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# Oral Pathology: Prevention and Treatment in Pediatric Dentistry

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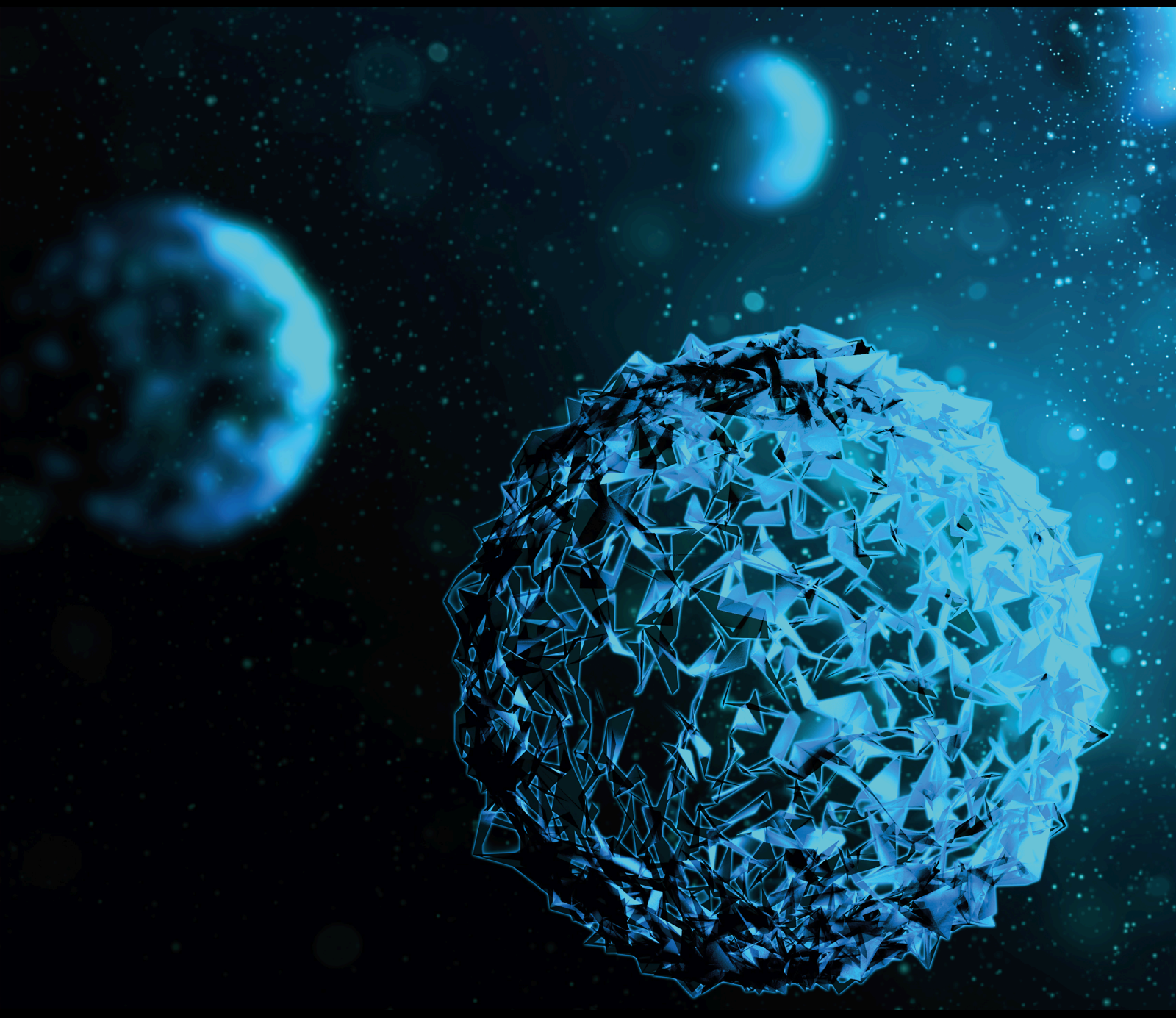

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*Research Article*

# Comparison between the Radiographic and Clinical Rates of Success for TheraCal and MTA in Primary Tooth Pulpotomy within a 12-Month Follow-Up: A Split-Mouth Clinical Trial

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*Background*. The present study was conducted for contrasting the efficacy of TheraCal and MTA for primary molar pulpotomy. *Methods*. During the current split-mouth randomized clinical trial, 90 bilateral primary molars from 45 healthy 5- to 8-year-old children were pulpotomized using TheraCal in one bilateral tooth and MTA in the other, randomly. Glass ionomer (GI) was used to cover these materials. Then, the treated teeth were restored with stainless steel crowns (SSC) and followed up clinically and radiographically at months 6 and 12 after treatment for any pulpotomy failure indications. Finally, data were analyzed by chi-square test considering $p$ value $< 0.05$ as statistically significant. *Results*. Among 82 teeth available at the final follow-up session, the total success rates were 98.1% and 99.3% for TheraCal and MTA, respectively, showing no significant difference between the two groups ($p > 0.05$). *Conclusion*. TheraCal can be used as an alternative material for pulpotomy of primary teeth instead of MTA.

## 1. Introduction

Pulpotomy is a clinical procedure widely applied for treating exposed pulps due to caries. After pulpectomy treatment, the radicular pulp should be kept vital, uninfected, and enclosed in the dentin chamber using a dressing material [1].

In the recent decades, MTA has been the gold standard material for pulp capping of primary molars after pulpotomy [2]. MTA has also antimicrobial properties and a dentinogenic effect on the pulp tissue by inducing the release of cytokines from the bone cells that stimulate hard tissue formation [3–5]. In addition, MTA preserves the integrity of pulp tissue with no cytotoxic effect [6]. However, the clinical application of MTA has got limited by some properties such as its long-time setting [6, 7], difficult handling [8], being washed out [9], discoloration, and its high price [5, 10].

Recently, a newer resin-based material has been introduced for endodontic treatments: TheraCal. This light-cured Portland cement-based resin consists of tricalcium silicate particles in a hydrophilic monomer that imitates the hydroxyapatite structure. Also, releasing calcium ions by TheraCal stimulates secondary dentin formation, as well as cell growth and proliferation [11]. Compared to MTA and other traditional materials based on calcium hydroxides ($Ca(OH)_2$), TheraCal is less soluble, and its handling is easier, can be dispensed from a syringe, and releases

higher calcium ions [12]. Handling TheraCal requires no mixing, clicking, or triturating. Then, it can be precisely placed and immediately set in the pulp chamber [11, 13].

Overall, the findings on using TheraCal compared to other standard biomaterials including MTA have been promising, and it is a potential bioinductive agent that can be easily applied especially in pediatric patients. Accordingly, this study is aimed at evaluating and contrasting the success rates of TheraCal and MTA in terms of clinical and radiographic outcomes of pulpotomies in primary molars. This study was designed as a split-mouth randomized controlled clinical trial with two follow-up sessions after 6 and 12 months.

## 2. Materials and Methods

This study was approved by the Ethics Committee of Tabriz University of Medical Sciences (approval no. IRCT2010 0125003168N7, 12 March 2019) and is compatible with the Helsinki Declaration of Human Rights. The study was carried out in Pediatric Dentistry Department, Dental Faculty, Tabriz University of Medical Sciences, starting on 23 March 2019 and continuing until 21 March 2020.

*2.1. Sampling.* The study group consisted of 45 patients aged 5-8 years old referring to the Department of Pediatric Dentistry for routine dental treatment. The inclusion criteria were (a) general physical and mental health; (b) healthy gingiva and periodontium; (c) no confounding medical history; (d) no pathologic symptoms including spontaneous pain, tooth mobility, redness, vestibulum swelling, root resorption, and draining sinus tracts; (e) no sensitivity to palpation in the vestibule; (f) no radiolucency observed in furcal and periapical regions; and (g) no pathology observed in the permanent tooth follicles.

Written informed consents were obtained and recorded from all parents/legal guardians for participating in this project and to be available during the 12-month follow-up. They were all informed about the study procedure, possible risks, and discomforts, as well as the expecting benefits.

*2.2. Sample Size.* To calculate the appropriate sample size, 20 pulpotomized primary molars were treated with TheraCal and MTA and then were followed up for three months. In terms of pain as the primary subsequence, a 12% outcome difference was observed between the two materials. Therefore, considering $\alpha = 0.05$, power = 80%, and 25% outcome difference between pulpotomized teeth using TheraCal and MTA, it was concluded that at least 39 teeth in each group were required for this study. However, it was increased to 45 to endorse the power of the study and compensate for any possible inconsistency in the follow-up program.

*2.3. Clinical Methods.* After a comprehensive dental examination, the clinical procedures were carried out in two consecutive sessions. In the initial appointment, children got familiar with the dental environment, instruments, and procedures through the standardized "tell-show-do" method. Thereafter, the children received prophylaxis followed by professional topical fluoride therapy. The next session started with a brief initial communication to establish rapport with the child (using simple statements and questions). Then, a chief postgraduate student provided all dental treatments to all included subjects under the supervision of an experienced pediatric dentist. Treatment included prophylaxis, local anesthesia (2% lidocaine with 1/80,000 epinephrine), TheraCal and MTA pulpotomy (according to the guidelines of the American Academy of Pediatric Dentistry), and SSC placement, respectively.

Since the materials used in the study needed different manipulation techniques, the operator could not be blinded to the treatment. However, all other contributors were kept blinded to the groups and procedures. Anesthesia in all groups was performed using rubber dam isolation. The pulp chamber and vital pulp exposure were obtained after the removal of caries using a #330 high-speed bur with a water spray. Subsequently, the coronal pulp tissue was removed down to the canal perforations using a sterile slow-speed round bur (#6 or #8). Then, irrigation of the pulp chamber was performed using a light water flow followed by evacuation. A sterile cotton pellet soaked in sterile saline was then placed against the stumps of the pulp at the openings of the root canals for a few minutes [14, 15]. After hemostasis, the materials were applied based on the study group as specified below. The bleeding was usually controlled by 5 minutes of pressure with moistened cotton pallet in normal saline. In the cases where the bleeding did not stop, the tooth was excluded from the study and subjected to more extensive treatment. In other subjects with normal blood clotting, sterile saline was used to rinse the pulp chamber to make sure there is no blood clot in the pulp chamber. After hemostasis establishment, the teeth were randomly treated with either TheraCal-LC® (BISCO Inc., Schaumburg, IL, USA) or white MTA (ProRoot; Dentsply, Tulsa, OK, USA). Randomization was performed with RandList software (version 1.2; DatInf GmbH, Tubingen, Germany). The symmetric tooth treatment with alternative experimental material was carried out in another session through the same preparation procedure. The whole procedure was accomplished in one session. Immediately after treatment, periapical radiographs were obtained from each treated tooth.

*2.4. MTA Group.* When hemostasis was established, MTA as a white powder (ProRoot; Dentsply, Tulsa, OK, USA) was prepared as was instructed by the manufacturers and then placed in the pulp chamber using a carrier (G. Hartzell & Son, Concord, CA, USA, #ISS52, 1.8 mm). After, it was gently squeezed with a moist cotton pellet for better adaptation to the pulp chamber. Then, it was covered with a thickness of 2 mm layer of low viscous cement type of GI (Fuji II, GC, Japan) [16]. All teeth were restored with SSC (3M, USA) [17].

*2.5. TheraCal Group.* In this group, TheraCal was placed over the pulp stump after hemostasis in one-millimeter thickness layers and underwent light curing for 20 seconds (Dentamerica, UK) [1]. Thereafter, low-viscosity GI cement was used to fill the remaining space of the pulp chamber, and the tooth restoration was performed by SSC (3M, USA) [17].

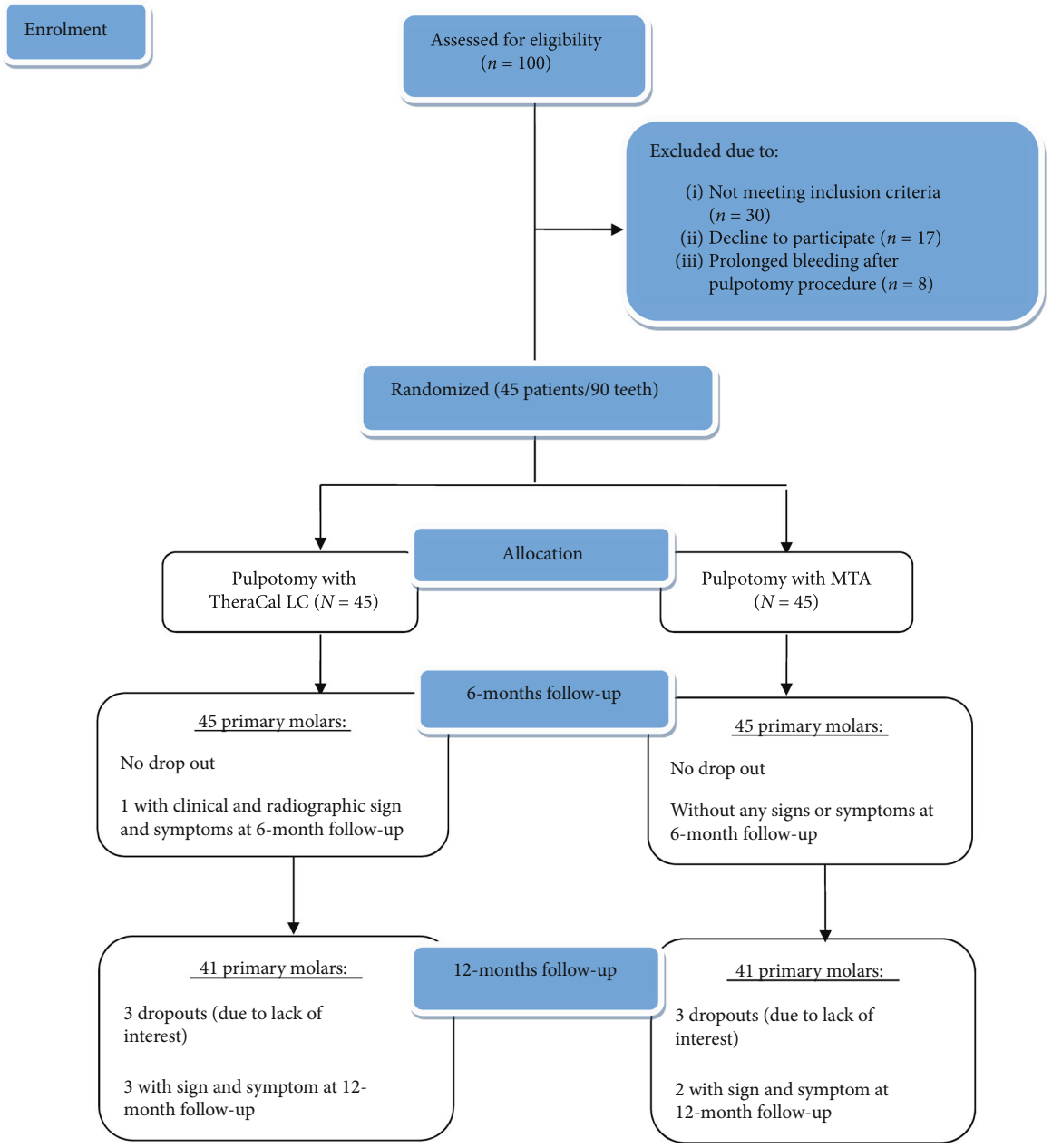



Figure 1: Flow chart of participants from baseline to 12-month follow-up.

Table 1: Characteristics of the assessed patients and teeth during the follow-ups.

| | | Follow-up 1 | | Follow-up 2 | |
|---|---|---|---|---|---|
| Age | | 5.5 ± 1.12 | | 5.8 ± 0.7 | |
| Gender | Male | 24 | 53.3% | 23 | 56.1% |
| | Female | 21 | 46.7% | 18 | 43.9% |
| Jaw type | Mandible | 44 | 48.9% | 40 | 48.8% |
| | Maxilla | 46 | 51.1% | 42 | 51.2% |
| Molar NO | First molar | 42 | 46.7% | 38 | 46.3% |
| | Second molar | 48 | 53.3% | 44 | 53.7% |

*2.6. Follow-Up Study.* Six and twelve months post treatment, patients were recalled for comprehensive radiographic and clinical examinations. Two experienced pediatric dentists were recruited for conducting each follow-up appointment who were blinded to the materials and calibrated with the study in an independent meeting before the follow-up sessions [18]. In every follow-up session, the radiographic and clinical data were evaluated for interexaminer reliability using the kappa agreement coefficient [19]. Presenting either of sinus tract, swelling, periapical lesion, spontaneous pain or long-lasting pain, tenderness to palpation and percussion, internal/external root resorption, or interradicular radiolucency was accounted as the treatment failure. However,

Table 2: Comparing the findings of 6- and 12-month follow-up sessions between the MTA and TheraCal groups.

| Evaluation criteria | After 6 months | | After 12 months | | Overall |
|---|---|---|---|---|---|
| | MTA | TheraCal | MTA | TheraCal | |
| External resorption | 0 | 0 | 0 | 0 | 0 |
| Internal resorption | 0 | 0 | 0 | 2.4 | 2.4 |
| Loss of integrity of lamina dura | 0 | 2.2 | 2.4 | 2.4 | 7 |
| Widening of PDL | 0 | 2.2 | 2.4 | 4.9 | 9.5 |
| Furcal radiolucency | 0 | 2.2 | 2.4 | 7.3 | 11.9 |
| Periapical radiolucency | 0 | 0 | 0 | 0 | 0 |
| Pain | 0 | 2.2 | 0 | 0 | 2.2 |
| Tenderness to percussion | 0 | 2.2 | 0 | 2.4 | 4.6 |
| Abscess | 0 | 2.2 | 0 | 0 | 2.2 |
| Mobility | 0 | 2.2 | 0 | 0 | 2.2 |

these symptoms were ruled out: gingival problem, food impaction, and other similar sources of pain that mimicked irreversible pulpitis. Tenderness to percussion and internal root resorption were considered radiographic and clinical criteria for evaluating the interexaminer reliability using the kappa agreement coefficient [1, 14].

*2.7. Statistical Analysis.* For comparing the qualitative data, chi-square or Fisher's exact tests were used and the Pearson correlation test was applied to compare means. All data analyses were conducted using SPSS 20 software (IBM, Chicago, IL, USA). The $p$ value $< 0.05$ was considered statistically significant.

## 3. Results

In total, 82 primary molars (40 mandibular molars and 42 maxillary molars) received pulpotomy in forty-one 5- to 8-year-old children (mean = $5.5 \pm 1.12$, 24 females and 21 males). Four patients were excluded due to missed follow-ups or missing data (Figure 1). There was no significant difference in the age and gender of children between the two study groups at the baseline and 12-month follow-up (Table 1). In Figure 1, the flow of participants from the baseline to the final follow-up session (after 12 months) and the reasons for dropouts are also illustrated.

Excellent agreement between the examiners was shown at the baseline (0.93) and follow-ups after 6 months (0.90) and 12 months (0.90) using overall Cohen's kappa coefficient. Also, no statistical difference was shown among their evaluations ($p > 0.05$).

The radiographic and clinical success rates at 6-month follow-up were shown to be 100% in the MTA group, whereas after the 12-month follow-up, the radiographic and clinical success rates were $98.8 \pm 7.7\%$ and 100%, respectively.

The radiographic and clinical success rates at 6-month follow-up in the TheraCal group were $98.9 \pm 7.4\%$ and $97.8 \pm 14.9\%$, respectively. Also, widening of PDL, furcal radiolucency, pain, tenderness, abscess, and pathologic mobility were recorded in one (2.2%) case. In the same group, after a 12-month follow-up session, internal resorption, loss of integrity lamina dura, widening of PDL, furcal radiolucency, and tenderness were, respectively, detected in 1 (2.4%), 1 (2.4%), 2 (4.9%), 3 (7.3%), and 1 (2.4%) subjects. Additionally, the radiographic and clinical success rates showed to be $97.2 \pm 11.6\%$ and $99.4 \pm 3.8\%$, respectively. Overall, the success rates of pulpotomy with TheraCal were 98.4% after 6 months and 98.1% after 12 months (Table 2).

Comparison of pulpotomy clinical success rate between the MTA and TheraCal groups using the Pearson correlation test revealed no significant differences over both 6-month ($p = 0.320$) and 12-month ($p = 0.320$) follow-up assessments. Also, comparing the pulpotomy radiographic success rates between the two study groups revealed no significant differences in either 6-month ($p = 0.320$) or 12-month ($p = 0.462$) follow-up sessions (Tables 3 and 4).

## 4. Discussion

To the extent of the authors' knowledge, few data are published on the effectiveness of TheraCal in pulpotomy of the primary molar. On the other hand, MTA is considered an ideal material for this purpose having excellent biocompatibility properties. However, its high cost, difficult handling, long setting time, and probability of discoloration have limited its usage expansion [8]. Accordingly, the present study is aimed at comparing TheraCal and MTA for primary molar pulpotomy in a split-mouth randomized controlled clinical trial model. The present findings demonstrated an excellent success rate with TheraCal similar to those reported in the literature with MTA for pulpotomy in a primary molar. The overall success rates were 99.3% and 98.1% in the MTA and TheraCal groups after the 12-month follow-up, respectively.

In the current study, MTA was applied as the control because of the numerous properties that have made it the gold standard material for pulpotomy in primary teeth. Its features include biocompatibility, bioinductivity, physical strength, antimicrobial effects, and proper sealing [2, 16, 20, 21].

According to the findings from the used evaluative methods in our study, the MTA-treated primary molars showed a 100% clinical success rate at both 6-month and

Table 3: Comparing the radiographic success rates of pulpotomy between the MTA and TheraCal groups.

| | MTA group | | TheraCal group | | $p$ value |
|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | |
| 6-month follow-up | 100 | 0 | 98.9 | 7.4 | 0.320 |
| 12-month follow-up | 98.8 | 7.7 | 97.2 | 11.6 | 0.462 |

Table 4: Comparing the clinical success rates of pulpotomy between the MTA and TheraCal groups.

| | | MTA group | | TheraCal group | | $p$ value |
|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | |
| 6-month follow-up | 100 | 0 | | 97.8 | 14.9 | 0.320 |
| 12-month follow-up | 100 | 0 | | 99.4 | 3.8 | 0.320 |

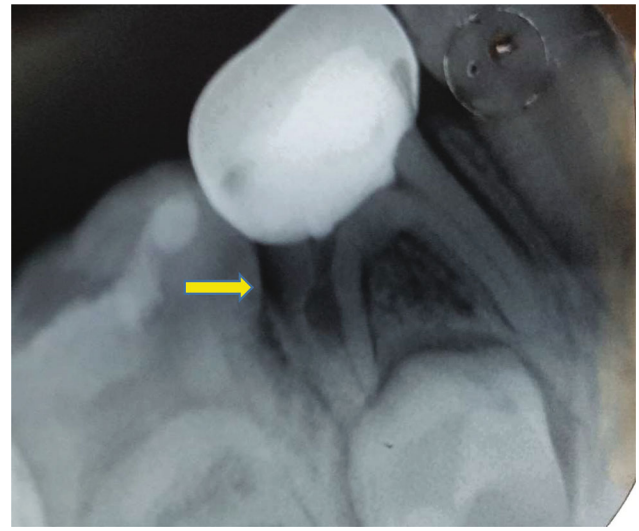


Figure 2: Internal root resorption in the tooth treated with TheraCal after 6 months.

12-month follow-up sessions. To the present findings, some other studies have mentioned similar clinical success rates for the MTA material [22, 23]. MTA's superiority over TheraCal in most clinical outcomes is probably because of its high sealing ability and biocompatibility that lead to hard tissue bridge formation and strong barrier creation against any future microbial permeation into the canals that preserves the remained pulp [24, 25]. On the other hand, the high clinical success rates of TheraCal in our study (99.4%) may be attributed to the cement's bacteriostatic properties which could be because of its high alkalinity.

In both MTA and TheraCal groups, radiographic failures were more common than clinical ones. However, MTA and TheraCal showed no periapical radiolucency. This can be explained by their appropriate coronal seal and high resistance against penetrating bacteria into the periapical areas. Parallelly, GI capped with SSC was used to reduce the microleakage along with the entire restoration interface [21].

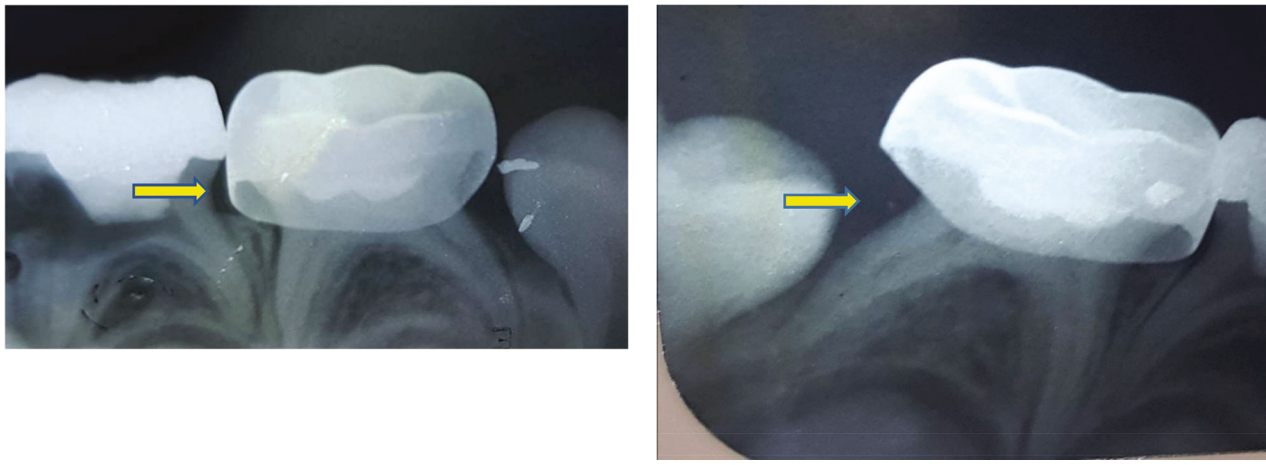
Similar to some other studies, no external or internal resorption was observed in the MTA-treated teeth [22, 26]. However, in one TheraCal-treated tooth, internal resorption was observed after 12 months and considered a failure of pulpotomy treatment (Figure 2). The precise reason underlying the internal resorption is not known; however, previous investigations suggest the association of eugenol in ZOE with a risk for subsequent internal resorption [27]. Nevertheless, this cannot explain the internal resorption in the present study because ZOE paste was not used as a base on the pulpal tissue. Another reason suggested for the internal resorption has been overstimulation of the primary pulp by the highly alkaline $Ca(OH)_2$ which could lead to the formation of odontoclasts by causing metaplasia within the pulp tissue [11].

The setting mechanism and calcium releasing properties of TheraCal are modified due to the presence of resin matrix in its formulation, for example, lower solubility and higher calcium release compared to the self-setting calcium hydroxide and ProRoot MTA [12]. Also, it has better mechanical properties due to further setting after its initial curing because of water penetration to the hydrophilic resin matrix. TheraCal was a very qualified agent and presented close rates to MTA. The frequency of hard tissue bridge formation in TheraCal was comparable with that of pure Portland cement which is higher in frequency and thickness than glass ionomer and resin-based calcium hydroxide. Interestingly, in our study, calcified bridge formation was observed in radiographies in some of the cases in both groups after 12 months (Figure 3). The concept of dentin bridge formation under pulpotomy medicaments has also remained a debated topic among the researchers who suggest it to be either a healing response or the pulp reaction to irritation [28, 29]. Therefore, dentin bridging cannot be considered a reliable response for the determination of success or failure [30].

The most important concern about biomaterials used in pulpotomy is their biocompatibility and cytotoxicity since the material contacts directly to the pulp. Free uncured resin monomers in resin-modified materials such as TheraCal may have a cytotoxic effect [31]. A reduced polymerization degree may cause elevated uncured resin monomers, which eventually may lower the cement's biocompatibility. In a study by Bakhtiar et al. [32] with a cavity depth of 2 millimeters, TheraCal showed poorer pulpotomy outcomes compared to MTA. To ensure complete curing and prevent the effects of free monomers, we reduced the thickness of the TheraCal to one millimeter. Similar to the study of Lee et al. which used 1 millimeter of cavity depth [31], satisfying pulpotomy outcomes were achieved in our study.

Overall, our findings demonstrated remarkable results of TheraCal in pulpotomy of primary teeth at the 12-month follow-up. Therefore, TheraCal can be considered as an alternative material in the pulpotomy of primary teeth.

Exposure location is an important determining factor; occlusal exposures are more successful than proximal ones. A five-minute guideline is provided by an expert, but there is evidence that failure to control bleeding may lead to



(a) (b)

Figure 3: Dentin bridge formation in teeth treated with TheraCal (a) and MTA (b) after 12 months.

reduced success in DPCs and reinforces the need to use direct clinical observation in diagnosis and decision-making. Absence of symptoms and preservation of pulp vitality after at least one year indicates a successful VPT. Another factor that affects the success of direct pulp cap is the ability to control pulpal bleeding after exposure and before pulpal bleeding. If the tooth is asymptomatic and well filled, even if the decay remains, the tooth's chances of survival are excellent [33, 34].

It is important to note the limitations of this study since they can determine the future direction of the present study. Firstly, it was the potential release of free monomers from TheraCal that its effect on reducing the biocompatibility was not well-known. Second, we did not observe the histological quality of dentin bridges under TheraCal. Meanwhile, with the precise histological examination of the treated teeth, a more understanding of the effect of TheraCal in pulpotomy milk teeth can be obtained. Finally, a larger sample size with long-term follow-ups until the exfoliation of treated teeth is suggested to assess the histologic results more accurately in different groups.

## 5. Conclusions

The current study confirmed TheraCal as a promising material for pulp capping in pulpotomy of primary teeth. The successful radiographic and clinical outcomes of TheraCal were repeated here in a 12-month follow-up in agreement with several other studies.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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*Research Article*

# Oral Hygiene Habits and Use of Fluoride in Developmental Age: Role of Parents and Impact on their Children

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*Introduction*. In healthcare, the need to pay more attention to the achievement of two objectives within the society arises: health promotion and prevention in terms of nutrition, good education, sport, and health education. Scientific evidence shows that adequate health standards must be learned since childhood through the help of parents and appropriate school projects. Parental intervention must be appropriate to support the responsibility of their children's health. In oral health, it has been established for many years that there is a correlation between parental behaviors and lifestyles and children's attitude. The aim of this study is to verify the close relation between behaviors, habits, lifestyles, and the knowledge of parents about their oral health and, consequently, their focus and care for their own children's oral health. Furthermore, the awareness of parents about the importance and use of fluorine was to be determined. *Materials and Methods*. The study lasted 15 months and was conducted from April 2018 to July 2019: an anonymous 29-question questionnaire was administered to all parents who accompanied their children (aged between 3 and 12 years) going under treatment in the Pediatric Dentistry Unit of the University Hospital Policlinico Umberto I, Rome. Anamnestic data, sociodemographic context (e.g., educational level and occupation), oral health habits, and prevention of parents and children and fluoride knowledge were investigated. The study received ethical approval. 204 questionnaires were collected. The data gathered were recorded with a specifically designed computer program and collected and analyzed using a Microsoft Excel 10 database. Data were evaluated using standard statistical analysis software; descriptive statistics including mean ± SD values and percentage were calculated for each variable. The relationship between the age of parents, between mother or father and the parents' degree of education levels, and the knowledge for their own children's oral health was explored using the chi-square test of homogeneity and Fisher's exact test ($P$ value of $< 0.05$ considered as statistically significant). *Results*. From the acquired data, it is possible to deduce that the major respondents were mothers aged from 36 to 45, while only a small part were fathers aged above 45 years. Questions related to parents' oral hygiene habits were included in the questionnaire, and from the sample taken into consideration, it emerges that 64.7% of the respondents (67.1% mothers and 57.7% fathers) periodically attend a dental office for a checkup, 20.9% tend to postpone the treatment, and 15.2% go there just for emergency. Some of the questions showed that 80% of the interviewed subjects use fluoride toothpaste for their child's oral hygiene. *Conclusion*. Prevention in childhood, in addition to being synonymous with monitoring the oral health of the child, means first of all to pay attention to parents who are the main behavioral reference. It emerged that there is no adequate knowledge about fluorine, especially when the subjects have a low educational level. A role of fundamental importance for the diffusion of adequate concepts in the field of oral hygiene is covered, according to the data received from the study carried out, by the dentist and dental hygienist.

## 1. Introduction

The industrialization of many countries has led to an increase in chronic degenerative diseases [1], which are the consequence of many behavioral factors and lifestyles indirectly imposed by the society for years [2]. In healthcare, therefore, the need to pay more attention to the achievement of two objectives within the society arises: health promotion and prevention in terms of nutrition, good education, sport, and health education. Even though dental caries significantly decreased in western countries, it is still considered one of the main public health problems affecting most of the

preschool children in many countries worldwide in the form of early childhood caries (ECC) [3].

Dental caries is a multifactorial disease that starts with microbiological changes within the complex biofilm and is affected by salivary flow and composition, exposure to fluoride, consumption of dietary sugars, etc. This affects humans of all ages all over the world and remains a major dental health problem among schoolchildren globally. [3]

The prevalence of ECC ranged from 1 to 12% in children living in developed countries [4]. The crucial reason for this situation is mainly ascribable to parents' or caregivers' lack of awareness of the potential disease [5]. Scientific evidence shows that adequate health standards must be learned since childhood through the help of parents [6] and appropriate school projects [7, 8].

Therefore, parental intervention must be appropriate to support the responsibility of their child's health. In oral health, it has been established for many years that there is a correlation between parental behaviors and lifestyle and children's attitude [5, 9]. It has also been established that the trend of the social and economic level of the family unit is also closely and proportionally to the interest of oral health itself [10]. Pediatricians' awareness regarding the process of dental caries is essential, as well as its prevention through early dental visits and available interventions, including fluorine [11]. The mechanisms of fluorine action are both topical and systemic, but the topical effect is the most important one. Fluorine has three main mechanisms of action: it reduces enamel demineralization; it promotes enamel remineralization; and it inhibits bacterial metabolism and acid production [12].

For this reason, this study aims to verify the close relation between behaviors, habits, lifestyles, and the knowledge of parents about their oral health and, consequently, the attention for their own children's oral health. In addition to this comparison, the second aim was to determine the degree of knowledge of parents about the importance of fluorine, whether they used fluoride products for the domestic oral hygiene of their children and their satisfaction about this choice.

## 2. Materials and Methods

The study was conducted from April 2018 to July 2019, and an anonymous questionnaire was administered to all parents who accompanied their children (aged between 3 and 12 years) going under treatment in the Pediatric Dentistry Unit of the University Hospital Policlinico Umberto I, Rome. The questionnaire validation was verified in a preliminary study [13].

The drafting of the questionnaire is derived from the analysis and subsequent combination of several scientifically validated questionnaires that have contributed over the years to determine the concept and importance of an individual's health:

(i) The HU-DBI questionnaire, Hiroshima University Dental Behavioral Inventory for the description of habits in oral health [14]

(ii) The EGOHID questionnaire derived from a European project of development of oral health indicators [15]

(iii) The TPB questionnaire theory of planned behavior [16]

(iv) The MHLC questionnaire, multidimensional health locus of control [17]

The final version of the questionnaire included a total of 29 questions which have been divided into four sections, to make it easier to understand.

The first section corresponded to the collection of anamnestic data (parental figures, nationality, age, province of residence, number, and age of children in the family) and data related to the sociodemographic context of the interviewed subjects (educational qualification and profession). Particularly, the educational qualification was assessed dividing it into the categories elementary school, middle school, high school, and degree, while the categories specified for the education part were manager/entrepreneur, freelance, employee, workman, housewife, student, unemployed, and others. This information helps to have a generic but complete picture of the physiological and economic-social aspects of the subject. The second section included questions related to the attention given to their oral health and prevention. The third section was completely focused on the habits and behaviors adopted by the children for oral hygiene. The fourth and last sections, on the other hand, were dedicated to fluoride, testing the parents' knowledge on this topic and the source of information acquired.

The inclusion criteria were the age of the children between 3 and 12 years old and that the questionnaire had to be completed in each part.

The study protocol complied with the Ethical Guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committee of Sapienza University of Rome.

The questionnaire included an informed consent to be signed, and this was obtained from all subjects participating in the study.

Three researchers took care of the creation of the manuscript, its administration and the collection of the data and results, while another one focused and developed the descriptive and statistical analysis.

*2.1. Statistical Analysis.* 204 questionnaires were collected, each one of them properly filled, and with children within the age range; therefore, none of them was excluded. The data gathered through the questionnaire were recorded with a specifically designed computer program and collected and analyzed using a Microsoft Excel 10 database. Data were evaluated using standard statistical analysis software (version 20.0, Statistical Package for the Social Sciences, IBM Corporation, Armonk, NY, USA). Descriptive statistics including mean ± SD values and percentage were calculated for each variable. The relationship between the age of parents, between mother or father and the parents' degree of education levels, and the knowledge for their own children's oral health was explored using the chi-square test of homogeneity and Fisher's exact test. A $P$ value of $< 0.05$ was considered as statistically significant.

## 3. Results

This trial collected 204 questionnaires completed by parents interviewed at the Pediatric Dentistry Unit of the University Hospital Policlinico Umberto I, Rome.

The medical history and sociodemographic characteristics of the sample are shown in Table 1.

From the acquired data, it is possible to deduce that the major respondents were mothers (74) aged from 36 to 45 and over 45 (56), while the remaining only 52 were fathers aged mainly between 36 and 45 or over 45.

The percentages of the sociocultural level, deduced from the qualification, were unchanged between the two parental figures as shown in Figure 1.

Questions related to parents' oral hygiene habits were included in the questionnaire, and from the sample taken into consideration, it emerges that 64.7% of the respondents (67.1% among all the mothers and 57.7% among all the fathers) periodically attend a dental office for a checkup, 20.9% tend to postpone the treatment, and 15.2% go there just for emergency.

To the question "How often do you usually attend a dental practice?", the prevailing answer was "Periodically, even just for a check" (64.7% of the total interviewees, 67.1% among all the mothers, and 57.7% among all the fathers), followed by "Periodically, but in case of problems that are not very relevant to me, I tend to postpone the treatment" (20.9% of the total respondents, 19.07% of whom are mothers, and 23.07% of the fathers) and finally "Only in cases of emergency, for example in the presence of pain" (15.19% of the total interviewees, 13.81% from mothers, and 19.23% from fathers). When questioned if "Don't the baby's milk teeth need a good cure because they will fall anyway?", a consistent result was found: 87.8% of the interviewed subjects did not agree, 7.3% were uncertain, and finally, 4.9% agreed not to perform treatment.

