ORIGINAL RESEARCH

Assessment of Interleukin-6 Levels and *Lactobacillus casei* Counts in Pediatric Stainless Steel and Zirconia Crowns: A Comparative Study

Nancy P Saharia[1], Manvi Malik[2], Pulkit Jhingan[3], Nikita Gulati[4], Shivani Mathur[5]

Abstract

Background: Pediatric dental crowns play an integral role as they maintain the form and function and prolong the life of the affected tooth. However, placing a crown in the oral cavity creates a new niche for the adhesion of microorganisms that can lead to plaque accumulation, gingival inflammation, and the development of secondary caries, which in the long term might determine the clinical success of the restored tooth. The present study allowed us to assess the changes caused by the full coverage restorations at a clinical, immunological, and microbiological level using enzyme-linked immunosorbent assay (ELISA) and microbial analysis.

Materials and methods: The *in vivo* analysis consisted of a total of 26 children aged 3–10 years. They were divided into two groups, group I (n = 13) children receiving preformed zirconia crowns and group II receiving stainless steel crowns (SSCs). Plaque index (PI) scores, gingival index (GI) scores, and interleukin-6 (IL-6) levels were assessed at baseline and at 45 days of follow-up. The *in vitro* part of the study consisted of 13 preformed zirconia crowns and 13 SSCs which were immersed in artificial saliva containing strains of *Lactobacillus casei* which were then processed for their microbial analysis.

Results: On mean comparison, preformed zirconia crowns performed superiorly both clinically and immunologically compared to SSCs. Microbial analysis using independent t-test revealed that the colony-forming units (CFU) per milliliter was statistically significantly higher for the SSCs, and the mean difference among the groups was statistically significant ($p < 0.01$).

Conclusion: Preformed zirconia crowns can be a relative replacement for SSCs in primary teeth with the advantage of esthetics and superior periodontal health.

Keywords: Colony count, Dental caries, Gingival inflammation, *Lactobacillus casei*, Pediatric crowns.

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Introduction

Throughout history, mankind has grappled with oral health challenges. Despite some progress in enhancing oral health across various nations, oral diseases remain a prevalent issue on a global scale. Dental caries, gingivitis, and periodontitis are some of the most commonly encountered oral diseases across all age groups, genders, and geographical locations.[1]

Dental caries, a chronic condition characterized by tooth decay, demonstrates notable fluctuations in its occurrence influenced by a myriad of factors and geographic regions. Worldwide, primary tooth caries afflicts around 486 million children, with approximately 2.4 billion individuals affected by caries in permanent teeth. Gingivitis has received less attention compared to dental caries despite the similar prevalence in understanding the long-term impact on overall health, especially in primary dentition.[2–4]

Following any intervention on a primary tooth, such as a proximal restoration or endodontic procedure, it is advisable to place a crown afterward. This action helps uphold the tooth's structure and function, mitigates the risk of residual root fractures, minimizes microleakage and reinfection, preserves esthetics, and aids in securing the final restoration. Hence, these crowns have become a salient factor in the restoration of extensively carious lesions.[4,5]

There are different types of restorations for complete crown coverage and each of these crowns presents technical, functional, or esthetic challenges that intricate their efficient and effective usage.[6,7] Stainless steel crowns (SSCs) and preformed zirconia crowns are among the most commonly used crowns in pediatric patients.

However, the placement of a crown in the oral cavity establishes a new environment for microbial attachment, potentially resulting in plaque buildup, gingival inflammation, and the formation of secondary caries. Over time, these factors play a pivotal role in determining the clinical outcome of the restored tooth.[8]

According to the literature, the primary cariogenic bacteria identified are *Streptococcus mutans* and *Lactobacillus*. *Lactobacillus*,

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recognized as the second most influential cariogenic bacterium within oral flora, significantly contributes to the advancement of caries. A strong correlation has been established between *Lactobacillus* count and caries.[9,10]

The effect of the full coverage restoration on the gingival health of an individual can be assessed by determining the levels of various inflammatory markers in the gingival crevicular fluid (GCF). Interleukin-6 (IL-6) plays a role in regulating the host's response to infection and tissue injury. It plays a principal role in the transition between acute and chronic inflammation and considerably remains longer in the plasma thus making it a good marker of inflammation.[11–13]

Hence, this comparative study was aimed at evaluating the adhesion of *L. casei* on SSCs and preformed zirconia crowns using microbial analysis and to further evaluate the effect of these full coverage restorations on gingival health by determining the gingival index (GI) scores, plaque index (PI) scores, and IL-6 levels in the GCF using enzyme-linked immunosorbent assay (ELISA). This study enabled us to assess the changes caused at clinical, microbiological, and immunological levels.

