Radhi et al. Scr Med. 2026 Mar-Apr;57(2):287-93. DOI:10.5937/scriptamed57-63279

ORIGINAL ARTICLE

The Effect of Gingival Inflammation on Salivary Cytochrome P450 Enzymes in Children: A Case-Control Study

Nada Jafer Radhi,[1] Heba N Yassin,[1] Ban Sahib Diab[1]

Abstract

Background/Aim: Gingival inflammation in children involves characteristic changes of the salivary composition that reflect not only weakened local defence factors but also increased defensive reflexes. This study aimed to compare the salivary parameters cytochrome concentration, density and flow rate between children with gingival inflammation and clinically healthy gingiva.

Methods: A total of 86 children were included and divided into two groups: 41 with gingival inflammation and 45 with healthy gingiva, which was gender matched for age. Unstimulated saliva was collected and cytochrome concentration, salivary density and flow rate were measured.

Results: Participants with gingival inflammation had significantly lower mean salivary cytochrome concentration and density but showed increased salivary flow rate compared to healthy controls. These differences were found in both sexes, being more marked in boys with respect to the age increase of flow rate. Correlation studies showed a strong negative correlation between salivary density and flow rate in both groups. These results indicate that there is a unique biochemical signature for gingival inflammation in children, which includes lower cytochrome and density, accompanied by an increase in flow rate.

Conclusion: In children, gingival inflammation was associated with reduced salivary CYP450 and density and increased flow rate in saliva, suggesting a unique biochemistry of oral disease.

Key words: Gingivitis; Cytochrome P450 enzyme system; Child; Case-control studies; Clinical enzyme tests; Oral health; Paediatrics.

1. Department of Pedodontics and Preventive Dentistry, University of Baghdad, College of Dentistry, Baghdad, Iraq.

Citation:
Radhi NJ, Yassin HN, Diab BS. The effect of gingival inflammation on salivary cytochrome P450 enzymes in children: a case-control study. Scr Med. 2026 Mar-Apr;57(2):287-93.

Corresponding author:
HEBA N YASSIN
E: hebaalkubaisy@codental.uobaghdad.edu.iq

Received: 6 December 2025
Revision received: 22 March 2026
Accepted: 29 March 2026

Introduction

Cytochrome P450 enzymes are critical in the synthesis of cholesterol, prostacyclin, steroids and thromboxane A2. They are also important in xenobiotic metabolism and drug metabolism. Although CYP450 enzymes are predominantly present in the liver, they are also present throughout the small intestines-where they reduce drug bioavailability well as in other organs of the body, including the lung, placenta, kidney and salivary glands.[1]

The name "cytochrome P450" stems from the fact that these haemoproteins are bound to the cellular membrane ("cyto") and they have gen-

eral absorption peaks at 450 nm and 490 nm (due to pyridine heme complexes) when reduced and liganded to carbon monoxide. More than 50 CYP450s have been described.[2, 3] They are a metabolically diverse group of enzymes, the activity of which can differ considerably between individuals because of genetic polymorphism, exposure to environmental insults and comorbidities.[4, 5]

Inflammation is a potent modulator of CYP450 enzymatic activity. The balance between proinflammatory and immunosuppressive properties can regulate the expression of CYP due to the release of cytokines during inflammatory responses, which could contribute to variability in drug response/toxicity.[6] However, prospective assessment of drug metabolism during inflammation is still a dilemma as it depends on the nature of the disease and its stage of progress well as CYP isoforms involved.[7] The effect of inflammation on CYP activity is typically an inhibition, although some isoforms are known to be induced or not to be affected.[8-10] Animal studies and primary hepatocyte models have shown that CYP expression is markedly changed by trauma, infection and exposure to cytokines.[9, 11, 12] The amplitude of such modulation varies according to the severity and nature of the inflammatory reaction and the type of mediators being considered. Resolution of inflammation frequently has the effect of returning CYP activity towards baseline.[6]

Salivary cytokines have been considered as biomarkers for oral diseases, including caries, gingivitis and periodontitis.[13, 14] Human saliva is a complex solution containing proteins, such as cytokines, especially suitable for non-invasive diagnostics in paediatric patients. Age and oral health status are related to salivary cytokine levels. Since CYP enzymes are expressed in salivary glands, even trace amounts of xenobiotics or their metabolites in saliva may influence the oral mucosa. This exposure, via systemic circulation as well as local salivary secretion, may result in oral mucosal diseases.[1] Furthermore, studies have shown that gene expression related to xenobiotic detoxification is downregulated in obese models.[15]

To date, no studies have been conducted in Iraq examining the relationship between oral health status and salivary CYP450 enzyme activity in children. Therefore, this study aimed to explore this important and understudied area. This study aimed to evaluate salivary cytochrome P450 amounts, salivary density and salivary flow rate in children aged 6–12 years with gingival inflammation and to compare these parameters with those observed in children with healthy gingiva.