While in the previous question, there was a similar percentage of responses between the two parental figures, in this case, 90.1% of mothers think that it is important to care of deciduous teeth, while for the fathers, the percentage was 78%.

Regarding the attitude to the oral hygiene care of the child, the questions and percentages of the respective answers are shown in Table 2.

Some of the previous questions showed that 80% of the interviewed subjects use fluoride toothpaste for their child's oral hygiene: of this, 80%, 57% declare to choose the toothpaste to prevent the onset of dental caries (and is therefore aware of the benefits of fluorine), while the remaining 43% buys fluoride toothpastes for reasons other than the ultimate end of the fluorine itself (and is therefore not aware of its action).

Referring to the last section of the questionnaire, of the 204 people interviewed, 52.4% are aware of information related to fluorine, while the remaining 47.6% say they have no notion about fluorine itself.

The sources from which the subjects learn fluorine notions more or less effectively are represented in Figure 2.

The subjects who said they were informed about this topic were asked which is, in their opinion, the role of the fluoride contained in the toothpaste. The results are shown in Table 3.

It is possible to observe how 81.28% (row 1 of Table 3), or almost all subjects who declared to be aware of the role of fluorine, know exactly what the action of this element is, while a much lower percentage (18.72%) believes he knows it (rows 2 and 3 of Table 3), but in reality, does not. More specifically, we can see that the quantity of outliers is much lower if the information about fluorine is provided by the dental hygienist or dentist, and we can deduce that the role of these professionals is much more effective than other tools in the diffusion and in learning about the role and effectiveness of this element.

Furthermore, the part of the sample that uses fluoridated products for the oral hygiene of their children has been asked whether they are satisfied with their use and, if not, to motivate their cause: 90.8% of their parents are satisfied, while 9.2% say they are not because the dental caries have appeared the same even if much attention has been paid to oral hygiene.

To deepen the analysis, we asked to use that part of the sample that used fluoridated products (80%) if

(i) they use both toothpaste with fluorine and a mouthwash with fluorine or only one of the two

(ii) for the type of fluoride product used, the concentration of fluorine is to be respected according to the age of the child

These results are shown in percentage values in Figures 3 and 4.

In support of the good knowledge about the role of fluoride by the parents highlighted above, these two figures illustrate how the parenting culture about this topic is reflected also in children.

Regarding the prevention of the carious lesion, as well as the use of topical fluoride, much importance has also been placed on the execution of dental sealants in T developmental age, for which the last three questions of the questionnaire are reserved.

67.15% of the interviewed subjects know what the dental sealants are, while the remaining 32.85% are not aware of the information in this regard: more in detail, from the statistically elaborated data, it emerges that the knowledge of dental sealants is greater in mothers (71.71%) compared to fathers (53.84%).

In addition, 162 questionnaires were selected out of a total of 204 that correspond to 162 parents with children aged between 6 and 12 years (that is, the age group in which the first permanent dental elements erupt as this performance it must be performed starting from these categories of teeth): to the question "Have your child ever had dental sealings done?", the answers were affirmative for 41.97% of which the totality proved to be satisfied (unlike the degree of satisfaction of the use of fluoridate products for which some respondents have responded negatively) and negative for 43.82%, and the remaining 14.19% of parents do not even know if they have ever had them done to their children.

Regarding the knowledge of children's oral health, no difference was found correlating it with the age of the parents ($P = 0.923$), between mother and father ($P = 0.293$) and the parents' educational level ($P = 0.817$).

Table 1: Medical history and sociodemographic characteristics of the sample.

| Anamnestic and socio-demographic characteristics | Mother | Father | Total |
|---|---|---|---|
| N. | 152 (74.5%) | 52 (25.5%) | 204 |
| Nationality | | | |
| Italian | 138 (90.7%) | 49 (94.23%) | 187 (91.6%) |
| Other | 14 (9.2%) | 3 (5.76%) | 17 (8.3%) |
| Province | | | |
| Roma | 126 (83%) | 48 (92.23%) | 174 (85.29%) |
| Viterbo | 3 (2%) | 0 | 3 (1.47%) |
| Latina | 5 (3%) | 2 (3.84%) | 7 (3.43%) |
| Frosinone | 11 (7.3%) | 1 (1.92%) | 12 (5.88%) |
| Rieti | 3 (2%) | 1 (1.92%) | 4 (1.96%) |
| Other | 4 (2.6%) | 0 | 4 (1.96%) |
| Age | | | |
| Up to 29 years | 10 (6.5%) | 1 (1.92%) | 11 (5.39%) |
| 30-35 years | 12 (8%) | 0 | 12 (5.88%) |
| 36-45years | 74 (48.6%) | 21 (40.38%) | 95 (46.56%) |
| Over 45 years | 56 (37%) | 30 (57.69%) | 86 (42.15%) |
| Education | | | |
| Primary school | 4 (2.6%) | 1 (1.92%) | 5 (2.45%) |
| Middle school | 21 (13.8%) | 6 (11.53%) | 27 (13.23%) |
| Upper secondary school | 73 (48%) | 25 (48.07%) | 98 (48.03%) |
| Graduation | 54 (35.6%) | 20 (38.46%) | 74 (36.27%) |
| Occupation | | | |
| Manager/businessman | 4 (2.6%) | 2 (3.84%) | 6 (2.94%) |
| Free professional | 16 (10.5%) | 8 (15.38%) | 24 (11.76%) |
| Employed/self-employed | 68 (45%) | 33 (63.5%) | 101 (49.5%) |
| Worker | 15 (9.8%) | 8 (15.8%) | 23 (11.27%) |
| Housewife | 33 (21.71%) | 0 | 33 (16.17%) |
| Student | 4 (2.7%) | 1 (1.9%) | 5 (2.45%) |
| Unemployed | 12 (7.8%) | 0 | 12 (5.88%) |
| Other | 0 | 0 | 0 |
| N. of children in the family | | | |
| 1 | | | 53 (25.98%) |
| 2-3 | | | 137 (67.15%) |
| 4-5 | | | 14 (6.86%) |

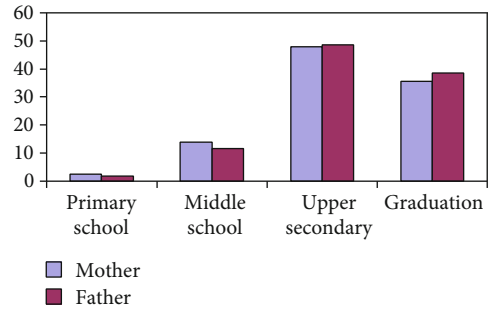


Figure 1: Sociocultural level, in percentage, of the interviewed subjects.

## 4. Discussion

From the statistical results obtained through the processing of the information collected, it was possible to make comparisons, setting variables, to compare what emerges from this study and what is evident from numerous studies conducted in literature with the same method, or data collection through the compilation of paper questionnaires by parents.

As already demonstrated by many articles in literature [5, 10, 18–20], the importance of oral care is often transmitted from parent to child with the same attitudes and attention that parents apply to themselves. For this reason, questions related to parents' oral hygiene habits were included in the questionnaire, and from the sample taken into consideration, it emerges that 64.7% of the respondents (67.1% mothers and 57.7% fathers) attend a dental office

Table 2: Child's attitude to oral hygiene care.

| Child's attitude to oral hygiene care | Percentage |
|---|---|
| When did you start cleaning your child's teeth? | |
| a. Immediately after the first milky tooth eruption | 32.8% |
| b. After the eruption of 4-6 teeth | 34.31% |
| c. After the eruption of all the teeth | 19.11% |
| d. I do not remember | 13.72% |
| What do you use to clean your child's teeth? | |
| a. Finger | 1.47% |
| b. Gauze | 0.49% |
| c. Toothbrush | 97.05% |
| d. I just rinse the mouth after meals | 1% |
| Which substance do you use during teeth cleaning? | |
| a. Fluoride toothpaste | 80% |
| b. Nonfluoride toothpaste | 18.6% |
| c. Water | 1.47% |
| d. Other | 0 |
| How many times? | |
| a. 3 times a day (after 3 meals) | 22.05% |
| b. 2 times a day (morning and evening) | 62.7% |
| c. 1 time a day | 12.7% |
| d. Only sometimes a week | 2.45% |
| In everyday life, who cleans the child's teeth? | |
| a. Mother | 23.03% |
| b. Father | 1.96% |
| c. Somebody else | 0.5% |
| d. The child cleans the teeth by himself (go to the next question) | 74.5% |
| While the child is brushing his teeth, is there anyone looking at him? | |
| a. Yes, even if he was well educated on the right movements to be done | 36.84% |
| b. There is no one with him, but later, I check if he has brushed his teeth | 54.60% |
| c. No control because no one in the family has time due to daily commitments | 8.55% |
| When does he/she eat sugary food products? | |
| a. Together with meals | 17.94% |
| b. Between meals during the day | 81.86% |
| c. Before going to sleep to facilitate sleep | 0.5% |
| Based on what do you choose the toothpaste for your child? | |
| a. Prevention of the onset of dental caries | 58% |
| b. Prevention of gingival inflammation | 5% |
| c. Prevention of bad breath | 1.9% |
| d. Depending on the design of the container or the color | 0.9% |
| e. A good taste of toothpaste | 13.2% |
| f. What we have at home | 7.8% |
| g. I have no specific reasons for choosing toothpaste | 13.2% |

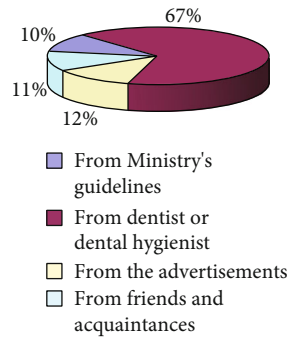


Figure 2: Sources from which the acquired information is derived.

periodically for a check, 20.9% tend to postpone the treatment, and 15.2% just for emergency.

By grouping the different educational levels of the parents into two groups (the first of which is composed by elementary and lower secondary school certificate and the second by upper secondary school and graduates), it emerges that there is a considerable difference regarding the acquisition of fluoride notions: the first band, composed of 32 subjects, is equally balanced between subjects who are correctly informed about the role of fluorine and not; on the contrary, in the second band, which includes 172 subjects with a medium-high educational level, it is found that 70% of the subjects are correctly informed, while 30% do not have the right notions on this element.

This finding is also supported by other studies, in which the knowledge and attitude of the parents on oral health were assessed stating that subjects with higher educational level have greater knowledge about the topic [11–14, 21, 22].

Regarding one of the studies mentioned above and conducted in Greater Noida, it is stated that the knowledge and attitude of the parents does not vary significantly with age as putting two group variables: parents aged under 25 and parents aged 26 or over [21].

The other way around, in this study, if on the one hand, it is true that different age groups were taken into consideration (even if not by much compared to the study conducted in Greater Noida), on the other, it is true that differences have been found: indeed, subdividing the respondents in subjects aged 35 years or less and those aged 36 or over, 47.82% of parents in the first age group has good knowledge about the role of fluorine, while up to 69% among subjects aged 36 or over, noting that in addition to the educational level is also the age of the subject to influence on the knowledge of this information.

In a study published in the Evidence-Based Dentistry database by Topping and Assaf, it has been observed that when the child brushes his teeth alone, parental supervision helps to reduce the occurrence of caries more than when such supervision is not carried out. Furthermore, it emerges that parents who do not exercise direct control over their child's oral hygiene at home and who are not very present in daily household activities due to work commitment are less aware of the importance of brushing and the use of fluoride toothpastes [23].

Table 3: Percentage of the responses of the subjects informed on the basis of the information source.

| | From ministry's guidelines | From dentist or dental hygienist | From the advertisement | From friends or acquaintance |
|---|---|---|---|---|
| Prevents the onset of dental caries | 7.47% | 57% | 9.34% | 7.47% |
| Prevents gum problems | 1.86% | 3.73% | 0 | 0 |
| It gives a sense of freshness | 0.93% | 5.69% | 2.79% | 3.72% |

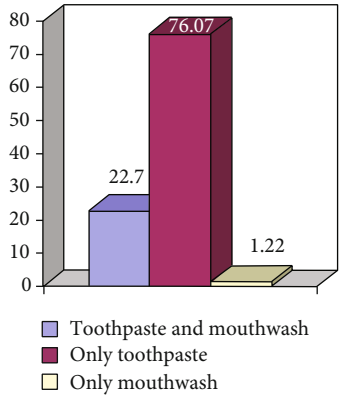


Figure 3: Fluorine products used for the child.

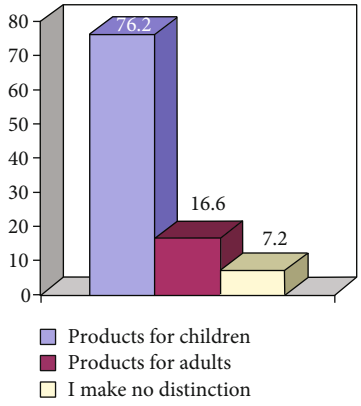


Figure 4: Type of fluoride products used for children, under the age of 6 years.

To be able whether to sustain this phenomenon or not, data from the abovementioned study [23] were compared with the results of our trial: out of 204 interviewed subjects, 74.5% said that their child clean their teeth by himself. When asked "While the child is brushing his teeth, is there anyone looking at him?", 8.55% say they do not control their child because they do not have time due to work commitments. Even if the percentage of those who do not supervise their children is very small, it is important to analyze how many of them know fluoride or not: of that 8.55%, only 15.4% say they are informed about the importance of fluorine itself, while the remaining 84.6% state the opposite; some of them declare that they have no specific reasons for choosing a toothpaste or using what they have at home for their children. In agreement with what has been deduced from the study of Topping and Assaf, it is also possible to affirm that parents who do not assist their children and who are not present in the routine of daily actions or who do not adopt direct control are uninformed about the importance of supervision and notions about fluorine, not considering the choice of toothpaste type as important.

In relation to what has been reported in this study and in the numerous scientific articles cited above regarding the positive effect of the educational level in the prevention of caries lesions, the mother's attitude and knowledge about oral health appears to be of considerable importance as they are statistically more present and they deal more frequently with their children's oral hygiene.

To support this data, together with the aim of understanding if there is a relationship between the strong incidence of caries (in 2010, it was found that 45% of children aged 5 years were affected by caries) and the behavior of parents about oral health, there is a study conducted in the Czech Republic. This study compares the positive effects that can be derived from the high educational levels of the mother with those of the father, asserting that the latter does not determine a significant influence on the oral health of the child as it is the mother, statistically, to take responsibility for the oral healthcare of the child himself [24]. Lencova and Duskova took the mid-high educational level of the parents (all parents who have acquired the high school diploma and the degree title) as the reference variable to verify, firstly, the percentage of mothers and fathers that really knew about fluorine and, secondly, if the awareness of mothers is actually more decisive and effective. Regarding the first hypothesis, 70% of mothers and 68% of fathers are correctly informed: as already stated above, the subjects with an adequate educational level are more aware about oral health, and in this case, there is no significant percentage difference between the two parental figures. Regarding the second hypothesis, it is possible to state that the awareness about oral health and the use of fluorine is more decisive in the education of the child if it is acquired by the mother, since the data processing shows that mothers are the parental figure that more frequently accompanies their children to various dental appointments. This phenomenon can be derived from the employment status as 30% of mothers are housewives or unemployed, while all paternal figures in the totality of the sample carry out a profession that does not allow a strong presence in everyday life. Precisely for these reasons, statistically, it is the mother, the main responsible for the oral health of the child and consequently having more time to devote to it, more frequently making decisions for him. In support of the argument, just exposed emerge from further statistical questionnaires about it: in fact, 25.5% of parents that claimed to brush himself their child's teeth consisted of 4 fathers and 48 mothers.

Within the prevention methods, dental sealants play an important role in preventing the onset and the development of dental cavities and became a worldwide prevention measure in the last decades [25, 26]. According to literature, mothers more than fathers have a positive opinion about dental sealants and that there is greater satisfaction with the use of fluoride products in larger families [19]. In the present study, it emerges that 67.15% of the subjects know what dental sealants are, of which 79.6% are mothers and the remaining 20.4% are fathers. Contrary to what emerges in the literature, both mothers and fathers are satisfied about the use of this benefit.

Regarding the use of fluorinated products, mouthwashes are a very popular additional oral hygiene element [27, 28], and in our study, a good percentage was found to use them together with a fluoride toothpaste, with high satisfaction regarding it.

## 5. Conclusions

From the ministerial guidelines, resulting from the November 2013 referred to the 2008 document, it was stated that "The individual risk of developing caries lesions should be evaluated through the experience of caries, dietary habits and oral hygiene, fluoride and the general state of health of each individual, as well as through the socio-economic status of the family" [29].

These factors can be assessed by studying the importance that the parent gives to oral healthcare, health education that will transmit to the child, and the attitudes about their oral hygiene at home. The elaboration of the data obtained from this study allowed to have a lot of information about these problems, crossing some variables among them.

It emerged, in fact, that most of the interviewees use fluoride products for the hygiene of their children, but only a part of these is aware of the positive effects that this has about the prevention of caries lesions: there is not adequate knowledge about fluorine, especially when the subjects have a low educational level.

The study also shows that mothers pay more attention and care to their children's oral health than fathers: the study shows that, if the educational level of the parents is medium-high, education related to oral hygiene will be more effective if it is given by the mother that is also considered the parental figure most available to perform performance to prevent the onset of carious lesions.

Moreover, age influences the acquisition of concepts, assuming that, if the subject understands the importance of prevention, with the passing of the years, he is increasingly determined to know as much information as possible for the maintenance of good oral health.

Even if minimal, a part of the interviewed subjects does not place the right importance on contemporary or subsequent brushing supervision if their child is autonomous in the management of oral hygiene: nonsupervision has also been synonymous with superficiality and less awareness. Therefore, prevention in childhood, in addition to being synonymous with monitoring the oral health of the child, means first of all to pay attention to parents who are the main behavioral reference model since the learning and adoption of good habits during childhood begins and is learned at home with the parental figures: a role of fundamental importance for the diffusion of adequate concepts in the field of oral hygiene is covered, according to the data received from the study carried out, by the dentist and dental hygienist.

## Data Availability

Data are available upon request to the corresponding author.

## Consent

Informed consent was obtained from all subjects participating in the study.

## Conflicts of Interest

The authors declare no conflict of interest.

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*Research Article*

# Peripheral Purinergic Modulation in Pediatric Orofacial Inflammatory Pain Affects Brainstem Nitroxidergic System: A Translational Research

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Physiology of orofacial pain pathways embraces primary afferent neurons, pathologic changes in the trigeminal ganglion, brainstem nociceptive neurons, and higher brain function regulating orofacial nociception. The goal of this study was to investigate the nitroxidergic system alteration at brainstem level (spinal trigeminal nucleus), and the role of peripheral P2 purinergic receptors in an experimental mouse model of pediatric inflammatory orofacial pain, to increase knowledge and supply information concerning orofacial pain in children and adolescents, like pediatric dentists and pathologists, as well as oro-maxillo-facial surgeons, may be asked to participate in the treatment of these patients. The experimental animals were treated subcutaneously in the perioral region with pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS), a P2 receptor antagonist, 30 minutes before formalin injection. The pain-related behavior and the nitroxidergic system alterations in the spinal trigeminal nucleus using immunohistochemistry and western blotting analysis have been evaluated. The local administration of PPADS decreased the face-rubbing activity and the expression of both neuronal and inducible nitric oxide (NO) synthase isoforms in the spinal trigeminal nucleus. These results underline a relationship between orofacial inflammatory pain and nitroxidergic system in the spinal trigeminal nucleus and suggest a role of peripheral P2 receptors in trigeminal pain transmission influencing NO production at central level. In this way, orofacial pain physiology should be elucidated and applied to clinical practice in the future.

## 1. Introduction

Orofacial inflammatory pain is a universal healthcare complaint in pediatric patients and a major concern of national public health [1]. In general, orofacial pain results from two pathological processes: (1) tissue injury and inflammation (nociceptive pain) or (2) a primary lesion or dysfunction of the nervous system (neuropathic pain) [2, 3].

Extracellular adenosine triphosphate (ATP) has been detected at high concentrations in injured tissues during acute inflammation state, whether in experimental animals or in humans [4–6]. ATP can act as an extracellular signalling molecule [7, 8], influencing various biological functions, and stimulating nociceptors to initiate pain sensation by inducing the synthesis and release of proinflammatory cytokines and nitric oxide (NO) [9, 10]. Because of short half-life of NO and its highly reactive nature, most studies have focused on the analysis of its synthesizing enzyme, nitric oxide synthase (NOS). To date, three distinct NOS isoforms are known, named constitutive neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) [11, 12]. Some studies have suggested that NOS/NO may play a role in pathological pain states [13, 14]. In this regard, nNOS activity appears to influence pain transmission [10] while iNOS is expressed only in pathological conditions and is induced by proinflammatory cytokines and/or endotoxins [15]. A specific population of nNOS-positive neurons mainly mediate nociception [2]. Peripheral inflammation has been shown to alter the expression of both nNOS and iNOS in the spinal cord [16, 17], suggesting that the NO level in the spinal cord is closely regulated during inflammation. Moreover, some studies revealed a role of NO in the mesencephalic trigeminal nucleus for proprioception [18, 19]. Nevertheless, few studies have examined the role of the NO pathway at trigeminal level in inflammatory states [20, 21]. Lee and coworkers [22] observed a time-dependent increase in nNOS and iNOS protein expression in the spinal trigeminal nucleus following capsaicin injection in the masseter muscle and a significant attenuation of hypersensitivity after the pretreatment with NOS inhibitors in rats.

The spinal trigeminal nucleus, area receiving somatosensory inputs from the orofacial district, is subdivided into three parts, i.e., subnucleus caudalis (Sp5C), interpolaris (Sp5I), and oralis (Sp5O). The characterization of trigeminal pain pathways in inflammatory states is an important biological and clinical question for the development of new therapeutic strategies, but the role of the three subnuclei in the trigeminal nociceptive mechanisms is not yet well defined. Nevertheless, some studies showed that the most caudal part of the trigeminal sensory complex, i.e., Sp5C, is the essential projection site for the nociceptive orofacial inputs [23] but NOS proteins did not result in significant changes at longer time points [24].

ATP acts through P2 receptors that are subdivided into P2X and P2Y families. They are purinoreceptors classified into G-protein-coupled receptors P2Y and ATP-gated cation channels, so-called P2X receptors. In spinal and trigeminal ganglia, P2X [25, 26] and P2Y receptors are present [27].

In this study, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) was used, a wide range P2 receptor antagonist [28, 29]. The receptors more sensitive to PPADS are $P2X_1$, $P2X_2$, $P2X_3$, and $P2X_5$. Although all of them are present in the nervous system, $P2X_1$, $P2X_3$, and $P2X_5$ receptors have been specifically described in trigeminal ganglia neurons [30, 31]. Besides, $P2X_4$, $P2X_7$, and $P2Y_1$ receptors, less sensitive to PPADS, have been described in sensory ganglia and at presynaptic terminals [30].

Orofacial pain conditions represent a challenge to pediatric clinicians because of the disease complexity and unclear etiology. However, the defined mechanisms of pain management are still largely unknown.

With this premise, the aim of the present research was to investigate the relationship among inflammation, peripheral purinergic receptors, and central nitric oxide alteration in a mouse model of pediatric orofacial inflammatory pain, evaluating the alteration of the nitroxidergic system in the trigeminal spinal nucleus.

## 2. Materials and Methods

*2.1. Animals.* Experiments were carried out on a total number of 60 C57BL/6J male mice (20-25 gr. Harlan, Italy), and, in particular, 48 for immunohistochemistry and remaining 12 for western blotting (WB). The animal model used was around 6 weeks of age, which can be considered, according to literature, representative of pediatric patients [32].

To minimize the circadian variations, the animals were housed in individual cages with food and water *ad libitum* and kept in an animal house at a constant temperature of 22°C with a 12 h alternating light-dark cycle. The experiments were performed between 08:00 h and 12:00 h. All effort was made to minimize animal suffering, and the number of animals used agreed with the good animal practice (GAP). The experimental procedures were approved by the Italian Ministry of Health (n° 105/2011–B) and in accordance with the ethical standards of the Helsinki Declaration and the principles presented in the "Guidelines for the Use of Animals in Neuroscience Research" of the Society for Neuroscience.

The animals were subdivided into 4 experimental groups of 15 animals each: the first group was injected with saline (control, CTR); the second group was injected with PPADS (PPADS); the third group was injected with formalin (FORM); the fourth group was treated with PPADS and after 30 minutes injected with formalin (PPADS+FORM).

*2.2. Injection Site and PPADS Treatment.* The formalin and PPADS injections were performed subcutaneously into the right upper lip, just lateral to the nose through a 27-gauge needle into the right vibrissa pad as quickly as possible, with only minimal animal restraint.

PPADS tetrasodium salt (Sigma-Aldrich, Milan, Italy) was dissolved in saline and used at dose 25 mg/kg (0.01 ml/10 g), according to Gourine et al. [33], Martucci et al. [10], and our previous experience in this field [13].

*2.3. Nociceptive Behavioral Response.* The formalin test was made by injecting 2.5% formalin (FORM) according to Luccarini et al. [34]. Following formalin injection, all animals

were immediately placed in the test box for a 60 minutes observation period. A nociceptive score was determined measuring the number of seconds that the animals spent rubbing the injected area with the ipsilateral forepaw or hindpaw. The recording time was divided into 20 blocks of 3 minutes. A video camera was used to record the grooming response.

*2.4. Immunohistochemical Analysis.* The nNOS and iNOS expressions were evaluated at 3, 6, 12, and 24 hours (h) after formalin injection.

All mice were anaesthetized with Zoletil (60 mg/kg i.p., Verbatic, France) and transcardially perfused with saline followed by 40 ml of 4% paraformaldehyde in phosphate buffer 0.1 M pH 7.4. After fixation, the brainstem of each animal was removed, postfixed in 4% paraformaldehyde in phosphate buffer for 2 h, and cryoprotected overnight in 30% sucrose at 4°C. Frozen serial transverse sections (40 $\mu$m thick) of all the brainstem were placed in TBS (Tris-Buffer-Saline) solution. Alternate sections were processed immunohistochemically or toluidine blue-stained for morphological control.

Briefly, the first series of sections were incubated in normal goat serum (10% in phosphate-buffered saline containing 0.1% Triton X-100) for 60 minutes and then incubated in rabbit polyclonal primary antiserum directed against nNOS (1 : 500, Chemicon, USA) or iNOS (1 : 500, Chemicon, USA) diluted in phosphate-buffered saline containing 3% normal goat serum and 0.1% Triton X-100, for 24 h at 4°C. After incubation in the primary antiserum, the sections were sequentially incubated in biotinylated goat anti-rabbit immunoglobulins and avidin-biotin-peroxidase complex (Vector Labs., Burlingame, CA, USA). The reaction product was visualized using hydrogen peroxide and diaminobenzidine (Sigma, St. Louis, MO, USA) as chromogen. The immunohistochemical control was performed by omitting the primary antibody, in the presence of isotype-matched IgGs, and performing preadsorption assay using the related peptide and gave negative results.

The distribution of the labelled cells of all animals was charted with the aid of an image analyzer (Immagini & Computer, Milan, Italy).

*2.5. Colocalization Immunofluorescence Assay.* Double immunofluorescence aided the morphological identification of neurons through colocalization of nNOS or iNOS with NeuN (a nuclear marker of neurons).

Frozen floating sections, obtained using the procedure described above, of the brainstem were processed for double immunofluorescence. Briefly, the sections were incubated in bovine serum albumin (BSA, Sigma-Aldrich, Saint Louis, USA) blocking solution (5% BSA, 0.25% Triton X-100 in TBS 1%) and then were incubated in mouse monoclonal primary antiserum against NeuN (1 : 100, Chemicon, Temecula, CA, USA) with rabbit polyclonal primary antiserum against nNOS (1 : 500, Chemicon, USA) or iNOS (1 : 500, Chemicon, USA) diluted in TBS containing 3% BSA and 0.1% Triton X-100, for 24 h at +4°C. After incubation in the primary antibodies, the sections were sequentially incubated with appropriated fluorescent secondary antibodies diluted in TBS (1 : 200, Alexa-Fluor 488, green fluorescent dye and Alexa-Fluor 555, red fluorescent dye; Invitrogen, Carlsbad, CA, USA). The immunofluorescence control was performed by omitting the primary antibody and incubating the sections with nonimmune rabbit serum. All floating sections were placed on slides and finally mounted using a special mounting medium (UltraCruz™ Mounting Medium, Santa Cruz Biotechnology, Santa Cruz, CA) with DAPI (4′, 6-diamidino-2-phenylindole). The colocalization was evaluated on digital images acquired with laser scanning confocal microscopy (LSM 510, Zeiss, Germany).

*2.6. Western Blotting Analysis.* The nNOS and iNOS expressions were evaluated at 3 h after formalin injection. All mice were anaesthetized with Zoletil (60 mg/kg i.p.) and sacrificed by cervical dislocation. The brainstem in the region of the spinal trigeminal nucleus of each animal was removed and immediately frozen in liquid nitrogen and stored at -80°C until the NOS content expression assay. On the day of NOS determination, tissues were defrosted at room temperature, weighed, diluted in lysis buffer (Tris HCl pH 8 50 mM, NaCl 150 mM, Triton 1% 100 $\mu$l/ml, PMSF 0.6 mM e aprotinina 1 $\mu$g/ml), homogenized, and centrifuged at 13000g at 4°C for 2 minutes. After protein assay, the supernatant was diluted in Laemmli buffer (0.3 M Tris–HCl, pH 6.8, containing 10% SDS, 50% glycerol, 5% dithiothreitol, and 0.05% bromophenol blue) to obtain 40 $\mu$g of proteins. The proteins were loaded onto an 8% SDS–polyacrylamide gel and then transferred onto a nitrocellulose membrane (Biosciences, Uppsala, Svezia) for 1 h at 4°C. The membrane was blocked with 5% BSA in TBST (20 mM Tris–base, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) at 4°C overnight. The next day, it was incubated with primary polyclonal antibody directed against mouse nNOS (Cayman Chemical, Ann Arbor, MI, USA) diluted 1 : 200 or iNOS (Santa Cruz, Biotechnology, Santa Cruz, CA) diluted 1 : 500 in blocking solution (1% serum albumin bovine) for 2 h at room temperature. The nitrocellulose membrane was also probed with a polyclonal anti-$\beta$actin antibody (1 : 3000; Cytoskeleton Inc., Denver; CO, USA) as loading controls. After two washing in TBST buffer, the blot was incubated with biotinylated goat anti-rabbit immunoglobulins (Vector Labs., Burlingame, CA, USA) for 1 h at room temperature. Subsequently, the blot was detected with the addition of avidin-biotin-peroxidase complex (Vector Labs., Burlingame, CA, USA). The reaction product was visualized using hydrogen peroxide and diaminobenzidine (Sigma, St. Louis, MO, USA) as chromogen.

*2.7. Data Analysis.* Behavioral analysis was made for 1 h after the formalin injection by three investigators (E.B., A.B, and S.C.) who were blinded to the group of animal assignment. The animal data were analyzed and compared by repeated ANOVA (analysis of variance) measurements followed by Tukey's post-test. The density of nNOS- and iNOS-positive neurons in the brainstem was evaluated by immunohistochemical analyses in Sp5C, Sp5I, and Sp5O using a quantitative method by blinded researchers. The neurons were recognized by their morphological characteristics [10] and

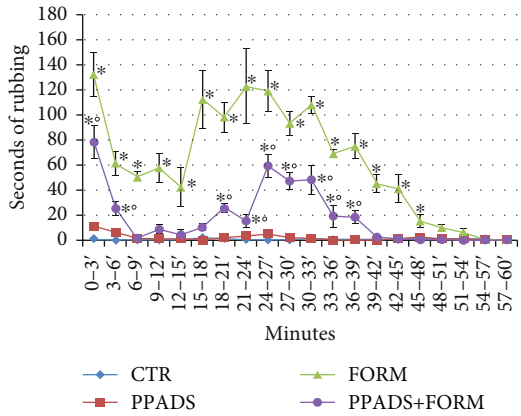



Figure 1: Time course of face-rubbing activity observed after subcutaneous injection of saline (CTR), formalin (FORM), PPADS (25 mg/kg), and formalin (PPADS+FORM) into the right upper lip. The mean number of seconds that each mouse spent rubbing was plotted for each 3 minutes block over the 60 minutes postinjection observation period. The experiments were performed in triplicate. Data represent mean ± S.D.; $^{*}p < 0.05$ versus CTR animals; $^{\circ}p < 0.05$ versus FORM animals.

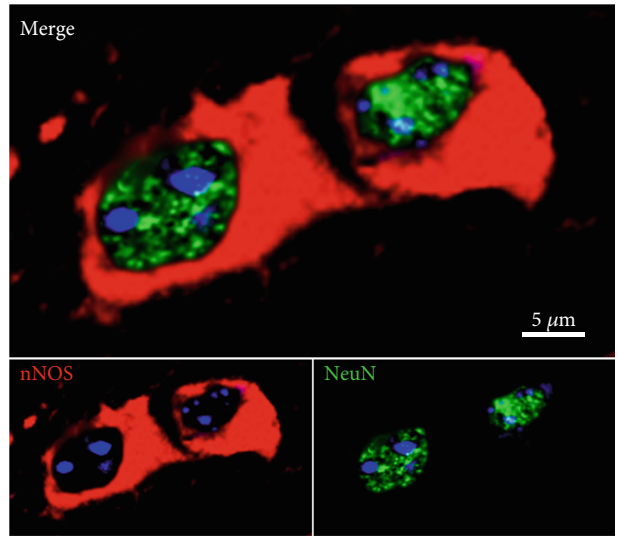



Figure 2: Double-label confocal images of spinal trigeminal nucleus stained neurons for NeuN (green) and nNOS (red). The nuclei were stained in blue (DAPI). Bar: 5 $\mu$m.

supported by a colocalization immunofluorescence assay with a NeuN. nNOS- and iNOS-positive cell counts were made in all the processed sections at a final 200x magnification. Total counts were taken from each section and assigned to specific components of the brainstem trigeminal complex. Cytoarchitecturally identified regions of the spinal trigeminal nucleus including Sp5C, Sp5I, and Sp5O were examined for nNOS- and iNOS-positive cells. Rostrocaudal levels of these subnuclei were referred to as bregma according to coordinates provided by Franklin and Paxinos [35]. Moreover, a set of serial transverse 40 $\mu$m sections stained with toluidine blue (Sigma, St. Louis, MO, USA) was prepared to identify the area of Sp5 subnuclei better. We analyzed the following according to the bregma coordinates: Sp5O sections were collected from -5.68 mm to -6.48 mm, Sp5I from -6.48 mm to -7.48 mm, and Sp5C from -7.48 mm to -8.48 mm. Immunoreactive bands of western blot analysis were analyzed using a computer-based densitometry image program. Grey levels were evaluated as integrated optical density (IOD) with an image analysis program (Image-Pro Premier 9.1, Milan, Italy).

The immunohistochemical and immunoblotting data were analyzed and compared by repeated-measures ANOVA followed by Tukey's post-test.

## 3. Results

*3.1. Behavior Analysis.* The evaluation of nociceptive threshold was performed for 1 h after the injection. Control animals showed only face-grooming episodes for full analysis time (Figure 1).

PPADS-treated animals displayed a nociceptive score not significantly different from control animals (Figure 1).

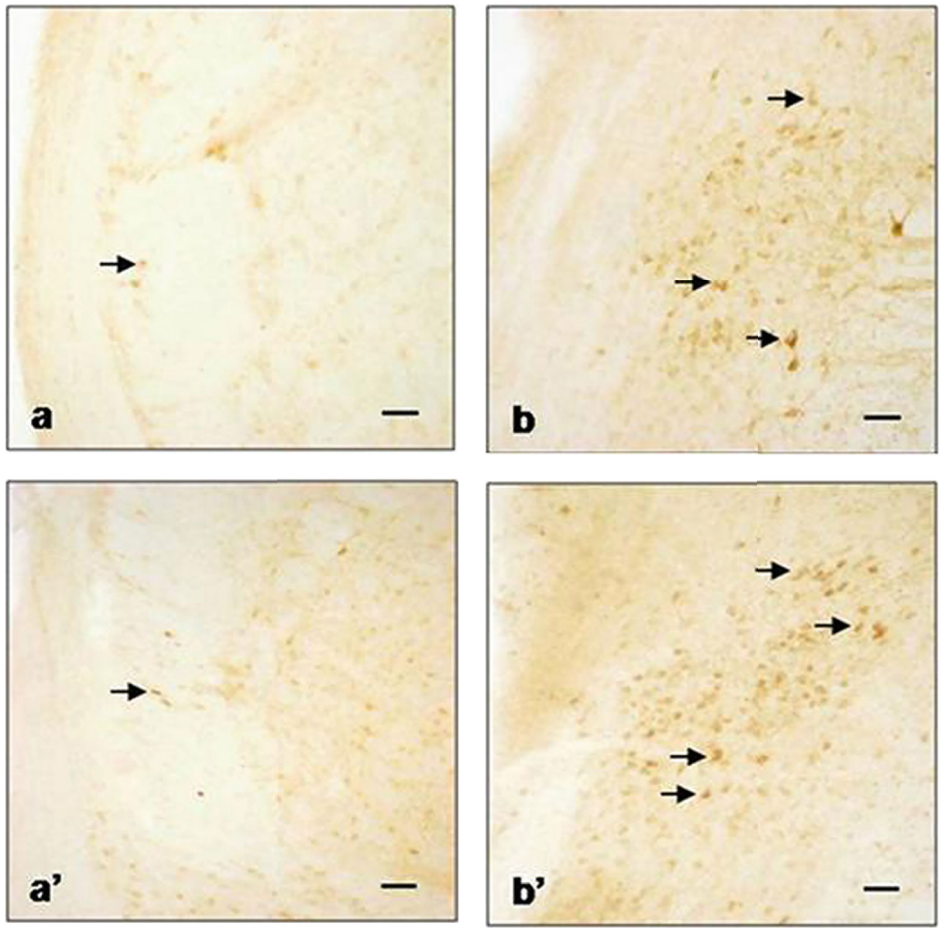
Formalin-injected animals showed sustained face-rubbing episodes with vigorous face-wash strokes directed to the perinasal area with the ipsilateral and, sometimes, contralateral forepaw. The forepaw was often accompanied in its movements by the hindpaw. This nociceptive response presented a typical biphasic time course interspersed with a period of quiescence (10-15 minutes): (1) an early and short-lasting first period of activity (3–5 minutes) and (2) a second prolonged (20–45 minutes) phase (Figure 1). Animals pretreated with PPADS and injected with formalin showed a significant decrease of nociceptive score with respect to the formalin animals. Particularly, they presented a less pronounced early phase and a less marked and lasting second rubbing period (Figure 1).