Materials and Methods

The present study employed a nonblinded, randomized, prospective controlled trial design. It was conducted in the Department of Pediatric and Preventive Dentistry, I.T.S. Centre for Dental Studies & Research, Muradnagar. Ethical clearance was granted by the Institutional Ethics Committee (approval number: ITSCDSR//IIEC/2020-2023/PEDO/05). The Clinical Trials Registry—India (CTRI) registration number for this study is CTRI/2022/03/041350. Before participation, parents and children were briefed on the study's objectives and procedures. Written consent was obtained from the parents, and verbal assent was sought from the children.

Sample Size Calculation

According to the results of the pilot study, the sample size was determined using the formula:

$$n = \frac{2(Z_\alpha + Z_\beta)^2 \left[s\right]^2}{d^2}$$

Where, Z_α is the z variate of α error, that is, a constant with value 1.96, $Z\beta$ is the z variate of β error, that is, a constant with value 0.84. Approximately, 13 subjects/crowns per group was derived as the sample size to completed the study to give a good external validity.

The present study was divided into two parts:

1. *In vivo* component (clinical and immunological analysis): Comparative evaluation of PI scores, GI scores, and IL-6 levels in the GCF of children receiving zirconia and SSCs.
2. *In vitro* component (microbiological analysis): Comparative evaluation of microbial adhesion onto the surfaces of preformed zirconia crowns and SSCs.

In Vivo Analysis: Recording of PI Scores, GI Scores, and IL-6 Levels in GCF in Children Receiving SSCs and Preformed Zirconia Crowns

Inclusion Criteria

- Children within 3–10 years of age group.
- Children indicated for full coverage restoration.
- Mandibular primary second molar with multiple carious surfaces requiring a full coverage restoration.
- Subjects having an intact contralateral primary molar for each crown type.
- Subjects with parental consent for the study.

Exclusion Criteria

- Mentally or physically challenged children.
- Children undergoing long-term antimicrobial therapy.
- Children having periodontal or systemic diseases.
- Children with unilateral chewing habits.
- Allergies to local anesthetics and/or nickel.

Blinding and Randomization

Statistician analyzing the data was blinded to the study arm. However, it was not possible to blind the investigator and the trial participants because each crown type has its own specification.

Patient Allocation

A total of 26 subjects aged between 3 and 10 years, who were being treated in the Department of Pediatric and Preventive Dentistry from January to September 2022 for dental caries and subsequently required full coverage restorations, were enrolled in the present study.

A total of 26 subjects and 52 mandibular primary molar teeth were included in the study. The participants were randomly allocated into two groups: group I and group II, with 13 children in each group. Group I comprised children receiving preformed zirconia crowns, and group II comprised children receiving SSCs. Group I was further subdivided into group I(A) and group I(B), which comprised carious teeth restored with preformed zirconia crowns and healthy contralateral teeth that served as controls, respectively. Similarly, group II was subdivided into group II(A) and group II(B), which comprised carious teeth restored with SSCs and healthy contralateral control teeth, respectively as shown in Figures 1 and 2.

Intervention

A single operator specialized in pediatric dentistry performed the study to reduce operator variability. Preparation of the teeth for SSCs and zirconia crowns was performed under local anesthesia. Tooth preparations for both crowns were conducted following the manufacturer's guidelines. SSCs of appropriate size were tried, trimmed, and contoured to adapt to the tooth. In the case of the zirconia crowns, try-in crowns were used to check for proper passive seating. The crowns were cemented in the same session under isolation using type I GIC (Fuji Plus, GC Inc., Tokyo, Japan).

