b. type: chronic or aggressive (Figures 2A, 2B)<sup>30</sup>;
  - c. presence of local facilitating factors for chronic periodontitis: caries or inadequate restorations (Figure 2A).<sup>31,32</sup>
14. number of sites with periodontitis.

All the GI, PI, and alveolar bone diagnoses were done by one author; previous studies indicated a >0.80 level of reliability by the same author in these measurements.

### Statistical examination

The *t* test analysis was utilized to examine the significance of the difference in age between males and females. Analysis of variance (ANOVA) was utilized to examine the significance of the differences in age, number of carious teeth, and number of sites with periodontitis by the leukocyte esterase

Table 1. Number of Children by Gingival Index (GI) and Plaque Index (PI)

Index value	Number of children by GI	Number of children by PI
1	0	3
2	12	15
3	10	6
4	8	6

or protein values. Chi-square analysis was utilized to examine the significance of differences in distribution of:

1. the leukocyte esterase or protein values by the GI and PI, number of sites with periodontitis, presence or type of dental materials, ethnic groups, and presence or absence of periodontitis and systemic condition (healthy/not healthy);
2. presence/absence of periodontitis by ethnic groups.

### Results

The sample included a total of 15 males (mean age=10 years, standard error [SE]=0.49 years) and 15 females (mean age=9.2 years, SE=0.52 years). No statistically significant difference in age was evident between males and females (*t* test, *P*>.05). Nineteen children had no evidence of systemic disease, and the other 11 had ≥1 systemic condition which required medication, such as asthma (N=5), attention deficiency syndrome (N=5), heart conditions (N=1), epilepsy (N=1), or other. The children with systemic diseases were distributed in both the control (N=6) and the test groups (N=5), and none of the children with asthma had periodontal disease. Eleven children (37%) were white, 10 (33%) were African American, and 9 (30%) were Hispanic. Most of the children (N=25, 83%) were in the mixed dentition period, 4 (13%) had a permanent dentition, and only 1 child (3%) was in the primary dentition period.

At the time of the examination, 11 children (37%) had pit and fissure sealants, 7 (23%) had amalgam restorations, and 6 (20%) had stainless steel preformed crowns. From these children, only 2 (6%) had both amalgams and stainless steel crowns. The mean number of decayed, missing, and filled tooth surfaces was 8.55 (SE=1.63); the mean for the carious component of this number was 4.57 (SE=5.1). Most of the children had GI or PI values of 2 or 3 (N=22, 73%, and N=21, 70%, respectively; Table 1). Seventeen children (57%) had no sign of periodontitis (control group), and 13 (43%) had periodontitis (study group).

Among the 13 children with periodontitis, 4 (31%) were African American, 10 (77%) had extensive proximal caries (Figure 2A), and 1 (8%) had an inadequate restoration. Two (15%) of the children with periodontitis had aggressive periodontitis (Figure 2B). Among the children with periodontitis, the range of affected sites was 1 to 6, with

**Table 2. Distribution of the Number of Sites With Periodontitis Per Child**

Number of sites with periodontitis per child	No. of children
0	17
1	7
2	2
3	2
4	1
5	0
6	1

most (69%) having only 1 or 2 affected sites (Table 2). Most of the children (N=24, 80%) had a protein value of 2 or 3, and the vast majority of the children (N=29, 97%) had a leukocyte esterase value of 3 or 4 (Figure 3).

No statistically significant difference was evident in the distribution of periodontitis by ethnic origin (chi-square,  $P>.05$ ). When the children were divided by their protein or leukocyte esterase values, statistically insignificant differences in the number of decayed, missing, or filled teeth or the number of restorations were found (ANOVA,  $P>.05$ ). The differences were not statistically significant (chi-square,  $P>.05$ ) in the distribution of the protein and leukocyte values by:

1. the presence/absence of the various dental materials;
2. the GI or PI values;
3. the systemic health status (healthy/not healthy or the different systemic conditions).

On the other hand, statistically significant differences in the distribution of protein values by the presence/absence of periodontitis or the number of sites with periodontitis were evident. A protein value of 4 was evident only in children with periodontitis, and protein values of 2 and 3 were evident (71%) mostly in children with no periodontitis (chi-square,  $P<.05$ , Tables 3 and 4). In addition, the number of sites with periodontitis was statistically significantly lower (ANOVA,  $P=.03$ ) in children with a protein value of 2 (mean=0.63, SE=0.41) and 3 (mean=0.61, SE=0.38) than in those with a protein value of 4 (mean=2.0, SE=0.56).

The differences in the distribution of leukocyte esterase and protein values by ethnic origin were not statistically significant (chi-square,  $P>.05$ ). It should be noted, however, that in 8 of 10 African American children, the maximum leukocyte esterase value was 4. Furthermore, when examining the distribution of the leukocyte esterase values among the African American children against all ethnic groups, the chi-square analysis indicated a significant difference ( $P=.01$ ).