*3.2. Immunohistochemical Evaluation.* Double immunofluorescence aided the morphological identification of neurons through colocalization of nNOS or iNOS with NeuN (Figure 2).

The time course of nNOS displayed a rapid increase of protein staining at 3 h in FORM animals, in Sp5C, Sp5I, and Sp5O (Figures 3(b), 3(b′), and 3(d)). Over 24 h, nNOS gradually decreased towards control values both for Sp5C, Sp5I, and Sp5O (Figures 3(e)–3(g)). The PPADS treatment partially limited the increase of nNOS in the trigeminal nucleus reaching a statistically decrease (Figures 3(c), 3(c′), and 3(d)–3(g)).

The time course of iNOS showed a rapid increase of protein staining at 3 h in FORM animals in Sp5C (Figure 4(d)). Over 24 h, iNOS immunostaining was low reaching control values (data not shown). The PPADS treatment partially limited the increase of iNOS reaching a statistically decrease (Figures 4(c), 4(c′), and 4(d)).

At 3 h, the expression of nNOS and iNOS was maximum in our experiment. At this time point, the nNOS and iNOS immunoreactivities were localized in the cytoplasm of neurons in all areas of the trigeminal subnuclei appearing as brown staining while the nuclei were unstained. In control and




(a) (b)

Figure 3: Continued.

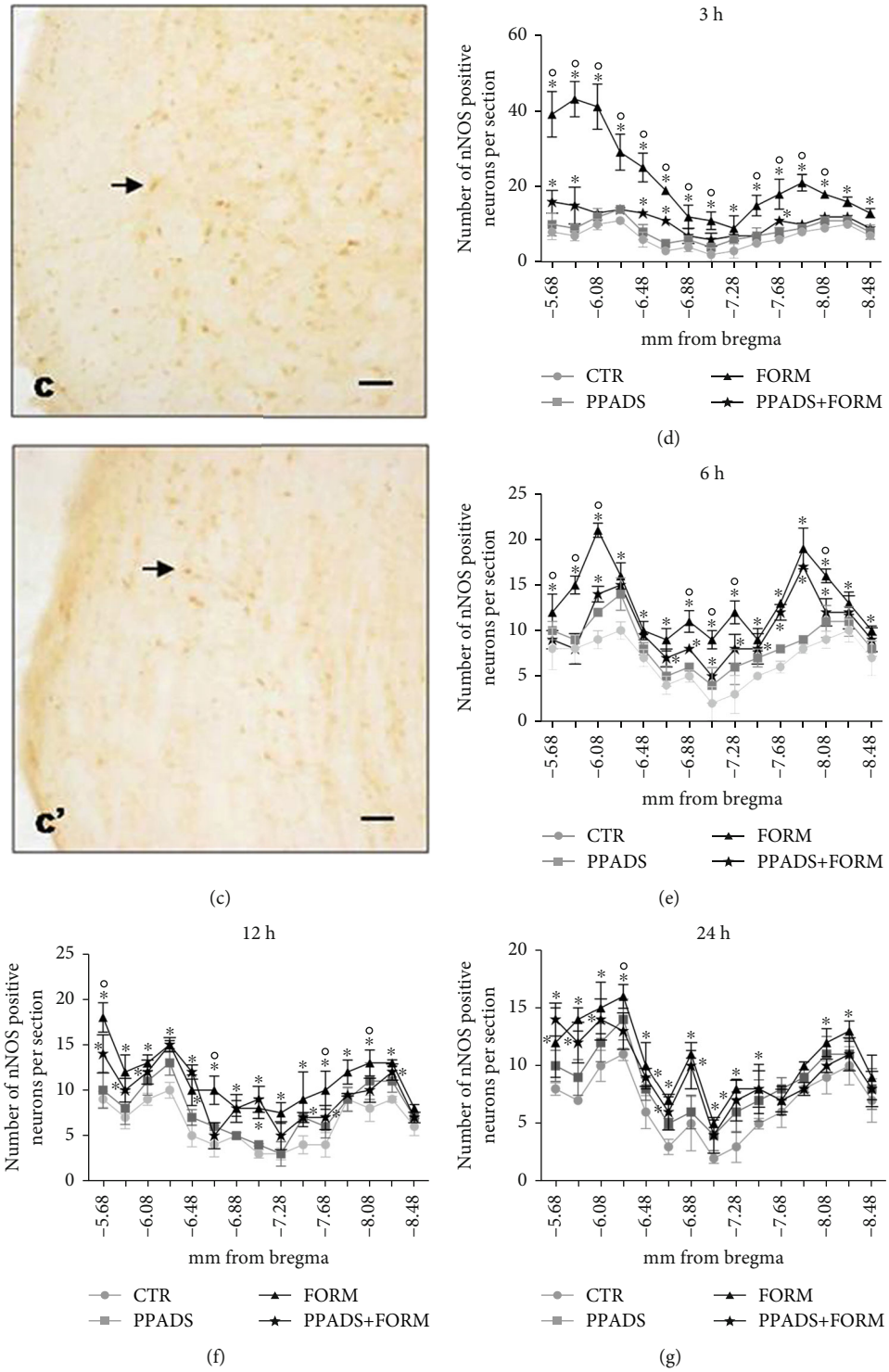
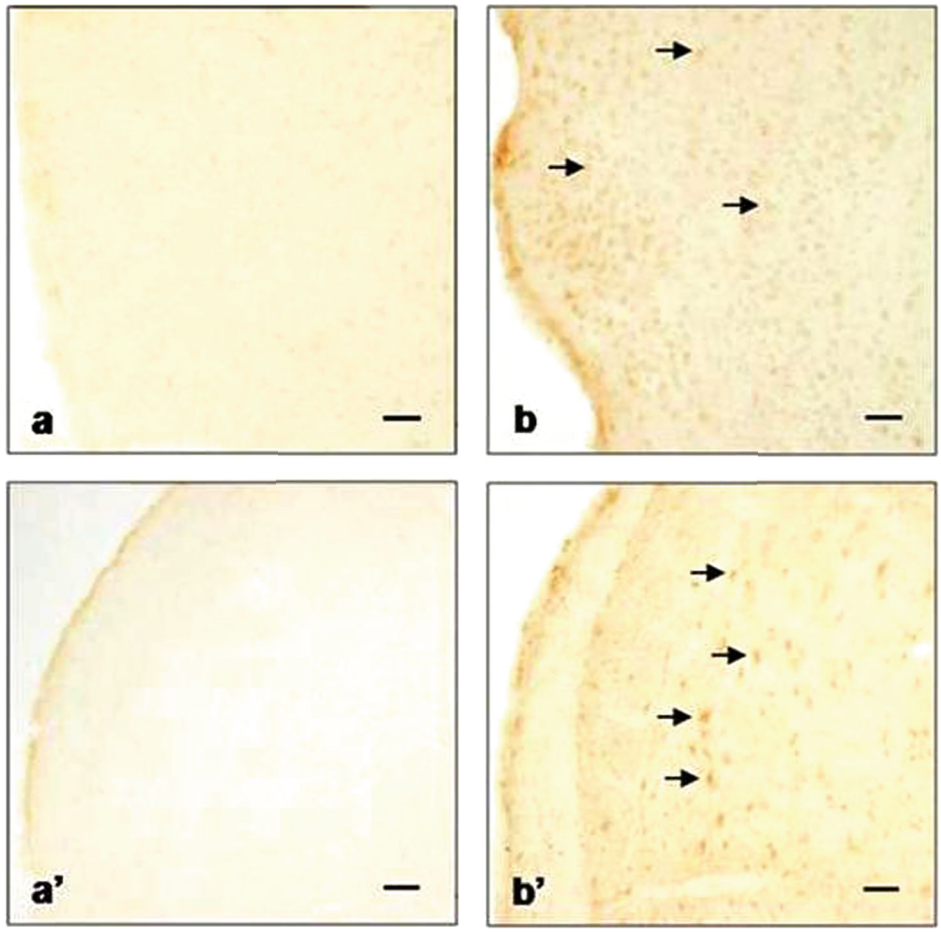



Figure 3: nNOS-positive neurons in the ipsilateral spinal trigeminal nucleus: (a) subnucleus caudalis (Sp5C) of saline-treated animals (CTR), (b) Sp5C of formalin-treated animals (FORM), (c) Sp5C of PPADS and formalin-treated animals (PPADS+FORM), (a′) subnucleus oralis (Sp5O) of saline-treated animals (CTR), (b′) Sp5C of formalin-treated animals (FORM), and (c′) Sp5C of PPADS and formalin-treated animals (PPADS+FORM). Arrows indicate nNOS-positive neurons. Bar 50 $\mu$m. Time course of nNOS immunopositive neurons in the ipsilateral spinal trigeminal nucleus in saline-treated animals (CTR), formalin-treated animals (FORM) and PPADS (25 mg/kg), and formalin-treated animals (PPADS+FORM) after (d) 3, (e) 6, (f) 12, and (g) 24 h from formalin injection. The experiments were performed in triplicate. Data represent mean ± S.D.$^{*}p < 0.05$ vs. CTR; $^{\circ}p < 0.05$: FORM vs. PPADS+FORM.




(a) (b)

Figure 4: Continued.

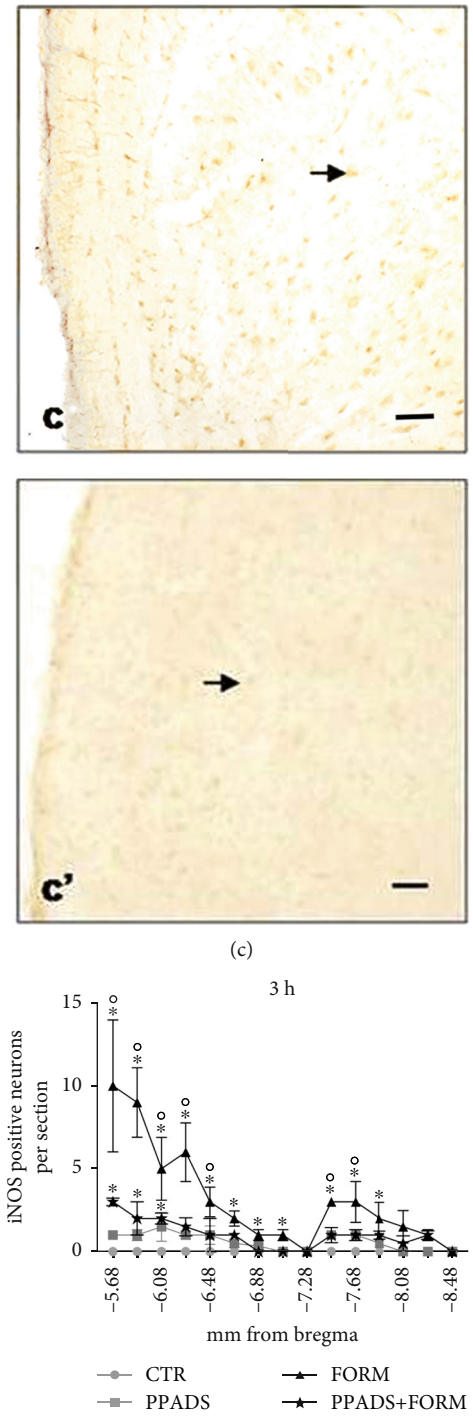



Figure 4: iNOS-positive neurons in the ipsilateral spinal trigeminal nucleus: (a) subnucleus caudalis (Sp5C) of saline-treated animals (CTR), (b) Sp5C of formalin-treated animals (FORM), (c) Sp5C of PPADS and formalin-treated animals (PPADS+FORM), (a′) subnucleus oralis (Sp5O) of saline-treated animals (CTR), (b′) Sp5C of formalin-treated animals (FORM), and (c′) Sp5C of PPADS and formalin-treated animals (PPADS+FORM). Arrows indicate iNOS-positive neurons. Bar 50 $\mu$m. Statistical evaluation of immunopositive neurons in the ipsilateral trigeminal nucleus in saline-treated animals (CTR), formalin-treated animals (FORM) and PPADS (25 mg/kg), and formalin-treated animals (PPADS+FORM) after 3 h from formalin injection. The experiments were performed in triplicate. Data represent mean ± S.D.* $p < 0.05$ vs. CTR; ° $p < 0.05$: FORM vs. PPADS+FORM.

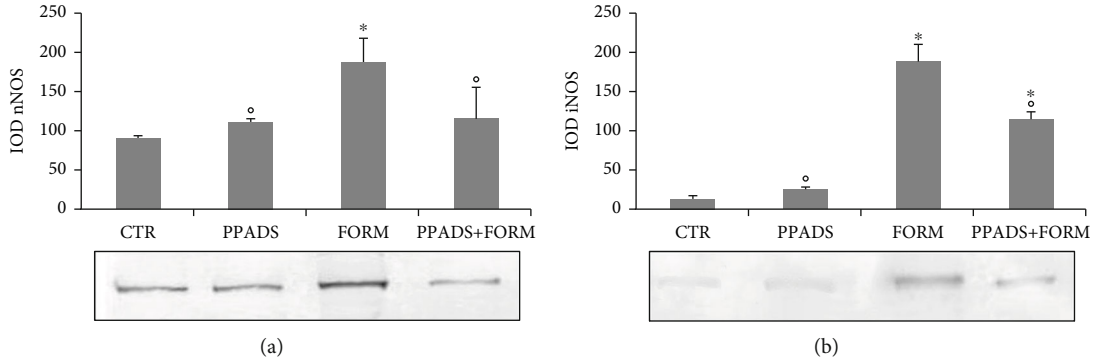



Figure 5: Statistical evaluation of nNOS (a) and iNOS (b) expression in the brainstem in saline-treated animals (CTR), PPADS-treated animals (PPADS), formalin-treated animals (FORM), and PPADS- and formalin-treated animals (PPADS+FORM) after 3 h from formalin injection. The experiments were performed in triplicate. Values are mean ± S.D. and represent the IOD (integrated optical density); $^{*}p < 0.05$ vs. CTR animals; $^{\circ}p < 0.05$ vs. FORM animals.

PPADS-treated animals, the number of nNOS-positive neurons in Sp5C, Sp5I, and Sp5O was very low without any significant difference between the sections (Figures 3(a), 3(a′), and 3(d)) while iNOS staining was not found (Figures 4(a), 4(a′), and 4(d)). On the contrary, in formalin-injected animals, the number of nNOS-positive cells greatly increased in the Sp5O and in the Sp5C areas compared to control and PPADS-treated animals (Figures 3(b), 3(b′), and 3(d)). The formalin-related increase of positivity was found mainly in Sp5O and in the ipsilateral side. In Sp5O, iNOS-positive neurons increased too (Figures 4(b′) and 4(d)). On the other hand, in animals pretreated with PPADS and injected with formalin, significantly fewer nNOS-positive neurons were observed bilaterally in all three parts of the spinal trigeminal nucleus compared to the formalin group (Figures 3(c), 3(c′), and 3(d)). Moreover, a recovery to normal iNOS staining was also reached (Figures 4(c), 4(c′), and 4(d)).

*3.3. Western Blotting Analysis after PPADS Treatment at 3 h: nNOS.* The nNOS expression in the brainstem decreased after PPADS treatment.

In control- and PPADS-treated animals, the nNOS expression was present and moderate (Figure 5(a)).

On the contrary, in formalin-injected animals, nNOS was overexpressed in the brainstem neurons compared to control animals (Figure 5(a)).

The pretreatment with PPADS showed a decrease of a nNOS expression in neurons (Figure 5(a)).

*3.4. Western Blotting Analysis after PPADS Treatment at 3 h: iNOS.* The iNOS expression in the brainstem decreased after PPADS treatment. In control and PPADS-treated animals, the iNOS expression was not found (Figure 5(b)).

On the contrary, in formalin-injected animals, iNOS expression in neurons was increased compared to control animals (Figure 5(b)).

## 4. Discussion

The results of this study suggest the correlation between the nitroxidergic system in the brainstem and peripheral P2 receptor modulation in orofacial inflammatory pain transmission contributing to the insight of this pathology [36].

Our results showed that the local application of PPADS in the inflamed site produces a reduction in pain-related behavior, as reported also in Borsani et al. [13]. P2 receptors are activated by ATP released in inflamed tissue promoting pain sensation. In this regard, acute peripheral inflammation induces an increase of extracellular ATP at the sites of tissue injury [4, 5] with consequent excessive activation of P2X receptors on primary sensory axons. An elevated P2X receptor activity can also result from the enhanced expression of this receptor in inflamed tissue and can contribute to abnormal pain responses associated with inflammatory injuries [37]. Indeed, in our previous experience, animals pretreated with A-317491, a P2X3, and P2X2/3 receptor antagonist and then injected with formalin in the perioral area had a statistically less pronounced early phase and a delayed second rubbing period compared to animals treated only with formalin [13]. These receptors are also involved in chronic inflammatory conditions such as rheumatoid arthritis [38]. Moreover, $P2Y_{14}$ receptor in trigeminal ganglia may contribute to the maintenance of orofacial inflammatory pain [39].

Furthermore, we showed a modulation of the nitroxidergic system in the spinal trigeminal nucleus, at Sp5C and Sp5O level. We observed an increase in nNOS immunostaining in the superficial laminae especially at 3 h decreasing at 24 h and an increase in iNOS immunostaining at 3 h after formalin injection. These observations suggest a role for NO in nociception also in the orofacial system at central level. In literature, there are poor knowledge about the relationship between orofacial nociception and NO [35, 40]. Nevertheless, it has been demonstrated that nNOS [17] and iNOS [41, 42] might play a critical role in central mechanisms of the development and/or maintenance of inflammatory pain, supporting our results. Other studies demonstrated the nNOS expression at trigeminal level not only in mammals [43–46] but also in birds and in reptiles [47]. The results of Fan and coworkers [48] displayed that NO plays an active role in both peripheral and central processing of nociceptive information following chronic tooth inflammation.

The significant decrease of nNOS and iNOS that we observed after the pretreatment with PPADS suggests an important role of NO-ATP in orofacial nociceptive transmission. Also interestingly, acute peripheral inflammation induces an increase of extracellular ATP at the sites of tissue injury [4, 5], with consequent excessive activation of P2X receptors on primary sensory axons. In addition, some works regarding peripheral inflammation have revealed that NOS expression in the central nervous system is differentially regulated, because of the target organ and the proinflammatory agent employed [49, 50], suggesting also a possible antinociceptive role.

Other studies have shown that the most caudal part of the trigeminal sensory complex, i.e., Sp5C, is the essential projection site for the nociceptive orofacial inputs [23, 24, 51]. In this specific subnucleus, our data showed an increase in the number of nNOS- and iNOS-positive neurons after formalin injection in the ipsilateral part and its decrease with PPADS pretreatment, demonstrating a correlation between peripheral purinergic receptor modulation and NO production in pain perception. We also observed an increase of nNOS- and iNOS-positive neurons in Sp5O too. These results suggest a role in nociceptive transmission of perioral area for both the subnuclei, and they are corroborated by other experimental observations reported in literature. Electrophysiological studies performed in rat and monkey [52–54] indicate that one or several of the three rostral divisions of the trigeminal sensory complex, i.e., the nucleus principalis (Pr5), Sp5O, and Sp5I, may also be involved in the transmission of orofacial pain. Some works focused the attention on the role of Sp5O as involved in intraoral nociceptive stimulation [55], even if it has been reported also a possible involvement of Sp5C in this area [56]. On the other hand, other experiments suggest the role of Sp5O in perioral nociceptive mechanisms [29]. In fact, abundant data indicate that the rostral relay for some oral/perioral nociceptive molecules is in Sp5O. The Sp5O lesions observed in humans or performed in animals induced a significant decrease in the nociceptive sensations or behaviors triggered by intraoral [56] but also perioral noxious stimuli [57].

Altogether, these data suggest a specific role and specialization of Sp5O and Sp5C in the processing of the nociception, confirming our previous results, even if some authors indicated only the Sp5C involved in formalin perioral stimulation [58, 59]. In particular, Sp5O is activated in transient nociception, while Sp5C in sustained nociception [60, 61].

## 5. Conclusions

In conclusion, our results suggest a key role for the endogenous ATP which can contribute at peripheral level to induct acute inflammatory pain processing. Moreover, we have demonstrated that the events after inflammatory induction involve ATP and NO, influencing the nociceptive pathways in the central nervous system. Based on our results, the PPADS could represent a therapeutic tool for the orofacial inflammatory pain in pediatric population. Large multicenter trials are required in order to study the biological behavior and formulate treatment strategies in the management of the same.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Authors' Contributions

E.B. and L.F.R. were responsible for the concept and design. E.B., A.B., B.B., and S.C. were responsible for the acquisition, analysis, and interpretation of data. E.B. and A.B. drafted the manuscript. M.D.D., M.B., S.S., and R.N. conducted bibliographic research. E.B., A.B., R.R., M.D.C., L.L.M., and S.C. critically revised the manuscript for important intellectual content. M.B., M.D.D., and R.N. were responsible for the technical and material support. E.B., A.B., and S.C. were responsible for the supervision and final approval. All authors have read and agreed to the published version of the manuscript. Elisa Borsani and Andrea Ballini contributed equally as co-first authors. Stefania Cantore, Luigi Fabrizio Rodella, and Michele Di Cosola contributed equally as co-last authors.

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*Research Article*

# Effectiveness of Visual-Tactile Examination and DIAGNOdent Pen in Detecting Early Enamel Caries and Its Remineralisation: An In Vitro Study

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*Background*. The caries preventive effect of Colgate Duraphat® and GC Tooth Mousse Plus® has been widely studied, but the remineralisation potential of initial occlusal caries using these two remineralisation materials remains unclear. *Aim*. This study is aimed at evaluating and comparing the remineralisation of early enamel caries on the occlusal surface of permanent posterior teeth using ICDAS II caries scoring system and DIAGNOdent Pen (DDPen) after remineralisation with Colgate Duraphat® and GC Tooth Mousse Plus®. *Materials and Methods*. Extracted posterior teeth ($N = 120$) with incipient occlusal caries were included in this study. The occlusal surface of each tooth was scored using DDPen and ICDAS II scoring before remineralisation. Then, remineralisation of the teeth of the experimental group was carried out using either CPP-ACP-F or fluoride varnish. After the remineralisation procedures, the occlusal surface of each tooth was again scored using DDPen and ICDAS II scoring. The teeth were then fixed in dental stone blocks and sectioned longitudinally for histological examination using a stereomicroscope. Statistical analysis was performed to calculate the sensitivity and specificity of DDPen and ICDAS II to detect remineralisation and compare with the gold standard histological examination. *Results*. According to ICDAS-II scores, a significant difference was noted in GC Tooth Mousse Plus® and Duraphat® study samples, whereas the difference between the pre-and post-remineralisation of the control group was not significant. According to the DDPen score criteria, a statistically significant difference was noted among all study groups; however, a greater significance level was noted in the GC Tooth Mousse Plus® and Duraphat® study samples compared with the control group. The Spearman's rank correlation of ICDAS-II and DDPen with Downer's histological score (gold standard) revealed a higher association of DDPen score (.738) as compared to ICDAS-II scores (.430). *Conclusion*. The study concluded that both ICDAS II and DDPen could detect remineralisation of early enamel occlusal caries. DDPen was more sensitive than ICDAS-II to detect remineralisation compared with the Downers histological scores.

## 1. Introduction

Dental caries is a gradually progressive disease that has been identified as the most commonly occurring oral disease/chronic infection [1, 2]. The diagnosis of a noncavitated carious lesion is considered challenging because this lesion can be disguised by the remineralisation effect of fluorides [3]. Visuo-tactile inspection of the carious lesion is an inexpensive and the most utilised method of caries assessment [4]. The accuracy and reliability of a diagnosis depend on the expertise and training of the assessor. A standardised caries assessment system is of paramount importance [5].

Several studies have reported that Duraphat® is highly effective in remineralising enamel from other mineralisation products [6–9]. Further research has found that a high concentration of fluoride treatments in acidic pH is more effective in reshaping the surface of noncavitated caries and encouraging fluoride absorption from those in neutral pH [10]. Casein phosphopeptide-amorphous calcium phosphate has been highlighted to enhance the stability of high calcium levels and enhance the delivery of ions to the tooth surface [11, 12]. A clinical trial involving CPP-ACP containing gum demonstrated decelerated progression and enhanced regression of carious lesions [13]. CPP-ACP application also showed postorthodontic white spot regression [14]. Furthermore, CPP-ACP has a significant remineralisation effect, and it can be considered a salivary biomimetic, as it shares many similarities with statherin [15].

ICDAS II supplies a consistent method of lesion detection and evaluation, leading to the diagnosis of caries [16]. ICDAS II provides good reproducibility and accurate detection in vivo and in vitro for initial caries lesions at different stages. The laser fluorescence method (DIAGNOdent) assists the detection of occlusal and approximal caries [2]. The use of the DDPen on white spot carious lesions [17] and an enamel window at the buccal surface in the middle one-third of the crown [18] has been reported. However, the use of DDPen to assess the remineralisation of the fissures, pits, and smooth surfaces has not been explored. Previous studies employed a relatively smaller sample size in their studies [19, 20]. Varying cut-off values have been used and recommended by previous studies [21, 22]. However, these values are dependent on various factors which include type of surface (smooth or fissure), physical properties (demineralisation or remineralisation), and the extent of the lesion (lesion confined to enamel only and lesion involving enamel and dentin).

Narrative and systematic reviews have shown that DDpen is more sensitive to the occlusal aspect of posterior teeth than traditional diagnostic methods, but the specificity is inferior to clinical visual examination [23–25]. DDPen also tends to overestimate deeper caries or dark (stained) lesions. Bhat et al. [26] conducted a systematic review assessing different cut-off values and concluded that the cut-off values based on the laboratory studies were not clinically correlated. The study suggested that the cut-off values may be selected based on the extent of the carious lesion, and different cut-off values were correlated with enamel and dentinal carious lesions.

Different cut-off values for DDPen have been described in the literature; however, no study has evaluated pre-and post-remineralisation relevance of cut-off values. Moreover, no previous studies have been carried out performing simultaneous comparison between the three assessment tools (ICDAS II, DDPen, and histological examination) on tooth surface remineralisation. Hence, this study is aimed at investigating the potential of ICDAS II and DDPen in detecting remineralisation of the early carious lesion after applying topical fluoride gel (Colgate Duraphat®) and casein phosphopeptide-amorphous calcium phosphate fluoride (Tooth Mouse Plus) at the occlusal surface of extracted human permanent posterior teeth. This study also compared the diagnostic cut-off values of DDPen corresponding with the histological examination after the remineralisation process.

## 2. Materials and Methods

Ethical approval for the current study was obtained from the Human Research Ethics Committee of Universiti Sains Malaysia (USM) (USM/JEPeM/18100516). Extracted permanent maxillary and mandibular posterior teeth for orthodontic, periodontal, or other reasons from adult patients that visited the outpatient dental clinics of the School of Dental Sciences, USM, were selected for this study.

A total of 120 freshly extracted teeth were included in this study. Before caries assessment, the occlusal surfaces were cleaned using a toothbrush with pumice slurry and water before randomly assigning numbers from 1 to 120. Unrestored mandibular or maxillary posterior teeth without any carious lesion or incipient carious lesion limited to the enamel, denoted as code 01, 02, and 03 according to the ICDAS II scoring system, were included. The teeth samples were examined under 10x magnification individually. Teeth were excluded from the study if there was the presence of dental fluorosis, tetracycline staining or any sources of staining, hypoplasia, and dentinal exposure.

Before the remineralisation process, two trained and calibrated examiners (T.Y.N and M.S.H) performed the visual assessment of each sample using ICDAS II. Visual examination was using a dental operating light, a WHO probe, and a 3-way syringe to score each tooth according to the ICDAS II scoring system. Sharp explorers were not used during the visual diagnosis. TYN and MSH scored the teeth individually with no discussion regarding the scores to ensure blinding. Interexaminer reproducibility was assessed using kappa analysis. The third examiner (M.F.R) used the DDPen to score each sample afterwards. A triple air syringe was used for five seconds to dry out the enamel surface before using the DDPen. Before scoring, the device was calibrated according to manufacturer's instructions. The probe tip was positioned on the occlusal surface of the tooth and rotated around its vertical axis until the highest value was found. ICDAS II scores and DDPen readings were repeated for two weeks for all samples to ascertain and evaluate intraexaminer reproducibility.

For the control group, the extracted teeth samples were washed with deionised water and then placed in artificial

saliva at 37°C. No material was used for remineralisation of the control group. For the CPP-ACP with fluoride (GC Tooth Mousse Plus®) group, the paste was applied once daily (every 24 h) for 21 days on the occlusal surfaces with a microbrush tip applicator left in place for three minutes which simulated at-home use of this cream. The specimens were then rinsed with deionised water and gently dried with an air syringe. The samples were then immersed in artificial saliva and incubated at 37°C [26]. This process was repeated for 21 days. For the fluoride varnish (Colgate Duraphat®) group, a thin layer of the Duraphat® was applied once using a microbrush tip applicator on tooth's enamel surface, which simulated the professional application in a dental visit. The samples were then immersed in artificial saliva at 37°C for 6 h [27] to simulate the oral environment. Then, the varnish was carefully removed using a toothbrush [27, 28] to ensure the complete elimination of the surface layer of varnish [6, 29]. The samples were then rinsed for one minute with deionised water. After that, the samples were immersed in artificial saliva and incubated at 37°C.

After the remineralisation process, MFR repeats the scoring using the DDPen post-remineralisation for each sample. To ensure blinding, the examiner did not have access to previous scores of ICDAS or DDPen, and the same procedure for performing the post-remineralisation ICDAS score was followed. The teeth were then fixed in dental stone blocks and sectioned longitudinally using a diamond-coated bandsaw (Exakt system) for histological examination using a stereomicroscope with 10x magnification. Parallel cutting of the samples was performed by using tweezer prongs. The parallelism of the block is of utmost importance to ensure accurate cutting direction. The teeth sections fixed in the blocks were then polished by using slurry pumice. The polished sections were then again examined at 10x magnification under the stereomicroscope. Each section was photographed using a digital camera. This gold standard histological examination was compared with the ICDAS II scoring and DDPen examination for validity assessment.

The descriptive statistical analysis was performed using SPSS software version 24 (IBM SPSS Statistics). Inter- and intraexaminer reproducibility was assessed by calculating the unweighted kappa coefficient [30]. Wilcoxon signed ranks for pre-and post-remineralisation using different caries diagnostic tests were performed to assess the potential to detect remineralisation. Spearman's rank correlation of ICDAS-II and DDPen with the histological gold standard was performed to check the association. Sensitivity, specificity, and area under the ROC were calculated for ICDAS II and DDPen scores to compare both caries diagnostic methods.

## 3. Results

Kappa statistics revealed almost perfect reproducibility of TYN (0.954), MSH (0.919), and MFR (0.885), whereas good interexaminer agreement (0.673) was observed [31]. Table 1 describes the difference between the pre- and post-remineralisation scores, which were assessed using ICDAS-II and DDPen. According to ICDAS-II scores, a significant difference was noted in GC Tooth Mousse Plus® and Duraphat® study samples, whereas the difference between the pre- and post-remineralisation of the control group was not significant. According to the DDPen score criteria, a statistically significant difference was noted among all study groups; however, a greater significance level was noted in the GC Tooth Mousse Plus® and Duraphat® study samples compared with the control group. Table 2 presents kappa values of the DDPen score with different cut-off values. The DDPen was noted to have the highest agreement compared with the histological gold standard described by Lussi et al. [32]. Table 3 presents the frequency of true-positive and false-positive findings of the carious occlusal lesion. The Spearman's rank correlation of ICDAS-II and DDPen with the histological gold standard revealed a higher association of DDPen score (.738) than ICDAS-II scores (.430).

Table 1: Wilcoxon signed ranks for pre- and post-remineralisation using different caries diagnostic tests.

| | $Z$ | $p$ value |
|---|---|---|
| ICDAS-II | | |
| Control | -1.342 | .180 |
| Tooth Mousse Plus® | -3.285 | .001** |
| Duraphat® | -2.592 | .010* |
| DDPen score | | |
| Control | -2.928 | .003** |
| Tooth Mousse Plus® | -4.379 | <.001** |
| Duraphat® | -4.411 | <.001** |

Figure 1(a) presents the ROC curves plotted for the ICDAS-II code 0 and DDPen score (0-13) measurements compared with Downer's histological level DI extent as a gold standard validation. Table 4 presents the $Az$ value of DDPen score (0-13) at D1 (0.972) was higher than ICDAS-II code 0 at D1 (0.811), showing the greater accuracy of the method. Rank correlations (Spearman's coefficient) with histology D1 were .692 for ICDAS-II code 0 and .950 for DDPen score (0-13). Figure 2(a) represents the histological section of the tooth at Downer's classification level D1.

Figure 1(b) presents the ROC curves plotted for the ICDAS-II codes 1 and 2 and DDPen score (14-20) measurements using Downer's histological level D2 extent as a gold standard validation. Table 4 presents the $Az$ value of DDPen score (14-20) at D2 (0.894) was higher than ICDAS-II codes 1 and 2 at D2 (0.667), showing the greater accuracy of the method. Rank correlations (Spearman's coefficient) with histology D2 were .769 for ICDAS-II codes 1 and 2 and .854 for DDPen score (14-20). Figure 2(b) represents the histological section of a tooth at Downer's classification level D2.

Figure 1(c) presents the ROC curves plotted for the ICDAS-II code 3 and DDPen score (>21) measurements using Downer's histological level D3 extent as a gold standard validation. Table 4 presents the $Az$ value of DDPen score (>21) at D3 (0.838) was higher than ICDAS-II code 3 at D3 (0.721), showing the greater accuracy of the method. Rank correlations (Spearman's coefficient) with histology D3 were .601 for ICDAS-II code 3 and .774 for DDPen score

Table 2: Kappa statistics for different cut-off values of DDPen score.

| DDPen criteria | Histological gold standard | | | | Kappa value |
|---|---|---|---|---|---|
| | Sound | Demineralised enamel | | Demineralised dentine | |
| | | Outer half | Inner half | | |
| [32] | 0-13 | 14-20 | | >21 | .725** |
| [44] | 0-14 | 15-21 | | >22 | .627 |
| [35] | 0-15 | 16-25 | | >25 | .437 |
| [33] | 0-4 | 5-10 | 11-18 | >18 | .457 |

Table 3: Percentage of occlusal carious lesions detected correctly utilizing different caries diagnostic methods at different histological levels.

| Caries diagnostic method | Histology score | Percentage of occlusal carious lesions detected correctly at different histological levels *n*% True positive-false positive/total | % | $r_S$ (SE) |
|---|---|---|---|---|
| ICDAS-II | | | | |
| Sound (code 0) | D1 | 33-20/53 | 62.26 | |
| ICDAS (codes 1 and 2) | D2 | 11-22/33 | 33 | .430 (.084) |
| ICDAS (code 3) | D3 | 15-19/34 | 44.117 | |
| DDPen score | | | | |
| 0-13 | D1 | 50-3/53 | 94.33 | |
| 14-20 | D2 | 26-7/33 | 78.78 | .738(.062) |
| >21 | D3 | 23-11/34 | 67.64 | |

%: percentage; $r_s$: Spearman rank correlation; *D*: Downer's classification.

(>21). Figure 2(c) represents the histological section of a tooth at Downer's classification level D3.

## 4. Discussion

The current study is aimed at evaluating the sensitivity and specificity of ICDAS II and DDPen to detect the remineralisation of early enamel caries lesion in extracted human permanent posterior teeth and compare with the gold standard histological examination to identify the most suitable cut-off values of DDPen for identification of remineralised carious lesions.

The current study utilised ICDAS-II and DDPen scoring, which are designed to enable the clinicians to diagnose the disease at the chairside, making the results of the current study more relatable to a clinical setup. The current study did not find a significant difference in the remineralisation potential of GC Tooth Mousse Plus® and Duraphat®, following previous literature [6]. However, the effect of GC Tooth Mousse Plus® on salivary constituents and pH value were not considered. Varying cut-off limits for DDPen have been proposed and adopted in the laboratory [33, 34] and clinical studies [32, 35, 36]. Heinrich-Weltzien et al. [37] conducted a systematic review assessing different cut-off values and concluded that the cut-off values might be selected based on the extent of the carious lesion, and different cut-off values were correlated with enamel and dentinal carious lesions.

The cut-off values for DDPen, which had the highest and a significant correlation (0.72*) with Downer's histological scores for the corresponding study samples, agree with the cut-off value suggested by Lussi et al. [32]. A possible explanation of this close agreement could be the assessment of freshly extracted teeth and the preservation medium (artificial saliva). The use of storage solutions such as formalin, sodium azide, or thymol-based mediums has been reported to affect the DDPen measurements [34, 38]. The values of fluorescence emission intensity decrease with time of immersion in all storage solutions, except for artificial saliva and glutaraldehyde [39]. The storage solutions tend to remove the organic compounds from the enamel surface. It is important to note that distilled water does not remove the organic compounds after 30 days of immersion [39]. Immersion in 0.1% thymol solution has shown a decline compared to the laser fluorescence values [40]. The area under the ROC curve (AUROC) can be defined as a composite measure of accuracy. Metz [41] stated that AUROC is a more meaningful measure of the value of a diagnostic test than the overall accuracy, as it does not depend on the disease prevalence in the population. The "diagnostic test" in the current study, being either ICDAS-II or DDPen, is compared with the gold standard Downers histological classification and "the disease" being remineralisation of the carious lesion.

