

Quantitative and Qualitative Methods for Caries Risk Assessment in Children: A Comparative Study

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Abstract

Aim: To compare the qualitative risk assessment of caries with Clinpro Cario L-Pop (CCLP) with the quantitative determination of lactic acid present in children's saliva using high performance liquid chromatography (HPLC).

Methods: 16 children were divided into 2 groups (high and low caries risk). For qualitative analysis of caries risk, the CCLP test was used, and for the quantitative analysis the concentration of lactic acid present in saliva was determined by HPLC. The results were analyzed using ANOVA and chi-square test with a significance level of 5%.

Results: The intraclass correlation between the values obtained by the CCLP test and the amount of lactic acid determined by HPLC was 0.040. Therefore, the correlation was not statistically significant ($p = 0.442$).

Conclusion: It can be concluded that CCPL alone was not an effective method to clinically determine the risk of caries.

Keywords: Caries risk; Children; Clinpro Cario L-Pop; Lactic Acid

Introduction

The determination of caries risk in children based on a single method of evaluation is questionable, considering the multifactorial etiology of this disease [1-3]. Thus, when tests are used together, an increase in sensitivity is observed, since some high-risk individuals can be detected by one test and not by another, resulting in the detection of a larger population of high-risk individuals. On the other hand, the specificity is reduced since each test can contribute individually with the total of false positives [4].

There are tests that are based on the evaluation of cariogenic microorganisms' activity by determining the production of acids [5-8]. In the oral cavity, the present bacteria that are associated with the consumption of fermentable sugars in sufficient concentration, will produce different carboxylic acids, among which the lactic acid, the main responsible for the decrease in pH and consequent demineralization of the enamel [9].

The degradation process of pyruvic acid from glucose follows different pathways, depending on the type of bacteria and the amount of sugar available in the oral environment, as well as the presence of oxygen and carbon dioxide. When the diet contains high amounts of sugars, the formation of lactic acid through lactate dehydrogenase is an important pathway for *Streptococcus*, *Lactobacillus* and *Actinomyces* under aerobiosis and anaerobic condition [10,11].

The Clinpro Cario L-Pop (3M ESPE - St. Paul-USA) biochemical test (CCLP) was recently launched in the market for qualitative analysis of caries risk in children and adults. According to the manufacturer, it is a test that determines the full potential for caries lesions development, as well as monitors the risk for caries individually for each patient. This test is based on the degradation of the lactic acid present in the saliva, which will be related to a color scale and scores (3M ESPE).

There are few studies in the specific literature that have evaluated the efficacy of this new test for caries risk assessment [5-7,12]. These studies demonstrated that CCLP was effective in determining risk. However, there are no studies evaluating this qualitative method in comparison to quantitative methods that precisely determine the amount of lactic acid produced.

Aim of the Study

The aim of the present study was to compare the qualitative risk assessment of caries with Clinpro Cario L-Pop (3M Espe) with the quantitative determination of lactic acid present in children’s saliva using high performance liquid chromatography (HPLC).

Materials and Methods

The institutional research ethics committee approved all the procedures. Sixteen healthy children of both genders, between ages 1 and 4 participated in the study. Children who did not undergo dental care and who did not use antimicrobials or antibiotics in the last 3 months were included. After obtaining the free and informed consent of the parents or guardians, the children selected for the study were divided into 2 groups according to the risk of caries, determined by clinical examination and specific form: Group 1 - 10 children with high caries risk; and Group 2 - 6 children with low caries risk. All children underwent both caries risk assessment (qualitative and quantitative).

For the qualitative analysis of caries risk the Clinpro Cario L-Pop test was used (3M ESPE Dental Products, St. Paul, USA). For this procedure an applicator with a cotton tip, provided by the manufacturer, was used, which was rubbed 4 times on the back of the tongue of each participant, in rotating movements, for the wetting of the device by the saliva. Then, according to the manufacturer’s instructions, the applicator was introduced into a device containing specific reagents that promote the enzymatic degradation of the lactic acid present in the saliva. The applicator was kept in contact with the reagents for 2 minutes. After the determined time, a color change occurred in the applicator that was compared to a specific scale. Each tonality present in the scale is related to scores from 1 to 9, with scores from 1 to 3 indicating low production of lactic acid, scores from 4 to 6 indicating moderate production, and scores from 7 to 9 indicating high production of lactic acid and, therefore, are associated with a low, medium or high risk to caries, respectively.