## Discussion

The American Academy of Pediatric Dentistry mission statement is "to [improve] and [maintain] the oral health

**Table 3. Distribution of the Presence/Absence of Periodontitis by the Protein Values\***

Protein value	Periodontitis		Totals
	Present	Absent (control)	
2	4	7	11
3	3	10	13
4	6	0	6
Totals	13	17	30

\*Statistically significant difference, chi-square,  $P=.001$ .

of infants, children, adolescents, and persons with special care needs."<sup>33</sup> Obviously, this statement relates to all oral diseases, including gingival and periodontal diseases. Most cases of gingival and periodontal diseases in childhood have less severe clinical appearance than the ones present in adulthood.<sup>4,34</sup> Nevertheless, periodontal diseases in childhood may also reach severe stages involving extensive alveolar bone resorption, swelling, and pain and even have the potential to jeopardize developing or erupted permanent teeth.<sup>4,34</sup> Furthermore, there is a significant possibility that periodontal disease in childhood may facilitate the establishment of periodontal diseases at older ages.<sup>7-9,14</sup>

The early diagnosis and treatment of periodontal diseases is preferred before irreversible damage takes place or before extensive treatments are required that have a decreased chance of success or which may elicit behavior management problems. However, incipient gingival or periodontal diseases in children may not be notable with conventional clinical diagnostic tools,<sup>4,13,35-37</sup> and the development of simple clinical diagnostic methods that allow for the disclosure of gingival or periodontal diseases as early as possible is desired.

Reagent strip diagnoses have been extensively examined in various fields, and have been found useful in the diagnosis of meningitis, *Helicobacter pylori* infections, peritonitis, urinary infections, and otitis media.<sup>16-22</sup> In the field of dentistry, reagent strips have the potential to diagnose and monitor periodontitis, either by the enhanced synthesis of acinar proteins due to the salivary gland response or by detection of destructive proteinases, associated with the progression of periodontitis, in gingival crevicular fluid.<sup>25,26</sup> In addition, the authors' hypothesis that leukocyte esterase or protein levels in saliva could be related to gingival or periodontal diseases was based on the fact the establishment and progress of periodontal diseases is dependent on the presence of bacterial pathogens, bacterial products, and the host characteristics, which include the protective responses.<sup>24</sup> More specifically:

1. In susceptible individuals or sites, the protective mechanisms are breached by the bacteria virulence factors, and polymorphonuclear leukocytes or neutrophils migrate from the bloodstream to the tissues towards the bacteria.<sup>24</sup>



**Table 4. Number of Sites With Periodontitis by the Protein Value\***

Protein value	Number of sites with periodontitis						Total
	0 (control)	1	2	3	4	6	
2	7	3	0	0	1	0	11
3	10	2	0	0	0	1	13
4	0	2	2	2	0	0	6
Total	17	7	2	2	1	1	30

\*Statistically significant difference, chi-square,  $P=.005$ .

2. The presence of leukocytes in gingival sulci as well as in gingival tissues have been well documented.<sup>38-41</sup>
3. Bacteria may:<sup>24</sup>
  - a. produce compounds that are recognized by specific neutrophils membrane receptors, proteins that can specifically bind to chemical agents, which in turn activate biochemical processes within the cell;
  - b. induce the gingival crevicular fluid, which is an inflammatory exudate that flows through the gingival crevice or pocket and contains serum antibody molecules;
  - c. produce proteases that destroy host proteins.

In the present study, the purpose of recording the demographic data, systemic condition, and the presence/absence of caries and dental treatments was to examine the possible influence of these variables on the leukocyte esterase or protein levels and not to relate them to gingival or periodontal diseases. The present findings indicated that none of the non-gingival/periodontal variables was significantly related to the saliva leukocyte esterase or protein values.

The failure to demonstrate that leukocyte values are related to the presence and severity of gingivitis or periodontitis may be related to the sensitivity of the diagnostic stick, which provided measurements of 3 and 4 for all the children regardless of the presence/absence of periodontitis or its extent. On the other hand, the sensitivity of the protein stick revealed that the highest scale value (No. 4=500 mg/dL) was limited to children with periodontitis. Lower protein values were more common among children with no periodontitis or periodontitis in only 1 site (Tables 3 and 4), confirming the authors' hypothesis. These findings are most likely related to the destructive characteristics of periodontitis and proteases and/or the salivary gland response.<sup>19,20</sup> By the same token, the lack of significance in the distribution of protein values by the presence or severity of gingivitis can be explained by the relatively diminished activity and severity of the disease, when compared to periodontitis.

In the present study, African American children had significantly higher leukocyte esterase saliva values. These values could be related to the fact that African Americans have been found to have a higher prevalence and severity of periodon-

tal diseases.<sup>41</sup> However, in the present study, the differences in the distribution of presence of periodontitis by ethnic origin was not statistically significant. Other factors not investigated in the present study may be related to this finding.

## Conclusions

Analyses of children's saliva with dip stick tests strips and pads saturated with dry reagent solutions (Serim Research Corporation, Elkhart, Ind) indicated that:

1. leukocyte esterase values do not indicate the presence or extent of gingival or periodontal diseases;
2. the highest protein value (500 mg/dL) was characteristic for children with periodontitis;
3. lower protein values were mostly found in children with none or only 1 site with periodontitis.

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