An advantage of the area under the ROC curve analysis is that it reflects the diagnostic performance more comprehensively than the sensitivity and specificity, determined by only one cut-off point. It also provides an overall validity of the methods [42, 43]. Diniz et al. [44] performed an in vivo evaluation of the DDPen utilising the area under the ROC curve and reported that the *Az* value at D1 (0.72), D2 (0.81), and D3 (0.93), whereas another clinical study by Heinrich-Weltzien et al. [37] reported higher *Az*

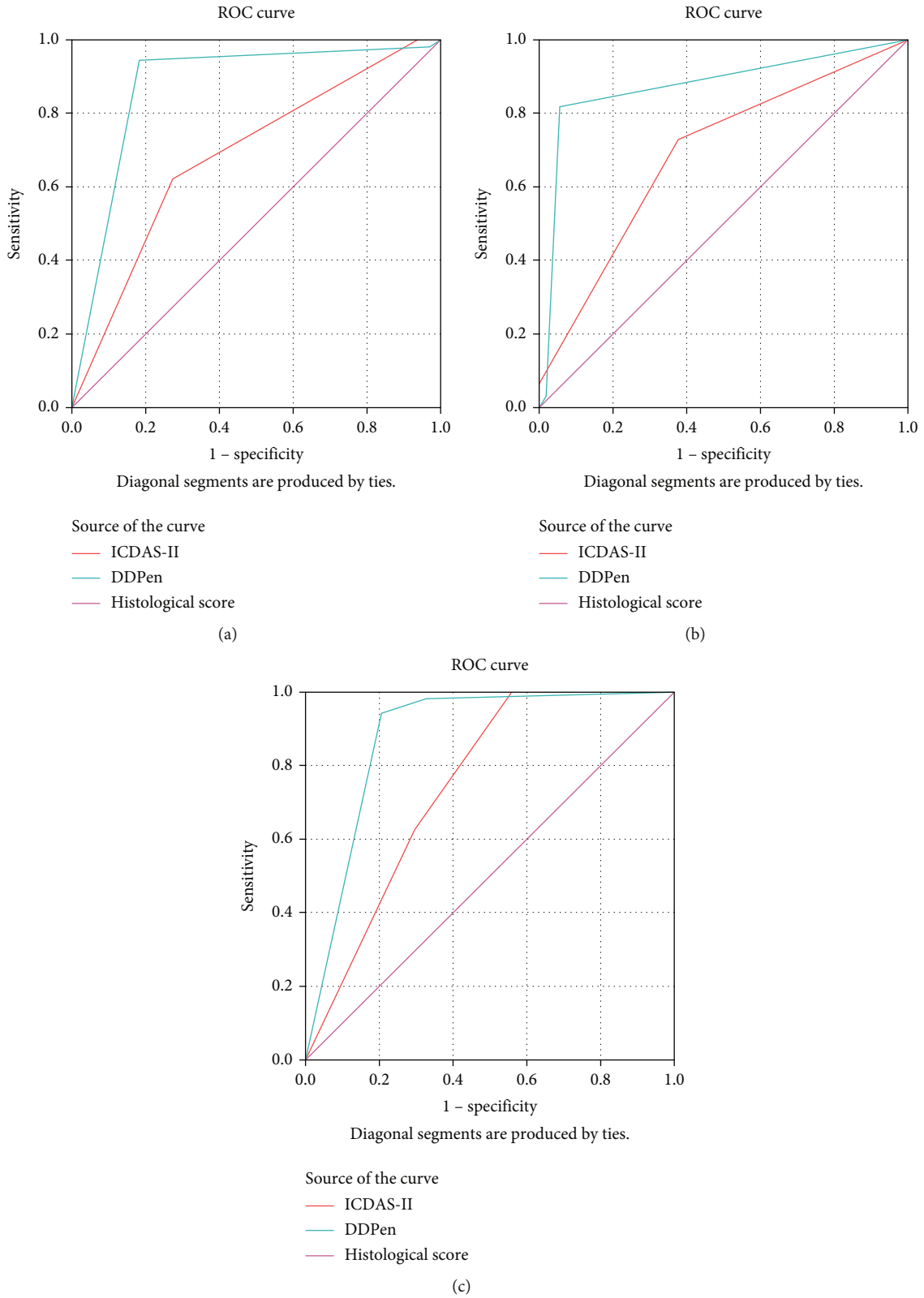



Figure 1: Area under the receiver-operating characteristic (AUROC) for International Caries Detection and Assessment System (ICDAS) codes under binary groups correlated with Downer's histologic classification at (a) D1 threshold, (b) D2 threshold, and (c) D3 threshold.

Table 4: Spearman's correlation coefficients, AUROC curve, specificity, and sensitivity for the mode ICDAS scores, DDPen when compared with the level D1, D2, and D3 Downer's histologic level.

| | Histo score | $r_s$ | Standard error | $A_z$ (95% CI) | Standard error | Sensitivity | Specificity |
|---|---|---|---|---|---|---|---|
| ICDAS II code 0 | D1 | .692 | .053 | .811 (.726-.897) | .044 | .623 | .284 |
| DDPen cutoff 0-13 | D1 | .950 | .028 | .972 (.935-1.000) | .034 | .943 | .194 |
| ICDAS II codes 1 and 2 | D2 | .769 | .059 | .667 (.557-.776) | .056 | .667 | .333 |
| DDPen cutoff 14-20 | D2 | .854 | .050 | .894 (.810-.978) | .043 | .788 | .069 |
| ICDAS II code 3 | D3 | .601 | .067 | .721 (.605-.837) | .059 | .441 | .023 |
| DDPen cutoff >21 | D3 | .774 | .058 | .838 (.740-.937) | .050 | .676 | .023 |

$A_z$: area under receiver-operator curve; CI: confidence interval; $D$: Downer's classification; $r_s$: Spearman's coefficient; $S_n$: sensitivity; $S_P$: specificity.

values for D2 (0.90) and D3 (0.83). The current study reported the highest Az value of DDPen score (0-13) at D1 (0.972) followed by DDPen score (14-20) at D2 (0.894) and then DDPen score (>21) at D3 (0.838). The difference in the performance of DDPen can be attributed to the determination of cut-off values or the number of cases reported in each study. Our study included the most significant number of cases having carious lesions at the D1 level, which could better represent higher accuracy in that category.

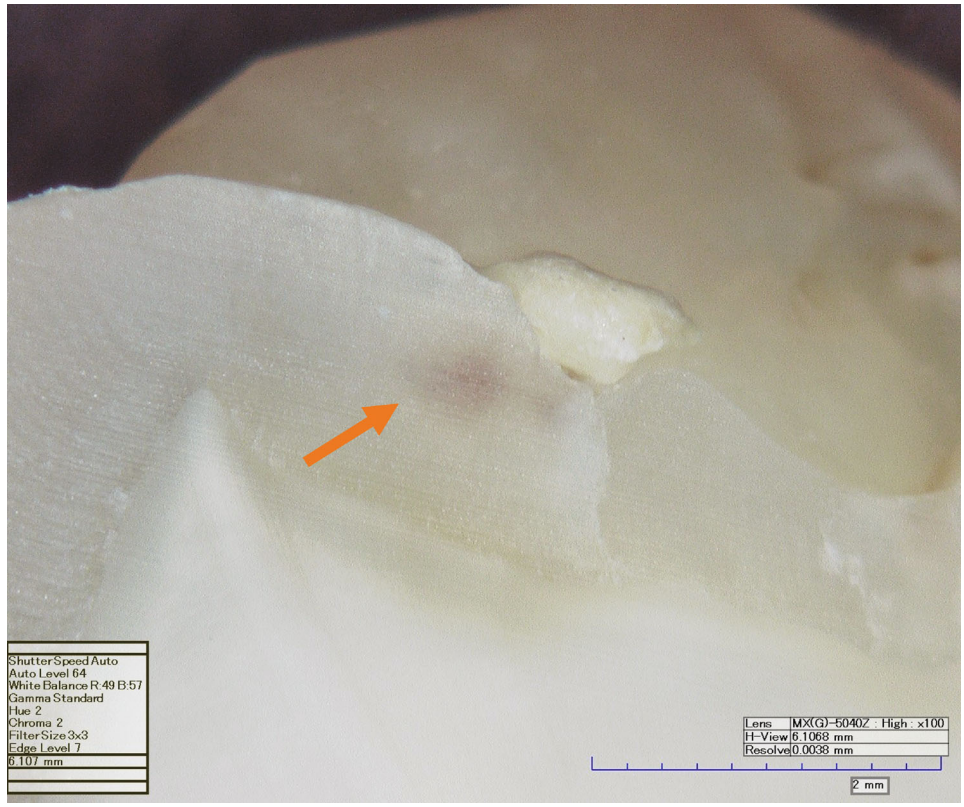
Iranzo-Cortés et al. [45] conducted a study focusing on occlusal caries by comparing the performance of ICDAS II and DDPen. The study concluded that the adjunct use of DDPen for the diagnosis of carious lesions was advisable. The results showed that DDPen demonstrated higher sensitivity, and ICDAS-II demonstrated higher specificity, following the current study results. A balance between sensitivity and specificity of a diagnostic tool is desired to ensure correct diagnosis and effective treatment planning. The current study found higher sensitivity of DDPen, which can sometimes lead to excessive treatment, which might not be deemed necessary when evaluated clinically.

On the other hand, a higher specificity is desirable to ensure minimal false-positive cases, which was found true in the case of ICDAS II. A possible explanation of this finding is the subjective nature of ICDAS criteria, which provides clinicians with a specific demarcation of disease progression. Similarly, Akgul et al. [1] performed a diagnostic evaluation of incipient carious lesion using visual inspection (VI), DDPen, DIAGNOdent Camera, and alternating-current-impedance-spectroscopy technique (ACIST). The study concluded that either of the diagnostic devices, when used alone, are not sensitive enough to diagnose incipient carious lesions, and these devices must be used as an adjunct to the traditional visual inspection method. The current study results also warrant the use of DDPen as an adjunct to the ICDAS II scoring system as both methods have their benefits, which add up together.

The spearman rank correlation coefficient was calculated for different lesion depths based on Downer's histological classification. The rank score of DDPen ranged from 0.774 to 0.950 compared to the ICDAS-II rank score ranging from 0.601 to 0.769. However, these results revealed the potential of ICDAS-II and DDPen to detect remineralisation. Another notable finding is that the most significant difficulty in diagnosing carious lesions was observed in deeper carious lesions with the lowest accuracy. The results revealed that DDPen could be utilised as a valuable clinical tool to detect remineralisation and monitor the progress of remineralisation of a particular lesion.

Alomari et al. [46] carried out a laboratory study evaluating the effectiveness by measuring the sensitivity and specificity of visual-only, visual + radiography, visual + radiography + DDPen for diagnosing noncavitated occlusodentinal caries. The study concluded that although the use of radiography and DDPen adjunctively had a benefit, the *Az* value did not present a statistically significant difference, meaning that the diagnosis and decision-making were not influenced in the three study groups. In comparison, our study focused on carious lesions and found that DDPen was significantly (rank correlation = 0.738) more effective in diagnosing cavitated carious lesions when compared with ICDAS-II (rank correlation = 0.430), which warrants the use of DDPen in cavitated lesions as an adjunct. The higher accuracy of DDPen and electronic caries monitor has also been previously reported [47]. A possible explanation of this difference might be the colour of the lesion, which can mask the true nature of a lesion, hence, making it difficult to diagnose using only visual examination (ICDAS-II). The DDPen uses fluorescence which makes the diagnosis of a lesion effortless; however, it can also lead to an under-or over-estimation. Another explanation is the subjective nature of ICDAS-II, which is not so in the case of DDPen, which uses a pinpoint location to quantify the extent of a lesion. Another notable finding is that the most significant difficulty in diagnosing carious lesions was observed in deeper carious lesions with the lowest accuracy. A possible explanation is the fewer samples in the D3 level. Different cut-off values can be tested to increase the specificity of DDPen at the dentine level. Lussi et al. [33] also found lower specificity of DDPen at dentine level; however, no explanation of this phenomenon was hypothesised. The depth of a carious lesion might influence the absorbance of the laser, influencing the fluorescence of deeper carious lesions.

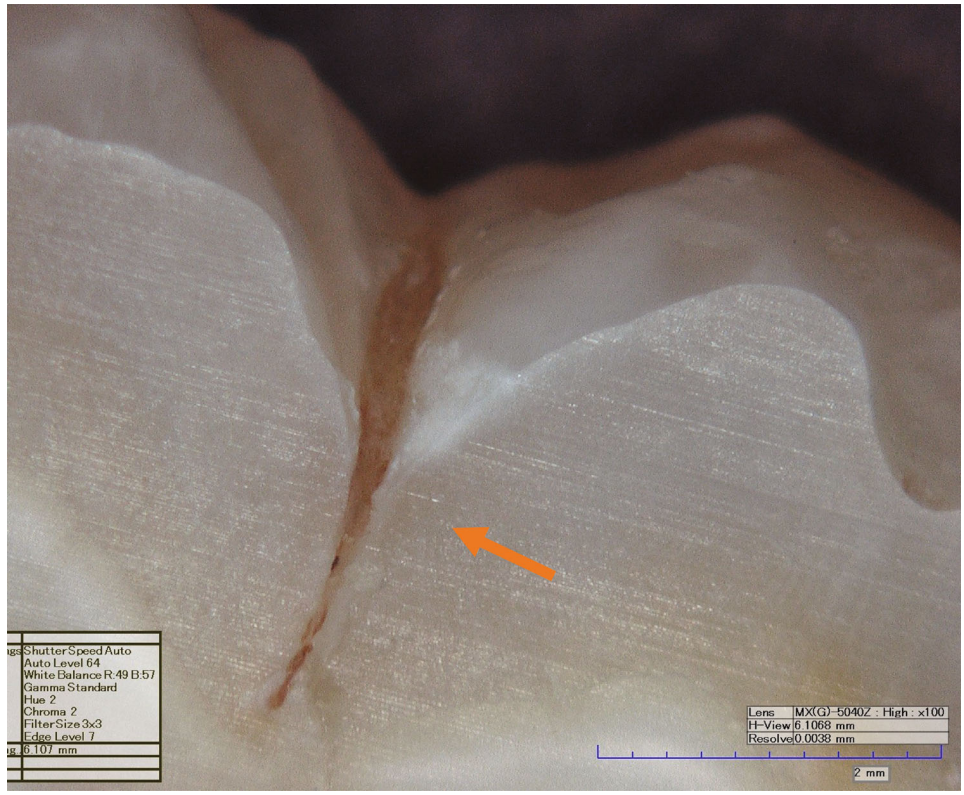
A change in either pH or the presence of free radical ions can create a desired anticariogenic environment, as previously noted in the literature [48, 49]. Another explanation is the limitation of assessing the effect of GC Tooth Mousse Plus® just by focusing on remineralisation. Other assessable properties include surface microhardness, pH cycling model, and salivary chemical analysis. These properties might reveal a benefit of using GC Tooth Mousse Plus® over conventional fluoride varnish. Although the current study did not find a




(a)


(b)

Figure 2: Continued.




(c)

Figure 2: Histological section from Hirox digital microscope (KH-7700) (100x magnification) of tooth showing lesion at Downer's classification level (a) D1, (b) D2, and (c) D3.

significant difference between GC Tooth Mousse Plus® and Duraphat® regarding the remineralisation of cavitated carious lesion, one of the CPP-ACP advantages is an extended periodic release of calcium and phosphate ions which pose an anticariogenic effect. However, the current study design did not consider the role of free radical ions or the long-term effect of CPP-ACP over a three- or six-month period, which has been explored previously in the literature [50]. Future studies can focus on a clinical study design that is relatable to real-life clinical practice. The parallel comparison of other diagnostic devices and the currently employed options would allow better comparability of sensitivity and specificity of diagnostic device options. Salivary constituents of pre- and post application of remineralising agents over more extended follow-up periods might reveal exciting findings that can contribute towards the prevention of the carious process.

## 5. Conclusion

The ICDAS-II scoring system and DDPen scoring were able to detect remineralisation. A cut-off value of 0-13 denotes sound tooth, 14-20 denotes enamel caries, and >21 denotes dentine caries. The DDPen scoring had a higher-ranked correlation with Downer's histological scoring as compared to ICDAS-II scoring. The area under the ROC curve revealed that DDPen scoring demonstrated higher sensitivity while ICDAS-II scoring demonstrated greater specificity to detect remineralisation.

## Data Availability

The data set used in the current study will be made available at the reasonable request.

## Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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*Research Article*

# The Use of Hall's Technique Preformed Metal Crown (HTPMC) by Pediatric Dentists in Malaysia

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*Introduction*. Hall's technique preformed metal crown (HTPMC) has been used widely by pediatric dentists in developed countries as a new approach for managing decayed primary molars without local anesthesia, caries removal, and tooth preparation. Currently, inadequate information is available regarding the implementation of this technique (HTPMC) in Malaysia. This study is aimed at evaluating the implementation of HTPMC by Malaysia's pediatric dentists and identify the co-occurrence frequencies of the HTPMC implementation with the respondents' demographic profile. *Materials and Methods*. A cross-sectional questionnaire-based research was conducted among 65 pediatric dentists in Malaysia. Online questionnaires were distributed to the pediatric dentists employed at public hospitals (MOH) and universities in Malaysia. *Result*. It was found that over half of the respondents (65.6%) employed HTPMC. The analysis of the co-occurrence network frequency revealed that a high frequency of female pediatric dentists who were within the age group of 31-40 years old had fulfilled their postgraduation overseas and was employed in the university mainly applied HTPMC. *Conclusion*. The application of HTPMC among respondent pediatric dentists in Malaysia was high. However, most respondents considered HTPMC a treatment option only to manage carious primary molar rather than a treatment of choice.

## 1. Introduction

Hall's technique preformed metal crowns (HTPMC) are used widely in developed countries, including the United Kingdom, United States, and Germany [1]. An increase in the application of HTPMC takes place worldwide despite past controversies regarding its success rate. Although many studies have demonstrated its high success rate in managing primary carious teeth, the resistance to using HTPMC remains among pediatric dentists [2]. This resistance among some practitioners is primarily due to complex technical and aesthetic concerns [2]. In 1997, a general dental practitioner, Dr. Norna Hall from North Scotland, applied an unconventional method to treat the carious primary molars with a preformed metal crown. In this case, a preformed metal crown was used without administrating local anesthesia,

removing caries, or preparing the tooth [1]. Conventionally, preformed metal crowns are placed on primary molars after local anesthesia and crown preparation in young children.

In contrast, provided that HTPMC requires minimum intervention for managing carious primary molars, this technique could be used on especially young children. HTPMC effectiveness is based on the sealing of carious tooth with a preformed metal crown, thus preventing microorganisms from their origin of nutrition, dietary carbohydrate, and the advancement of caries [3, 4]. Besides, a systematic review has demonstrated that the avoidance of caries removal could prevent pulpal exposure [5].

Based on a comparison by Santamaria et al.'s (2018) study comparison between HTPMC and the conventional methods, it was found that HTPMC showed a better outcome and more extended longevity compared to the conventional restorations [6]. It has been proven that the HTPMC shows successful management of dental caries in a primary molar. In contrast, another research has demonstrated that GDP, the patients, and their parents preferred HPTMC compared to the conventional restorative approaches for managing primary carious molar [7]. A recent study was performed in Germany to assess the implementation and perspectives of the preformed metal crown by pediatric dentists. It was found that 77% of the respondents did not have familiarity with HTPMC, while 66% of the respondents did not employ HTPMC as this technique was found to be challenging and aesthetic [6].

Malaysian pediatric dentists have been practicing HTPMC in the management of primary carious molar. However, the information regarding the implementation of HTPMC in Malaysia is inadequate. Following a literature review, it was seen that no research had been carried out on the use of HTPMC in Malaysia. This study is aimed at examining the generalizability of the implementation of HTPMC by pediatric dentists in Malaysia. This study would assist in identifying the use rate and the acceptability of HTPMC among dentists, as mentioned above.

## 2. Materials and Method

*2.1. Ethical Consideration.* The research was approved by the responsible committee on human research and ethics, with the research protocol code of USM/JEPeM/19010099. Informed consent was acquired from all the parties involved in the research. Given that the information and responses were regarded as fully confidential and anonymous, the research team members were the only parties allowed to access the information.

*2.2. Subjects.* A total of 45 pediatric dentists offered services under the Ministry of Health (MOH)/Kementerian Kesihatan Malaysia (KKM). On the other hand, the Malaysian Dental Council retained 33 pediatric dentists from private and government universities. It was predicted that the pediatric dentists registered under the hospitals and universities in Malaysia would amount to 78. After incorporating 10% of the nonresponse rate, the purposive selection was performed on 65 pediatric dentists from Malaysia's hospitals and universities to evaluate the application of HTPMC.

*2.3. Sample Size Calculation.* A single proportion formula was applied to compute the sample size to identify the prevalence of HTPMC usage among 78 pediatric dentists in Malaysia. With a predicted prevalence of 96%, the population of pediatric dentists who employed HTPMC as an option of treatment to manage caries in the primary molar was according to Roberts et al.'s (2018) research.

$$n = \left(\frac{Z}{\Delta}\right)^2 p(1-p), \tag{1}$$

where n is the sample required, Z is the normal standard deviation corresponding to 95%confidence interval = 1.96, $\Delta$ is the research precision, which would be 5% of this research as per the recommendation by WHO guidelines for oral health surveys, and $P$ is the population proportion needed according to past research findings.

Following past research findings, 96% of pediatric dentists were employing HPTMC as an option of treatment to manage caries in primary molars [8]. Therefore, the precision of 0.05 at 95% of the confidence interval the total sample size ($n$) was as follows:

$$n = 0.96\,(1 - 0.96)\,(1.96/0.05)^{2=5}9. \tag{2}$$

The sample size with a precision value of 0.05 amounted to 59. Taking into account the 10% nonresponse rate, the final sample size amounted to 65.

*2.4. Study Design.* This cross-sectional research was conducted among the pediatric dentists in Malaysia who work under hospitals and universities. An online survey questionnaire, which comprised 16 close-ended questions, was adopted from the previous research by Roberts et al. 2018 [8]. Pretest and modification of the questionnaire were conducted accordingly. The questionnaire consisted of three sections: demographic characteristics, the use of HTPMCs, and the last section contained questions for the pediatric dentists to answer.

*2.4.1. Demographic Section.* This sociodemographic section presented the participants' characteristics, including age, gender, province, education history, the clinical setting the respondents were employed in, job title, and the years of their practice as pediatric dentists.

*2.4.2. Usage of HTPMC.* The second segment highlighted the questions regarding HTPMC use, which addressed whether this approach was the preferred treatment modality, treatment methods for various categories of carious lesions, and the duration for using the approach. They were also asked whether they would place HTPMC under inhalation sedation and general anesthesia for special-needs children. A question was also included on whether the respondents agreed that HTPMC was suitable to be used by undergraduate students, postgraduate students, dental officers, and postbasic staff nurses.

*2.4.3. Perceived Barriers.* In the last section, a single question is provided that represents the perceived barriers during the use of Hall's technique preformed metal crown. This is a continuation of the first question in the second section. In this section, they need to mention the barriers that could cause the reluctance to use HTPMC.

*2.5. Data Collection.* Data collection began with distributing the data collection sheet (conversion of the sheet into an online survey) to the respondents through the email chosen based on inclusive and exclusive standards. Anonymity was maintained for the entire questionnaires employed in this research, while data entry into the SPSS software was performed. Furthermore, the research team members were the sole parties accessing the data, while data was displayed in grouped data and would not classify the respondents as individuals. Notably, ethical approval from National Medical Research, Malaysia (NMR), was required for respondents from MOH and Malaysia's universities. In this case, data collection would be performed through questionnaire distribution if the respondents did not present a response within 30 days. The respondents were called to be reminded of this matter. A cover letter was included in the survey to elaborate on the purpose of the data collection and the questionnaire distribution to all pediatric dentists in Malaysia.

## 3. Data Entry and Statistical Analysis

Data entry and statistical analysis were performed using SPSS version 24.0. Descriptive analysis was conducted, including the distribution of percentage for demographic profiles and questions associated with HTPMC. Multiple response questions were scored following the frequency of the chosen responses. This was followed by calculation of the frequencies and interpretation of results. Moreover, SPSS software was employed to analyze the co-occurrence frequencies.

## 4. Result

The response rate in this study amounted to 49%, representing 32 out of 65 respondents who submitted the responses online.

*4.1. Demographics.* Most of the respondents (50%) were aged 31 to 40 years old, while nine respondents (28.1%) were aged 41 to 50 years old. The majority of the respondents, which amounted to 27 (84.4%), were presented by female pediatric dentists. Furthermore, 14 respondents (43.8%) served in hospitals that have been registered under the Ministry of Health (MOH), while 18 respondents (56.3%) served in private and government universities. Eleven respondents (34.4%) served as lecturers in universities, nine respondents (28.1%) were employed as specialists, and five respondents (15.6%) served as consultants in Malaysia's hospitals. Nine respondents (28.1%) worked for over 20 years, while eight (25%) gained 11-15 years of work experience in practice. Following that, 24 respondents (77.4%), the majority, had fulfilled postgraduation overseas, while seven respondents (22.6%) had fulfilled postgraduation from Malaysia.

*4.2. Usage of HTPMC.* 21 out of 32 pediatric dentists are using HTPMC in their regular practice, which indicated the frequent use of HTPMC among the respondent pediatric dentists in Malaysia in their regular practice. However, this result did not apply to all pediatric dentists in Malaysia. Meanwhile, 14 respondents (66.7%) mentioned using HTPMC as a treatment option for managing a carious primary molar. On the other hand, four respondents (19%) want to use HTPMC as a treatment of choice for managing carious primary molar, and seven respondents (33.3%) want to use HTPMC upon facing failure in managing the carious tooth with the conventional approach. Generally, treatment of choice refers to choosing HTPMC as a primary treatment for managing carious primary molar over the conventional restorative methods, whereas treatment option means they only choose HTPMC as an alternative or secondary treatment when conventional treatment with the bur was impossible.

Among the 21 respondents, 10 (47.6%) mentioned that they would prefer to apply HTPMC in cavitated interproximal carious teeth, while four respondents (19.0%) mentioned using HTPMC in cavitated occlusal carious teeth. However, five respondents (23.8%) did not prefer the application of HTPMC for noncavitated occlusal carious teeth, while two respondents (9.5%) did not wish to employ it for noncavitated interproximal carious teeth (Table 1). Following that, Table 2 demonstrates that most respondents (20 respondents, 95.2%) would like to use HTPMC for special needs children. However, 16 respondents (76.2%) preferred fitting the metal crown under inhalation sedation, while five respondents (23.8%) wished to apply it under general anesthesia.

From the answers by 19 pediatric dentists (95%), it was perceived that HTPMC is suitable for postgraduation, while 12 (60%) agreed that it is acceptable for the undergraduate curriculum. It was observed that dental officers 95% (19) and postbasic staff nurses 40% (8) could be trained using HTPMC. Table 3 illustrates that 44.4% of the respondents did not wish to use the separators or obtain X-Rays when HTPMC was used.

*4.3. Co-Occurrence Frequency Analysis.* The co-occurrence frequencies of HTPMC and demographic profile demonstrated a high-frequency network (indicated by dark line) between respondent female pediatric dentists aged from 31 to 40 years old, fulfilled their postgraduation overseas, and applied HTPMC upon work in the university (Figure 1).

## 5. Discussion

In this study, the sample size consisted of 65 pediatric specialists, and a low response rate of 49% was recorded. However, 41% and 38.9% response rates were recorded in past questionnaire-based research studies on using HTPMC [6, 8]. Therefore, the response rate from this study was comparatively higher. A study by Cunningham et al. (2015) highlighted that 35% of the response rate is comparable for online surveys to eliminate the issue of response bias [9]. For the current study, the response rate mentioned earlier

Table 1: Frequency of using HTPMC in a given situation.

| | *a* | *b* | *c* | *d* |
|---|---|---|---|---|
| Never | 23.8% (5) | 0% | 9.5% (2) | 0% |
| Rarely | 47.6% (10) | 19.0% (4) | 19.0% (4) | 0% |
| Sometimes | 28.6% (6) | 62.0% (13) | 62.0% (13) | 52.4% (11) |
| Always | 0% | 19.0% (4) | 9.5% (2) | 47.6% (10) |
| Total | 100% (21) | 100% (21) | 100% (21) | 100% (21) |

a: the use of HTPMC in noncavitated occlusal carious teeth; b: the use of HTPMC in cavitated occlusal carious teeth; c: the use of HTPMC in noncavitated interproximal carious teeth; d: the use of HTPMC in cavitated interproximal carious teeth.

Table 2: The situation for the use of HTPMC.

| | Special need children | Under inhalation sedation | Under general anesthesia |
|---|---|---|---|
| Yes | 95.2% (20) | 76.2% (16) | 23.8% (5) |
| No | 4.8% (1) | 23.8% (5) | 76.2% (16) |

Table 3: Frequency of pediatric dentists who do not use the following steps for HTPMC placement.

| Variable | Protocol | Responses | |
|---|---|---|---|
| | | N | Percentage |
| Take consent from parents (verbal or written) | | 1 | 11.1% |
| Placement of separators | | 4 | 44.4% |
| X-rays (OPG, bitewing, PA view) | | 4 | 44.4% |
| Total | | 9 | 100.0% |

was 49%, which seems low and could have been higher had we met the pediatric dentists personally for data collection. The sample size of the present survey was restricted to pediatric dentists employed in universities, public hospitals, and private clinic practitioners excluded from the survey. Several research works were performed among the general dental practitioners and private clinic practitioners (Dean et al., 2011; John, 2016; Santamaría et al., 2018), while this study involved a sample size of 65. The predicted number of registered pediatric dentists under universities and public hospitals in Malaysia was 78, which was adequate for conducting this research by omitting private practitioners.

The study results suggested that there was high usage of HTPMC amongst the respondent pediatric dentists in Malaysia. However, most of them would apply HTPMC to manage carious primary molars instead of the chosen treatment or primary treatment option. This research highlighted that several practitioners perceived the "second-best" feature about the HTPMC, which is applied only when other techniques are not applicable. The online survey performed by Hussein et al. (2020) to evaluate global pediatric dentist opinions/use of HTPMC was only used by over half of those respondents. Conventional restorations remained the preferred option even among HT users [10]. On the other hand, in a recently conducted systematic review, the authors mentioned that HT remained the preferred option among the general dentist. Moreover, the study also mentioned that the questionnaire-based study showed better acceptance than face scale-based evaluation [11].

Approval was made by specialists on the general use of HTPMC, with most of them suggesting the training for dental officers. Furthermore, half of the specialists highlighted the importance of HTPMC training among the undergraduate and postgraduate students for the early exposure of HTPMC. Given that HTPMC is a noninterventional treatment, convenient to conduct, and practical, few pediatric dentists recommended training primary staff nurses on HTPMC. It was believed that the use of HTPMC would encourage more practice and could be a favorable treatment option for the management of carious primary molars.

It was found that the use of HTPMC in cavitated lesions and interproximal lesions is more frequent based on Hall's technique manual, which suggests the use of this technique in the presence of two surface lesions or extensive one surface lesions. Contrary to the manual, which recommends partial caries removal and sealant for noncavitated occlusal cavities, including sealant only for cavitated occlusal cavities, a notable amount of respondents employed HTPMC for noncavitated and cavitated occlusal cavities [8]. This condition could be attributed to the convenience of fitting HTPMC in comparison to partial caries removal. A majority of the children in Malaysia are classified as high caries risk; thus, the placement of HTPMC for noncavitated and cavitated occlusal cavities is more reasonable for preventing sealant failure than sealants.

A majority of the children arranged for oral rehabilitation under general anesthesia (GA) were recorded with negative behavior towards dental treatment, which led to challenging extraction of radiographs during the arrangement for treatment [12]. Based on Hall's technique manual, a thorough assessment that includes a radiograph must be performed to omit the irreversible pulpal involvement before the Hall crown is placed. It was observed from this research that the respondents were not willing to employ HTPMC under the GA setting; although, they were highly accepting of the success rate of HTPMC. The absence of radiographs could cause this condition during GA.

Malaysia's Ministry of Health currently promotes oral healthcare services for special needs children [13]. Given that these children belong to the group with high caries risk, they require a durable restorative material to restore carious teeth. Furthermore, most special-needs children display challenging behavior, which was a factor for the majority of the respondents (95.2%) in this research to employ HTPMC for special needs children due to the convenience in conducting HTPMC and its remarkable longevity. In a recent study on European postgraduate pediatric dentistry students, HTPMC was chosen as an option more often for anxious children than children who were not anxious, i.e., not as the treatment of choice for nonanxious children [14].

Notably, HTPMC is a treatment option for anxious children with a high success rate, suggesting that future

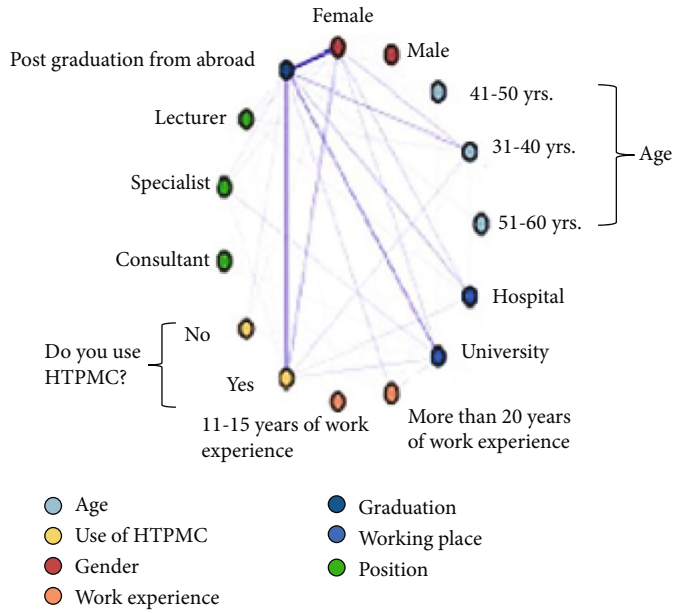


Figure 1: Co-occurrence frequency network between the use of HTPMC with the demographic profile of the respondents.

specialists are more pragmatic in employing this method. However, it has been observed that a low number of specialists (34.4%) remain reluctant to use HTPMC as they do not prefer to fit the crown without the removal of caries. In a questionnaire-based study performed in Scotland, it was found that 48% of respondents employed HTPMC, while the respondents who did not do so had a preference for further training [15]. Despite identifying the effectiveness of HTPMC compared to conventional restorations, the factors of specialists' refusal to use it could not be determined. The current systematic review of Jesmin et al. (2020) demonstrated that HTPMC led to responses ranging from "no discomfort" to "mild discomfort" compared to conventional restorations [16].

According to the co-occurrence frequency analysis, an established relationship was present between the demographic profile of the respondents upon the application of HTPMC. The frequency network (Figure 1) demonstrated that the respondent female pediatric dentists aged from 31 to 40 years old, having fulfilled their postgraduation overseas and worked in the university, showed more frequent use of HTPMC. Moreover, the respondents of this research, who had fulfilled their postgraduation from Europe, showed confidence regarding this technique. Besides, they reported an equal use of HTPMC in their routine practice.

In a recent study, the author mentioned that most pediatric dentists across the globe do not use HT. Moreover, only half of the pediatric dentists who participated in that study used HT for treating carious primary molar [10]. The current study result has shown a similar output pattern; the Malaysian pediatric dentists showed reluctance to use HTPMC to treat carious molar after having enough evidence of its success rate.

Previously, Midani et al., a retrospective study was performed to assess the success rate of standard HT and modified HT. The modified HT involved proximal tooth slicing, allowing the PMC to fit without separation and providing minimal occlusal cusps reduction without caries removal and local anesthesia. The study result showed that comparing crowns performed with no tooth preparation to crowns performed with proximal slicing, and no differences were observed [17]. Identifiable barriers such as lack of training, substandard dentistry, and perceived lack of evidence reduced its use. Therefore, it was believed that the HTPMC is not used primarily due to inadequate training or confidence in the available evidence.

*5.1. Limitations.* As explained in the discussion, the response rate for this study was 49%, which could have been improved if the data collection had been done physically instead of using an online survey. Also, in this case, online data collection was the only feasible method for data collection, considering the current situation. It has been seen that health professionals usually offer a low number of responses to online surveys [15]. Moreover, the dentists who responded to the survey are more likely to be interested in HTPMCs than those who did not respond. In addition to that, we can assume that the nonrespondent of this study may have less interest in using HPTMC for managing carious primary molars. The nonrespondent bias may affect the overall outcome of the current study.

*5.2. Recommendations.* For future studies, general dental practitioners in Malaysia can be included in the sample to assess the knowledge, attitude, and practices regarding HPTMC usage among Malaysian dentists. The data obtained from this study can also be utilized for carrying out multicultural studies to analyze differences in HTPMC usage across different countries.

## 6. Conclusion

A notable result was found in this study that the respondent pediatric dentists in Malaysia preferred HTPMC. In addition to that, from the demographic assessment, we found that the respondents using HTPMC to manage carious premolars are mostly aged from 31 to 40 years old, having fulfilled their postgraduation overseas and working under the university.

## Data Availability

Any data used in the current article can be provided upon request from the corresponding author.

## Ethical Approval

Ethical approval was gained regarding the research processes by the committee in charge of human research and ethics, with the study protocol code of USM/JEPeM/19010099.

## Disclosure

The manuscript has been presented as a thesis at the School of Dental Sciences, Universiti Sains Malaysia, towards the fulfillment of master of science in dentistry course requirement.

## Conflicts of Interest

There was no conflict of interest nor significant financial support present in this work to affect its results.

## Supplementary Materials

The Supplementary file consists of the questionnaire used in the current study. *(Supplementary Materials)*

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*Research Article*

# Impact of Oral Health Educational Interventions on Oral Hygiene Status of Children with Hearing Loss: A Randomized Controlled Trial

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*Introduction*. Oral health is considered as one of the essential components of the overall health of every individual. Maintaining oral health is a gradual process that requires commitment. Children who require special care such as hearing impairment experience difficulty in maintaining oral health primarily due to communication difficulties. This study is aimed at using different interventions to evaluate the improvement of oral hygiene in hearing impaired children. *Materials and Methods*. Fifty-nine children were recruited in this study that were allocated randomly into each group with twenty children as follows: group 1: pictorial, group 2: video, and group 3: control. Mean plaque and gingival scores were noted before and after the use of different interventions. Oral hygiene was categorized as "excellent," "good," and "fair." Gingival health was categorized as "healthy," "mild gingivitis," and "moderate gingivitis." *Results*. Thirty-four children (57.6%) were from 12-13 years of age bracket, and 25 (42.4%) belonged to 14-16 years of age. Regarding gender, there were 37 (62.7%) males and 22 (37.3%) females. About comparison of mean gingival and plaque scores before and after interventions in each group, a significant difference was found in group 1 ($p < 0.001$) and group 2 ($p < 0.001$), as compared to group 3 where the difference in scores was not significant ($p > 0.05$). *Conclusion*. Maintaining oral health requires the compliance of individuals to perform different methods of preventive dentistry, such as tooth brushing and use of dental floss. The use of different oral hygiene educational interventions such as pictorial and video methods have been proven and useful for hearing impaired children in improving oral health.

