# LACK OF A SIGNIFICANT RELATIONSHIP BETWEEN TOENAIL FLUORIDE CONCENTRATIONS AND CARIES PREVALENCE

S Charone, a,c MM Bertolini, BRM Gonçalves, AC loivos, L Grizzo, AMAR Buzalaf, S Groisman Rio de Janeiro and São Paulo, Brazil

SUMMARY: The relationship between fluoride (F) concentrations in toenails and prevalence of caries using the International Caries Detection and Assessment System (ICDAS-II) criteria was evaluated. Fifty-four children (4–13 years of age) from Rio de Janeiro, Brazil, had their teeth surfaces examined and toenails clipped and analyzed for F. Toenail F concentrations in children presenting ICDAS-II≤10 or >10 were compared by unpaired t test with Welch correction. Dichotomized data were analyzed by Fisher's exact test. Children presenting ICDAS-II≤10 (n=23) had 1.85±1.32 (Mean±SD) µg/g [F]; these values were higher than children having ICDAS-II>10 (n=31), whose toenails had 1.58±0.78 µg/g [F], a nonsignificant difference. The sensitivity and specificity of toenail F concentrations in identifying children with ICDAS-II≤10 were 0.22 and 0.77, respectively. We conclude that children with low caries prevalence tend to have higher toenail F concentrations, but the validity of this biomarker as a diagnostic tool for caries prevalence is low, possibly owing to the fact that the mechanism of action of F on caries control appears to be essentially topical.

Keywords: Brazilian children; Caries prevalence; Fluoride concentration; ICDAS-II; Toenails.

## INTRODUCTION

A recent review of 29 caries detection criteria systems concluded that the majority of the current caries detection systems do not measure the disease process at its different stages. More recently several new criteria systems have been proposed and evaluated, but they still vary in how the disease is measured. Based on the work of Ekstrand et al., which tried to integrate the best features of the other caries systems, and restorative dentists proposed a new system, named the International Caries Detection and Assessment System (ICDAS-II). Before this proposal, caries detection in surveys has been performed at a cavitation level. The ICDAS-II is intended to be feasible for use in epidemiological surveys and to detect cavitated and non-cavitated stage lesions with acceptable reliability. In Brazil, 56% of 12-year-old children have dental decay and the mean DMFT is 2.1. Oral and dental health programs in Brazil and in many countries are planned and based on similar data, which have considered only cavitated caries.

Since caries experience is widely viewed as being related to levels of fluoride (F) intake, the search for biomarkers of exposure to F, which are relatively easy to collect and analyze, has intensified. Fingernails and toenails have been used as biomarkers of exposure to F, 16-20 since they offer a simple, noninvasive bioassay method. Since children with caries experience have generally slightly lower intakes of fluoride, the aim of this study was to examine the relationship

<sup>&</sup>lt;sup>a</sup>Bauru School of Dentistry, University of São Paulo, Bauru, Brazil. Alameda Doutor Octávio Pinheiro Brisolla, 9-75. CEP.: 17012-901 - Bauru - SP; <sup>b</sup>School of Dentistry, Federal University of Rio de Janeiro, Brazil, Avenida Professor Rodolpho Paulo Rocco, 325. Ilha do Fundão, Rio de Janeiro, RJ, Brazil CEP.: 21941 617 - Rio de Janeiro - RJ. <sup>c</sup>For correspondence: Senda Charone. Rua Doutor Olimpio de Macedo, 2-32 apt 33. Bauru - São Paulo - Brazil. E-mail: sendacharone@yahoo.com.br

between F concentrations in toenails and prevalence of caries using the ICDAS-II criteria.

#### **MATERIALS AND METHODS**

*Volunteers:* Fifty-four healthy children (4–13 years old) were recruited in a selected Health Center, with recommended levels of F in the drinking water, in the municipality of Rio de Janeiro, RJ, Brazil. This cross-sectional study was approved by the Ethical Committee of School of Dentistry, Federal University of Rio de Janeiro, Brazil.

Dental Examinations and toenails sampling: All the children had their teeth surfaces examined by two calibrated examiners (Kappa inter-examiner = 0.72–0.80). The ICDAS-II scores for several grades of dental caries in all surfaces have previously been described in detail. Ten toenails clippings from both big toes were pooled and stored at room temperature until analysis.