Methods

Study design

A case-control study was conducted to examine the relationship between gingival inflammation and salivary cytochrome P450 enzyme levels in children. A total of 86 children, ranging in ages from 6 to 12 years, were recruited for this study and subsequently allocated to one of two groups:

- The study group consisted of 41 children diagnosed with gingival inflammation based on WHO criteria (2013).[16]
- The control group included 45 children with clinically healthy gums, matched to the study group by age, gender and weight.

Exclusion criteria: Children were not included if they had systemic diseases (such as diabetes, hepatic or renal impairment or autoimmune disorders) or oral soft tissue disorders (including active mucosal lesions), used dental prostheses (fixed or removable), or orthodontic devices, had taken any medications in the previous month, or had consumed caffeinated drinks within the last 48 hours, to ensure a baseline metabolic state, as caffeine is a known substrate and modifier of CYP enzyme activity.

Clinical assessment: All oral examinations were performed in standardised situations. Gingival bleeding was assessed using the WHO criteria (2013).

Sample collection

Participants were instructed not to eat or drink (except for water) for at least 1 h prior to sample collection. Fresh saliva was obtained by the spitting technique as described by Khurshid et al.[17] Children were instructed to stay quiet, reduce physical activity and let saliva gather in their mouth before expectorating into a sterile disposable cap every 30 s. The samples were then dipped in ice and stored for further analysis.

Salivary flow rate was determined by collecting unstimulated saliva over a timed period of 3 min-

utes. The total volume of saliva produced was measured and divided by the collection time to calculate the flow rate (mL/min).[18] Salivary density was measured using a calibrated densitometer, following standard laboratory procedures to assess the concentration of solutes in the saliva sample.[19]

Biochemical analysis

Salivary cytochrome P450 2E1 (CYP2E1) amounts were quantified utilising a sandwich ELISA kit (*YLBIONT*, China), according to the manufacturer's instructions. Briefly, samples and standards were added to wells pre-coated with CYP2E1 monoclonal antibody and incubated at 37 °C for 60 minutes. After washing, biotin-labelled anti-CYP2E1 antibodies and streptavidin-HRP were added, followed by chromogen solutions for colour development. The reaction was ended and absorbance was measured at 450 nm using a microplate reader. CYP2E1 concentrations were calculated from a standard curve generated with known concentrations. All methods and reagent handling were completed according to the manufacturer's instructions.

Statistical analysis

The statistical analysis was directed using the Statistical Package for the Social Sciences (SPSS version 22; Chicago, Illinois, USA). Descriptive statistics include frequency and percentage for qualitative variables, mean and standard deviation for quantitative variables. Inferential statistics involve an independent sample t-test. The significance level was established at $p \leq 0.05$.

Results

Sample of the present study includes 41 children (study group) with gingival inflammation compared with the control group that includes 45 children with healthy gingiva; however, the results showed no significant association between gender or age with the study and control groups. Table 1 presents the mean values and statistical comparisons of salivary cytochrome concentration, density and flow rate between children with gingival inflammation (study group) and those with clinically healthy gingiva (control group). A statistically significant difference was found between the two groups for all parameters.

The mean salivary cytochrome concentration was significantly lower in the study group compared with the control group. Also, salivary density was significantly reduced in the study group relative to the control group. Conversely, salivary flow rate was significantly higher in children with gingival inflammation than in those with clinically healthy gingiva.

Table 2 shows that in boys and girls, the mean cytochrome concentration was significantly lower in the study group compared with the control group. For salivary density, the same table illustrates a significant reduction in both boys and girls in the study group compared with the control group. However, other results found concerning salivary flow rate as it was boys in the study group who demonstrated a higher mean flow rate than those in the control group, while the difference in girls did not reach statistical significance. No significant differences were found between boys and girls within the same group for all salivary variables, indicating that the effect of gingival condition on salivary parameters was generally independent of gender.