## 1. Introduction

Oral health is one of the most fundamental elements for maintaining the general physical health and well-being of every individual. A healthy mouth performs many of the vital functions such as eating, speaking, and participating in facial expressions effortlessly. The most visible part of the mouth in terms of esthetics is the teeth. Teeth are the first thing that people notice when it comes to socializing. Healthy and decay-free teeth are able to perform many of

their functions such as mastication and speech, along with providing self-confidence to individuals, as esthetics is equally important to many individuals [1].

The children are more prone to develop oral health problems primarily due to the lack of attention being given by them, as compared to adults who are well mature to understand the importance of healthy oral hygiene. Furthermore, children who require special care in order to maintain their oral hygiene are even more vulnerable to develop oral health difficulties because of the illness they suffer from [2].

Hearing disability is one of the major disabilities that is faced by children globally [3]. According to one study, there are 23 to 25 thousand children aged between 0 and 15 years in the United Kingdom who have a hearing disability [3]. Children who are born with deafness as a disability or those who acquire it at any period of time undergo a series of traumatic episodes which makes taking care of oral hygiene an insignificant aspect of life [4]. A child whose hearing has been impaired due to any reason endures a difficult time socializing with people, learning, communicating, and cognitively lacks behind normal children [5].

Educating children as well as adults has been a vital part of preventive dentistry that has evolved over a period of time in order to improve the oral hygiene of individuals [5]. Preventive dentistry begins at the level of primary school that includes a visual presentation of brushing and the importance of oral health through various educational videos [6]. Furthermore, children are also encouraged to participate in various school-based activities which are part of their school curriculum such as poster competitions, essay writing, and oral presentation on various aspects of the importance of oral health [7]. However, such strategies for children with impaired hearing have not been very useful. Many different strategies have been explored and put to work that is described in various studies such as using playful learning interventions for children [8].

Normally, many oral diseases such as dental caries, gingivitis, periodontitis, and even dental fracture can easily be prevented in children, but due to hearing disability, these children are more prone to suffer from such dental diseases [9]. Numerous methods can be used to maintain adequate oral hygiene such as toothbrushes, dental floss, chewing gums, interdental toothbrushes, mouthwashes, and dentifrices as well [9]. However, for these interventions to be beneficial, manual dexterity is the main hindering factor for the children with impaired hearing as communicating with them to explain the importance of each of these interventions is a challenging task [10]. According to American Dental Association (ADA) and British Dental Association (BDA), dental floss along with toothbrushes has been recommended to be daily used by all individuals [11].

In this study, we aimed to compare the efficacy of pictorial and video demonstration methods as oral health education interventions, to evaluate changes in oral hygiene of children who had a hearing impairment.

## 2. Materials and Methods

*2.1. Study Setting and Sample Size Calculation.* This randomized controlled trial study was carried out from October 2019 to January 2020 in 3 different schools of Karachi, Pakistan, that were dedicated to the education of children who require special care. To calculate the sample size, OpenEpi software was used keeping the confidence interval at 95% and power of test 80%. The sample size was calculated to be 60 participants (20 in each of the 3 groups) [12].

*2.2. Ethical Consideration and Participant Recruitment.* The ethical approval was granted by the ethical review committee of Altamash Institute of Dental Medicine, Pakistan; number (AIDM/ERC/02/2019/07). The trial was registered under "clinicaltrials.gov" (United States National Library of Medicine). This study is composed in line with CONSORT guidelines for reporting clinical trials [13]. The protocol of the study was explained to parents of all the children included in this study, as well as the administration of the school. After explaining the protocols of this study, written and verbal consent was taken. Hearing-impaired children who were aged between 12 and 16 years of age who showed a willingness to participate in this study were recruited. Furthermore, children with no systemic illness, with plaque adhering to the free gingival margins more than one-third and more than two-thirds of the tooth surface. The children with mild, moderate, and severe gingivitis and those being able to comply with the protocols of this study were included in this study. Those children who required antibiotics prior to the start of the study and with extensive dental calculus were excluded from this study.

*2.3. Sampling Technique.* In this study, a purposive sampling technique was used; there were twelve schools for disabled children in Karachi city. Out of these twelve, three schools IDAREU, Deaf Reach, and JS Academy for deaf agreed to participate in the study that is why these three were selected. The Deaf Reach School had a total of one-hundred and eighty-six hearing impaired children; out of them, sixty were between 12 and 16 years of age; IDAREU had three hundred and fifty hearing impaired children; out of them, sixty were 12-16 years of age; and JS Academy had one-hundred and fifty hearing impaired children; out of them, 51 were 12-16 years of age.

*2.4. Grouping and Randomization of the Participants.* The eligibility criteria were applied to all the two-hundred and seventy-one hearing impaired children with 12-16 years of age. Seventy-four children were found eligible for this trial. Out of these seventy-four children, through randomization, sixty children were selected using a `random number table by creating a numbered list. Out of the sixty children selected, randomly, each of the twenty children were allocated to three different oral health educational interventions such as group 1 (pictorial), group 2 (video), and group 3 (control) as shown in Figure 1. Participants in group 1 were assigned a pictorial method of oral hygiene intervention. They were shown various pictures of ways to maintain oral hygiene such as brushing techniques, use of mouthwash, and dental floss. Participants in group 2 were assigned a video playing method of oral hygiene intervention, and

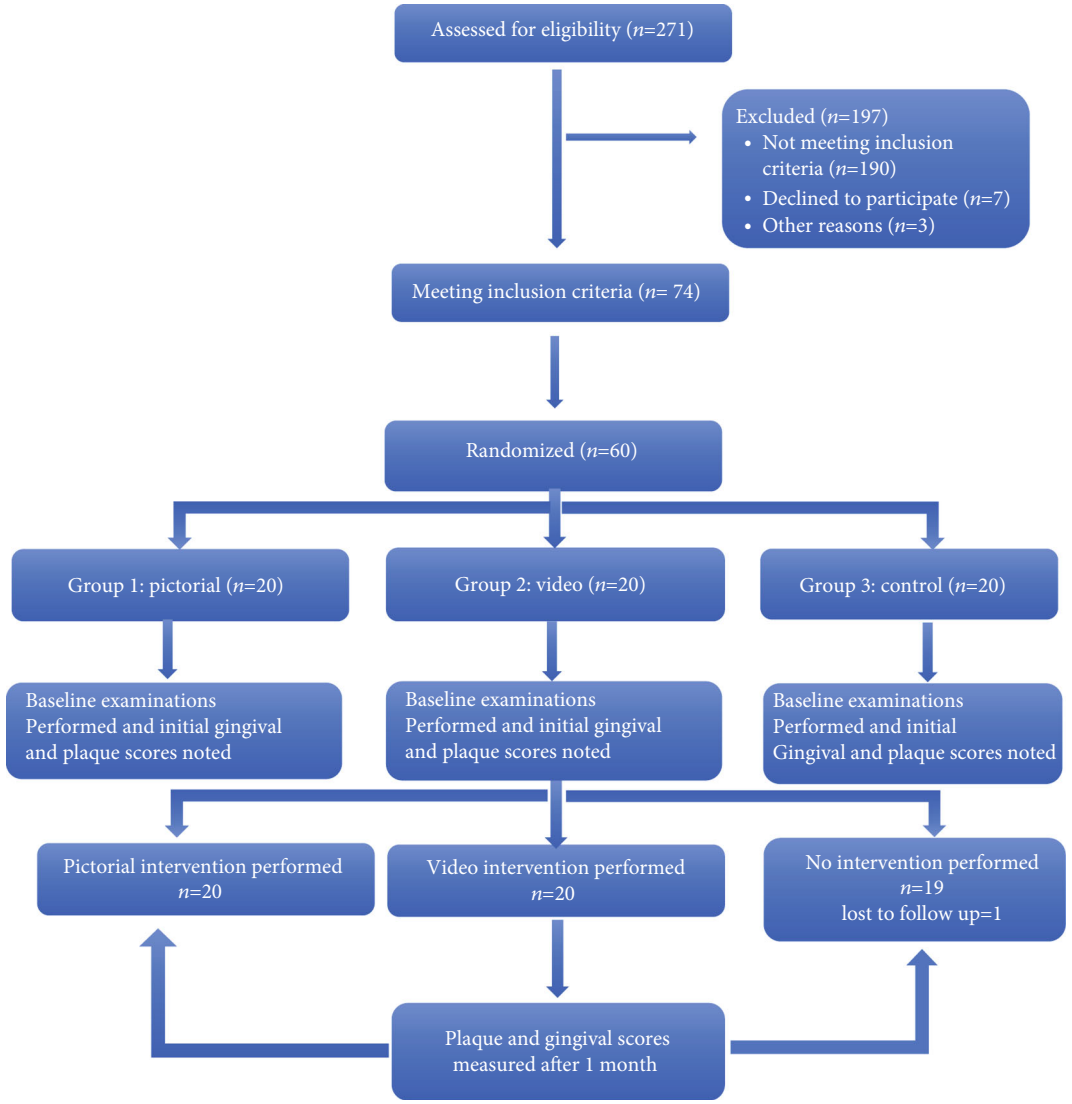


Figure 1: CONSORT flow diagram of the study.

participants in group 3 did not receive any intervention during the study.

*2.5. Application of Oral Hygiene Educational Intervention.* To assess the current oral hygiene of the participants in each group, baseline examinations were performed using sterilized dental instruments on a wooden chair using natural sunlight. A proforma was designed that included personal details of the participants along with oral examination tools. The current plaque and gingival scores of the participants were recorded using plaque index (PI) [14] and gingival index (GI) [15] with the Michigan O probe that is recommended by the World Health Organization (WHO). The plaque and gingival scores were documented on the mesial, distal, and middle surface of the buccal and lingual surface of six index teeth which are 16, 12, 24, 44, 32, and 36, respectively. A total of six measurements were taken, and their average score was then calculated. The clinical oral examination of each of the participants was performed by two examiners (M.M.) and (S.R.), with the examination of all index teeth done, and an average score was calculated for each participant in this study. The oral hygiene of each individual was categorized by oral hygiene score as follows: 0 = excellent, 0.1-0.99 = good, 1.0-0.99 = fair, and 2.0-3.0 = poor. The gingival status of each individual was categorized by gingival score as follows: 0 = healthy gingiva, 0.1-1.0 = mild gingivitis, 1.1-2.0 = moderate gingivitis, and 2.1-3.0 = severe gingivitis.

After the clinical oral examination of the children was performed, they were randomly assigned to each of the three groups. All of the children in each of the 3 groups received a toothbrush as a standard. For children in group 1, they received tooth brushing instructions by formulating laminated cards that presented each step of performing tooth brushing as they were asked to take the cards with them home and mimic the steps shown in the pictures while they brush their teeth. For group 2, a video demonstration method of oral hygiene intervention was used where the children were shown a video of 10 minutes where a cartoon character presented ways of tooth brushing and showed the

importance of having good oral hygiene, and the children were then asked to see the video and mimic the steps shown to them while they brush their teeth. In both pictorial and video demonstration methods, the recommended tooth brushing technique was "horizontal scrub technique" and the duration and frequency of the toothbrush were set at twice daily for two minutes. For group 3, these children only received a toothbrush, and they were not provided with any sort of brushing technique instructions.

*2.6. Motivation Sessions.* In order to keep the children motivated and encouraged to follow their assigned intervention, motivational sessions were kept for them by making them repeat either the pictorial or video method depending on their groups. Two motivational sessions were kept, with a gap of 12-14 days between each of the two sessions for one month. However, children in group 3 did not receive any motivational sessions. The motivation sessions were performed in the first, second, and third month of data collection. The sessions were performed in the three different schools.

*2.7. Follow-Up Examination.* After a period of 1 month, the plaque and gingival scores in all of the participants in the 3 groups were recorded to assess the impact of oral hygiene education interventions, and these scores were then compared to the baseline scores that were recorded at the beginning of the study. The changes in plaque index and gingival index before (preintervention) and after (postintervention) the oral hygiene education were compared in each of the 3 groups.

*2.8. Statistical Analysis.* For the statistical analysis, Statistical Package for the Social Sciences software (IBM, SPSS Statistics, version 25, Chicago, Illinois, United States) was used. The mean values and standard deviation were calculated for plaque and gingival scores along with descriptive analysis such as frequencies and percentages of the given data. In each of the groups, preintervention and postintervention scores were recorded. A one-way ANOVA test was used to see any statistically significant difference between preintervention and postintervention scores within and between each group. A $p$ value of ≤0.05 was considered to be as statistically significant.

## 3. Results

In this study, a total of sixty children were recruited; out of which, fifty-nine successfully completed the study due to one child being lost in follow-up after 1 month, due to unknown reasons. Of the 59 children, there were 37 males (62.7%) and 22 females (37.3%). Regarding age, 34 (57.6%) belonged to 12-13 years of age; 25 (42.4%) belong to 14-16 years of age. In group 1 (pictorial), there were 20 hearing impaired children; in group 2 (video) there were 20 children, and 19 children in group 3 (control). In both groups 1 and 2, there were 12 children aged between 12 and 13 years and 8 children between 14 and 16 years of age. For group 3, there were 10 children aged between 12 and 13 years and 9 children between 14 and 16 years of age. About the gender of each group, there were 11 males and 9 females in groups 1 and 2 and 15 males and 4 females in group 3 (as presented in Table 1).

Table 1: Age and gender distribution of different groups ($n = 59$).

| Demographics | Group 1 $n$ (%) | Group 2 $n$ (%) | Group 3 $n$ (%) |
|---|---|---|---|
| Age | | | |
| 12-13 years | 12 (60.0%) | 12 (60.0%) | 10 (52.6%) |
| 14-16 years | 8 (40.0%) | 8 (40.0%) | 9 (47.4%) |
| Gender | | | |
| Male | 11 (55.0%) | 11 (55.0%) | 15 (78.9%) |
| Female | 9 (45.0%) | 9 (45.0%) | 4 (21.1%) |

For group 1, the mean plaque score preintervention for group 1 was $1.53 \pm 0.20$, group 2: $1.48 \pm 0.26$, and group 3: $1.50 \pm 0.23$. After the intervention, the mean plaque scores were as follows: group 1: $0.36 \pm 0.26$, group 2: $0.60 \pm 0.40$, and group 3: $0.94 \pm 0.43$. The mean difference in reduction of plaque scores for each group was as follows: group 1: 1.17, group 2: 0.88, and group 3: 0.56. The mean score difference was higher for group 1 (1.17) as compared to group 2 and group 3. A statistically significant difference ($p < 0.001$) was noted between the postintervention levels in the study and control groups, whereas no significant difference ($p = 0.807$) was found in plaque scores at preintervention levels (as presented in Table 2).

For group 1, the mean gingival score preintervention for group 1 was $1.29 \pm 0.22$, group 2: $1.39 \pm 0.30$, and group 3: $1.47 \pm 0.26$. After the intervention, the mean gingival scores were as follows: group 1: $0.29 \pm 0.23$, group 2: $0.44 \pm 0.37$, and group 3: $0.80 \pm 0.42$. The mean difference in reduction of gingival scores for each group was as follows: group 1: 1.00, group 2: 0.95, and group 3: 0.67. The mean score difference was higher for group 1 (1.00) as compared to group 2 and group 3. A statistically significant difference ($p < 0.001$) was found between the gingival scores at postintervention levels (as presented in Table 3).

About the comparison of OHI and GI scores among the 3 group postinterventions, a statistically significant difference was found between group 1 and group 3 ($p < 0.001$). For group 1 and group 2 OHI and GI scores, no statistically significant difference was found ($p = 0.290$). Similarly, a statistically significant difference was found between OHI and GI scores of groups 2 and 3 ($p = 0.015$) (as presented in Table 4).

About the mean plaque scores in terms of gender disparity in study and control groups. The mean plaque scores before and after the interventions in females were as follows: group 1: $1.52 \pm 0.20$ and $0.36 \pm 0.25$, group 2: $1.38 \pm 0.24$ and $0.50 \pm 0.37$, and group 3: $1.58 \pm 0.25$ and $0.89 \pm 0.23$. Furthermore, for males, the mean plaque scores before and after the interventions were as follows: group 1: $1.54 \pm 0.21$ and $0.36 \pm 0.29$, group 2: $1.57 \pm 0.26$ and $0.69 \pm 0.42$, and group 3: $1.48 \pm 0.23$ and $0.96 \pm 0.47$. For females, a statistically significant difference was noted in mean plaque scores amongst the 3 groups after the provision of educational intervention ($p = 0.004$). Similarly, a statistically significant

Table 2: Comparison of mean plaque scores at pre- and postintervention levels in study groups.

| Groups | Plaque scores | | |
|---|---|---|---|
| | Preintervention Mean ± SD | Postintervention Mean ± SD | Pre-post Mean difference |
| Group 1 (pictorial) | 1.53 ± 0.20 | 0.36 ± 0.26 | 1.17 |
| Group 2 (video) | 1.48 ± 0.26 | 0.60 ± 0.40 | 0.88 |
| Group 3 (control) | 1.50 ± 0.23 | 0.94 ± 0.43 | 0.56 |
| $p$ value | 0.807 | <0.001 | — |

SD: standard deviation.

Table 3: Comparison of mean gingival scores at pre- and postintervention levels in study groups.

| Groups | Gingival scores | | |
|---|---|---|---|
| | Preintervention Mean ± SD | Postintervention Mean ± SD | Pre-post Mean difference |
| Group 1 (pictorial) | 1.29 ± 0.22 | 0.29 ± 0.23 | 1.00 |
| Group 2 (video) | 1.39 ± 0.30 | 0.44 ± 0.37 | 0.95 |
| Group 3 (control) | 1.47 ± 0.26 | 0.80 ± 0.42 | 0.67 |
| $p$ value | 0.144 | <0.001 | — |

SD: standard deviation.

difference ($p = 0.007$) was found in mean plaque scores amongst the 3 groups for males. However, at preinterventional levels, no significant difference ($p > 0.05$) was found amongst all 3 groups in both sexes (as presented in Table 5).

About mean gingival scores in terms of gender between the groups, for females, the mean gingival scores before and after the interventions were as follows: group 1: 1.24 ± 0.12 and 0.34 ± 0.24, group 2: 1.31 ± 0.30 and 0.30 ± 0.39, and group 3: 1.52 ± 0.41 and 0.79 ± 0.54. For males, the mean gingival scores before and after the interventions were as follows: group 1: 1.34 ± 0.27 and 0.24 ± 0.22, group 2: 1.45 ± 0.29 and 0.56 ± 0.33, and group 3: 1.45 ± 0.23 and 0.80 ± 0.40. For females, no statistically significant difference was noted in mean gingival scores amongst the three groups ($p = 0.095$). However, a statistically significant difference was found in mean gingival scores amongst the 3 groups ($p < 0.001$) for males (as presented in Table 6).

Regarding OHI status of the 3 groups postintervention, "excellent" oral hygiene was noted in 4 (20%) hearing-impaired children in group 1, 2 (10%) in group 2, and none reported having "excellent" oral hygiene in group 3. "Good" oral hygiene was noted in 15 (75%) children belonging to group 1, 14 (70%) in group 2, and 10 (52.63%) in group 3, respectively. Moreover, 1 (5%) child in group 1, 4 (20%) in group 2, and 9 (47.36%) in group 3 were found to have "fair" oral hygiene.

About the health of the gingiva (GI), 5 hearing impaired children (25%) both in groups 1 and 2 reported having "healthy" gingiva along with 3 children (15.78%) from group 3. Furthermore, "mild" gingivitis was found in 15 (75%) children in group 1, 14 children (70%) in group 2, and 9 children (47.36%) in group 3.

Lastly, "moderate" gingivitis was not established in any of the children belonging to group 1, but it was found in 1 child (5%) in group 2, and 7 children (77.77%) in group 3 (as presented in Table 7).

## 4. Discussion

Maintaining good and healthy oral hygiene is of vital importance that plays a pivotal part in maintaining general physical health [16]. Normally, children are prone to have less than healthy oral health since at this age it is overlooked. Since children do not pay attention to their oral health, parents' guidance and education introduce many preventive dentistry methods such as tooth brushing and the use of dental floss [17]. However, for children with disabilities such as hearing impairment, difficulty in communication primarily hinders in maintaining healthy oral health most of the time [18].

In literature, many studies have evaluated different strategies to improve oral health such as giving direct or indirect personal tasks, written instructions, and audio-visual modalities provision [19]. In our study, the first intervention that we used was video as an oral hygiene education intervention, to help children with hearing impairment improve their oral health. According to our findings, video-based interventions significantly decreased both the mean plaque and gingival scores for the children as compared to no interventions being implemented. These findings correspond with a study by Widati and Nurmala, where a decrease in plaque scores was noted by using audio-visual aid and pamphlets [20]. Similarly, another study by Baliga et al. reports a decrease in plaque scores when oral health educational video was used as an oral hygiene education intervention [21]. The advantages associated with video-based interventions are that they can be repeatedly seen and mimicked by the children when they are brushing their teeth and using dental floss, along with being cost-effective.

The second intervention that was used in this study was the pictorial method as oral hygiene education to improve oral health in hearing impaired children. In this study, a significant reduction in mean plaque and gingival scores were noted when children were shown pictures of oral hygiene instructions. These findings correspond to various studies where improvement in oral health education was noted in hearing impaired children by showing pictures in a storybook [22, 23] Moreover, the use of sign language and lip-reading has also been used as a strategy in literature to guide hearing impaired children in managing their oral health [24]. A study in literature concludes a mean reduction in simplified oral hygiene index, gingival index, and plaque index by the use of sign language as a method of giving dental health education [25].

Tooth brushing can be performed using various techniques described in the literature such as horizontal scrub technique, bass techniques, and modified bass technique

Table 4: Intergroup OHI and GI scores comparison postinterventions.

| Groups | OHI scores | | | GI scores | | |
|---|---|---|---|---|---|---|
| | Mean difference | 95% CI | $p$ value | Mean difference | 95% CI | $p$ value |
| Group 1 (pictorial) vs. group 2 (video) | -0.097 | -0.27, 0.07 | 0.537 | -0.124 | -0.31, 0.05 | 0.290 |
| Group 1 (pictorial) vs. group 3 (control) | -0.274 | -0.45, -0.09 | 0.001 | -0.342 | -0.52, -0.15 | 0.001 |
| Group 2 (video) vs. group 3 (control) | -0.177 | -0.35, 0.01 | 0.052 | -0.218 | -0.40, -0.03 | 0.015 |

OHI: oral hygiene index; GI: gingival index; CI: confidence interval; $p$ value of ≤0.05 was considered as statistically significant.

Table 5: Comparison of mean plaque scores preintervention and postintervention in both sexes.

| | $n$ | Plaque scores | |
|---|---|---|---|
| | | Preintervention Mean ± SD | Postintervention Mean ± SD |
| *Gender* | | | |
| Females | 22 | | |
| Group 1 (pictorial) | 9 | 1.52 ± 0.20 | 0.36 ± 0.25 |
| Group 2 (video) | 9 | 1.38 ± 0.24 | 0.50 ± 0.37 |
| Group 3 (control) | 4 | 1.58 ± 0.25 | 0.89 ± 0.23 |
| $p$ value | | 0.608 | 0.004 |
| Males | 37 | | |
| Group 1 (pictorial) | 11 | 1.54 ± 0.21 | 0.36 ± 0.29 |
| Group 2 (video) | 11 | 1.57 ± 0.26 | 0.69 ± 0.42 |
| Group 3 (control) | 15 | 1.48 ± 0.23 | 0.96 ± 0.47 |
| $p$ value | | 0.719 | 0.007 |

SD: standard deviation.

Table 6: Comparison of mean gingival score preintervention and postintervention in both sexes.

| | $n$ | Gingival scores | |
|---|---|---|---|
| | | Preintervention Mean ± SD | Postintervention Mean ± SD |
| *Gender* | | | |
| Females | 22 | | |
| Group 1 (pictorial) | 9 | 1.24 ± 0.12 | 0.34 ± 0.24 |
| Group 2 (video) | 9 | 1.31 ± 0.30 | 0.30 ± 0.39 |
| Group 3 (control) | 4 | 1.52 ± 0.41 | 0.79 ± 0.54 |
| $p$ value | — | 0.242 | 0.095 |
| Males | 37 | | |
| Group 1 (pictorial) | 11 | 1.34 ± 0.27 | 0.24 ± 0.22 |
| Group 2 (video) | 11 | 1.45 ± 0.29 | 0.56 ± 0.33 |
| Group 3 (control) | 15 | 1.45 ± 0.23 | 0.80 ± 0.40 |
| $p$ value | — | 0.523 | 0.001 |

SD: standard deviation, $p$ value of ≤0.05 was taken as significant.

[26]. For children, horizontal scrub techniques have been recommended for the effective removal of plaque [27, 28]. Similarly, in our study, we recommended the children to use the horizontal scrub technique to brush their teeth. However, compliance to a particular tooth brushing technique can be a challenging factor to overcome especially for children. Toothbrushing along with mouthwash can be used to improve oral health according to one study where the use of mouthwash resulted in a decrease in gingival and plaque index scores [29].

It has been reported multiple times in the studies that females tend to have better oral hygiene as compared to males [30]. This might be due to females tend to visit dentists more often as compared to males and are more concerned about their oral hygiene [31]. In our study a mean reduction in plaque scores was reported in both males and females in postintervention; however, the reduction was slightly greater in females as compared to males. Furthermore, the reduction in the mean gingival scores of males was slightly more significant as compared to females. These results contrast with a study in literature where a significant difference between gingival scores of females and males was noted, with females having healthier gingiva [32].

Oral hygiene has been categorized as excellent, good, and fair in our study. In all of the 3 groups included in this study, the majority of the children belonging to group 1 (pictorial), and group 2 (video) had excellent and good oral hygiene at the end of the interventions assigned to each of the two groups. However, group 3 where no intervention was implemented mainly averaged fair in the oral hygiene status. Such results correspond to a study in literature where similar oral hygiene improvement has been found in intellectually disabled children by using oral hygiene educational interventions [33]. The use of electronic toothbrushes has been studied and proved effective for the better removal of plaque in children. However, a study showed no reduction in plaque when an electronic toothbrush was used in a child with special needs [34].

Oral hygiene educational interventions such as pictorial and video methods have been proven to be useful tools in improving the oral health of hearing-impaired children. However, other motivation techniques such as video games, sign language, and dental models have been used to improve the oral hygiene of hearing-impaired children [8, 35]. Despite the strengths of this study such as allocation of children to interventional groups randomly to avoid biases and following ADA (American Dental Association) and CONSORT guidelines, there were few limitations. Firstly, only a visual examination was used to assess the dental plaque without using plaque disclosing agents. Secondly, a few participants were recruited in this study, and lastly, only 2 motivational sessions were organized for the children.

Due to significant reduction of dental plaque in hearing-impaired children's students after oral hygiene education in this study. Properly planned educational programs are

Table 7: Distribution of oral hygiene index and gingival index amongst participants at postintervention levels in study groups.

| | Group 1 (pictorial) *n* (%) | Group 2 (video) *n* (%) | Group 3 (control) *n* (%) |
|---|---|---|---|
| Post-OHI status | | | |
| Excellent | 04 (20) | 02 (10) | 00 (0) |
| Good | 15 (75) | 14 (70) | 10 (52.63) |
| Fair | 01 (5) | 04 (20) | 09 (47.36) |
| Post-GI status | | | |
| Healthy | 05 (25) | 05 (25) | 03 (15.78) |
| Mild | 15 (75) | 14 (70) | 09 (47.36) |
| Moderate | 0 (0) | 01 (5) | 07 (77.77) |

OHI: oral hygiene index; GI: gingival index; %: percentage; *n*: frequency.

recommended to target oral health improvement to overcome the communication barriers. We also recommend a similar study on a large scale, with a longer duration greater than six months, maybe conducted in hearing impaired children. The participants may be exposed to the oral health educational interventions at least twice a week for three months to ensure the effectiveness of the educational interventions.

## 5. Conclusion

Oral hygiene is a vital aspect of the well-being of every individual due to functional and esthetic reasons. Children who have special needs such as being hearing impaired have difficulty in various aspects of life with oral health maintenance being one of them. Oral hygiene educational interventions such as the use of pictorial and video methods to educate hearing impaired children have been proven to be effective in improving the oral health of such children by a reduction in mean plaque and gingival scores.

## Data Availability

The raw data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare no conflict of interest.

## Authors' Contributions

MM, SS, NA, and MKA planned and designed the present work, and MM, AM, and SS were responsible for realizing the work. NA, AL, SR, and MM were responsible for the data acquisition and analysis. AM, FV, AL, and MKA drafted and revised the manuscript. MM, AM, FV, MKA, and NA approved the final version of the manuscript. All authors read and approved the final manuscript. NA and MKA contributed equally to this work and are corresponding authors.

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*Review Article*

# Overview of *Candida albicans* and Human Papillomavirus (HPV) Infection Agents and their Biomolecular Mechanisms in Promoting Oral Cancer in Pediatric Patients

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Oral carcinoma represents one of the most common malignancies worldwide. Oral squamous cell carcinomas (OSCCs) account over 90% of all oral malignant tumors and are characterized by high mortality in the advanced stages. Early diagnosis is often a challenge for its ambiguous appearance in early stages. Mucosal infection by the human papillomavirus (HPV) is responsible for a growing number of malignancies, particularly cervical cancer and oropharyngeal carcinomas. In addition, *Candida albicans* (*C. albicans*), which is the principal fungi involved in the oral cancer development, may induce carcinogenesis through several mechanisms, mainly promoting inflammation. Medical knowledge and research on adolescent/pediatric patients' management and prevention are in continuous evolution. Besides, microbiota can play an important role in maintaining oral health and therefore all human health. The aim of this review is to evaluate epidemiological and pathophysiological characteristics of the several biochemical pathways involved during HPV and *C. albicans* infections in pediatric dentistry.

## 1. Introduction

Oral carcinoma is the fifth most common malignant tumor worldwide and accounts for the majority of head and neck tumors [1, 2]. During the last decades, there has been a progressive increase in proportion of incidence of oral cancer not related to a known etiologic factor, such as the so-called "oral cancer in young," a relevant disease in nonsmoker nondrinker (NSND) patients [3]. The topic is matter of long standing debate, and adequate study models to analyze this entity are lacking [4].

Squamous cell carcinomas (OSCCs) are the most common type of oral tumor and can occur in larynx and pharynx too, with over 90% of oral cancers and represents 2-3% of all cancers [5]. Besides, OSCCs are more common in people over the age of 50, while some studies report that 1%-6% occur under the age of 40. There is a special preference for men: in fact, the ratio of men to women in Western societies is 2 : 1 [6]. On the other hand, the incidence of injury in young adults has been noted to be increasing worldwide [4, 7]. In recent years, despite advances in diagnosis and oncologic therapy, the 5-year survival rate of oral carcinoma is still 50–60%, with a slight increase in the United States (US) during the last decade (66%) [6].

The current model of oral and pharyngeal carcinoma development suggests a progressive multistep transition from normal mucosa to OSCCs through a series of progressive histological changes (oral epithelial dysplasia) reflecting the accumulation of genetic and epigenetic abnormalities and genetic susceptibility [5, 6].

The evaluation of the literature and surveillance data concerning oral carcinoma is difficult because this neoplasia often is reported associated with other head and neck malignancies, and report of anatomic subsites is often unclear or can create confusion between its localization between oral cavity and oropharynx.

*Candida albicans* is a commensal fungal species commonly colonizing the human mucosal surfaces [7]. Carriage rates, corresponding to 18.5–40.9% in healthy individuals, are usually higher in individuals with compromised immunity, such as human immunodeficiency virus-positive individuals, diabetes patients, and infants and elder populations [7]. Besides, *C. albicans* can strongly interact with additional oral microorganisms and enforce noteworthy impact on the virulence of polymicrobial biofilms [7]. Furthermore, it is recognized that coinfection of *C. albicans* is strongly associated with increasing diseases in pediatric dentistry, such as severe caries in children (S-ECC) [7].

Thus, the purpose of the present paper is to review the current evidence on the role of *C. albicans* and human papillomavirus (HPV), as well their biomolecular mechanisms, in the pathogenesis of potentially malignant oral disorders (PMODs) and oral carcinoma (Figure 1) in pediatric dentistry.

## 2. Risk Factors for Oral Cancer in Pediatric Dentistry

Several risk factors have been involved in the pathogenesis of these lesions, but the mechanisms and the causes of malignant transformations remain unknown. Lifestyle factors, PMODs, modification of the microbiome, systemic sclerosis, genetic disease with dysregulation of DNA metabolism (Zinsser–Engman–Cole syndrome, Fanconi anemia, and Xeroderma pigmentosum), mucosal inflammation and oral mucosa chronic trauma, and hematinic and micronutrient deficiency are the most important causes associated with oral carcinoma [8].

In several Asian countries, the combination of tobacco and/or betel nuts increases the risk for PMODs and oral cancer. In addition, the synergistic effect in consuming large quantities of alcohol and/or tobacco has a high risk associated with consumption and is proportional to the amount of alcohol consumed [9, 10]. Cigarette smoke from combustion releases several carcinogenic chemicals substances such as benzopyrene, dimethylbenzanthracene, nitrosazine, and free radicals [11, 12]. On the other hand, large amounts of alcohol can cause the mitochondrial salivary suppression of aldehyde dehydrogenase ALDH 2 allele gene which leads to high levels of serum acetaldehyde (derived from the ethanol metabolism and turn to acetic acid by ALDH), increasing the risk of carcinogenesis [13, 14].

Virus infections such as HPV (mainly type 16) have been linked to oral carcinomas [15, 16]. Chronic *Candida* spp. infections also appear to be a major risk factor [17, 18]. The risk by HPV strain infections is involved in a growing percentage of oral cancer, but other infectious agents such as the *Candida* spp. (more specifically *C. albicans*) could be involved for their production of endogenous nitrosamines starting from dietary nitrites present in the mouth, particularly in saliva [19, 20].

*2.1. The Role of Microbiota Dysbiosis in Candidiasis.* The terms of oral microbiome, oral microbiota, or oral microflora are used for the microorganisms present in the human mouth [21]. The mouth is the second largest and diverse microbiota niche after the gut, harboring over 700 species of bacteria. Every person has his or her own microbiome signature [22]. The human microbiota consists of archaeal cells, bacteria, fungi, viruses, and protozoa. Thus, various bacteria of the oral microbiota have a cross talking with *C. albicans* and HPV by modifying its pathogenicity or the behavior of bacterial population [23].

The over presenting relative phyla, according to the Human Oral Microbiome Database (eHOMD), as reported in Figure 2, are the *Streptococcus* spp. [24].

The core microbiome is shared to all the persons, while variable microbiome is distinctive for single subject and is due to the lifestyle and to the physiological differences [22]. The mouth has two different types of surfaces on which bacteria can colonize: the hard and the soft tissues, respectively, of the teeth and the oral mucosa [22]. Moreover, the model environment for the development of microorganisms is presented by the oral cavity and related nasopharyngeal regions. In fact, the usual temperature of mouth is about 37°C without significant changes, lead in this way to a stable environment to bacterial survive [23]. In addition, in normal conditions, saliva too has a pH between 6.5 and 7, favorable for most bacteria species [8]. The *Streptococcus mitis* is one

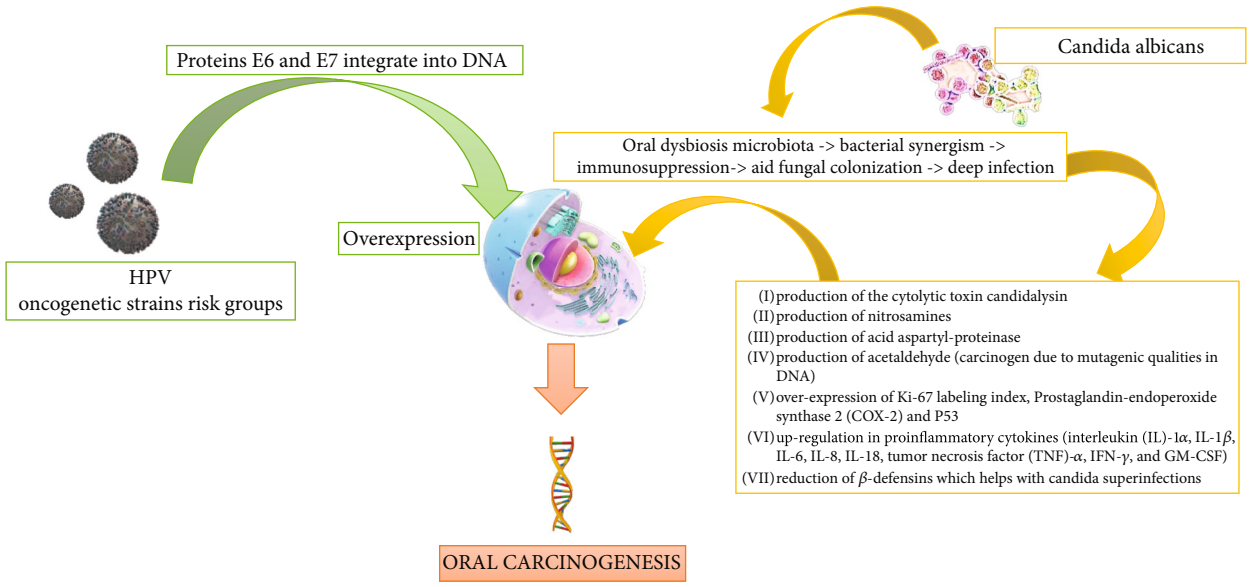


Figure 1: The pathways of carcinogenesis in the oral cavity through the oncogenetic HPV virus strain and *Candida albicans*. Several hypothetical mechanisms have been proposed for the fungal infection that can induce PMODs and malignant lesions in oral epithelium. The dysbiosis of the microbiota can play an important role with the subsequent alteration of the microbiome. There follows a synergism between "bad" bacteria with *Candida albicans* and HPV.