Analytical procedure: Each toenail clipping was cleaned briefly with deionized water using an interdental brush and then sonicated in deionized water for 10 min, dried and weighed. The F concentrations were determined after overnight hexamethyldisiloxane-facilitated diffusion<sup>22</sup> as modified by Whitford,<sup>23</sup> using an ion-specific electrode (model 9409, Orion Research, Cambridge, Mass., USA) and a miniature calomel reference electrode (Accumet, No. 13-620-79), both coupled to a potentiometer (model EA 940, Orion Research). F standards (0.0095, 0.019, 0.095, 0.190 and 0.950 μg F as NaF were prepared by serial dilution of a stock standard containing 0.1 M of F as NaF (Orion 940906). Comparison of the millivolt readings demonstrated that the F in the diffused standards had been completely trapped and analyzed (recovery 99%). The millivoltage potentials were converted to μg of F/g of sample using a standard curve with a coefficient correlation of r >0.99.

Statistical Analysis: The software GraphPad InStat version 3.0 for Windows (GraphPad, San Diego, CA, USA) was used. The data are presented as mean±SD. Toenail F concentrations in children presenting ICDAS-II  $\leq 10$  or >10 were compared by unpaired t test with Welch correction. Dichotomized data (toenails F concentrations  $\leq 2$  or >2 µg/g) were analyzed by Fisher's exact test. This allowed calculations of sensitivity, specificity, and predictive values regarding the use of toenail F concentrations as indicators of caries prevalence. The significance level was set at 5%.

#### **RESULTS**

The results of ICDAS-II were dichotomized into two categories: ICDAS-II  $\leq$ 10 and ICDAS-II >10 representing children with low and high caries prevalence, respectively. Wean ( $\pm$ SD) toenail F concentrations in children (n=23) presenting ICDAS-II  $\leq$ 10 (1.85 $\pm$ 1.32  $\mu$ g/g) were higher than levels found in children (n=31) having ICDAS-II >10 (1.58 $\pm$ 0.78  $\mu$ g/g). The difference, however, was not significant (non-paired t test, p=0.38). The corresponding 95% CIs were 1.28–2.42 and 1.29–1.87  $\mu$ g/g, respectively. The Table shows the data dichotomized according to toenail F concentrations ( $\leq$ 2 or >2  $\mu$ g/g) and ICDAS-II scores ( $\leq$ 10 or >10). The sensitivity and specificity of toenail F concentrations in identifying

children with ICDAS-II  $\leq$ 10 was 0.22 (95% CI 0.07–0.44) and 0.77 (0.59–0.90), respectively. The positive and negative predictive values were 0.42 and 0.57, respectively.

**Table**. Number of children according to dichotomized values for toenail F concentrations and ICDAS-II scores

Toenail [F]s	ICDAS-II	
	 ≤ 10	>10
≤ 2 μg/g > 2μg/g	5	7
> 2µg/g	18	24
Total	23	31

### DISCUSSION

Since nails have been used as biomarkers of exposure to F, <sup>16-18,20,25-29</sup> and a longitudinal study revealed that children with high caries experience have generally slightly less intakes of F, <sup>21</sup> the present study was designed to examine the relationship between toenail F concentrations and prevalence of caries.

In the study by Braga et al.<sup>24</sup> it was reported that a mean DMFT of 1.9 would be correspond to an ICDAS-II score of 7.0 in Brazilian schoolchildren. Considering that the mean DMFT at 12 years recently reported for Brazil was 2.1,<sup>14</sup> it was decided to adopt a cut-off point at ICDAS-II score of 10 as indicative of low or high caries experience. However, determining the caries prevalence of the children was not the aim of the present study. Our goal was to examine the relationship between toenail F concentrations and prevalence of caries assessed using ICDAS-II criteria.