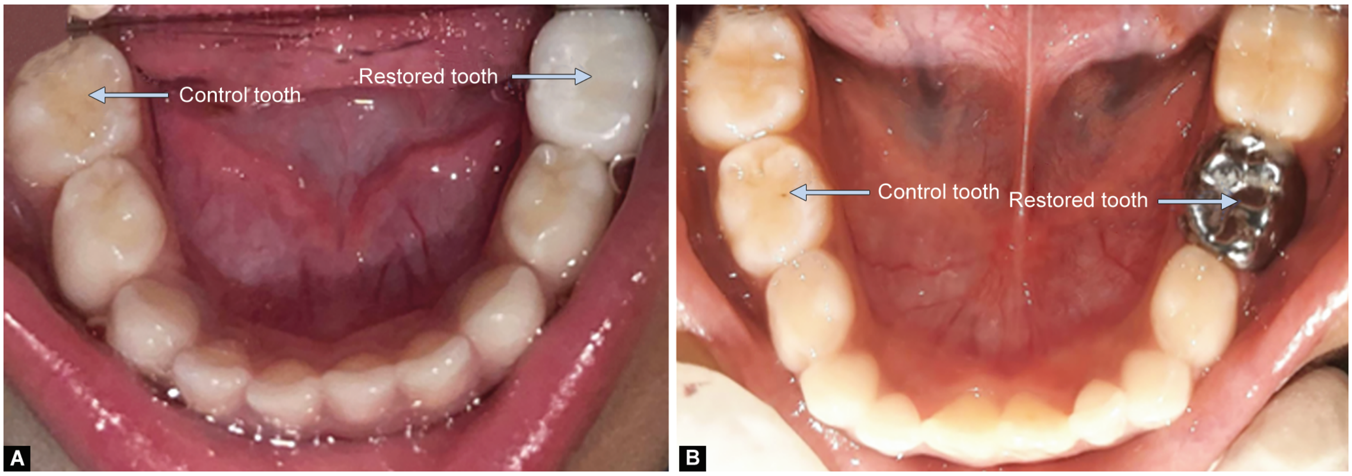
Restored and control teeth were comparatively evaluated by assessing PI, GI scores, and IL-6 levels at baseline and at day 45 of follow-up. Baseline measurements were scored 1 day after cementation in order to prevent blood contamination during GCF collection.

Clinical Analysis

Periodontal health and oral hygiene were clinically evaluated using the PI and GI given by Silness and Loe.[14–16] Shepherd's Hook No. 23 explorer tip was carefully maneuvered along the sulcus of buccal, lingual, mesial, and distal surfaces of both restored and control teeth, with scoring conducted accordingly.

Immunological Analysis

Immunologically, periodontal health was assessed by comparatively evaluating the IL-6 levels in the GCF of the



Figs 1A and B: (A) Tooth restored with preformed zirconia crown and its respective contralateral control tooth; (B) Tooth restored with SSC and its respective contralateral control tooth