Correlation analyses (Table 3) explored the relationships between cytochrome concentration, salivary density and flow rate within each group. In the study group, cytochrome concentration showed no significant correlation with either

Table 1: Salivary variables in children in the study and control group

Salivary variables	Study group Mean ± SD	Control group Mean ± SD	T-test between groups	
			t-value	p-value
Cytochrome concentration (ng/mL)	10.20 ± 1.69	12.74 ± 4.52	-3.387	0.001
Density (mg/mL)	0.35 ± 0.20	0.83 ± 0.58	-4.954	< 0.001
Flow rate (mL/min)	0.39 ± 0.15	0.27 ± 0.13	3.720	< 0.001

Table 2: Salivary variables in children by gender in study and control group

Salivary variables	Gender	Study group Mean ± SD	Control group Mean ± SD	T-test between groups	
				t-value	p-value
Cytochrome concentration (ng/mL)	Boys	10.13 ± 1.78	12.12 ± 4.18	-2.275	0.027
	Girls	10.33 ± 1.56	13.68 ± 4.97	-2.416	0.022
t-test between genders		t = -0.365, p = 0.710	t = -1.138, p = 0.261		
Density (mg/mL)	Boys	0.37 ± 0.21	0.84 ± 0.53	-4.222	< 0.001
	Girls	0.32 ± 0.20	0.82 ± 0.67	-2.710	0.011
t-test between genders		t = 0.828, p = 0.413	t = 0.081, p = 0.936		

Table 3: Correlation of salivary variables in both study and control group

Salivary variables	Group	Cytochrome concentration (ng/mL)		Density (mg/mL)		Flow rate (mL/min)	
		r	p-value	r	p-value	r	p-value
Cytochrome concentration (ng/mL)	Study	----	----	-0.073	0.648	-0.125	0.437
	Control	----	----	-0.047	0.760	-0.147	0.335
Density (mg/mL)	Study	-0.073	0.648	----	----	-0.421 *	0.006
	Control	-0.047	0.760	----	----	-0.470 *	0.001

**Significant p ≤ 0.05*

salivary density. However, a significant negative correlation was found linking salivary density and flow rate. The same result was found in the control group, but a significant negative correlation was observed between density and flow rate.

Discussion

In children with healthy and inflamed gingiva, salivary cytochrome concentration, density and flow rate were measured in the study. The findings evidenced group-specific modifications in all salivary parameters were analysed, revealing a local biochemical response to gingival disease in child patients.

There were no significant demographic differences nor a significant age or sex bias between the study groups, which increases the reliability of presented findings. That is, the variations found in salivary parameters seem to be a result of the deployment in its pro-inflammatory condition rather than of interpersonal differences, corroborating other epidemiological findings that associate oral inflammation more with hygiene status and less with age or sex.[20, 21]

Salivary flow rate was determined based on the Navazesh and Kumar criteria[18] because this procedure has been employed in Iraqi studies in the past.[22-24] A marked decrease in mean salivary cytochrome concentration and density was detected in children with inflamed gingiva. Especially cytochromes, involved in the defence against cellular metabolism, might be depleted because of increased consumption or impaired production during inflammation. This is in line with previous research indicating lower protective salivary factors under pathological conditions such as periodontal disease[25] and contrary to studies describing higher levels of inflammatory biomarkers in these cases.[26] The discrepancy between cytochromes (those that are consumed) and immune cell markers (those that are released) may be due to the different biological roles they play, underlining the importance of more accurate biochemical assays in future studies. The reduction in salivary dilution is reinforced by the diminished density of saliva.

The highest recorded salivary flow rate was in children with gingivitis. This hyper-salivation is probably a reflex protective measure to irritation and inflammation, facilitating elimination of bacteria and inflammatory factors from the oral cavity.[27-29] This response was greater in boys, implying sex differences in the parasympathet-

ic reflex arc. Although little is known regarding gender-related differences in salivary flow in childhood, there is some suggestion that girls have a greater threshold of challenging stimulus to produce an equal response,[30] which requires further attention as far as neurologic and hormone influences are concerned on oral defence mechanisms.

The results of the correlation analysis showed that there are strong negative relationships between the salivary density and flow rate in both groups, indicating the diluting effect was pervasive: higher flow resulted in lower density.[31] Cytochrome concentration did not have a significant association with density in the gingivitis group, but rather it appears to fall due to localised inflammatory consumption (ie, not just being diluted).[32] This suggests that the reduction of cytochrome in oral inflammation may have a multifactorial and more complex aetiology.

In the young child, an association between gingival inflammation and a specific pattern of cytochrome and density decrease and flow rate increase can be summarised. The sex differences in flow rate and the fact that the reduction of cytochrome was independent of dilution point to complexity in underlying mechanisms. The cytochrome subtype identification further works in clinical application, validating gender divergences on salivary response and re-evaluating the dilution effect of the oral defence components will be intriguing to clarify.