Toenails were chosen instead of fingernails because they provide enough mass to be analyzed and also are usually less prone to external contamination. <sup>16,20</sup> Our thought was that the mean toenail F concentrations might be higher in children presenting ICDAS-II≤10 compared with those having ICDAS-II>10. This would be indicative of higher F intake in the former group, which could be expected to occur in cases of lower caries experience. <sup>21</sup> However, the difference did not reach statistical significance. This could be attributed mainly to the fact that although lower F intakes have been associated with higher caries experience, it is widely recognized that the mechanism of action of F for caries control occurs essentially through its topical contact with the teeth. <sup>30</sup> In other words, it is not necessary to ingest F to have F protection against caries. However, nail F concentrations represent the amount of F that was ingested along a period of time. <sup>19</sup>

In addition, when attempting to correlate toenail F concentration with ICDAS-II scores, we decided to establish a cut-off point for toenail F concentrations around 2  $\mu$ g/g. This was done because it was recently reported that a significantly higher risk of developing fluorosis is observed when fingernail F concentrations are higher than 2  $\mu$ g/g.<sup>31</sup> When this was done, no significant association between the

dichotomized variables could be seen. Moreover, specificity, sensitivity, and predictive values were very low.

In conclusion, in a population of Brazilian children who had a high caries experience when the ICDAS-II index was employed, it was observed that the children with low caries prevalence tended to have only slightly, nonsignificantly higher toenail F concentrations. Thus, the validity of this biomarker as a diagnostic tool for caries prevalence is low, probably due the fact that, among various possible reasons, the mechanism of action of F on caries control occurs essentially though its local contact with the teeth.

#### **REFERENCES**

- 1 Ismail AI. Visual and visuo-tactile detection of dental caries. J Dent Res. 2004;83 Spec No C:C56-66
- 2 Pitts NB, Fyffe HE. The effect of varying diagnostic thresholds upon clinical caries data for a low prevalence group. J Dent Res. 1988 Mar;67(3):592-6.
- 3 Ismail AI, Brodeur JM, Gagnon P, Payette M, Picard D, Hamalian T, et al. Prevalence of non-cavitated and cavitated carious lesions in a random sample of 7-9-year-old schoolchildren in Montreal, Quebec. Community Dent Oral Epidemiol. 1992 Oct;20(5):250-5.
- 4 Ekstrand KR, Ricketts DN, Kidd EA. Reproducibility and accuracy of three methods for assessment of demineralization depth of the occlusal surface: an *in vitro* examination. Caries Res. 1997;31(3):224-31.
- 5 Fyffe HE, Deery C, Nugent ZJ, Nuttall NM, Pitts NB. Effect of diagnostic threshold on the validity and reliability of epidemiological caries diagnosis using the Dundee Selectable Threshold Method for caries diagnosis (DSTM). Community Dent Oral Epidemiol. 2000 Feb;28(1):42-51.
- 6 Ismail AI, Gagnon P. A longitudinal evaluation of fissure sealants applied in dental practices. J Dent Res. 1995 Sept;74(9):1583-90.
- 7 Ricketts DN, Ekstrand KR, Kidd EA, Larsen T. Relating visual and radiographic ranked scoring systems for occlusal caries detection to histological and microbiological evidence. Oper Dent. 2002 May-Jun;27(3):231-7.
- 8 Chesters RK, Pitts NB, Matuliene G, Kvedariene A, Huntington E, Bendinskaite R, et al. An abbreviated caries clinical trial design validated over 24 months. J Dent Res. 2002 Sep;81(9):637-40.
- 9 Nyvad B, Machiulskiene V, Baelum V. Construct and predictive validity of clinical caries diagnostic criteria assessing lesion activity. J Dent Res. 2003 Feb;82(2):117-22.
- 10 WHO: Oral health surveys: basic methods. 4th ed. Geneva: WHO; 1997.
- 11 Pitts N. "ICDAS"—an international system for caries detection and assessment being developed to facilitate caries epidemiology, research and appropriate clinical management. Community Dent Health. 2004 Sep;21(3):193-8.
- 12 Assaf AV, de Castro Meneghim M, Zanin L, Tengan C, Pereira AC. Effect of different diagnostic thresholds on dental caries calibration—a 12 month evaluation. Community Dent Oral Epidemiol. 2006 Jun;34(3):213-9.
- 13 Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, Hasson H, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. Community Dent Oral Epidemiol. 2007 Jun;35(3):170-8.
- 14 Brazil. Ministry of Health. SB Brasil 2010. Available from: http://dab.saude.gov.br/cnsb/sbbrasil/resultados.htm. [in Portuguese].
- 15 Clarkson, J. International collaborative research on fluoride. J Dent Res. 2000 Apr;79(4):893-904. [No authors listed in the PubMed listing of the reference].
- 16 Fukushima R, Rigolizzo DS, Maia LP, Sampaio FC, Lauris JR, Buzalaf MA. Environmental and individual factors associated with nail fluoride concentration. Caries Res. 2009;43(2):147-54.