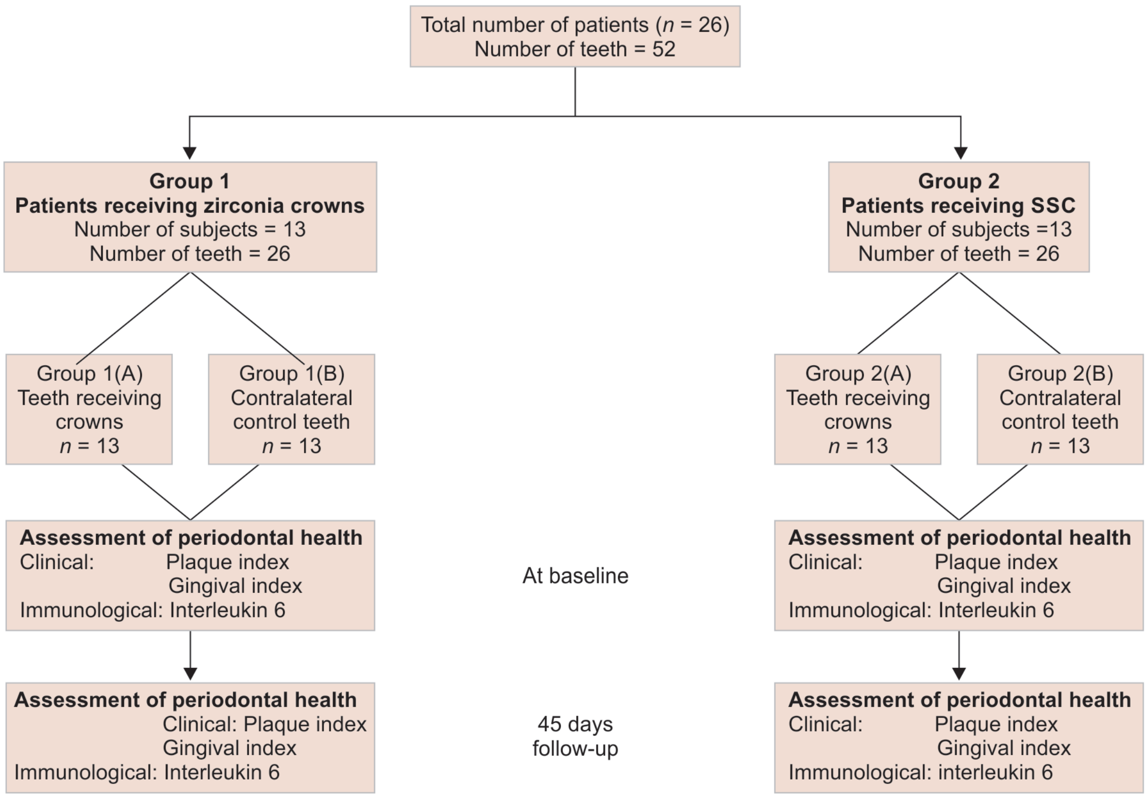


Fig. 2: *In vivo* study design

restored and control teeth. The site was readied by eliminating the supragingival plaque under isolation, followed by gentle air drying. GCF was acquired utilizing prefabricated 2 × 13 mm PerioPaper strips (Ora flow Inc., New York, United States). These strips were gently inserted into the gingival crevice until encountering slight resistance. Following a 30-second interval, the strips were transferred into Eppendorf tubes containing 1 mL of phosphate buffer solution (PBS) and preserved at –70°C for future assessment. ELISA was conducted following the manufacturer's instructions, utilizing the Diaclone Human IL-6 ELISA Kit. At the end of the analysis, the color change was noticed after the addition of the stop reagent (H_2SO_4) in some of the samples in which IL-6 was detected as shown in Figures 3A and B. The enzyme-linked immunosorbent assay with automated intelligent detection (ELISA-AiDTM) method was employed to compute the results. Plate readings were taken at 450 nm initially, and then at 490 nm with reference filter set at 650 nm.

In Vitro Analysis of *Lactobacillus* Counts on the Surfaces of Preformed Zirconia Crowns and SSCs

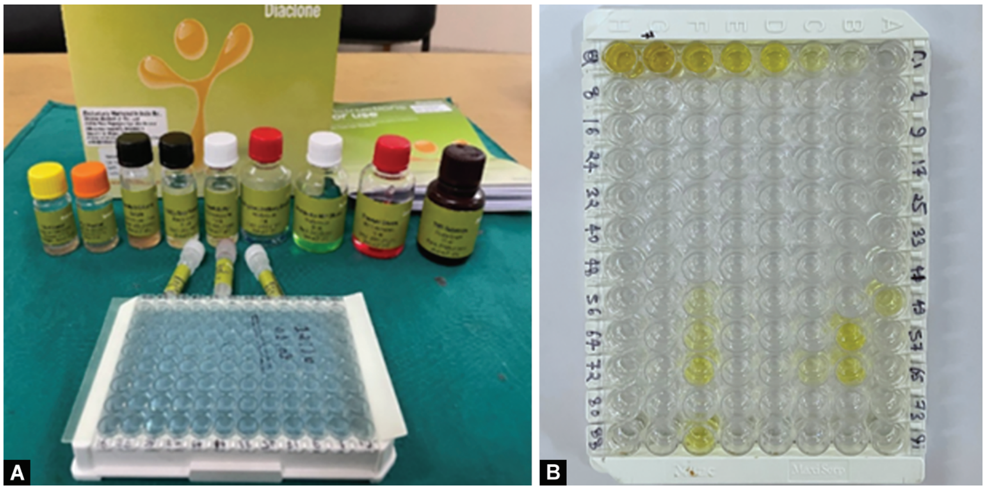
In the *in vivo* analysis, 26 crowns were examined, comprising 13 preformed zirconia crowns and an equal number of SSCs. A freeze-dried bacterial culture strain of *L. casei* with Microbial Type Culture Collection and Gene Bank (MTCC) number 1408 was used as shown in Figures 4A and B.