Conclusion

This investigation describes a unique biochemical profile of children's gingival inflammation, which manifests itself with decreased salivary cytochrome concentration and density; however, is associated with its increased flow rate. The consistent alterations in these parameters indicate that local immune defences (decreased cytochrome and density) as well as neurological protective reflexes (increased flow rate) are actively and simultaneously taking place in gingivitis. Additional studies are warranted to identify the specific cytochrome types involved and replicate these findings in wider and more diverse paediatric populations.

Ethics

The Scientific Committee of the Pedodontics and Preventive Dentistry Department and the Central Ethical Committee of the College of Dentistry at the University of Baghdad, Iraq, gave approval for this study (project No 1118325, dated 27 November 2025.

Acknowledgement

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

Author ORCID numbers

Nada Radhi (NR):
0000-0002-8186-5553
Heba N Yassin (HNY):
0000-0002-5928-9908
Ban Sahib Diab (BSD):
0000-0002-9310-9210

Author contributions

Conceptualisation: NR, BSD, HNY
Methodology: NR, HNY, BSD
Software: NR, HNY, BSD
Validation: NR, HNY, BSD
Formal analysis: NR, BSD
Investigation: NR, HNY, BSD
Resources: NR, BSD
Data curation: NR, HNY
Writing - original draft: HNY, BSD
Writing – review and editing: NR, HNY, BSD
Supervision: NR
Project administration: NR, HNY, BSD
Funding acquisition: NR, HNY, BSD.

References

1. Kragelund C, Hansen C, Torpet LA, Nauntofte B, Brøsen K, Pedersen AML, Buchwald C, Therkildsen MH, Reibel J. Expression of two drug metabolizing cytochrome P450 enzymes in human salivary glands. Oral Dis. 2008;14(6):533–40. doi: 10.1111/j.1601-0825.2007.01415.x.
2. Slaughter RL, Edwards DJ. Recent advances: the cytochrome P450 enzymes. Ann Pharmacother. 1995;29(6):619–24. doi: 10.1177/106002809502900612.
3. Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med. 2005;352(21):2211–21. doi: 10.1056/NEJMra032424.
4. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician. 2007 Aug 1;76(3):391–6. PMID:17708140.
5. Zhou Y, Ingelman Sundberg M, Lauschke VM. Worldwide distribution of cytochrome P450 alleles: a meta analysis of population scale sequencing projects. Clin Pharmacol Ther. 2017 Oct;102(4):688–700. doi: 10.1002/cpt.690.
6. Lenoir C, Rollason V, Desmeules JA, Samer CF. Influence of inflammation on cytochromes P450 activity in adults: a systematic review of the literature. Front Pharmacol. 2021 Nov 16;12:733935. doi: 10.3389/fphar.2021.733935.
7. Christmas P. Role of cytochrome P450s in inflammation. Adv Pharmacol. 2015;74:163–92. doi: 10.1016/bs.apha.2015.03.005.
8. Morgan ET. Regulation of cytochrome P450 by inflammatory mediators: why and how? Drug Metab Dispos. 2001 Mar;29(3):207–12. Erratum in: Drug Metab Dispos. 2001 Jun;29(6):932. PMID:11181485.
9. de Jong LM, Jiskoot W, Swen JJ, Manson ML. Distinct effects of inflammation on cytochrome P450 regulation and drug metabolism: lessons from experimental models and a potential role for pharmacogenetics. Genes (Basel). 2020 Dec 16;11(12):1509. doi: 10.3390/genes11121509.
10. Stanke Labesque F, Concordet D, Djerada Z, Bouchet S, Solas C, Mériglier E, et al. Neglecting plasma protein binding in COVID 19 patients leads to a wrong interpretation of lopinavir overexposure. Clin Pharmacol Ther. 2021 Apr;109(4):1030–3. doi: 10.1002/cpt.2196.
11. Aitken AE, Morgan ET. Gene specific effects of inflammatory cytokines on cytochrome P450 2C, 2B6 and 3A4 mRNA levels in human hepatocytes. Drug Metab Dispos. 2007 Sep;35(9):1687–93. doi: 10.1124/dmd.107.015511.
12. Stavropoulou E, Pircalabioru GG, Bezirtzoglou E. The role of cytochromes P450 in infection. Front Immunol. 2018;9:89. doi: 10.3389/fimmu.2018.00089.
13. Dikova VR, Principe S, Bagan JV. Salivary inflammatory proteins in patients with oral potentially malignant disorders. J Clin Exp Dent. 2019 Jul;11(7):e659–64. doi: 10.4317/jced.55917.
14. Diesch T, Filippi C, Fritschi N, Filippi A, Ritz N. Cytokines in saliva as biomarkers of oral and systemic oncological or infectious diseases: a systematic review. Cytokine. 2021 Jul;143:155506. doi: 10.1016/j.cyto.2021.155506.
15. Tomofuji T, Ekuni D, Irie K, Azuma T, Endo Y, Tamaki N, et al. Preventive effects of a cocoa enriched diet on gingival oxidative stress in experimental periodontitis. J Periodontol. 2009 Nov;80(11):1799–808. doi: 10.1902/jop.2009.090270.
16. World Health Organization. Oral Health Surveys: Basic Methods. 5th ed. Geneva: WHO; 2013.
17. Khurshid Z, Zohaib S, Najeeb S, Zafar MS, Slowey PD, Almas K. Human saliva collection devices for proteomics: an update. Int J Mol Sci. 2016 Jun 6;17(6):846. doi: 10.3390/ijms17060846.
18. Navazesh M, Kumar SK; University of Southern California School of Dentistry. Measuring salivary flow: challenges and opportunities. J Am Dent Assoc. 2008 May;139(Suppl):35S–40S. doi: 10.14219/jada.archive.2008.0353.
19. Humphrey SP, Williamson RT. A review of saliva: normal composition, flow, and function. J Prosthet Dent. 2001 Feb;85(2):162–9. doi: 10.1067/mpr.2001.113778.
20. Davidovich E, Ccahuana Vasquez RA, Grender J, Timm H, Gonen H, Zini A. A 4 week randomized controlled trial evaluating plaque and gingivitis effects of an electric toothbrush in a paediatric population. Int J Paediatr Dent. 2024;34(3):246–55. doi: 10.1111/ipd.13130.
21. Sanchis Sanchis A, Grau MD, Moliner AR, Morales Murillo CP. Effects of age and gender in emotion regulation of children and adolescents. Front Psychol. 2020;11:946. doi: 10.3389/fpsyg.2020.00946.
22. Ajeel N, Diab B. Dental caries as a health risk for visual acuity in relation to salivary transforming growth factor beta 1 among students aged 8–10 years: a cross sectional study. J Int Soc Prevent Community Dent. 2024;14(2):144–51. doi: 10.4103/jispcd.jispcd_205_23.
23. Ahmed AA, Diab BS, Razeghi S. Sleep disorders and dental caries related to salivary alpha amylase among dental students. J Baghdad Coll Dent. 2024;36(2). doi: 10.26477/jbcd.v36i2.3679.
24. Taher AK, Radhi NJMH. Salivary irisin in relation to recurrent aphthous ulcer and weight status in Diyala City, Iraq. J Baghdad Coll Dent. 2024;36(1):19–26. doi: 10.26477/jbcd.v36i1.3587.
25. Skutnik Radziszewska A, Zalewska A. Salivary redox biomarkers in the course of caries and periodontal disease. Appl Sci. 2020;10(18):6240. doi: 10.3390/app10186240.
26. Eberhard J, Grote K, Luchtefeld M, Heuer W, Schuett H, Divchev D, et al. Experimental gingivitis induces systemic inflammatory markers in young healthy individuals: a single subject interventional study. PLoS One. 2013;8(2):e55265. doi: 10.1371/journal.pone.0055265.