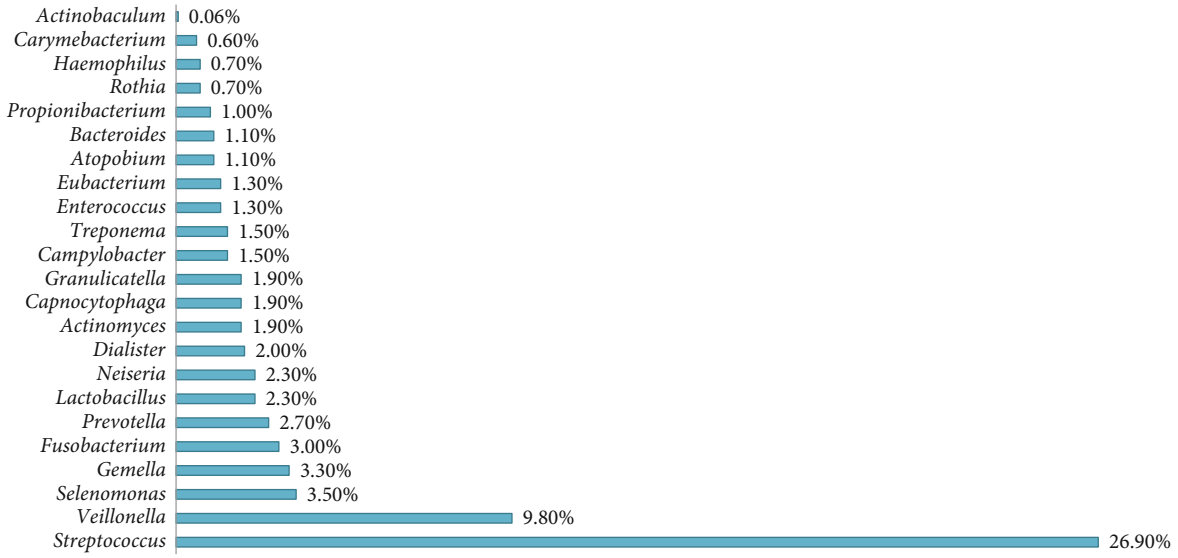


Figure 2: The main relative percentage (%) phyla of oral microbiome reported in eHOMD (source eHOMD, http://www.homd.org/).

of the first bacteria to colonize the oral cavity, establish dental plaque and, under certain conditions (such as the tooth extraction), may cause systemic infection such as endocarditis [25]. *Streptococcus salivarius* electively colonizes the oral mucosa and is over presenting in saliva, acting as an opportunistic pathogen that produce polysaccharides in the presence of fructose, increasing in this way salivary pH [26]. *Streptococcus mutans* (based on the antigens, there are 8 different serotypes), producing bacteriokines and extracellular and intracellular polysaccharides (thus acidifying the oral environment), seems to have a role in the genesis of caries, as well influencing the composition of dental plaque [27]. *Vestibular streptococcus* produces hydrogen peroxide and urease from which ammonia is produced, with a consequent increases of local pH [28]. On the other hand, *Streptococcus anginosus*, usually isolated from the mucous membranes, gingival fissure, and dental plaque, do not secrete polysaccharides playing a protective role in caries development [28]. *Staphylococci* spp. are isolated to a small extent from saliva, gingival cleft of the mucous membranes, and dental plaque (mainly from immunocompromised subjects), with over represented the *S. aureus* [26]. *Lactobacillus species* are members of the oral flora in a small percentage (less than 1%) but increase in tooth areas showing caries,

also for the characteristic that it could grow in a low pH environment [23, 27]. Several species of potential opportunistic pathogens, such as *Propionibacterium* and *Corynebacterium*, have been isolated from the oral environment [28]. *Actinomyces* spp. are also common members of dental plaque flora [29]. Other pathogens, such as the *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Treponema denticola*, which are anaerobic bacterium Gram-negative (-), can cause periodontitis and are linked to systemic diseases [30, 31]. Archaeal or archaic cells are only an exceedingly small percentage of the oral microbiome with a limited diversity (very few species and phytotypes) that can be adapted as a small minority of organisms in this environment. *Methanes* have been isolated from the oral cavity and, in fact, in 36% of patients with periodontitis archaeal were detected by in situ fluorescent hybridization [27]. It was found that the archaeal were confined to a subset of human beings and consisted of two different *Rinna fililipi* within the genus *Methanobrevibacter*. The archaeal community in periodontal disease was dominated by a *Methanobrevibacter oralis* type phytologist and a separate subspecies of *Methanobrevibacter* such as *Methanobrevibacter cuticularis*, *Methanobrevibacter filiformi*, *Methanobrevibacter ruminantium*, and *Methanobrevibacter arboriphilius* [21, 22]. Fungi are a small part of the oral microbiome. The predominant species is *Candida albicans*. Other fungal species present in the mouth are *Cladosporium*, *Saccharomycetes*, *Aspergillus* spp. (such as *A. Penicillium*), *Gibberella*, *Cryptococcus*, *Fusarium*, *Rhodotorula*, and *Schizophyllum* [21, 25]. Two species of protozoa were found in physiological flora of the mouth: the *Entamoeba gingivalis* amoeba and the *Trichomonas tenax* [21, 25]. The number of these organisms is high in people with poor oral hygiene and gingivitis and was once considered potential pathogens [28]. At present, saprophytes are considered harmless, and the apparent association with the disease is linked to diet, because a poor oral hygiene can allow an increase in the quantities of food intake and bacterial residues, which are the main nutrients for the protozoa [28]. The conditions of dysbiosis can lead to the imbalance of growth and reduction of some microorganisms present in the oral microbiota. Bacteria are not the only microorganisms present in the periodontal area, and there are also several fungi members. Recent studies in patients with periodontal infections found at least 150 species of fungi belonging to the generate *Candida albicans*, *Ascomycota*, *Basidiomycota*, *Glomeromycota*, and *Chytridiomycota* [29, 30]. This subsequently damages the periodontal tissue, in turn creating nutrients for the bacteria of the dysbiotic microbiome [31]. It has been noted that *Mitis* Group *Streptococci*, *Salivarius* Group *Streptococci*, *S. gordonii*, *S. mutans*, *S. oralis*, *S. sanguinis*, and *S. parasanguini* can interact with *C. albicans*. The *S. mutans* adhering to *C. albicans* interact through three mechanisms. Firstly, *C. ablicans* metabolizes carbohydrates and improves the sugar metabolism of *S. mutans* and *S. mitis*; secondly, *S. mutans* can influence the coding of adhesins in *C. albicans*; finally, the synergism of *S. mutans* and *C. albicans* can promote an increase of growth and invasion of mucous tissues in coinfection [32, 33]. The *S. salivarius* K19 may play a protective role in *C. albicans* infection because it can inhibit adhesion and fungal filamentation. The *S. aureus* may be helped by *C. albicans* in an infection, such as *R. dentocariosa*, and *S. mitis* aids fungal colonization [34, 35]. It has been noted that *Fusobacterium nucleatum*, *S. salivarius*, *A. actinomycetemcomitans*, and *E. faecalis* can have a protective role in a *C. albicans* infections, because it can inhibit the overall virulence and fungal biofilm formation (Figure 3) [36, 37].

Finally, some studies reported several interactions between *C. albicans* and *P. gingivalis* that could be used to increase infectivity [38, 39]. In some patients, the inflammatory reaction in candidiasis can be self-limiting, but in other patients, multiple genetic, epigenetic, or external factors (tobacco, alcohol, diet, diabetes, etc.), as well in synergetic crosstalk with oral bacterial, can cause excessive and chronic inflammation, leading to PMODs and alveolar bone injure [40–42].

Moreover recent evidence points out a causal relationship between specific bacterial infections, such as by *P. gingivalis*, and the development of oral and esophageal carcinoma [43].

*2.2. Candidiasis Can Generate Oral Precancerous Conditions?* Major predisposing factors to oral candidiasis could be diabetes, mobile prosthesis, antibiotics, antiblastic or inhaled corticosteroid therapy, xerostomia by radiations, or Sjógren's syndrome and HIV infection, presenting clinical forms as angular cheilitis, mucocutaneous (rare), pseudomembranous, hyperplastic, and erythematous (atrophic) [44, 45]. Moreover, current evidence revealed correlation between *Candida* infection and malignant transformation of the oral cavity [46, 47]. In fact, several hypothetical mechanisms have been proposed for *C. albicans interactions with* oral epithelium, leading to PMODs and malignant lesions [48–50]:

(a) Production of the cytolitic toxin candidalysin

(b) Production of nitrosamines

(c) Production of acid aspartyl-proteinase

(d) Production of acetaldehyde (carcinogen inducing mutations in genes)

(e) Overexpression of Ki-67 labeling index, prostaglandin-endoperoxide synthase 2 (COX-2), and p53

(f) Upregulation of proinflammatory cytokines, such as interleukin- (IL-) 1$\alpha$, IL-1$\beta$, IL-6, IL-8, IL-18, tumor necrosis factor- (TNF-) $\alpha$, interferon gamma (IFN-$\gamma$), and granulocyte-macrophage colony-stimulating factor (GM-CSF)

(g) Reduction of $\beta$-defensins which facilitate Candida superinfections

In a prospective cohort study (between 2007 and 2009) on 103 patients, *Candida* spp. was isolated from 31 (30%) patients with carcinoma and from 33 (32%) patients with PMODs [51]. In another prospective cohort study, it was noted that the presence of *Candida* infections was related

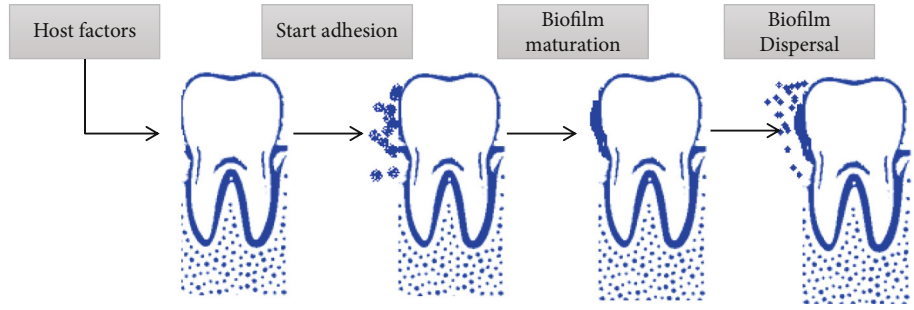


Figure 3: Example stages of biofilm formation process on dental surface (this mechanism occurs throughout the oral cavity). Biofilms are an organized community of microorganisms. Microbes with weak and reversible forces attach first, dental surface. All bacteria that are not immediately removed are rigidly attached to special structures, such as fibrils. Bacteria multiply and offer additional attack sites for other microorganisms. The ability to maintain the consistency of microbes in dental plaque is based on the balance between cooperative and competitive relationships between microorganisms and their host. This microbial homeostasis in combination with host defense prevents the formation of pathogenic microorganism colonization. However, when this community is unbalanced quantitatively and qualitatively, dysbiosis occurs.

to an increased risk of cancer [52]. In addition, some bacteria such as S. *viridans* are able to convert ethanol into acetaldehyde for the presence of the alcohol-dehydrogenase enzyme in them [53–55]. The ability to switch among different phenotypic forms has been thought to contribute to *C. albicans* virulence, and phenotypic switching events in *C. albicans* can be induced by hydroxyurea [55, 56]. Besides, some chemotherapeutic agents, such as 5-fluorouracil, could reduce the susceptibility of *C. albicans* to the antifungal drugs [57]. The genotype "A" of *C. albicans* is more represented in the OSCCs, while the genotype "C" of *C. albicans* is more represented in the leucoplakia [58, 59]. *Candida* leukoplakia (CL) lesions are complex to discriminate from non-*Candida* leukoplakias (NCLs) clinically, but the presence of invading *Candida* hyphae in the superficial layer of epithelium accompanied by infiltratration of polymorphic neutrophils histologically discriminates CL lesions [60, 61]. Moreover, *Candida* was isolated by exfoliative cytology and periodic acid–Schiff (PAS) staining of biopsies in a study that analyzed 44 cases with 59.1% presenting oral leucoplakia, showing *Candida* detection in 62.5% of the cases [62]. Furthermore, in the observed cases with *Candida*, the DNA alterations were higher [62]. In a retrospective study on 136 patients with oral leukoplakia divided into two groups, it was found that lesions had higher degrees of cell abnormalities (epithelial dysplasia) with *Candida* coinfection. The first group presenting multiple oral leucoplakia lesions, *Candida* infection was detected in the 47.9% of the sample (with 28.6% of dysplasia), while in the second group with single oral leucoplakia lesion, *Candida* infection was detected in the 19.0% of the sample (with 20.0% of dysplasia) [63].

*2.3. The Role of Human Papillomavirus (HPV).* HPVs are members of the Papillomaviridae (PV) family, presenting a circular, with supercoiled and double-stranded DNA, and some are considered as oncogenic promoters [64–66]. The virus has a hexahedral symmetry capsid (consisting of pentameric capsomeres) and has two structural proteins, L1 and L2 [67, 68]. Oral HPV is often sexually transmitted, but nonsexual modes of transmission should be considered, including autoinoculation from skin lesion HPV in adolescent and pediatric patients [69, 70]. The HPV infection is commonly is commomly associated to benign lesions (vulgar warts, warts, focal epithelial hyperplasia, squamous cell papilloma, Bowen's papillomatosis), or to cancerous lesions such as squamous cell carcinoma (SCC) [64]. There must be chafing or small lesions for the virus to enter the epithelium, and direct contact with the skin or mucosa is required for virus transmission. The strain of virus determines the different types of lesion that will be developed, as well as the location of the infection. Hence, HPV can be transmitted in many ways through different abrasions, with sexual intercourse, when the newborn passes through an infected genital tract, and from a variety of self-inoculation positions, for example, by scratching the skin [68].

Since HPV does not have a protein or ribosomal synthesis, for its proliferation, it employs the genetic mechanism of the host cell [69, 70]. The virus uses, to direct the metabolic functions of the host cell in its favor, the production of viral messenger RNA, which is produced by transcription of viral genetic material. The genome is divided into three parts. The "E" (early region) which codes for proteins necessary for the viral DNA genome duplication. It includes 7-9 open reading frames (ORFs), coding regions for proteins E1, E2, E4, E5, E6, E7, and E8 (although E5 and E8 ORF are not present in the genomes of all HPV types). The "L" (late region) which encodes the structural proteins of the HPV capsid virus (L1 and L2). Finally, the URR or NCR (upstream regulatory region or noncoding region) comprised between "E" and "L" regions and regulates the function of the viral genome, in addition to four binding sites to E2 proteins and multiple binding sites of the transcription [69–71].

The differences between different strains of the virus are evident when comparing lesions of the same epithelial area. In fact, cell proliferation obtained from the expression of E6 and E7 proteins, due to infection with oncogenic HPV virus strains, facilitates expansion of lesion, with a higher risk of metastasis [71, 72].

*2.4. Which Are the Pathways of Oncogenesis in HPV Infection?* The receptor that HPV uses to enter cells is

Table 1: There are over 450 types of HPV. The HPV's genotypes are divided into 4 groups according to the associate oncogenic risk by IARC/WHO (source from https://monographs.iarc.who.int/agents-classified-by-the-iarc/).

| Group | HPV virus |
|---|---|
| 1 | Carcinogenic to humans: human papillomavirus types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 (the HPV types that have been classified as carcinogenic to humans can differ by an order of magnitude in risk for cervical cancer) |
| 2A | Probably carcinogenic to humans: human papillomavirus type 68 |
| 2B | Possibly carcinogenic to humans: human papillomavirus types 26, 53, 66, 67, 70, 73, and 82; human papillomavirus types 30, 34, 69, 85, and 97 (classified by phylogenetic analogy to the HPV genus alpha types classified in group 1); human papillomavirus types 5 and 8 (in patients with epidermodysplasia verruciformis) |
| 3 | Not classifiable as to its carcinogenicity to humans: human papillomavirus genus beta (except types 5 and 8) and genus gamma; human papillomavirus types 6 and 11 |

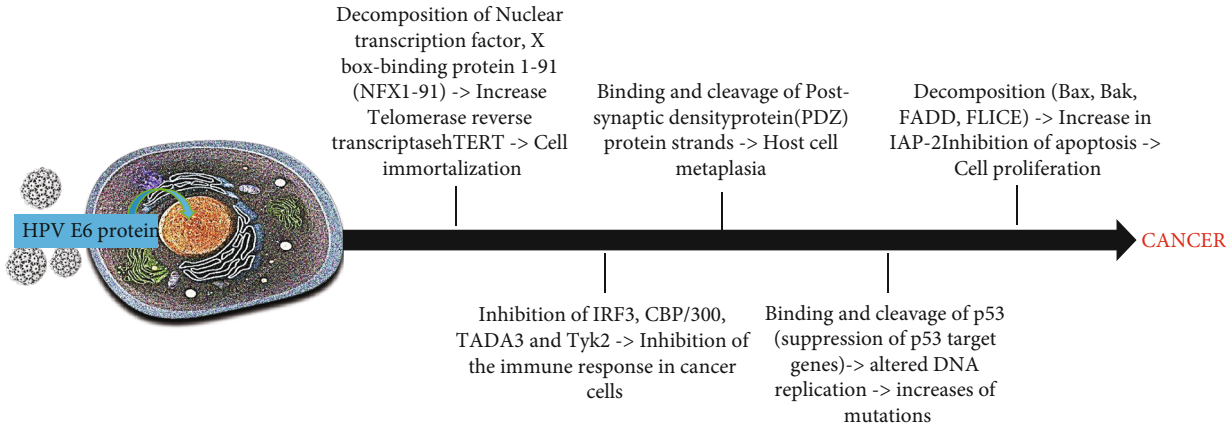


Figure 4: Integration of HPV DNA into the host genome leading to the E6 protein overexpression and to carcinogenic processes.

integrin A6 [71]. On one hand, there are many types of HPV such as 16, 18, 31, and 52, and on the other hand, they are characterized by a high potential for malignancy and also have a high risk of metastasis (Table 1) [73, 74].

The role of the virus on carcinogenesis is due to proteins E6 and E7 that integrate into host DNA. These two proteins could induce the neoplastic growth, since they block the two tumor suppressor proteins, the retinoblastoma protein (pRb) and p53, which regulate the transition from the G1 phase of the cell cycle to S. E7 binds to p53 blocking its ability to interact with the transcription factor E2F [75, 76]. Therefore, cells characterized by E7 overexpression lose the control of the transition from G1 to S phase, which causes continuous cell cycles and therefore cell proliferation without suppression, as well, degradation of the pRb [76].

Levels of p53 in normal cells are exceptionally low. During the E7 overexpression, an increase in p53 was shown for the inhibition of its breakdown in normal cells which is in turn regulated by MDM2 (mediator of DNA damage 2) [77, 78]. E7 also interacts with many other factors; among these, the CDK (cyclin-dependent kinase) inhibits p27 and p21, which induce the dysregulation of cell cycle [78]. E7 forms protein complexes with pRb, p107, and p130 and participates in E6 cleavage of p53 via the E6 complex, E6AP (associated protein E6), and p53 [79, 80]. Dysregulation of pRb phosphorylation-dephosphorylation is an early event. There is a gradual reduction of pRb expression in dysplastic lesions and in neoplastic process [81]. Briefly, the Rb protein pathway could induce carcinogenesis by inactivating cell cycle regulators [82]. Activation of p53 turns on the production of the CD-kinase inhibitor protein, p21WAF1 which contributes to cell cycle regulation by acting on different CD-kinase/cyclin complexes [83]. Thus, failure of p53 has been observed to cause carcinogenesis. This is attributed to the mutation of the genetic locus 17p13. Furthermore, E6 sequentially causes the cleavage of p53 [84]. Oncoprotein E7 causes p16 overexpression. In fact, the gene that codes for the regulatory protein p16 is found in chromosome 9p21, and its mutation is correlated with OSCCs [85, 86] (Figure 4).

Besides, E6 inhibits the activity of p73, a type 16 homologous p53. HPV blocks cell apoptosis by inhibiting Bax gene expression in keratinocytes. This results in increased mutations in the DNA of the cells. The apoptosis can be inhibited by E7 binding to the tumor necrosis factor 1 receptor (TNF-R1) [87], originating a variety of bimolecular effects that promote carcinogenesis (Figure 5).

According to carcinogenesis model proposed by Califano et al., healthy tissues near the lesions show the same pattern of mutations (loss of heterozygosity) of neoplastic cells, since all population come from a single mutant progenitor cell [88]. Another model reports that the progenitor cell of the basal layer acquires a genetic mutation, which it transfers to its daughter cells. The result is the creation of a mass of cells that grows, strains, and also affects nearby tissues, in the development of lesions (clinically, they appear as

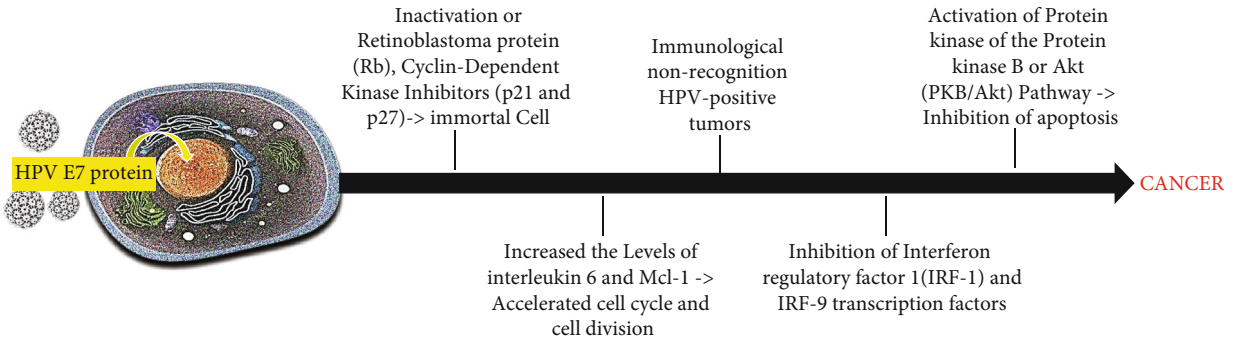


Figure 5: Integration of HPV DNA into the host genome leading to E7 overexpression and to carcinogenic processes.

leukoplakia or erythroplakia). In the final stage, specific clone cells after further mutations acquire a tumoral genotype. In this context, loss of heterozygosity at loci 9p21 and 3p21 appears to increase the risk of malignant recurrence [89, 90]. A mutation in the type of gene duplication has been shown to be involved in oral carcinogenesis [91].

Replication of genetic material in the TERT (telomerase reverse transcriptase) protein coding gene results in overexpression of the TERT human protein (hTERT). Studies conducted on OSCCs have shown an increase in both telomerase activity and hTERT expression [92, 93]. This hTERT expression occurs not only in cancerous cells but also around in the normal epithelium in the early course of carcinogenesis [94]. The spindle assembly checkpoint (SAC) describes the way point to control mitosis, and Cdc20 is a SAC protein able to activate the anaphase promoting complex (APC) which is a key pathway factor and is also responsible for the formation of aneuploidy cells [95–97].

It has been noted that in 70% of oral tumors there is an overexpression of the Cdc20 mRNA [98, 99]. Besides, as further bimolecular mechanisms in the etiology of oral cancer, several studies reported the role of the epidermal growth factor (EGF), the mitogen-activated protein kinase (MAPK) pathway, the PI3K/AKT/mTOR pathway, the signal transducer and activator of transcription (STAT) pathway, TGF (transforming growth factor), NF-$\kappa$B (nuclear factor $\kappa$B), and Wnt/$\beta$-catenin [100–102]. The ERbB1 gene, responsible for EGF encoding, is found on chromosome 7p12 and has been associated with increased activity in oral cancer [103, 104]. ERbB1 dysregulation in head and neck squamous epithelial neoplasms is attributed more to its overexpression than to the presence of mutations [105, 106]. TGF promotes tumor progression by increasing angiogenesis and reducing sensitivity to the immune system. This action is caused by the tolerance of tumor cells to TGF-induced apoptosis [107, 108]. The TGF$\beta$R-II mutation is important, and thus, a reduction of the TGF$\beta$R-II/TGF$\beta$R-I ratio (suspension/development) is determined, and thus, the protective role of TGF is canceled [109]. A gradual decrease in expression of TGF-$\beta$, TGF$\beta$R-I, and TGF$\beta$R-II was observed in the different stages of carcinogenesis. In cancer cells, there is a reduction in TGF$\beta$R-II levels, and this can promote the development and growth of oral cancer, acting as an indicator of differentiation and therefore of aggressive behavior [110]. Activation of the Wnt/$\beta$-catenin pathway induces $\beta$-catenin release and subsequently cytoplasmic aggregation and its transport to the nucleus, through the interaction with certain genes (with inhibition of apoptosis and increase of cell proliferation) such as COX-2, cyclin D1, and cMyc [105, 111]. Furthermore, this pathway increases the expression of metalloproteinases, which catalyze the basement membrane and the dense structure of the epithelium, favoring infiltration [111]. Lastly, HPV proteins E6 and E7 can regulate the epigenetic mechanisms, as DNA methylation, histone modification, chromatin remodeling, and miRNA production in cell host or viral genes. Therefore, on one hand, E6/E7 induced DNA methylation shut out normal epigenetic processes, and on the other hand, E7 binds and modulates methyltransferase activity [112, 113].

## 3. Conclusions

Nowadays, several suggestive and consistent correlations between *C. albicans* and HPV infection in oral cancer progression in adolescent/pediatric patients have been reported worldwide. *Candida* spp. and in particular *C. albicans* can produce carcinogens such as nitrosamine or promote the development of oral carcinoma. HPV are involved in the pathogenesis of different types of cancer. In particular, from literature data, it emerges that HPV infection plays an important role in the risk of PMODs of the oral mucosa and consequently in the dysplastic and malignant transformation of these lesions. Based on existing evidence, we can also conclude that, in the composition of the microbiome associated with OSCCs, there are no specific species to implicate in its etiology, of course excluding oncoviruses (i.e., HPV) or fungi (i.e., *C. albicans*) that are associated with oral cancer. Large multicenter trials are required in order to study the biological behavior and formulate treatment strategies in the management of the same. Perhaps, these findings will produce interest in the possible association among oral microbiota dysbiosis, *C. albicans*, HPV, and oral cancer and spur controlled prospective clinical studies in this field.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

## Authors' Contributions

A.B., I.A.C., and L.S. contributed to the concept and design. M.A., L.B., I.A.C., and A.B. contributed to the acquisition, analysis, and interpretation of data. I.A.C., E.B., and A.B. contributed to the drafting of the manuscript. S.C., E.B., A.P.C., and A.B. contributed to the bibliographic research. M.D.C., S.C., and L.B. contributed to the critical revision of the manuscript for important intellectual content. A.M., R.N., and M.D. contributed to the data interpretation, technical, and material support. L.S., A.B., M.D.C., and L.L.M contributed to the supervision and final approval. All authors have read and agreed to the published version of the manuscript. Lorenzo Lo Muzio, Andrea Ballini, and Stefania Cantore contributed equally as co-first authors. Michele Di Cosola, Luigi Santacroce, and Edoardo Brauner contributed equally as co-last authors.

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*Research Article*

# The Association between Nutritional Alterations and Oral Lesions in a Pediatric Population: An Epidemiological Study

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The oral conditions of an individual are the result of different factors, including the subject's genotype, oral hygiene habits, the type of diet, and lifestyle, such as smoking. Nutrition in the first years of life can affect dental health for a long time. To prevent mouth diseases, it is also important to eliminate unfavorable eating behaviour and to amplify protective ones. Eating habits, especially in pediatric age, are an easily modifiable and controllable factor, and diet, in addition to influencing the health of the oral cavity, plays a fundamental role in systemic health. Indeed, a sugar-rich diet can lead to conditions, such as diabetes, being overweight, and obesity. The present research was an epidemiological study, with the aim of highlighting some of the associations between nutrition and oral health. In particular, we studied those lesions of hard and soft tissues that are diagnosed most frequently by dentists: caries, enamel hypoplasia, periodontal disease, and aphotoxic lesions and their associations with nutritional deficiencies and excesses including proteins, vitamin A, vitamin D, B vitamins, and iron and calcium minerals. To perform this study, we recruited 70 patients from the pediatric and orthodontic clinics, aged between 3 and 15 years (y), with mean age of 10.4 y.o. The study was conducted by providing a questionnaire to pediatric patients' (supported from their parents or guardians) on individual eating habits, followed by an accurate oral cavity specialistic examination. The nutritional data were processed by using Grana Padano Observatory (OGP) software, freely provided online by the OPG. The statistical tests performed were the chi-square ($\chi^2$) for independence, and Cramér's V test was used to evaluate the associations between eating habits and oral pathologies. The results showed that certain nutritional vitamin deficiencies and nutritional excesses were associated with definite oral pathologies.

## 1. Introduction

The oral conditions of an individual are the result of various factors, such as the subject's genotype, oral hygiene habits (i.e., toothbrushing), type of diet, and any smoking habits [1–3]. Unhealthy eating habits adversely affect both oral and general health. Nutrition in the early years of life can influence long-term dental health [4–7]. Foods that are harmful to general health can also damage teeth and *vice versa*. Research has been focusing on the effect of nutrition on mucous membranes and hard dental tissues, which can be a systemic effect or a local effect, such as the effect of acidic foods and drinks on the teeth, which can be responsible for dental erosion in patients with good oral hygiene [8–11]. Changing one's eating habits and lifestyle can lead to the improvement of oral and systemic conditions [12–15].

To prevent mouth diseases, it is, therefore, advisable to change the modifiable factors, eliminating unfavorable ones and amplifying the protective ones. Eating habits represent an easily modifiable and controllable factor, knowing that diet, in addition to influencing the health of the oral cavity, plays a fundamental role in systemic health, acting also in the autologous self-renewal stem cell niche, related to the oral mucous membrane trophism [16, 17]. It is well known that a diet rich in sugars can lead to conditions, such as diabetes, being overweight, and obesity [18]. The present research was an epidemiological study, with the aim of highlighting some of the associations between nutrition and oral health.

In particular, our attention was directed towards those lesions of hard and soft tissues more frequently diagnosed by a dental practitioner: caries, enamel hypoplasia, periodontal disease, and aphthous lesions. The nutrients we assessed for excesses or deficits in pediatric patients, based on the frequency of intake of certain foods, were proteins, vitamin A, vitamin D, vitamins of group B, and the minerals iron and calcium. Our main questions were as follows: (1) "What eating habits can influence the onset of these statuses?" and (2) "At what point can a correct diet act on the maintenance of a good trophism of the oral mucous membranes and on the formation and safeguarding of dental structures?"

## 2. Materials and Methods

This research was conducted in collaboration with Elbasan University "A. Xhuvani" (School of Technical Medical Sciences), Elbasan, Albania, a dental community cabinet (Sorriso & Benessere—Ricerca e Clinica SRL, Bari, Italy), the University of Bari Aldo Moro (Italy), and the University of Foggia (Italy).

To perform this study, we recruited 70 pediatric patients from the pediatric and orthodontic private practice clinics. The Institutional Ethics Committee of the Faculty of Technical Medical Sciences of Elbasan "Aleksandër Xhuvani" approved the application to conduct the clinical trial in the faculty (protocol identification: INTL_ALITCOOP/DentPath/2020_SLK). Informed consent was obtained from all subjects' parents or guardians involved in the study. Written informed consent was obtained from the patients' parents or guardians to publish this paper.

The inclusion criteria were as follows:

(i) Age from 3 to 15 years, with a mean age of 10.4 years, a standard deviation of 2.75, and a median (value that leaves 50% on the left and 50% on the right of a series of data sorted in a nondecreasing way) of 10

(ii) Gender: both male and female

(iii) The absence of situations requiring special diets

The exclusion criteria include all situations that required specific diets, including diabetes and hereditary metabolic diseases such as phenylketonuria, glycogenosis, galactosemia, celiac disease, severe food intolerances, and chronic inflammatory bowel diseases.

During the first phase of the study, we administered a questionnaire to patients that inquired about their eating habits. To obtain more reliable answers, we typically asked for the help of the parents. For the purposes of this research, the software freely available online, following registration at the website http://www.osservatorio.granapadano.it (OGP), was used (i) to evaluate if the patients' food intake was appropriate and (ii) to provide them personalized nutritional advice, highlighting any nutritional deficiencies or excesses caused by their ordinary diet [19].

The first phase, that of the interview, allowed an active healthcare professional- (dentist) patient relationship, strengthened by the emotional elements linked to nutrition (nourishing means, first of all, "taking care" of someone), and the subsequent phase of delivery of the press of the nutritional advice involved communicating things never said before, and do this in a way where the patient feels empathy from the healthcare professional, facilitated by the "counseling" approach. Through this, thanks to the healthcare professional, the patient becomes the protagonist of the change process, acquiring awareness of their own eating habits and lifestyle. This was a useful and powerful tool of improving compliance and achieving expected results more easily.

For the calculation of nutritional needs, the software refers to age groups expressed as follows:

(i) From 3 to 6 years, male and female

(ii) From 7 to 10 years, male and female

(iii) From 11 to 15 years, male and female

(iv) From 11 to 15 years, female

Data collection was conducted with the help of an electronic questionnaire to evaluate the frequency of weekly or, alternatively, monthly intake of the most common foods of the interviewee's diet, whose nutrient content was "weighed."

The data collected by the questionnaire were as follows:

(i) Essential personal data for the inclusion of the subject within a specific cluster

(ii) The weight and height for calculating the body mass index (BMI)

(iii) The abdominal circumference (the weight, height, and abdominal circumference data can be reported by the subject or detected by the healthcare professional, and the software automatically checks "therefore if the healthcare professional decides to detect the data, the corresponding item must be ticked")

(iv) Lifestyle data (hours spent close to television and/or at personal computer/notebook for free time, smoking habits, physical activity, etc.)

Before moving on to the analysis of individual foods, we asked for the weekly frequency of intake of certain food families (side dishes, fruits, dairy products, meats, and salami). The collected data were compared with those collected during the administration of the detailed questionnaire, in order to reduce the risk of underestimating/overestimating the consumption of relevant foods.

During the detailed interview, a number was inserted for each food that responds to weekly or monthly assumptions; if the food in question was not taken, the corresponding space was left blank. The single intake refers to the quantity of food, expressed in grams, which represents the standard portion for age and sex, which was automatically extrapolated from the dedicated software. When the questionnaire reports more than one food in the same row (for example, beans, lentils, fava beans, chickpeas, and boiled/canned cooked soybeans), it was necessary to sum the intake of the individual foods and report their total value in the box with the relative frequency.

Once the data were acquired through the questionnaire, it was possible to proceed to the oral cavity specialist pathological anamnesis. We asked patients if they suffered from particular mucous diseases, almost always not detectable at the time of the visit, as in the case of vesicular-bullous lesions or aphthous lesions. We investigated the onset of any burning or painful symptoms associated with the ingestion of particular foods and the possible remission of the symptoms when avoiding the intake.

The third step consisted of the objective examination of the patients' oral cavity to assess the presence of caries, earlier restorative treatments, endodontic therapies, dental erosions, missing teeth, enamel hypoplasia, and calcifications, as well the gingival status and the presence of plaque/calculus.

Therefore, the information available for each patient included the following:

(i) The nutritional status

(ii) The nutritional deficiencies or excesses

(iii) The clinical history

(iv) The current presence of caries, already restored teeth, enamel hypoplasia, enamel erosion, gingivitis, and periodontal disease

(v) Information reported on the possible appearance of aphthous lesions

We collected the data acquired through the OGP software, the anamnesis, and the physical examination, and, in particular, for each of the 70 patients, we assessed the presence or absence of the following:

(i) Excesses of proteins, carbohydrates, lipids, saturated fats, and cholesterol

(ii) Deficiencies of carbohydrates, lipids, vitamin C, vitamin A, vitamin D, calcium, iron, and omega 3

(iii) The presence of four types of lesions: caries, gingivitis, enamel hypoplasia, and aphthosis

The above elements were the variables available to us to conduct a study on the association between nutritional alterations and oral lesions.

The statistical analysis between the variables was performed with the chi-square test ($\chi^2$) for the evaluation of the significance of the association or independence. The degree of association between the nominal variables was verified with Cramér's V index ($C$).

## 3. Results

The results showed the following deficiencies in the diet (expressed as percentages): calcium (67%), vitamin D (66%), iron (56%), vitamin A (30%), carbohydrates (23%), omega 3 (20%), lipids (17%), and vitamin C (16%). The excessive amounts of nutrients introduced with diet were cholesterol (64%), saturated fats (63%), proteins (58.56%), lipids (44%), and carbohydrates (20%) (Tables 1(a)–1(c)).

As reported in Table 2, it shows the average ages, and most of the alterations (9 out of 13) were present at a slightly lower average age. The excesses of carbohydrates and saturated fats and the deficiencies of carbohydrates, lipids, vitamin C, vitamin D, calcium, iron, and omega 3 were present in subjects with a lower average age compared with those who were not characterized by such alterations. Only the excesses of proteins, lipids, and cholesterol and deficiency of vitamin A were found in patients with a higher average age compared to patients who did not have these alterations.

From this, we consider that the diet tended to improve with advancing growth. Clearly, we are talking about children who, having an age greater than 3 years, begin to select foods of their preference, avoiding quality (Table 3).

Regarding the sample's oral lesions, the following were found:

(i) 50% had caries

(ii) 34% had periodontal disease, represented by mild or moderate gingivitis

(iii) 33% had hypoplasia of the enamel

(iv) 20% reported the more or less frequent onset of canker sores

Table 1

(a) Population and distribution of subjects (total = 70), with an excess of each of the variables considered

| Excess | $n$ | % |
|---|---|---|
| Protein | 41 | 58.6 |
| Lipids | 31 | 44.3 |
| Carbohydrates | 14 | 20.0 |
| Saturated fats | 44 | 62.9 |
| Cholesterol | 45 | 64.3 |

(b) Population and distribution of subjects (total = 70), with a deficiency of each of the variables considered

| Deficiency | $n$ | % |
|---|---|---|
| Carbohydrates | 16 | 22.9 |
| Lipids | 12 | 17.1 |
| Vitamin C | 4 | 5.7 |
| Vitamin A | 21 | 30.0 |
| Vitamin D | 46 | 65.7 |
| Calcium | 47 | 67.1 |
| Iron | 39 | 55.7 |
| Omega 3 | 14 | 20.1 |

(c) Population and distribution of subjects (total = 70), with a deficiency of each of the variables considered

| Oral lesions | $n$ | % |
|---|---|---|
| Caries | 35 | 50.0 |
| Gingivitis | 24 | 34.2 |
| Hypoplasia | 23 | 32.9 |
| Aphthae | 14 | 20.0 |

The rejection region for the $\chi^2$ test of independence (Table 4), between the explanatory variable (presence or absence of nutritional alteration) and dependent variable (presence or absence of a lesion), showed dependence between the following:

(i) Caries and excess lipids and carbohydrates

(ii) Periodontal disease and excess saturated fats and carbohydrates

(iii) Periodontal disease and deficient vitamin C and iron

(iv) Enamel hypoplasia and deficient vitamin D

(v) Aphthae and deficient vitamin A

Through Cramér's V index, these associations were classified based on their degree of significance (Table 5).