Microbial Analysis

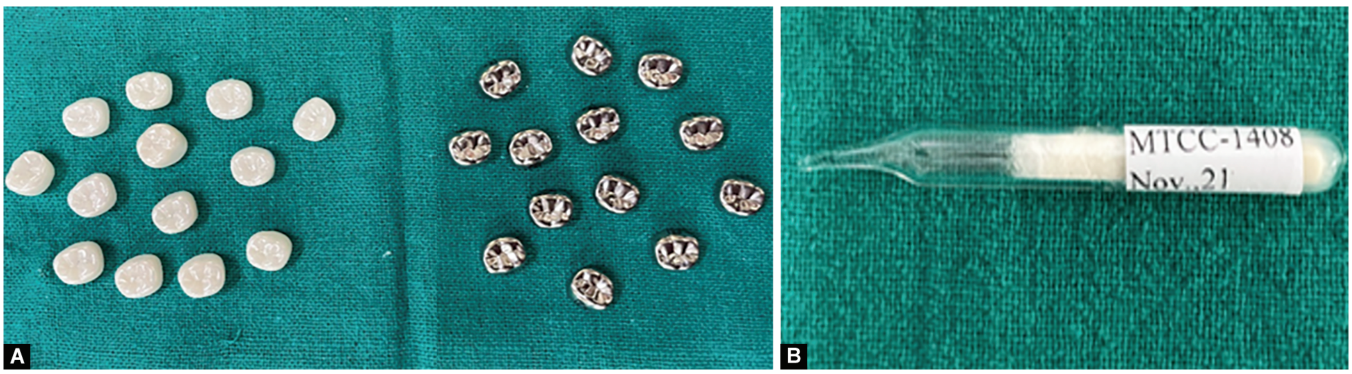
Following the pilot study, the remaining bacteria were revived by introducing the *L. casei* strain into 500 mL of brain heart infusion (BHI) broth. The cultivation was then placed in an incubator set at 37°C for 24 hours, adhering to MTCC guidelines. A turbid growth in the BHI broth was observed after 24 hours of incubation, implying the bacterial strain's revival.

All 26 crowns were immersed in a solution containing 20 mL of revived broth cultivation and 10 mL of commercially available artificial saliva in order to simulate the environment of the oral cavity. It was then incubated at room temperature for 72 hours.