27. Sreebny LM. Saliva in health and disease: an appraisal and update. Int Dent J. 2000;50(3):160–71. doi: 10.1111/j.1875-595X.2000.tb00554.x.
28. Haug SR, Birkeland JM, Olsen I. Acute dental pain and salivary biomarkers for stress and inflammation in patients with pulpal or periapical inflammation. J Oral Facial Pain Headache. 2023;33(2):227–34. doi: 10.3290/j.ofph_33_2_227.
29. Proctor GB. Disease induced changes in salivary gland function and the composition of saliva. J Dent Res. 2021 Nov;100(11):1201–9. doi: 10.1177/00220345211004842.
30. Inoue H, Ono K, Masuda W, Morimoto Y, Tanaka T, Yokota M, et al. Gender differences in unstimulated whole saliva flow rate and salivary gland sizes. Arch Oral Biol. 2006;51(12):1055–60. doi: 10.1016/j.archoralbio.2006.06.010.
31. Gotouda H, Sasai H, Taguchi C, Wang J, Arikawa K, Kuyama K, et al. Study of salivary volumes by the modified ion dilution method. Int J Oral Med Sci. 2004;3(3):121–7. doi: 10.5466/ijoms.3.121.
32. Tóthová L, Kamodyová N, Červenka T, Celec P. Salivary markers of oxidative stress in oral diseases. Front Cell Infect Microbiol. 2015 Oct 20;5:73. doi: 10.3389/fcimb.2015.00073.