For the quantitative analysis, 1 mL of saliva of each participant was collected in plastic tubes (Eppendorf), which were stored in a refrigerator until the moment of the analysis. The concentration of lactic acid present in saliva was determined by high performance liquid chromatography (HPLC) [13]. For this methodology, the mobile phase of 90% of 0.1% phosphoric acid (pH = 2.82) and 10% of methanol was used. The lactic acid solutions were then prepared for the construction of the 8-point calibration curve according to the following concentrations: 15,0 mg/mL; 7,5 mg/mL; 5,0 mg/mL; 2,5 mg/mL; 1,25 mg/mL; 0,625 mg/mL; 0,312 mg/mL; 0,156 mg/mL (Figure 1).

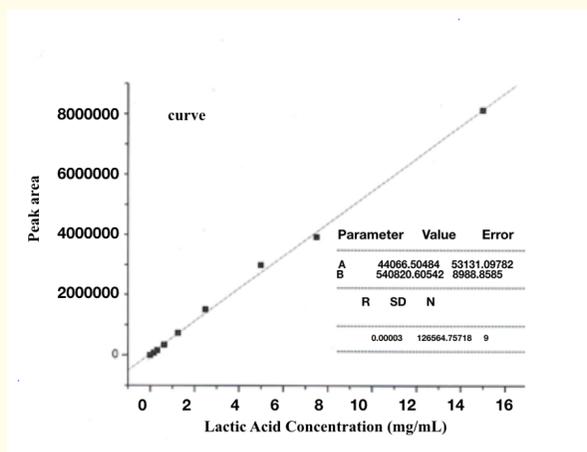


Figure 1: Calibration curve of lactic acid.

To determine the amount of lactic acid present in the saliva of each patient, 10 μ l of the sample were used and the lactic acid concentration was calculated based on the equation of the line (calibration curve).

The results were analyzed using the intraclass correlation test, ANOVA and chi-square test with a significance level of 5%. The data were analyzed in the SAS (Statistical Analysis System) software for Windows version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

The intraclass correlation between the values obtained by the CCLP test and the amount of lactic acid determined by HPLC was 0.040 (Confidence Interval 95%: -0.467 to 0.527). Therefore, the correlation was very low and was not statistically significant ($p = 0.442$).

When the amount of lactic acid determined by HPLC was correlated with the risk of caries determined by clinical parameters using the ANOVA test, it was possible to observe that low risk subjects presented a mean of lactic acid of 1.13 (Standard Deviation = 0.62), while high risk individuals presented a mean of 2.08 (SD = 1.67). Although it was not possible to find a statistically significant difference ($p = 0.2458$), there was a numerical tendency of a positive correlation between the risk of caries and the amount of lactic acid determined by HPLC, as can be observed in figure 2.

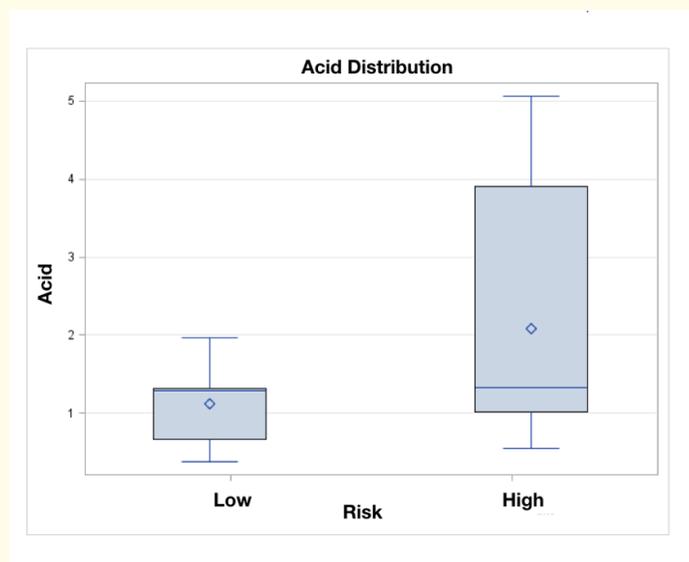


Figure 2: Box plot showing the average amounts of lactic acid determined by HPLC found in low-risk and high-risk individuals.