(1) Vitamin A deficiency and canker sores ($C = 0.37$)

Table 2

(a) Ratio of the number of individuals with the presence (P) or absence (A) of excesses for each nutritional variable into the sample size (70 patients). Upper (U.L.) and lower (L.L.) limits can differ, within the sample population

| Excess | A ($n$) | P ($n$) | U.L. | L.L. |
|---|---|---|---|---|
| Protein | 0.41 | 0.59 | 0.70 | 0.47 |
| Lipids | 0.56 | 0.44 | 0.56 | 0.33 |
| Carbohydrates | 0.80 | 0.20 | 0.29 | 0.11 |
| Saturated fats | 0.71 | 0.63 | 0.74 | 0.52 |
| Cholesterol | 0.36 | 0.64 | 0.76 | 0.53 |

(b) Ratio of the number of individuals with the presence (P) or absence (A) of deficiencies for each nutritional variable into the sample size (70 patients). Upper (U.L.) and lower (L.L.) limits can differ, within the sample population

| Deficiency | A ($n$) | P ($n$) | U.L. | L.L. |
|---|---|---|---|---|
| Carbohydrates | 0.77 | 0.23 | 0.33 | 0.13 |
| Lipids | 0.83 | 0.17 | 0.26 | 0.08 |
| Vitamin C | 0.94 | 0.06 | 0.14 | 0.02 |
| Vitamin A | 0.70 | 0.30 | 0.41 | 0.19 |
| Vitamin D | 0.34 | 0.66 | 0.77 | 0.55 |
| Calcium | 0.33 | 0.67 | 0.78 | 0.56 |
| Iron | 0.44 | 0.56 | 0.67 | 0.44 |
| Omega 3 | 0.80 | 0.20 | 0.29 | 0.11 |

(c) Ratio of the number of individuals with the presence (P) or absence (A) of specific oral lesions to the sample size (70 patients). Upper (U.L.) and lower (L.L.) limits can differ, within the sample population

| Oral lesions | A ($n$) | P ($n$) | U.L. | L.L. |
|---|---|---|---|---|
| Caries | 0.50 | 0.50 | 0.62 | 0.38 |
| Gingivitis | 0.66 | 0.34 | 0.45 | 0.23 |
| Hypoplasia | 0.67 | 0.33 | 0.44 | 0.22 |
| Aphthae | 0.80 | 0.20 | 0.29 | 0.11 |

(2) Excess carbohydrates and caries ($C = 0.35$)

(3) Vitamin C deficiency and periodontal disease ($C = 0.34$)

(4) Iron deficiency and periodontal disease ($C = 0.28$)

(5) Excess lipids and caries ($C = 0.258$)

(6) Excess of saturated fat and periodontal disease ($C = 0.254$)

(7) Carbohydrate deficiency and periodontal disease ($C = 0.251$)

(8) Vitamin D deficiency and enamel hypoplasia ($C = 0.24$)

Table 3: Patients' mean age in the presence (P) or absence (A) of each nutritional alterations and oral lesions. The table also highlights whether the alteration characterizes a trail of greater or lower age (*P* major/minor).

| | A | P | *P* major/minor |
|---|---|---|---|
| Excess | | | |
| Protein | 9.93 | 10.12 | +0.19 |
| Lipids | 9.31 | 10.97 | +1.66 |
| Carbohydrates | 10.09 | 9.86 | -0.23 |
| Saturated fats | 10.08 | 10.02 | -0.05 |
| Cholesterol | 9.92 | 10.11 | +0.19 |
| Deficiency | | | |
| Carbohydrates | 10.09 | 9.88 | -0.22 |
| Lipids | 10.26 | 9.00 | -1.26 |
| Vitamin C | 10.18 | 7.75 | -2.43 |
| Vitamin A | 9.98 | 10.19 | +0.21 |
| Vitamin D | 10.42 | 9.85 | -0.57 |
| Calcium | 10.48 | 9.83 | -0.65 |
| Iron | 10.10 | 10.00 | -0.10 |
| Omega 3 | 10.57 | 7.93 | -2.64 |
| Oral lesions | | | |
| Caries | 10.20 | 9.89 | -0.31 |
| Gingivitis | 9.98 | 10.17 | +0.19 |
| Hypoplasia | 10.11 | 9.91 | -0.19 |
| Aphthae | 10.05 | 10.00 | -0.05 |

## 4. Discussion

The results obtained in this study regarding the association between nutritional alterations and oral manifestations have found confirmation in various studies [19–21]. Several studies have shown the existence of an association between nutritional alterations and tooth decay: studies that have evaluated the distribution of the body mass index (BMI) and the D(3+4)MFT index in a sample of children and have compared the different regression models by analyzing the association between these two indices [22–24]. Chen et al. in a cross-sectional study stated that an excess of fats does not predispose to caries [25], data confirmed by subsequent studies [26–28].

Bowen, on the other hand, reported how the significant presence of fats in the diet influenced cariogenicity, as fats would increase the clearance of sugars in the oral environment. It would also be conceivable that many fatty acids would exhibit a powerful antibacterial effect [24]; also, changes in the lipid levels and fatty acid composition could, therefore, be associated with caries development [29–32]. Earlier studies confirmed the association between caries and a sugar diet in accordance with the results of our study [33–36]. Gerdin et al. in a study aimed at evaluating the association between dental caries, body mass index, and socioeconomic status in Sweden concluded that the prevalence of being overweight and having caries was (weakly) associated in Swedish children [33].

Murty et al. reported in their study that saliva played a very important role in the prevention and development of tooth decay in enamel [35]. The study involved a comparison between two samples, one with caries-resistant subjects and the other with caries-sensitive subjects, showing that lipid concentration in the parotid saliva was higher in subjects with caries susceptibility [35].

Special cases are represented by the baby bottle syndrome: in the context of pathologies affecting deciduous teeth, we refer to the baby bottle tooth decay syndrome, which derives from incorrect use of the bottle [37–39]. For instance, letting the child fall asleep with the bottle by sucking milk or other sweet substances, or leaving the child for hours with a pacifier soaked in cariogenic substances (i.e., honey or sugar), is strongly discouraged as it often causes the formation of caries so extensive as to reduce the tooth to a small dark stump with consequent, painful abscesses, leading in this way to extracting the decayed dental elements. Statistically, children under 3 years of age are the most affected (in particular the incisor area) [40–43].

For the association between canker sores and vitamin A deficiency, the control function of vitamin A in the keratinization, maturation, and hydration of mucous membranes and skin was implicated [44–47]. Various studies confirm the association between aphthosis and nutritional deficiencies; however, they principally concerned deficiencies of iron, folate, and vitamins of the B group [48–51].

In the literature, we did not find clinical studies in which the association between the onset of aphthosis and the low vitamin A diet was highlighted [52–55]. In our findings, this association was established. Only Scully and Boyle describe, in 1992, in a review, the role of vitamin A in the prevention of potentially malignant lesions by indicating the protective effects but do not describe their pathogenic mechanisms [56].

Associations between vitamin C and D deficit and periodontal disease are described in the literature [56–59]. In our study, only four patients demonstrated a deficit of vitamin C (contained deficit), but all had a sustained degree of gingivitis [60, 61]. These were patients who had undergone more than one oral hygiene session due to the increased susceptibility to plaque buildup and inflammatory responses of the gums that led to edema and bleeding on probing more easily [62–64].

Regarding the association between a high-fat diet and periodontal disease, we can reiterate that, as adipose tissue is a source of inflammatory cytokines, an increase in body fat increases the risk of an increased inflammatory response in periodontitis [65, 66]. Several studies have shown the association between obesity and periodontitis [67–69].

Obesity is characterized by abnormal or excessive deposition of fat in the adipose tissue. The consequences go far beyond negative metabolic effects on health, causing an increase in oxidative stress, which leads not only to endothelial dysfunction but also to negative effects in relation to periodontitis, due to the increase in the inflammatory cytokines that are produced [70]. Thus, obesity appears to participate in the multifactorial phenomenon of the causality of

Table 4: Chi-square test. Values rejecting the null hypothesis of independence supportive alternative association (∗).

(a)

| Excess | Protein | Lipids | Carbohydrates | Saturated fats | Cholesterol |
|---|---|---|---|---|---|
| Caries | 0.0589 | 4.6898* | 8.9286* | 0.2448 | 0.5600 |
| Gingivitis | 0.2323 | 0.0355 | 0.2536 | 4.5335* | 0.0507 |
| Hypoplasia | 3.3226 | 0.3690 | 1.0361 | 0.0580 | 0.0130 |
| Aphthae | 0.5299 | 1.7514 | 1.8080 | 0.0153 | 0.0000 |

(b)

| Deficiency | Carbohydrates | Lipids | Vit. C | Vit. A | Vit. D | Calcium | Iron | Omega 3 |
|---|---|---|---|---|---|---|---|---|
| Caries | 0.0000 | 3.6207 | 1.0606 | 0.0680 | 2.2826 | 1.6189 | 2.8371 | 3.2143 |
| Gingivitis | 4.4410* | 0.3502 | 8.1313* | 0.4348 | 1.3977 | 0.0038 | 5.5054* | 0.5707 |
| Hypoplasia | 0.5804 | 0.0015 | 0.5651 | 0.2498 | 4.3395* | 1.9193 | 0.1740 | 0.0648 |
| Aphthae | 1.6406 | 1.6092 | 0.0663 | 9.7959* | 0.0159 | 1.0361 | 3.7055 | 2.7009 |

Table 5: Cramér's V index values (∗ indicates significant association).

(a)

| Excess | Protein | Lipids | Carbohydrates | Saturated fats | Cholesterol |
|---|---|---|---|---|---|
| Caries | 0.0290 | 0.2588* | 0.3571* | 0.0591 | 0.0894 |
| Gingivitis | 0.0576 | 0.0225 | 0.0602 | 0.2545* | 0.0269 |
| Hypoplasia | 0.2179 | 0.0726 | 0.1217 | 0.0288 | 0.0136 |
| Aphthae | 0.0870 | 0.1582 | 0.1607 | 0.0148 | 0.0000 |

(b)

| Deficiency | Carbohydrates | Lipids | Vit. C | Vit. A | Vit. D | Calcium | Iron | Omega 3 |
|---|---|---|---|---|---|---|---|---|
| Caries | 0.0000 | 0.2274 | 0.1231 | 0.0312 | 0.1806 | 0.1521 | 0.2013 | 0.2143 |
| Gingivitis | 0.2519* | 0.0707 | 0.3408* | 0.0788 | 0.1413 | 0.0073 | 0.2804* | 0.0903 |
| Hypoplasia | 0.0911 | 0.0046 | 0.0899 | 0.0597 | 0.2490* | 0.1656 | 0.0499 | 0.0304 |
| Aphthae | 0.1531 | 0.1516 | 0.0308 | 0.3741* | 0.0150 | 0.1217 | 0.2301 | 0.1964 |

periodontitis through an increase in the production of reactive oxygen species [70, 71].

Another association that emerged from our study was between a low-iron diet and periodontal disease. Enhos et al. led a study aimed at assessing the periodontal health status in patients with iron deficiency anemia, through the detection of ferritin levels in the crevicular fluid before and after periodontal therapy and concluded that iron deficiency was not a factor of direct risk for periodontal disease. There are, however, many other lesions associated with iron deficiency, such as atrophy of the lingual papillae, atrophic glossitis, angular cheilitis, and hyposalivation [72].

Regarding the association we found between periodontal disease and the low introduction of carbohydrates, the literature does not provide us with much data. Merchant et al.'s study showed an inverse correlation between the intake of whole grains and periodontitis [73]. Finally, regarding the last association between vitamin D deficiency and enamel hypoplasia, we can confirm this strong association widely described in the literature [74]. Vitamin D is strongly implicated as it is a direct protagonist of the deposition of calcium and phosphorus in the bones and teeth [75–78].

Throughout physical/oral examinations and the study of each patient's medical records, we collected data regarding the presence of oral lesions.

Statistical analysis confirmed many associations already reported in literature so far, introducing new original data. In this study, being a preliminary study, the results can be used as a baseline data for future studies with similar study design. For the issues related to "excess carbohydrates and caries," "vitamin C deficiency and periodontal disease," and "vitamin D deficiency and enamel hypoplasia," the associations already established were confirmed [79–82]. Therefore, in these three specific investigations, our findings have

the role only to strengthen and confirm current concepts. In contrast, the issues related to "vitamin A deficiency and canker sores," "iron deficiency and periodontal disease," and "carbohydrate deficiency and periodontal disease," more attractive results are introduced. In particular, association between "vitamin A deficiency and canker sores" not only confirmed but also showed a higher statistical significance. This could be due to the role that vitamin A plays in epithelialisation and keratinisation.

For the last association related to iron-periodontopathic deficiency, with regard to points "excess lipids and caries" and "excess of saturated fats and presence of periodontal disease," the relationships are according to literature reports [83–85]. However, studies of longer duration with crossover study design and wider sample size would have been more authenticating as it eliminates the bias of viable host.

## 5. Conclusions

The most relevant data emerging from the current research was the presence of an association between vitamin A deficiency and canker sores ($C = 0.37$) that has been not often described in scientific literature. The obtained results lead us to conclude that a correct and balanced diet, without excess and without nutritional deficiencies, contributes to the well-being of overall human body, including oral cavity, laying the fundamentals for proper tooth structure, maintaining the tropism of the mucosal membranes.

In addition, conclusions on an association of nutrient deficiency with periodontal disease cannot be assessed if the results are adjusted for comorbidity, such as smoking, diabetes mellitus, and other systemic diseases.

To produce conclusive evidence on the subject of this study, longitudinal cohort studies and follow-up randomized controlled trials are needed.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Authors' Contributions

A.P.C, M.D.C., and V.C. were responsible for the concept and design. A.B., L.S., R.L., and S.C. were responsible for the acquisition, analysis, and interpretation of data. M.D., A.B., and S.C. drafted the manuscript. S.C., N.F.T., V.L., and A.D.F. conducted bibliographic research. M.D.C., L.L.M., V.C., and M.D. critically revised the manuscript for important intellectual content. G.T., M. Di Comite., and R.N. were responsible for the data interpretation and technical and material support. S.C., M.D.C., L.S., A.B., and L.L.M. were responsible for the supervision and final approval. All authors have read and agreed to the published version of the manuscript. Angela Pia Cazzolla, Michele Di Cosola, and Andrea Ballini contributed equally as co-first authors. Vito Crincoli and Mario Dioguardi contributed equally as co-last authors.

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*Research Article*

# Comparative Evaluation of Adaptation of Esthetic Prefabricated Fiberglass and CAD/CAM Crowns for Primary Teeth: Microcomputed Tomography Analysis

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Adaptation is an important factor for the clinical success of restorations. However, no studies are available evaluating the adaptation of primary crowns. The aim of this study was to compare the adaptation of crowns fabricated by CAD/CAM technology versus prefabricated fiberglass primary crowns. Typodont maxillary central, canine, and mandibular molar teeth were prepared to serve as master dies after the size of Figaro crowns was determined ($n = 10$). Master dies were scanned with an intraoral scanner, and 10 identical CAD/CAM crowns were fabricated from resin-ceramic blocks. Figaro and CAD/CAM crowns were placed on the corresponding master dies and scanned via micro-CT. Three-dimensional volumetric gap measurements were performed to evaluate the overall adaptation. A total of 255 location-based linear measurements were allocated into 4 categories: marginal, cervical-axial, middle-axial, and occlusal. Statistical analyses were performed with factorial ANOVA, repeated measure ANOVA, and LSD tests ($\alpha = 0.05$). CAD/CAM crowns showed significantly lower overall and location-based gap measurements than Figaro crowns regardless of tooth number ($p < 0.05$). For all groups, mean marginal discrepancies were lower than occlusal measurements ($p < 0.05$). Both crown types showed higher marginal gaps for molar teeth than for canine and central incisors with no significant difference between them ($p > 0.05$). CAD/CAM-fabricated crowns showed better marginal and internal adaptation than prefabricated Figaro crowns.

## 1. Introduction

Early childhood caries (ECC) is defined as the presence of decay and decay-related filled or lost tooth surfaces in one or more teeth of children aged 71 months or younger [1, 2]. ECC begins with white lesions along the margin of the maxillary primary incisors and can progress rapidly, leading to the destruction of the crown [1, 3]. Besides esthetic, nutrition, and phonation problems, ECC may cause detrimental effects on general health [4]. If treatment for ECC is delayed, serious disorders such as pain dysfunction, negative effects on growth and development, psychological problems, and a decrease in quality of life may occur [1, 2, 5].

Depending on the progression of the disease, different treatment modalities for ECC can be applied from preventive techniques to crown restorations [4]. Primary teeth with widespread crown damage have been successfully treated with stainless steel crowns (SSCs) for many years [6, 7]. However, SSCs could not meet the esthetic expectations of paediatric patients and parents [8, 9]. Restorations that can satisfy increasing expectations have been obtained as a result of the developments in technology and esthetic material science for crowns in paediatric dentistry [8, 10, 11]. Veneered SSCs, composite strip crowns, and prefabricated zirconia crowns were the first materials introduced to accomplish an esthetic outcome [10–12]. The most preferred esthetic

crown nowadays is prefabricated zirconia crowns which are available from different manufacturers. The most important advantage of zirconia crowns is that gingival and plaque indices are lower among these crowns than other crown types [8]. However, these crowns have certain disadvantages such as (i) they are very technique sensitive and (ii) they require excessive tooth preparation to provide a passive fit [12, 13].

One of the newly launched materials to overcome such disadvantages is Figaro crowns made of fiberglass [14]. They are tooth colored and require less tooth reduction than paediatric preformed zirconia crowns with its flex-fit technology [14]. It is less technique sensitive than both composite strip crowns and zirconia crowns with a similar technique to place a SSC [15]. However, a previous study indicated failures in terms of crown retention, fracture resistance, and color deterioration for Figaro crowns compared to SSCs after 6 months of clinical evaluation period [14].

Another method of note to achieve esthetics in paediatric dentistry is the computer-aided design and computer-aided manufacturing (CAD/CAM) technology. Developments in CAD/CAM technics have enabled the production of esthetic and functional restorations for both permanent and primary dentition [16]. Customized crowns can be manufactured chairside by using CAD/CAM in a single appointment. Among a wide variety of blocks available for CAD/CAM, resin-ceramic blocks stand out with advantageous features for primary dentition including the wear prevention of opposing dentition due to their low hardness values and absorption of functional stresses because of their low modulus of elasticity [17, 18]. Another beneficial outcome of low modulus of elasticity was reported as the accurate adaptation of the restoration [18].

The marginal and internal adaptations are critical factors that determine the success of the restoration. While the marginal misfit was related to cement dissolution, microleakage, plaque accumulation, secondary caries, and periodontal disease, the internal misfit was associated with poor mechanical retention and reduced fractural strength [7, 18–20]. The adaptation may vary depending on the restorative material or production method of the restoration [20, 21]. No study to date has focused on the adaptation of crowns applied on primary teeth.

Therefore, the present in vitro study was aimed at comparing the adaptation of two types of esthetic paediatric crowns, the prefabricated fiberglass and custom-made resin-ceramic crowns, for primary teeth. The null hypothesis tested was that prefabricated fiberglass and CAD/CAM crowns would not differ in terms of adaptation.

## 2. Materials and Methods

This study has followed the CRIS guidelines for in vitro studies as discussed in the 2014 concept note.

*2.1. Master Die Preparation.* The marginal and internal adaptations of CAD/CAM crowns and fiberglass primary crowns were compared by using microcomputed tomography. A sample size of 10 per group was determined based on a power analysis (expected difference = 0.01, standard error of the mean = 23.85, $\alpha = 0.05$, $1 - \beta = 0.8$) [21]; 10 identical fiberglass crowns (Figaro crowns, Size XS; Figaro Crowns Inc., Minnesota, US) were selected considering the size of typodont primary central incisor (#51), canine (#53), and molar (#75) teeth prior to preparations as suggested by the manufacturer ($n = 10$). Typodont teeth were placed on a typodont model (Frasaco Dental Model, AK-6; Frasaco GmbH, Tettnang, Germany) and prepared by the same operator (TB) according to the manufacturer's preparation guide and suggestions for Figaro crowns [22]. The finished preparations and seating of the chosen Figaro crowns were approved by 2 operators (EİO and TB). The margin lines of the 3 master dies were marked by using a permanent marker.

*2.2. CAD/CAM Process.* The prepared #51, #53, and #75 master dies were placed on the typodont model one by one and digitized with an intraoral scanner (CEREC Omnicam; Dentsply Sirona, York, US). To replicate the external form of fiberglass crowns, the "biocopy" tool of the CEREC software (SW 4.6, Dentsply Sirona) was used. For this purpose, prefabricated fiberglass crowns were placed on the corresponding master dies and scanned with the CEREC Omnicam. The scanning process took approximately 5 min for each tooth. Preparation margins were drawn by the "automatic margin finder" tool, and deviations from the marked margin line were corrected manually. The die spacer parameter was set as 120 $\mu$m for all teeth, and the software automatically designed virtual crowns based on the scans of the fiberglass crowns. Ten CAD/CAM crowns for each master die (#51, #53, and #75) were milled from resin-ceramic blocks (CERASMART 270; GC Dental Products, Tokyo, Japan) by using a clinical type milling unit (CEREC MC XL; Dentsply Sirona) ($N = 30$). The milling time of each crown was about 10 min.

The sample size and test groups of the study are presented in Table 1.

*2.3. Micro-CT Evaluation.* Figaro and CAD/CAM crowns were placed on the corresponding master dies one by one with finger pressure until complete seating, maintained in that position under an axial load of 5 kg for 10 min in a seating pressure device, and were fixed with a parafilm (Parafilm M film; Bemis Company, Inc., Oshkosh, WI, US). The master dies were scanned with and without crowns by using a high-resolution desktop micro-CT (Bruker Skyscan 1275, Kontich, Belgium). Each stabilized specimen was positioned perpendicularly to the X-ray beam to ensure standardized positioning in the scanning tube and scanned with the following conditions: beam current at 100 kVp, 100 mA, 0.5 mm Al/Cu filter, 10.1 $\mu$m pixel size, rotation at 0.5 step, and 360° within an integration time of 10 min. The mean scanning time for each specimen was about 1 hour. Air calibration of the detector was done before each scan to minimize the ring artifacts. Beam-hardening correction and input of optimal contrast limits according to the manufacturer's instructions were carried out based on the former scanning and reconstruction.

Table 1: Test groups of the study.

| Tooth number | Crown type | |
|---|---|---|
| | CAD/CAM ($n$) | Prefabricated fiberglass ($n$) |
| 51 | 10 | 10 |
| 53 | 10 | 10 |
| 75 | 10 | 10 |

Visualization and quantitative measurements were utilized by using NRecon (ver. 1.6.10.4, SkyScan, Kontich, Belgium), DataViewer (version 1.5.6.2, SkyScan), and CTAn (version 1.17.7.2, SkyScan) software. For the reconstruction parameters, ring artifact correction and smoothing were fixed at zero, and the beam artifact correction was set at 30%. First, the reconstructed images were superimposed with the DataViewer software. The scans of the master die alone were used as a reference for the standardization of the measurement points. The master die images without a crown (reference) and with a crown (target) were superimposed, generating a volume of subtracting image. This image represented the entire area and volume of the gap between the crown and the master die. Then, the CTAn software was used for the 3-dimensional (3D) volumetric gap measurements ($mm^3$) to evaluate the overall adaptation.

A semiautomatic global thresholding (binarization) process was applied with CTAn software to distinguish the gap from other structures by processing the range of grey levels and to obtain imposed images of black and white pixels only. In this procedure, a Gaussian low-pass filter for noise reduction and an automatic segmentation threshold was used. Then, 5 fixed regions of interest (ROI) with the same dimensions ($1.5 \times 1.5$ mm for the central and canine teeth and $2.0 \times 2.0$ mm for molar tooth) were determined separately for each master die and for each slice to include the crown entirely. Forty equidistant vertical cuts from axial images were made in the mesiodistal direction. This procedure ensured the standardization of the location-based measurements. Seventeen measurement points were determined, and 85 measurements were done from 5 predesignated ROIs. Moreover, the observer repeated the measurements for each point 3 times. The mean values of all measurements were noted and were included in the statistical analysis. The observer also performed the study twice with an interval of 2 weeks to detect intraobserver variability. In total, 255 measurements were done for each crown. These 2D linear measurements ($\mu$m) were allocated into 4 location categories as follows: marginal (absolute marginal discrepancy: the average of the linear distances from the finish line of the preparation to the outer margin of the restoration) [21, 23], cervical-axial (the average of horizontal gap measurements performed in the cervical third of the axial walls), middle-axial (the average of horizontal gap measurements performed in the middle third of the axial walls), and incisal/occlusal discrepancies (the average of vertical gap measurements performed in the incisal/occlusal surface). The reconstructed images were also processed in SkyScan CTVox (ver. 3.3.0, SkyScan) for visualization (Figures 1(a)–1(f)).

*2.4. Statistical Analysis.* To assess intraobserver reliability, the Wilcoxon matched pairs signed rank test was used for repeated measurements. The mean values of these measurements were considered to be the final data. The normality of the data was verified using Shapiro-Wilk test ($p > 0.05$). The overall volumetric gap measurements were statistically analyzed with factorial analysis of variance (ANOVA) and least significant difference (LSD) tests. Location-based linear measurement data were evaluated with repeated measure ANOVA and LSD tests. The statistical analyses were performed using R v.3.5.3 (Microsoft Corporation, Redmond, WA, US) ($\alpha = 0.05$).

## 3. Results

Repeated measurements indicated no significant intraobserver difference for the observer ($p > 0.05$). Overall intraobserver consistency was rated at 92.6% between the two measurements, and all measurements were found to be highly reproducible.

Factorial ANOVA results and descriptive statistics for overall gap measurements are shown in Tables 2 and 3, respectively. CAD/CAM crowns showed lower overall mean gaps than fiberglass crowns irrespective of the tooth number ($p < 0.05$). Both crown types showed the highest volumetric gap for #75 ($p < 0.05$). The lowest overall volumetric gap for fiberglass crowns was obtained for the central incisor ($p < 0.05$), whereas for CAD/CAM crowns, no statistical difference was found between central and canine incisors ($p > 0.05$).

Repeated ANOVA results for linear measurements showed that the interactions between tooth number, crown type, and measurement location were significant ($p < 0.001$) (Table 4). Considering the differences between the location-based measurements for a certain tooth and crown type (Table 5), all groups showed lower mean gap values for the margin than that for the occlusal surface ($p < 0.05$). Both crown types applied on #51 and CAD/CAM crowns applied on #53 showed similar mean values for the marginal discrepancy and cervical-axial location ($p > 0.05$), whereas the other groups showed lower gap measurements for marginal discrepancy than that for the cervical-axial location ($p < 0.05$). Regardless of the crown type, middle-axial and incisal gap measurements were comparable for #51 and #53 ($p > 0.05$). The highest gap measurement for both crown types was obtained for the occlusal surface of #75 ($p < 0.05$).

Gap measurements for CAD/CAM crowns were lower than fiberglass crowns regardless of the location and tooth number ($p < 0.05$) with an exception of #51 for which comparable incisal gap measurements were found for CAD/-CAM and fiberglass crowns ($p > 0.05$) (Figure 2).

When the gap measurements obtained for different teeth were compared, #75 showed higher gap measurements than #51 and #53 irrespective of the location and for both crown types ($p < 0.05$). Considering fiberglass crowns, #53 showed higher middle-axial and occlusal gaps than #51 ($p < 0.05$). However, no significant differences were found between #51 and #53 at other locations either for CAD/CAM or for fiberglass crowns ($p > 0.05$).

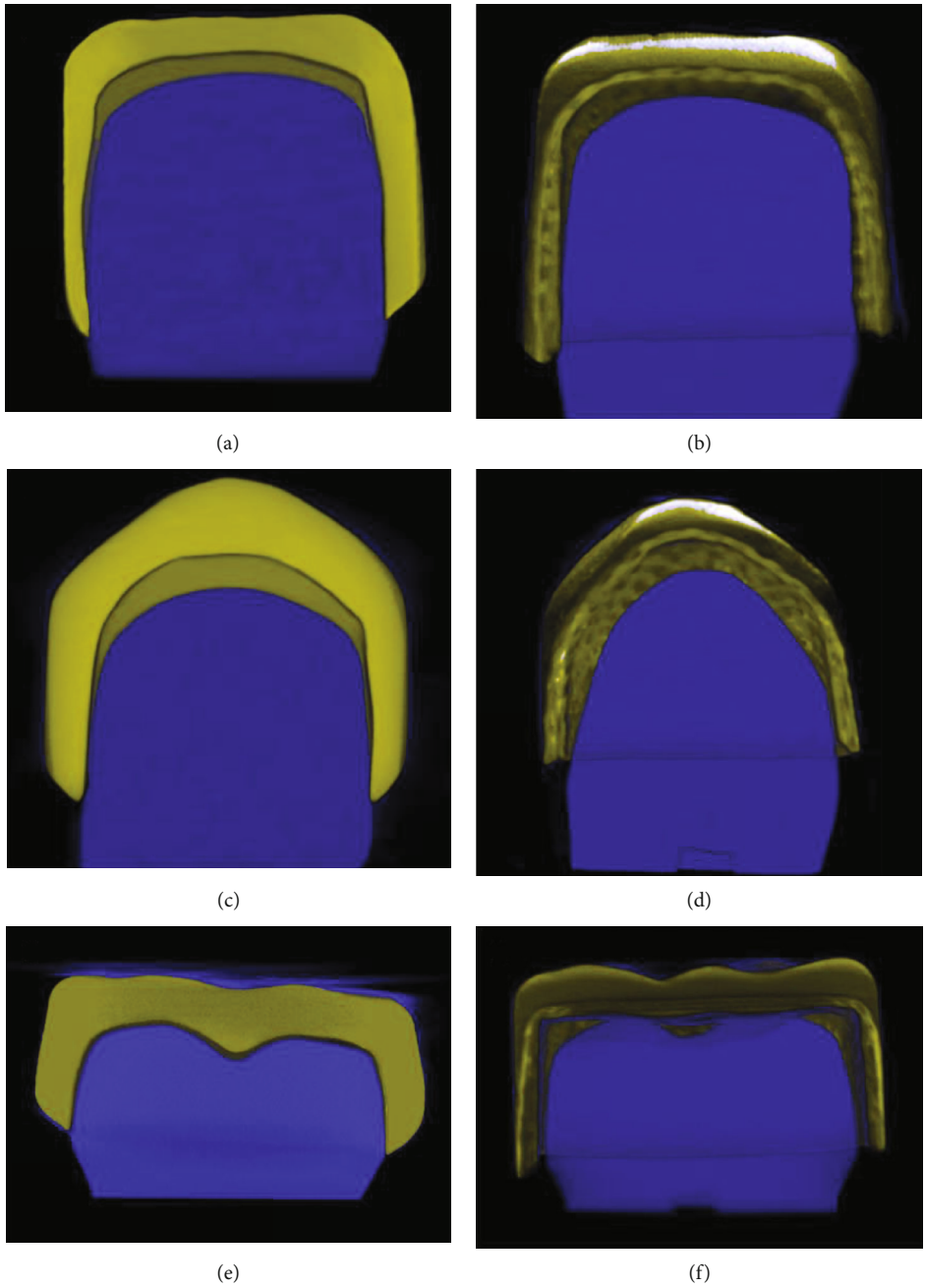


Figure 1: Representative micro-CT images of crowns applied on the corresponding dies. (a) CAD/CAM crown for #51; (b) Figaro crown for #51; (c) CAD/CAM crown for #53; (d) Figaro crown for #53; (e) CAD/CAM crown for #75; (f) Figaro crown for #75.

Table 2: Factorial ANOVA results for overall gap measurements.

| | SS | df | MS | $F$ | $p$ value |
|---|---|---|---|---|---|
| Tooth number | 185.777 | 2 | 92.888 | 279.999 | <.0001 |
| Crown type | 365.585 | 1 | 365.585 | 1102.003 | <.0001 |
| Tooth number ∗ crown type | 39.124 | 2 | 19.562 | 58.967 | <.0001 |

SS: sum of squares; df: degree of freedom; MS: mean squares.

Table 3: Mean and standard deviations (±SD) for overall gap measurements ($mm^3$).

| Tooth number | Crown type | |
|---|---|---|
| | CAD/CAM | Prefabricated fiberglass |
| 51 | 3.08 (0.38)$^{Aa}$ | 8.15 (0.74)$^{Ab}$ |
| 53 | 2.93 (0.48)$^{Aa}$ | 5.82 (0.61)$^{Bb}$ |
| 75 | 5.15 (0.56)$^{Ba}$ | 11.99 (0.61)$^{Cb}$ |

Different superscript uppercase letters (A, B, C) in the same column and different superscript lowercase letters (a, b) in the same line indicate statistically significant difference ($p < 0.05$).

## 4. Discussion

The adaptation of a crown is of importance for primary teeth as well as permanent dentition, considering that poor-fitting crowns may cause secondary caries or gingivitis [23]. This in vitro study compared the adaptation of CAD/CAM resin-ceramic and prefabricated fiberglass primary crowns by calculating overall, marginal, and internal gaps via micro-CT. The results showed significant differences between the gap measurements for CAD/CAM and fiberglass crowns concerning both overall and location-based evaluations. Therefore, the null hypothesis suggesting that the adaptation of fiberglass and resin-ceramic CAD/CAM crowns would be similar was rejected.

The adaptation can be evaluated by measuring the gap between the restoration and preparation with various methods such as direct microscopic measurement [18, 24], silicone replica technique [18], virtual seating of the crown and die by using their 3D scan data via reverse engineering software [20], and, as the newest technique, micro-CT imaging [21]. Precise linear 2D and volumetric 3D measurements can be performed in micron-level precision by using micro-CT, which was recommended as an innovative and nondestructive method for the in vitro evaluation of the adaptation [25]. Micro-CT allows for a great number of measurement points with close sectioning of the specimen, which ensures the reliability of the results [25]. In the present study, 5 ROIs were determined with equal sectioning in slices, and 255 measurements for each specimen were performed from 17 standardized points to ensure a comprehensive evaluation of internal and marginal adaptation of the crowns.

The present study compared the adaptation of two different esthetic crown types for primary teeth. Figaro crowns are composed of fiberglass, aramid, carbon, and quartz filaments embedded within a composite resin material [14]. The combination of these materials brings flexibility which enables a slight elastic deformation while placing the crown on the prepared tooth [26]. This flex-fit technology allows minimal tooth reduction, unlike zirconia esthetic crowns which require excessive preparation to compensate for the lack of flexibility [13, 14, 26]. To ensure the passive fit of zirconia crowns as recommended, retention and adaptation problems are frequently encountered [13]. Based on these considerations, zirconia esthetic crowns were not included in this study. SSCs are the most appropriate restorative materials in paediatric dentistry [8]. In addition, the tooth preparation is minimal, and their adaptation is flex-fit. However, SSCs were not included in this study as a control group because micro-CT does not allow the scanning of materials with high atomic number such as metals [27]. On the other side, CAD/CAM crowns were fabricated from resin-ceramic blocks considering the advantages for primary teeth and the similarity in composition to Figaro crowns. Therefore, custom-made CAD/CAM crowns were included as the control group.

In the present study, the overall adaptation was evaluated based on the 3D volumetric analysis and should be regarded as the total cement space [21, 28]. However, location-based linear 2D measurements provide data indicative of increased cement thickness at particular internal measurement points, as well as marginal adaptation [28]. CAD/CAM crowns showed better adaptation than Figaro crowns for both overall and location-based gap measurements and for all teeth. CAD/CAM crowns were designed based on the scans of Figaro crowns, and both crown types had identical outer forms. However, the internal contours were different as Figaro crowns have prefabricated, nonanatomical, and standardized inner surfaces while CAD/CAM crowns were custom-made. Micro-CT images for the mandibular molar showed that the CAD/CAM crown had rounded inner corners which were in harmony with the preparation outline (Figure 1(e)). On the other hand, right-angled internal corners that did not fit the preparation outline at the axioocclusal transition areas of the Figaro crown were observed (Figure 1(f)). Therefore, according to the present findings, it can be suggested that despite Figaro crowns allowing the restoration to adapt on the prepared tooth with flex-fitting, custom-made crowns fabricated with CAD/CAM technology provide better adaptation for primary teeth.

The uniformity of the gap between the preparation and the crown is important to ensure the retention form as well as fracture strength [21, 29]. Overall adaptation gives a general overview of the entire gap between the preparation and the crown; however, to evaluate the uniformity, location-based analysis is essential [21]. Previous studies reported that increased gap spaces at axial walls and the occlusal surface may reduce resistance to fracture [21, 29, 30]. Considering location-based adaptation, all groups showed a tendency for increased gap measurements from the marginal region to the occlusal surface. This finding corroborates with previous studies that reported the highest location-based gap measurements for the occlusal surface [31, 32]. For CAD/CAM restorations, the diameter and shape of the milling tools might limit the machining ability which would adversely affect the internal adaptation, especially at the occlusal surface [29]. On the other hand, frictional contacts that exceeded the flexibility limit of the Figaro crowns in the cervical region may have prevented proper fitting, resulting in an increased occlusal gap. Since disadvantages related to excessive occlusal gap include stress concentration and restoration fractures, clinicians should be cautious about occlusal adaptation when restoring primary teeth with Figaro or CAD/CAM crowns [19, 32].

Previous studies reported that the marginal gap values of ceramic crowns may range from 50 to 200 $\mu$m [18, 20, 21].

Table 4: Repeated measure ANOVA results for linear gap measurements.

| | SS | df | MS | $F$ | $p$ |
|---|---|---|---|---|---|
| Tooth number | 9859436 | 2 | 4929718 | 315.871 | <.0001 |
| Crown type | 2867286 | 1 | 2867286 | 183.721 | <.0001 |
| Tooth number ∗ crown type | 258328 | 2 | 129164 | 8.276 | <.0001 |
| Measurement location | 6145911 | 3 | 2048637 | 255.888 | <.0001 |
| Measurement location ∗ tooth number | 4239697 | 6 | 706616 | 88.261 | <.0001 |
| Measurement location ∗ crown type | 150605 | 3 | 50202 | 6.271 | <.0001 |
| Measurement location ∗ tooth number ∗ crown type | 474306 | 6 | 79051 | 9.874 | <.0001 |

SS: sum of squares; df: degree of freedom; MS: mean squares.