- 17 Whitford GM, Sampaio FC, Arneberg P, von der Fehr FR. Fingernail fluoride: a method for monitoring fluoride exposure. Caries Res. 1999 Nov-Dec;33(6):462-7.
- 18 Corrêa Rodrigues MH, de Magalhães Bastos JR, Rabelo Buzalaf MA. Fingernails and toenails as biomarkers of subchronic exposure to fluoride from dentifrice in 2- to 3-year-old children. Caries Res. 2004 Mar-Apr;38(2):109-14.
- 19 Clarkson J, Watt RG, Rugg-Gunn AJ, Pitiphat W, Ettinger RL, Horowitz AM, et al. Proceedings: 9th World Congress on Preventive Dentistry (WCPD): "Community Participation and Global Alliances for Lifelong Oral Health for All," Phuket, Thailand, September 7-10, 2009. Adv Dent Res. 2010 Jun;22(1):2-30.
- 20 Buzalaf MA, Pessan JP, Alves KM. Influence of growth rate and length on fluoride detection in human nails. Caries Res. 2006;40(3):231-8.
- 21 Warren JJ, Levy SM, Broffitt B, Cavanaugh JE, Kanellis MJ, Weber-Gasparoni K. Considerations on optimal fluoride intake using dental fluorosis and dental caries outcomes—a longitudinal study. J Public Health Dent. 2009 Spring;69(2):111-5.
- 22 Taves DR. Determination of submicromolar concentrations of fluoride in biological samples. Talanta. 1968 Oct;15(10):1015-23.
- 23 Whitford GM. Some characteristics of fluoride analysis with the electrode. In: Myers HM, editor. The metabolism and toxicity of fluoride, Monographs in oral science, volume 16. Basel, Switzerland: S Karger AG; 1996. p. 303-33
- 24 Braga MM, Oliveira LB, Bonini GA, Bönecker M, Mendes FM. Feasibility of the International Caries Detection and Assessment System (ICDAS-II) in epidemiological surveys and comparability with standard World Health Organization criteria. Caries Res. 2009;43(4):245-9
- 25 Schamschula RG, Sugár E, Un PS, Tóth K, Barmes DE, Adkins BL. Physiological indicators of fluoride exposure and utilization: an epidemiological study. Community Dent Oral Epidemiol. 1985 Apr;13(2):104-7
- 26 Machoy Z. Effects of environment upon fluoride content in nails of children. Fluoride 1989 (22):169-73
- 27 Czarnowski W, Krechniak J. Fluoride in the urine, hair, and nails of phosphate fertilizer workers. Br J Ind Med. 1990 May;47(5):349-51.
- 28 Schmidt CW, Leuschke W. Fluoride content in finger nails of individuals with and without chronic fluoride exposure. Fluoride 1990;23(2):79-82
- 29 Czarnowski W, Stolarska K, Brzezinska B, Krechniak J. Fluoride in urine, hair and nails of phosphate fertilizer workers. Fluoride. 1996 (29):163-165.
- 30 ten Cate JM. Current concepts on the theories of the mechanism of action of fluoride. Acta Odontol Scand. 1999 Dec;57(6):325-9.
- 31. Buzalaf MAR, Rodrigues MHC, Almeida BS, Levy FM, Fukushima R, Pessan JP, et al. Validation of fingernails fluoride concentrations as biomarker of dental fluorosis in children [abstract 1 in session 1, Fluoride, at the 58th Annual ORCA Congress; 2011 July 6-9; Kaunas, Lihtuania]. Caries Res. 2011;45(2):174-242. Available from: http:// content.karger.com/ProdukteDB/produkte.asp?doi=10.1159/000328514 and http:// content.karger.com/ProdukteDB/ produkte.asp?Aktion=ShowPDF&ArtikelNr=328514&Ausgabe=254989&ProduktNr=22421 9&filename=328514.pdf