With regard to the comparison of the values obtained by the CCLP and the risk of caries, by means of the chi-square test, it was not possible to find a statistically significant association ($p = 0.2043$), nor was there a numerical tendency of positive correlation.

Discussion

The determination of patients at high risk for caries and the early detection of active lesions are of fundamental importance in pediatric dentistry [5,14,15]. Current clinical methods of assessing caries risk involve a combination of factors including diet, fluoride exposure, host susceptibility and microbiota, which interact with a variety of social, cultural, and behavioral factors [15,16]. In addition, some tests based on the presence, number and metabolic activity of cariogenic microorganisms have been proposed with the aim of improving the efficacy and precision in determining caries risk.

CCLP is a biochemical test that qualitatively detects the production of lactic acid metabolically produced by cariogenic bacteria. The mechanism of action is based on the enzymatic oxidation of lactic acid by the enzyme lactate dehydrogenase bound to a cascade of “redox” indicators that generate a signal of blue color [6].

This test has not been vastly studied in the literature and previous work [1,5-7] have shown satisfactory results, indicating to be an effective method. However, the present study demonstrated opposite results, and it was not possible to find any association between the measurements obtained by the CCLP and those determined by the quantitative chemical method (HPLC). Possibly, the divergence of results can be attributed to the differences in the methodologies used and the main objective of each study.

Schiffner and Torres-Quintero (2005) [1] aimed to evaluate the reproducibility of CCLP under known oral conditions, by applying the test several times in the same patient. Under these conditions, the test exhibited high reproducibility (82.0%); however, in situations in which oral conditions were altered, the reproducibility was lower (60,0%). Gerardu., *et al.* (2006) [5] conducted a clinical study concluding that the CCLP was able to detect changes in acidogenicity after the use of antimicrobials, and could be used for monitoring and control of treatments performed.

In 2010, a study was carried out to evaluate the association between elevated scores obtained by CCLP and the occurrence of caries lesions in patients treated with orthodontic appliances after a 12-month follow-up period [7]. Based on the results, it was concluded that the CCLP is a test that can be applied clinically to determine the risk of caries in adolescents who are under orthodontic treatment.

A study conducted by Saraiva., *et al.* (2015) [17] aimed to compare, through a clinical study (dental students), the qualitative caries risk assessment with Clinpro Cario L-Pop (3M Espe) with conventional semiquantitative colony counting culture-based technique. As a result it was observed that clinically both tests Clinpro Cario L-Pop and Saliva-Check Mutans achieved satisfactory results.

In the study by Bretz., *et al.* (2007) [6], the efficacy of CCLP was assessed by association with the prevalence and severity of caries lesions in children. Briefly, the test results were effective in differentiating caries-free patients from those with active lesions. In general, prevalence levels of carious surfaces and lesion severity tended to be higher in the presence of moderate and high lactic acid production indicators.

On the other hand, in the present study an evaluation of the efficacy of CCLP was carried out in comparison to an accurate method (HPLC) to determine the amount of lactic acid present in the saliva of children with low and high risk to caries. Differently from the results of Bretz., *et al.* [6] the values obtained by the CCLP test were not correlated with the clinical picture, nor was there a significant association with the amount of lactic acid.

Although in the present study there was no statistically significant association between any of the methods employed (clinical, CCLP and chemical), there was a numerical trend of positive correlation between the risk of clinically assessed caries and the level of chemically measured lactic acid (HPLC) in the saliva samples. Possibly, if the number of participants had been higher this correlation could have statistical significance, which demonstrates the reliability of the methods employed.