Table 5: Mean and standard deviations (±SD) for location-based gap measurements ($\mu$m).

| Tooth number | Crown type | Location | Mean (SD) | Range |
|---|---|---|---|---|
| 51 | CAD/CAM | Marginal | 197.8 (30.7)$^{A}$ | 157.89-261.41 |
| | | Cervical-axial | 235.9 (19.79)$^{AB}$ | 199.80-257.1 |
| | | Middle-axial | 297.03 (49.12)$^{BC}$ | 230.15-393.29 |
| | | Incisal | 356.11 (103.31)$^{C}$ | 241.72-613.87 |
| | Figaro | Marginal | 336.53 (22.59)$^{A}$ | 297.76-366.87 |
| | | Cervical-axial | 400.44 (48.5)$^{AB}$ | 295.27-464.57 |
| | | Middle-axial | 454.01 (85.37)$^{B}$ | 362.44-621.71 |
| | | Incisal | 445.21 (180.73)$^{B}$ | 288.36-763.2 |
| 53 | CAD/CAM | Marginal | 170.98 (22.11)$^{A}$ | 148-218.21 |
| | | Cervical-axial | 213.09 (17.87)$^{A}$ | 189.52-249.88 |
| | | Middle-axial | 301.44 (23.45)$^{B}$ | 276.43-342.69 |
| | | Incisal | 371.41 (50.91)$^{B}$ | 284.39-459.88 |
| | Figaro | Marginal | 313.17 (32.39)$^{A}$ | 252.95-373.85 |
| | | Cervical-axial | 454.26 (40.31)$^{B}$ | 386.98-501.17 |
| | | Middle-axial | 580.43 (64.71)$^{C}$ | 496.07-676.08 |
| | | Incisal | 590.84 (129.51)$^{C}$ | 461.73-897.04 |
| 75 | CAD/CAM | Marginal | 295.17 (26.48)$^{A}$ | 241.77-327.34 |
| | | Cervical-axial | 538.55 (74.31)$^{B}$ | 418.21-651.8 |
| | | Middle-axial | 672.18 (115.99)$^{C}$ | 533.05-881.19 |
| | | Occlusal | 1043.47 (254.81)$^{D}$ | 673.32-1589.18 |
| | Figaro | Marginal | 520.55 (47.55)$^{A}$ | 461.54-613.36 |
| | | Cervical-axial | 668.74 (55.39)$^{B}$ | 587.72-747.95 |
| | | Middle-axial | 933.65 (74.73)$^{C}$ | 795.15-1041.24 |
| | | Occlusal | 1618.56 (239.58)$^{D}$ | 1358.85-2131.47 |

Different superscript uppercase letters (A, B, C, D) in the same column indicate statistically significant difference ($p < 0.05$). $\mu$m: micrometer.

Only CAD/CAM crowns fabricated for central and canine incisors were within these limits. The marginal gap for CAD/CAM molar crowns was above 200 $\mu$m, yet lower than the marginal gap for Figaro crowns. All Figaro crowns exhibited marginal gap values exceeding the clinically acceptable range irrespective of tooth number. In the present study, the preparation design recommended for Figaro crowns was performed for all teeth, and optical impressions of the same prepared teeth were obtained to fabricate CAD/-CAM crowns. Therefore, for both crown types, marginal adaptations were evaluated for knife-edge margin which was reported to result in the greatest marginal discrepancy compared to other margin designs [24]. Furthermore, the present study evaluated marginal adaptation based on the absolute marginal discrepancy which considers both the horizontal and vertical directions [20]. Marginal design and marginal adaptation evaluation method employed in the present study may be the reason for high marginal gap values. Furthermore, micro-CT images of the Figaro crowns showed an overextension in the outer margin line which would have increased the gap measurements for the absolute marginal discrepancy (Figures 1(b), 1(d), and 1(f)). Based on

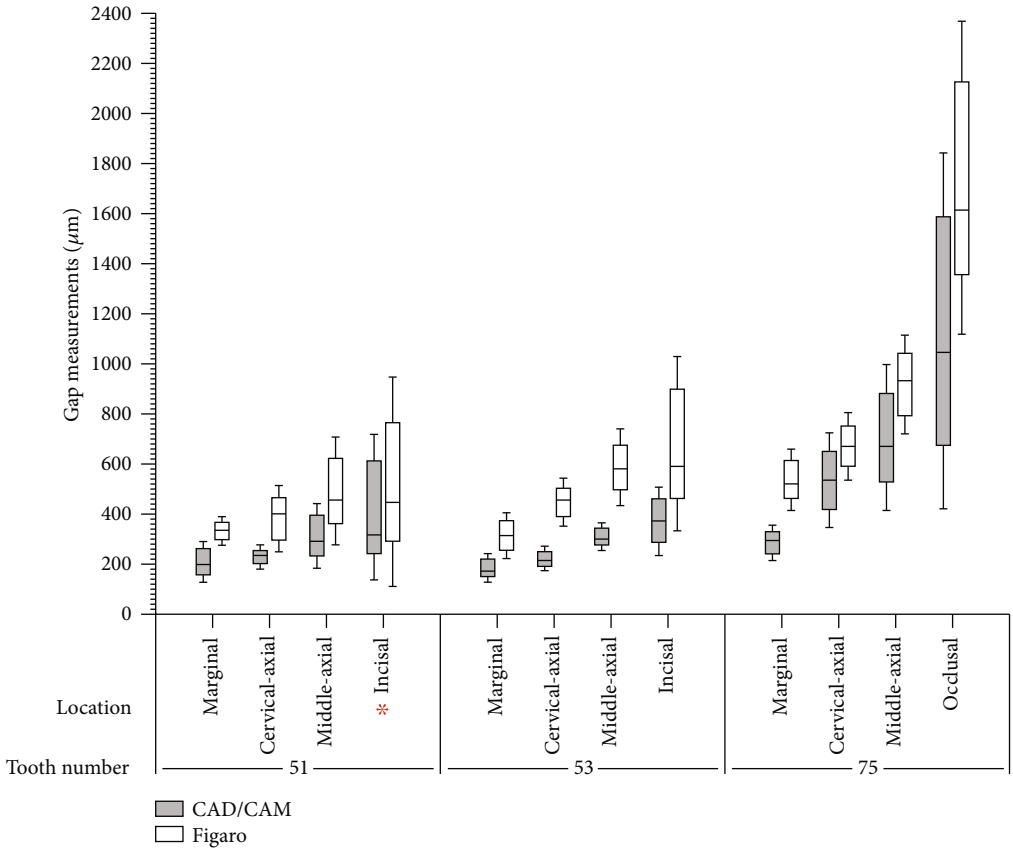


Figure 2: Comparison of gap measurements of different crown types based on location for each tooth. The asterisks (∗) indicate no statistical difference between groups ($p > 0.05$).

these findings, CAD/CAM crowns may be preferred over Figaro crowns considering the clinical significance of marginal adaptation.

In the present in vitro study, consistency of the results was ensured with standardized test conditions. Location-based gap measurements were performed by using the same ROIs and measurement points for all scans. To implement micro-CT measurements under the same conditions, CAD/CAM crowns were fabricated by scanning the same preparations on which Figaro crowns were adapted. Therefore, the preparations were standardized for both groups. Also, to eliminate differences in crown geometry, CAD/CAM crowns were designed based on the scans of Figaro crowns by utilizing the "biocopy" tool of the CEREC software. Nevertheless, limitations exist as in any in vitro study. Gap measurements were executed without cementation which may influence the fit of the restoration [24]. However, if the crowns were cemented on the corresponding master dies, the adaptation evaluation should have been performed on different preparations. To use a single standardized master die for each tooth, adaptation evaluation was performed without cementation. In addition, intraoral conditions such as soft tissue, saliva, and gingival fluid may affect the quality of the digital impression, thus adaptation. Further in vivo studies are warranted to evaluate the applicability of the CAD/CAM and Figaro crowns in paediatric dentistry and the effect of intraoral variables on the adaptation.

## 5. Conclusion

In this study, microcomputed tomography was first used to evaluate the adaptation of crowns for primary teeth, and the results showed that resin ceramic CAD/CAM crowns showed better overall, marginal, and internal adaptation compared to prefabricated fiberglass primary crowns for all primary teeth.

All crowns showed lower gap measurements at the marginal region compared to the occlusal surface, which is important for the clinical prognosis. A modality to define the clinically acceptable adaptation parameters for crowns applied to primary teeth can be developed based on the findings of this study.

## Data Availability

All data of the present article are available on request by contacting the corresponding author.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Authors' Contributions

E.İ.O., T.B., and K.O. conceived the ideas; E.İ.O., T.B., and A.I.O. collected the data and conducted the experiments; A.I.O. and K.O. implemented micro-CT measurements and analyzed the data; and E.İ.O. and T.B. led the writing.

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*Research Article*

# Evaluation of Residual Monomers Eluted from Pediatric Dental Restorative Materials

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Unreacted monomers eluted from resin-based restorative materials have been considered a reason of local and systemic adverse reactions. This study was designed to determine the effect of finishing and polishing procedures on the elution of Bis-GMA, TEGDMA, UDMA, and HEMA monomers from compomer and bulk-fill composite resins. Bulk-fill composite (3M ESPE GmbH, Seefeld, Germany) and compomer (Dentsply DeTrey GmbH, Konstanz, Germany) specimens with $3 \times 4$ mm diameters were prepared. The specimens were randomly divided into two groups, and finishing-polishing procedures were applied only to the experimental groups. Release of residual monomers was analyzed by using High-Performance Liquid Chromatography (HPLC) after 24, 48, and 72 hours. Repeated measures ANOVA and Tukey post hoc tests were used for comparisons. Finishing and polishing procedures had a significant effect on reducing the quantity of UDMA release in the Filtek™ Bulk Fill composite and Bis-GMA, HEMA, and TEGDMA in the Dyract XP compomer ($p < 0.05$). The restorative materials investigated here are not chemically stable after polymerization, and concentrations of eluted monomers may reach critical toxicity levels even after one restoration placement. Finishing and polishing procedures are mandatory to reduce residual monomers.

## 1. Introduction

In the early 1990s, polyacid-modified composite resins (compomers) are introduced to combine the superior mechanical properties of composite resins and fluoride release of glass-ionomer cements to overcome the disadvantages such as water sensitivity and physical strength of glass ionomer cements [1]. In recent years, bulk-fill composite resins are developed with the promise of placing single bulk increment up to 4-5 mm, thus providing lesser risk of contamination and reduced chair time which are important while working on pediatric patients [2].

Resin-based restorative materials consist of an organic polymerizable matrix, filler materials, molecules initiating the polymerization reaction, and silane coupling agents [3]. Mainly used constituents of the organic matrix are cross-linking dimethacrylates such as bisphenol-A-glycidyl dimethacrylate (Bis-GMA), triethylene glycol dimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), and hydroxyethyl methacrylate (HEMA) [4].

The polymerization of resin-based restorative materials is the end product of a chemical reaction between the methacrylate resin monomers that results in the formation of a rigid and highly cross-linked polymer network [5]. It is known that atmospheric oxygen inhibits the polymerization of monomers. This results in an "oxygen inhibition layer (OIL)" on the surface of resin-based restorative materials which are rich in unreacted monomers [6]. The degree of conversion of monomer to polymer in resin-based restorative materials varies between 55% and 80%, and this rate decreases to 35% in the presence of an OIL [7]. It has been reported that the degree of conversion further increased to nearly 95% when the OIL was removed by finishing and polishing techniques [8]. OIL could be minimized by blocking air contact with the use of matrix strips or glycerin before curing; however, since there is oxygen already present within the resin material, the most effective method to eliminate the OIL is to finish and polish the surface after curing [8, 9]. Finishing and polishing procedures are also essential for the elimination of the resin-rich outer surface [8–12].

Unreacted monomers eluted from resin-based restorative materials have been considered a reason of local and systemic adverse reactions, such as estrogenicity, cytotoxic effects on cell metabolism, genotoxicity, and mutagenicity [13, 14]. Quantity of the residual monomers in resin-based restorative materials was investigated in many studies [15–17]. However, there is no study in the literature investigating the effect of finishing and polishing procedures on monomer elution from resin-based restorative materials. Therefore, the present study was aimed at evaluating the effect of finishing and polishing procedures on monomer elution in the compomer that is commonly used in pediatric patients and in bulk-fill restorative resins which has become popular in pediatric dentistry, using High-Performance Liquid Chromatography (HPLC). The null hypothesis to be tested was that finishing and polishing procedures do not cause difference at elution of residual unreacted monomers from resin-based restorative materials.

## 2. Materials and Methods

*2.1. Specimen Preparation.* This study has followed the CRIS guidelines for in vitro studies as discussed in the 2014 concept note. Two types of resin-based restorative materials, bulk-fill composite (3M ESPE GmbH, Seefeld, Germany) and compomer (Dentsply DeTrey GmbH, Konstanz, Germany), in A2 shade were used. Power analysis indicated that a minimum of 60 teeth were required based on an effect size of 0.5, an alpha significance level of 5% (0.05), and a beta of 20% (0.20) to achieve an 80% power to detect a difference of 20% based on a previously conducted research [16]. The compositions of the tested restorative materials are given in Table 1.

The test specimens were prepared by using a cylindrical plexiglass mold of 3 mm in diameter and 4 mm in height [16]. The volume of test specimens was calculated as 28.3 $mm^3$, which approximately simulates the mesio-occluso-distal cavity volume of primary teeth [18]. Bulk-fill composite resins were applied as a single bulk increment of 4 mm, and compomers were applied in two increments with the height of 2 mm. A plexiglass mold is covered with Mylar strips (SNA, Universal Strips, Cologne, Germany) and with 1 mm thick glass slides at the top and bottom. Glass slides were then finger-pressed to the height of the mold to extrude the excess material. Bulk-fill composite specimens were cured by using a LED light curing unit (Elipar S10; 3M Unitek, Monrovia, Calif) with irradiance of 1000 $mW/cm^2$ for 20 seconds. Every increment of compomer specimens was cured for 10 seconds according to the manufacturer's instructions. The consistency of the curing light intensity was verified using a radiometer (PMA2100, Solar Light, Pennsylvania, USA) for each irradiation. After curing, the specimens were randomly divided into two groups, and finishing-polishing procedures were applied to the experimental groups only (Filtek™ Bulk Fill+ ($n = 15$), Dyract XP + ($n = 15$)). No finishing or polishing procedures were applied to the control groups (Filtek™ Bulk Fill- ($n = 15$), Dyract XP- ($n = 15$)).

In the experimental groups, finishing procedures were performed with a 12-fluted carbide finishing bur (Hager & Meisinger GmbH, Neuss, Germany) in a high-speed handpiece under water cooling. The tungsten carbide burs were changed every 4 specimens. The specimens were then polished with coarse, medium, fine, and superfine grit Sof-Lex discs (3M ESPE, St. Paul, MN, USA) with a low-speed handpiece for 15 seconds, respectively. Each disc was discarded after each use. After each step of polishing, all specimens were rinsed with water for 10 seconds and air dried for 5 seconds. All specimen preparations and finishing and polishing procedures were carried out by the same investigator to provide standardization.

Each specimen was immediately immersed in amber-colored HPLC vials containing 1.5 mL 75% ethanol/water solution and stored at room temperature [16]. Fifteen test specimens were divided into 3 groups, and each of the 5-specimen group was retained in the solution for 24, 48, and 72 hours, respectively. Samples of 0.5 mL ethanol/water were collected from each vial at the end of the observation periods.

*2.2. HPLC Analysis.* The analysis was performed by using the HPLC system (Agilent Technologies, Palo Alto, CA) with a C18 column (150 × 4.6 mm; 5 $\mu$m, ACE, Aberdeen, Scotland). Standard solutions of HEMA, Bis-GMA, UDMA, and TEGDMA (Sigma-Aldrich, St. Louis, MO, USA) were used for calibration. 5, 10, 25, 50, and 100 $\mu$g/ml solutions of each monomer were prepared and injected into the HPLC system. The injection volume was 10 $\mu$l on the column. The mobile phase was a solution of 80% acetonitrile (Sigma-Aldrich, St. Louis, MO, USA) and 20% water. UV detection was performed at 204 nm for HEMA and 193 nm for HEMA, TEGDMA, and UDMA. A calibration curve for each monomer was constructed from the injection standard solutions as an external standard. Correlation coefficients and linear range mathematical equations of monomers were obtained by linear regression analysis of concentration in standard solutions. Retention times, correlation coefficients, regression equations of the calibration curves, limit of detection (LOD), and limit of quantitation (LOQ) values of the monomers are given in Table 2.

*2.3. Statistical Analysis.* The statistical analyses were performed with R Studio (RStudio: Integrated Development for R. RStudio, Inc., Boston, MA). The results of the Shapiro-Wilk normality test showed that the data was normally distributed. Therefore, repeated measures ANOVA and Tukey post hoc tests were used for comparisons. The confidence interval was set to 95%, and values of $p < 0.05$ were considered to be statistically significant.

## 3. Results

The concentrations of eluted monomers were determined as a mean ± standard deviation ($\mu$g/mL) using peak areas. The mean values and standard deviations of the monomers eluted from test specimens are shown in Tables 3–6 and Figures 1–4.

Table 1: Restorative materials used in the study.

| Material type | Material | Composition | Manufacturer |
|---|---|---|---|
| Compomer | Dyract XP compomer | (i) Urethane dimethacrylate (UDMA)<br>(ii) Carboxylic acid-modified dimethacrylate (TCB resin)<br>(iii) Triethylene glycol dimethacrylate (TEGDMA)<br>(iv) Trimethacrylate resin (TMPTMA)<br>(v) Dimethacrylate resins<br>(vi) Camphorquinone<br>(vii) Ethyl-4(dimethylamino)benzoate<br>(viii) Butylated hydroxytoluene (BHT)<br>(ix) UV stabilizer<br>(x) Strontium-alumino-sodium-fluoro-phosphor-silicate glass<br>(xi) Highly dispersed silicon dioxide<br>(xii) Strontium fluoride<br>(xiii) Iron oxide pigments and titanium oxide pigments | Dentsply DeTrey GmbH, Konstanz, Germany |
| Bulk-fill composite | Filtek™ Bulk Fill composite | (i) Aromatic dimethacrylate (AUDMA)<br>(ii) Addition-fragmentation monomers (AFM)<br>(iii) UDMA<br>(iv) 1,12-Dodecanediol dimethacrylate (DDDMA)<br>(v) Nonagglomerated/nonaggregated 20 nm silica filler<br>(vi) Nonagglomerated/nonaggregated 4 to 11 nm zirconia filler<br>(vii) Aggregated zirconia/silica cluster filler<br>(viii) Ytterbium trifluoride filler consisting of agglomerate 100 nm particles | 3M ESPE GmbH, Seefeld, Germany |

Table 2: Regression equations of the calibration curves, detection limits, and retention times of the monomers.

| | Regression equation | $R^2$ | LOD ($\mu$g/mL) | LOQ ($\mu$g/mL) | Retention time (minute) |
|---|---|---|---|---|---|
| Bis-GMA | $y = 0.0148x - 1.7497$ | 0.9978 | 0.680 | 2.053 | 2.85 |
| TEGDMA | $y = 0.0229x - 1.012$ | 0.9997 | 0.788 | 2.363 | 2.32 |
| HEMA | $y = 0.0235x - 19.753$ | 0.9999 | 0.482 | 1.445 | 1.82 |
| UDMA | $y = 0.0351x - 0.1227$ | 0.9999 | 0.412 | 1.236 | 2.59 |

LOD: limit of detection; LOQ: limit of quantitation.

Table 3: Mean values and standard deviations (±SD) of UDMA ($\mu$g/mL) eluted from restorative materials.

| | Dyract XP- | Dyract XP+ | Filtek™ Bulk Fill- | Filtek™ Bulk Fill+ |
|---|---|---|---|---|
| 24hours | $3.981 \pm 0.260^{aA}$ (0.0084) | $1.630 \pm 0.348^{aA}$ (0.0034) | $65.682 \pm 6.078^{bA}$ (0.1395) | $23.898 \pm 14.356^{cA}$ (0.0507) |
| 48 hours | $4.746 \pm 0.354^{aA}$ (0.01) | $1.993 \pm 0.533^{aA}$ (0.0042) | $85.974 \pm 18.299^{bB}$ (0.1826) | $26.090 \pm 15.577^{cA}$ (0.0554) |
| 72 hours | $4.547 \pm 0.592^{aA}$ (0.0096) | $2.304 \pm 0.329^{aA}$ (0.0048) | $89.101 \pm 9.325^{bB}$ (0.1893) | $30.212 \pm 16.750^{cA}$ (0.0641) |

Different superscript uppercase letters indicate a significant difference ($p < 0.05$) within the same column; different superscript lowercase letters indicate a significant difference ($p < 0.05$) within the same row (Tukey post hoc test). The numbers in parentheses are expressed as mmol/L.

Table 4: Mean values and standard deviations (±SD) of HEMA ($\mu$g/mL) eluted from restorative materials.

| | Dyract XP- | Dyract XP+ | Filtek™ Bulk Fill- | Filtek™ Bulk Fill+ |
|---|---|---|---|---|
| 24 hours | $36.939 \pm 1.413^{bA}$ (0.2838) | $14.473 \pm 1.805^{aA}$ (0.1112) | $5.281 \pm 0.481^{a}$ (0.0405) | $3.344 \pm 0.943^{a}$ (0.0256) |
| 48 hours | $44.418 \pm 2.240^{aAC}$ (0.3413) | $18.769 \pm 3.518^{bA}$ (0.1442) | Below LOD | Below LOD |
| 72 hours | $49.939 \pm 3.811^{aBC}$ (0.3837) | $24.238 \pm 2.504^{bA}$ (0.1862) | Below LOD | Below LOD |

Different superscript uppercase letters indicate a significant difference ($p < 0.05$) within the same column; different superscript lowercase letters indicate a significant difference ($p < 0.05$) within the same row (Tukey post hoc test). The numbers in parentheses are expressed as mmol/L. LOD: limit of detection.

Table 5: Mean values and standard deviations (±SD) of Bis-GMA (μg/mL) eluted from restorative materials.

| | Dyract XP- | Dyract XP+ | Filtek™ Bulk Fill- | Filtek™ Bulk Fill+ |
|---|---|---|---|---|
| 24 hours | $30.209 \pm 0.895^{bA}$ (0.0589) | $15.386 \pm 2.058^{aA}$ (0.03) | $8.996 \pm 0.721^{aA}$ (0.017) | $4.482 \pm 2.255^{aA}$ (0.0087) |
| 48 hours | $37.401 \pm 2.135^{bAC}$ (0.0729) | $20.306 \pm 3.261^{cdA}$ (0.0396) | $12.509 \pm 1.580^{adA}$ (0.0244) | $5.674 \pm 2.289^{aA}$ (0.011) |
| 72 hours | $42.312 \pm 3.823^{bBC}$ (0.0825) | $25.435 \pm 1.699^{cdA}$ (0.0498) | $15.317 \pm 2.113^{adA}$ (0.0298) | $10.278 \pm 4.011^{aA}$ (0.02) |

Different superscript uppercase letters indicate a significant difference ($p < 0.05$) within the same column; different superscript lowercase letters indicate a significant difference ($p < 0.05$) within the same row (Tukey post hoc test). The numbers in parentheses are expressed as mmol/L.

Table 6: Mean values and standard deviations (±SD) of TEGDMA (μg/mL) eluted from restorative materials.

| | Dyract XP- | Dyract XP+ | Filtek™ Bulk Fill- | Filtek™ Bulk Fill+ |
|---|---|---|---|---|
| 24 hours | $20.249 \pm 0.943^{aA}$ (0.0707) | $6.342 \pm 1.310^{bA}$ (0.022) | Below LOD | Below LOD |
| 48 hours | $22.137 \pm 0.806^{aA}$ (0.0773) | $7.667 \pm 1.794^{bA}$ (0.0267) | Below LOD | Below LOD |
| 72 hours | $24.476 \pm 3.323^{aA}$ (0.0854) | $9.791 \pm 1.145^{bA}$ (0.0341) | Below LOD | Below LOD |

Different superscript uppercase letters indicate a significant difference ($p < 0.05$) within the same column; different superscript lowercase letters indicate a significant difference ($p < 0.05$) within the same row (Tukey post hoc test). The numbers in parentheses are expressed as mmol/L. LOD: limit of detection.

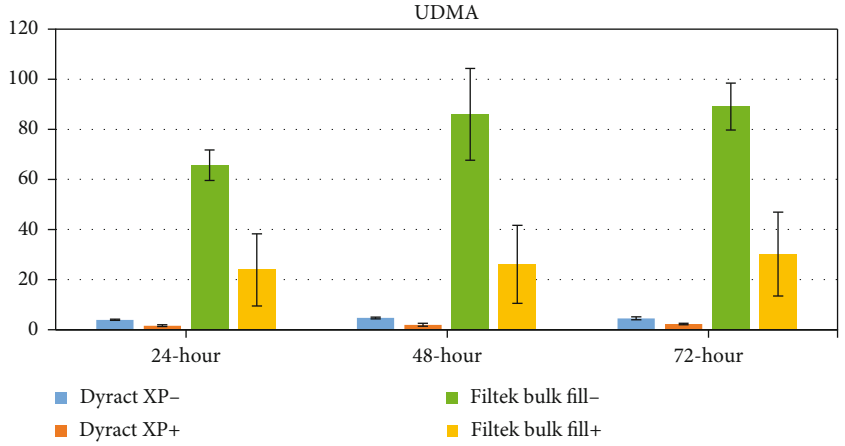


Figure 1: Graphical view of UDMA release from tested restorative materials.

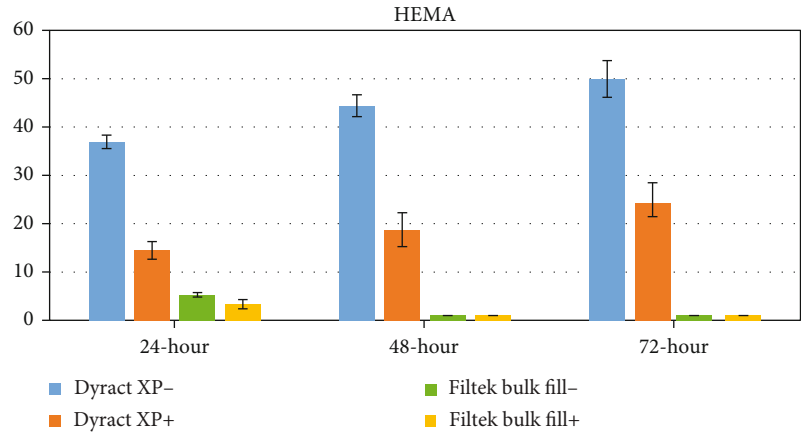


Figure 2: Graphical view of HEMA release from tested restorative materials.

*3.1. UDMA.* Elution of UDMA was reduced after finishing and polishing procedures at all time periods in both Filtek™ Bulk Fill groups and Dyract XP groups; meanwhile, the decrease was significant only in Filtek™ Bulk Fill groups ($p < 0.001$). The cumulative quantities of UDMA in Filtek™ Bulk Fill- at the 48th and 72nd hours were significantly higher than those at the 24th hour ($p < 0.001$) (Table 3, Figure 1).

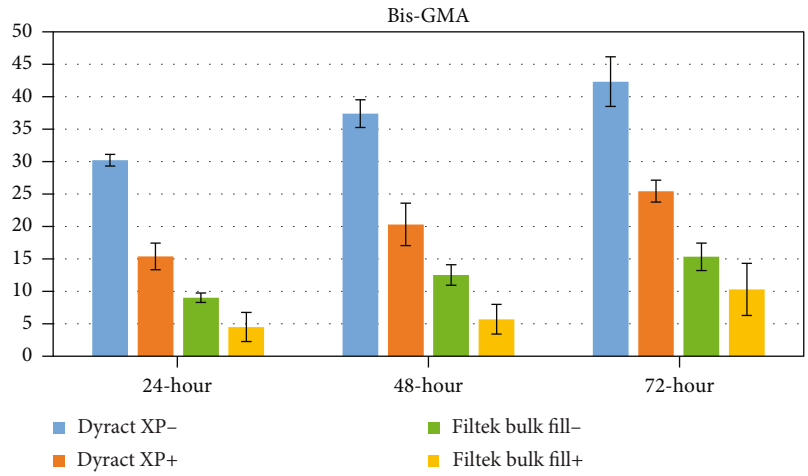


Figure 3: Graphical view of Bis-GMA release from tested restorative materials.

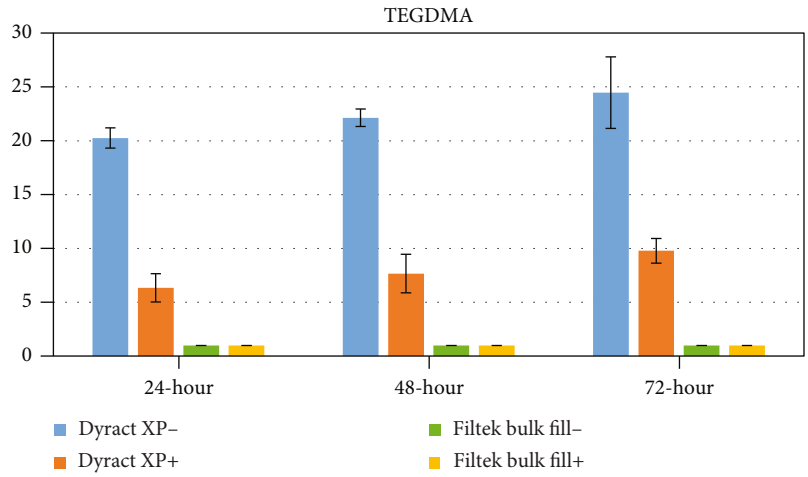


Figure 4: Graphical view of TEGDMA release from tested restorative materials.

*3.2. HEMA.* HEMA elution dropped significantly in Dyract XP+ when compared to Dyract XP- at all time periods ($p < 0.001$). The highest cumulative quantity of HEMA was detected at the 72$^{nd}$ hour in Dyract XP-, which is significantly higher than the quantity at the 24$^{th}$ hour ($p < 0.05$). Elution of HEMA was not detected in Filtek™ Bulk Fill- and Filtek™ Bulk Fill+ at the 48$^{th}$ and 72$^{nd}$ hours. The decrease in the elution of HEMA in Filtek Bulk Fill- was not significant in comparison to that in Filtek Bulk Fill+ (Table 4, Figure 2).

*3.3. Bis-GMA.* The reduction in the quantity of Bis-GMA was not significant for both Filtek™ Bulk Fill- and Filtek™ Bulk Fill+ at any time period. However, Bis-GMA elution was significantly lower in Dyract XP+ in comparison to Dyract XP- at all time periods ($p < 0.05$; $p < 0.001$). The cumulative quantity of Bis-GMA in Dyract XP- at the 72$^{nd}$ hour significantly increased when compared to that at the first 24-hour span ($p < 0.05$) (Table 5, Figure 3).

*3.4. TEGDMA.* There was no release of TEGDMA in both Filtek™ Bulk Fill composite groups at any time periods. In Dyract XP groups, finishing and polishing procedures led to a significant decrease in the quantity of TEGDMA at all time periods ($p < 0.05$) (Table 6, Figure 4).

## 4. Discussion

In the present study, HPLC analysis demonstrated that residual monomers were leached from resin-based restorative materials and the quantity of residual monomers could be reduced with finishing and polishing procedures.

Resin-based restorative materials may cause hazards due to the release of unreacted monomers after polymerization. It was reported that the resin monomers are able to increase the amount of reactive oxygen species and oxidative stress which results in apoptosis of the cell. They also have been found to be related to DNA strand breaks, caspase activation, and delay in the cell cycle [14]. In many studies, toxic

doses of monomers have been investigated and various results have been obtained by the test method and cell type dependence. The toxicity for the following monomers was ranked as Bis-GMA > UDMA > TEGDMA > HEMA (least toxic) [19, 20]. In another study, exposure of dental pulp cells to Bis-GMA at concentrations of 0.075 mmol/L markedly affected the viable cell number with 40% of inhibition [19]. Reichl et al. [21] reported the concentration that causes 50% reduction of cell viability which is named half maximal effect concentration ($EC_{50}$) of UDMA and Bis-GMA as 0.106 mmol/L and 0.087 mmol/L on human gingival fibroblasts, respectively. Toxic concentration$_{50}$ ($TC_{50}$) of HEMA ranged from 3.6 mmol/L to 11.2 mmol/L with different cell lines in various studies [22–24]. The effective dose that reduced the number of cell viability to 50% for TEGDMA was reported as 0.26 mmol/L on human pulp fibroblasts [19] and 3.46 mmol/L on human gingival fibroblasts [21]. In our study, Bis-GMA concentration in Dyract XP- at the 72$^{nd}$ hour was found either equal or greater than the toxic concentrations obtained in some previous studies [21, 22, 25]. UDMA concentrations in Filtek™ Bulk Fill- for all retention times were also higher than the toxic concentration reported in the study of Reichl et al. [21]. However, after finishing and polishing procedures were applied, neither of the monomer concentrations in groups was above the toxic doses.

HEMA and Bis-GMA were detected in both of the restorative materials tested. However, these monomers were not listed as ingredients in the Material Safety Data Sheet (MSDS) of the products. Manufacturers are not obligated to reveal the components with concentrations lower than 1% in their products as trade secret. Furthermore, it was shown that ingredients in MSDS are sometimes insufficient [26, 27]. Botsali et al. [28] also confirmed the presence of HEMA in the Dyract XP compomer. Another reason for HEMA elution in this study could be that it was a degradation product from UDMA, which is an ingredient in both restorative materials [29]. Therefore; HEMA elution detected from Filtek Bulk Fill- and Filtek Bulk Fill+ groups at the 24$^{th}$ hour may have been going down below the detection limits at the 48$^{th}$ and 72$^{nd}$ hours. However, to the best of our knowledge, no previous study has ever investigated monomer elution from the Filtek™ Bulk Fill composite with or without finishing and polishing procedures by using HPLC analysis, which makes it difficult for us to compare the results of our study.

Time is also a significant factor on monomer elution. Some studies have reported that acute release of monomers occurs in the first 24 hours [30, 31]. However, some recent researches have shown that monomer elution is not completed within the first 24 hours and leaching in certain monomers continued for a longer time [16, 31]. Therefore, 72 hours of elution was investigated in this study and found that monomers mostly leached in the first 24 hours which is consistent with previous studies, but the elution of UDMA in Filtek™ Bulk Fill- significantly increased in time. Similarly, the elution of HEMA and Bis-GMA from Filtek™ Bulk Fill- was also higher at the 72$^{nd}$ hour when compared to the level of elution from Dyract XP- at the 24$^{th}$ hour. However, this increase in the quantity of monomers over time was not observed in restorative material groups when finishing and polishing procedures were applied. Therefore, since unreacted monomers were removed by finishing and polishing agents, monomer elution in Filtek™ Bulk Fill+ and Dyract XP+ was observed in lower quantities and it did not increase significantly over time. Also, it is possible to say that the surface of test specimens was still rich in unreacted monomers when finishing and polishing were not performed, although clinicians usually think that the use of matrix strips prevented the formation of an oxygen inhibition zone. In line with previous studies [11, 12], this study showed that Mylar strips may minimize the formation OIL, but finishing and polishing procedures are still essential for the elimination of the resin-rich outer layer that can be the source of the unreacted monomers eluted to the oral cavity.

Finishing and polishing procedures had a significant effect on reducing the quantity of UDMA in the Filtek™ Bulk Fill composite and Bis-GMA, HEMA, and TEGDMA in the Dyract XP compomer. Because of these differences, the null hypothesis had to be rejected. The test specimens in this study simulated the mesio-occluso-distal cavity volume of primary teeth [18]. Therefore, the quantity of elution demonstrated in this study was from a single restoration. The quantity of unreacted monomers may reach dangerous levels when more than one restoration is performed in the same treatment session. Furthermore, compomer and bulk-fill composites are frequently used in dental restorations of pediatric patients, and the restorations are expected to last for a reasonable time. There are few studies that investigate the long-term elution of monomers for 1, 3, and 12 months [16, 17]. However, long-term effects of residual monomers on biocompatibility are still unclear. Due to constant flow of saliva in oral environment, it is believed that monomer concentrations may not reach cumulative values found in this study, whereas long-term chronic exposure and systemic adverse effects must also be considered when assessing the potential toxicity of the eluted compounds. Thus, finishing and polishing procedures play an important role in the elimination of unreacted monomers to the highest possible extent to prevent health effects from long-term exposure.

This research has several limitations. Firstly, only one brand of the compomer and bulk-fill composite was used for the assessment of monomer elution. The differences in composition of resin-based restorative materials could result in variability at release of amount and type of monomers. Secondly, finishing and polishing procedures were implemented using 12-fluted carbide finishing bur and Sof-Lex discs. It was reported that the use of carbide burs and Sof-Lex discs provided the smoothest surface [32]; therefore, they are preferred in this study. However, further in vitro researches may focus the amount of monomer elution by using different restorative materials and finishing-polishing techniques.

According to the results of the present study, the elution of residual monomers was higher if finishing and polishing were not performed. The bulk-fill composite showed lesser monomer elution compared to the compomer restoration

except UDMA elution. In addition, the bulk-fill composites have the advantage of shorter chair time which can be an important factor in pediatric dentistry. Therefore, it can be concluded that this type of restorative material can be a good alternative for pediatric patients [33]. However, long-term clinical studies are needed to evaluate its success.

## 5. Conclusion

(1) Since the results of the present study demonstrated that the restorative materials investigated here are not chemically stable after polymerization and concentrations of eluted monomers may reach critical toxicity levels even after one restoration placement, further research is needed to understand potential long-term toxicity of resin-based restorative materials, especially for pediatric patients

(2) This study showed that Mylar strips did not prevent the formation of the oxygen inhibition layer, and finishing-polishing is still essential for the elimination of the resin-rich outer layer that can be the source of the unreacted monomers eluted to the oral cavity

(3) When placing multiple restorations at a single session, it is highly recommended to follow the instructions of manufacturers during polymerization and apply finishing and polishing procedures

## Data Availability

All data of the present article are available on request by contacting the corresponding author.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

## Authors' Contributions

TB was responsible for the conceptualization, methodology, data curation, project administration, formal analysis, validation, and writing of the original draft. CC was responsible for the conceptualization, methodology, data curation, project administration, investigation, formal analysis, validation, and writing of the original draft. NO was responsible for the conceptualization, methodology, data curation, project administration, formal analysis, validation, and review and editing of the manuscript.

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