Further studies are needed with a larger number of individuals in order to clarify the divergences found and to establish the performance of this test.

Conclusion

Therefore, it can be concluded that CCPL alone was not an effective method to clinically determine the risk of caries.

Authors Contribution

Marília Pacífico Lucisano, Lea Assed Bezerra Silva and Paulo Nelson-Filho: conceptualized and designed the study, drafted the manuscript, reviewed and approved the final manuscript as submitted.

Marília Pacífico Lucisano, Amanda Silva Bertasso and Mónica Verónica Escalante Romero: carried out the experimental phase with children (application of the Clinpro Cario L-Pop test and saliva collection), reviewed and approved the final manuscript as submitted.

Raquel Assed Bezerra Silva, Roberto Santana da Silva and Fabiana de Souza Oliveira: carried out the lactic acid determination in saliva, the statistical analysis, interpreted and elaborated the results, drafted the manuscript, reviewed and approved the final manuscript as submitted.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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Bibliography

1. Schiffner U and Torres-Quintero A. "Reproducibility of a new caries risk test under different oral conditions". *Clinical Oral Investigations* 9.3 (2005): 187-191.
2. Fontana M., *et al.* "Evidence-based caries, risk assessment, and treatment". *Dental Clinics of North America* 63.1 (2019): 119-128.
3. Gannam CV., *et al.* "Caries risk assessment". *General Dentistry* 66.6 (2018): 12-17.
4. Alaluusua S., *et al.* "Salivary caries-related tests as predictors of future caries increment in teenagers. A three-year longitudinal study". *Oral Microbiology and Immunology* 5.2 (1990): 77-81.
5. Gerardu V., *et al.* "Comparison of Clinpro Cario L-Pop estimates with CIA lactic acid estimates of the oral microflora". *European Journal of Oral Sciences* 114.2 (2006): 128-132.
6. Bretz WA., *et al.* "Microbial acid production (Clinpro Cario L-Pop) and dental caries in infants and children". *Quintessence International* 38.4 (2007): e213-217.
7. Chaussain C., *et al.* "Interest in a new test for caries risk in adolescents undergoing orthodontic treatment". *Clinical Oral Investigations* 14.2 (2010): 177-1185.
8. Grigalauskiėnė R., *et al.* "Biological approach of dental caries management". *Stomatologija* 17.4 (2015): 107-112.
9. Marsh PD. "Are dental diseases examples of ecological catastrophes?" *Microbiology* 149.2 (2003): 279-294.
10. Abbe K., *et al.* "Oxygen and the sugar metabolism in oral streptococci". *Proceedings of the Finnish Dental Society* 87.4 (1991): 477-487.
11. Peltroche-Llacsahuanga H., *et al.* "Assessment of acid production by various human oral micro-organisms when palatinose or leucrose is utilized". *Journal of Dental Research* 80.1 (2001): 378-384.
12. Schiffner U and Torres-Quintero A. "Reproducibility of a new caries risk test under different oral conditions". *Clinical Oral Investigations* 9.3 (2005): 187-191.

13. Qureshi MS, *et al.* "Determination of organic acid impurities in lactic acid obtained by fermentation of sugarcane juice". *Journal of Chromatography A* 1218.40 (2011): 7147-7157.
14. Kraljevic I, *et al.* "Risk indicators of early childhood caries (ECC) in children with high treatment needs". *Swiss Dental Journal SSO* 127.5 (2017): 398-410.
15. Corrêa-Faria P, *et al.* "Incidence of dental caries in primary dentition and risk factors: a longitudinal study". *Brazilian Oral Research – SciELO* 30.1 (2016): S1806-83242016000100254.
16. Featherstone JD. "The caries balance: The basis for caries management by risk assessment". *Oral Health and Preventive Dentistry* 2 (2004): 259-264.
17. Saravia ME, *et al.* "Evaluation of Chair-Side Assays in High Microbiological Caries-Risk Subjects". *Brazilian Dental Journal* 26.6 (2015): 592-595.

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