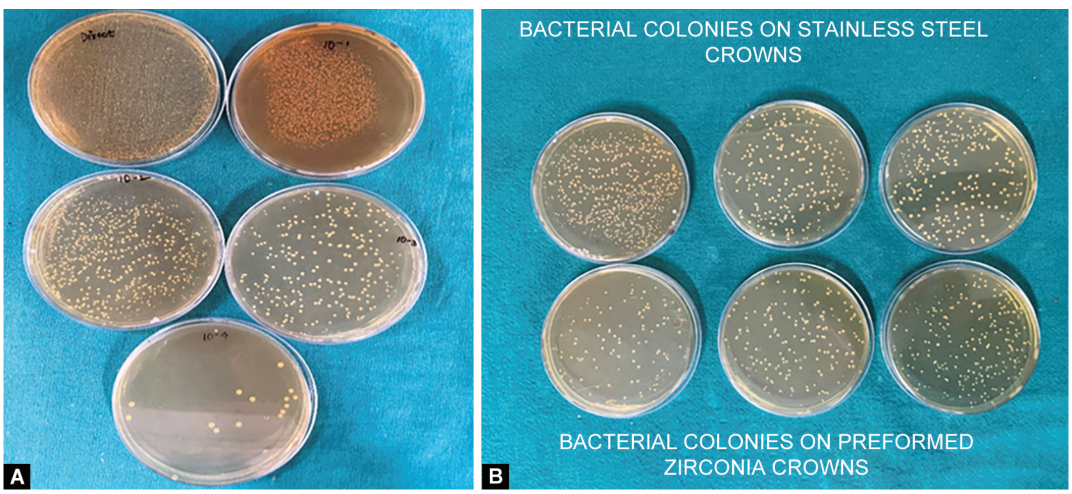
On day 4, swabs from both the buccal and lingual surfaces of these crowns were taken and inserted into the Eppendorf tubes containing 1,000 µL of PBS. To assess microbial counts, samples were vortexed for 10 seconds. Following this, each sample was diluted three times in a 10-fold manner (10^{-3} dilutions) as outlined in Figure 5A based on insights gained from the pilot study. A total of 100 µL of 10^{-3} dilutions from each sample were seeded



Figs 3A and B: (A) Diaclone IL-6 ELISA kit; (B) Addition of 100 µL of H_2SO_4 stop reagent into all wells of the ELISA microtiter plate



Figs 4A and B: (A) Preformed zirconia crowns and preformed SSCs; (B) Freeze-dried bacterial strain containing *L. casei* with MTCC number 1408



Figs 5A and B: (A) Bacterial growth at direct, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions; (B) Microbial growth on SSCs and preformed zirconia crowns onto the Man Rogosa agar plates for bacterial growth. The plates were left to incubate at 37°C for a period of 48–72 hours until colonies became visible, as illustrated in Figure 5B. Observations were made regarding the growth, and the colonies were quantified using a colony counter to estimate the colony-forming units (CFU) per milliliter of volume. The data obtained were tabulated and further sent for statistical analysis.

Statistical Analysis

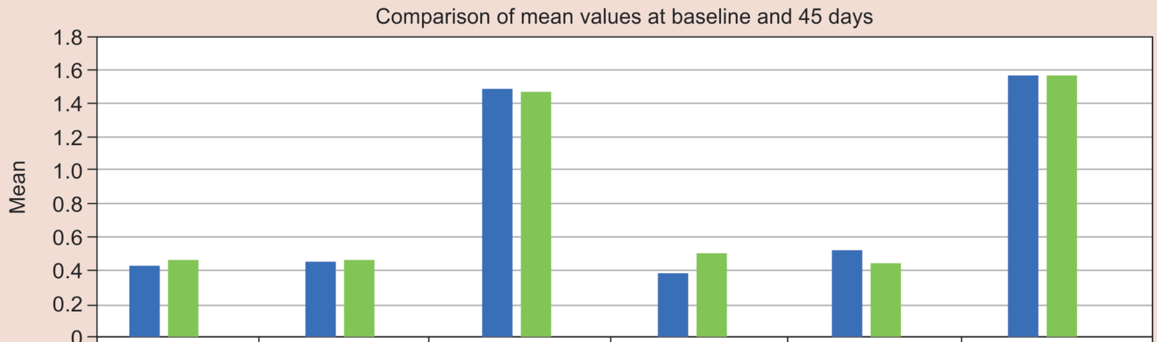
The collected data was recorded in Microsoft Excel and then subjected to analysis using Statistical Package for the Social Sciences (SPSS) 16.0 for Windows (SPSS Inc., Chicago, Illinois, United States, 2001). In the *in vivo* analysis, the significance of mean differences within groups (intragroup comparison) was assessed using the Wilcoxon signed-rank test, while comparisons between groups (intergroup comparison) were conducted using the Mann–Whitney *U* test. In the *in vitro* analysis, the mean values of parameters between groups were examined using an independent *t*-test, with a significance level of 5% and a confidence interval of 95%.

*Significant $p < 0.05$, **Highly significant $p < 0.001$, NS = Not significant $p > 0.05$.

Results

In Vivo Analysis

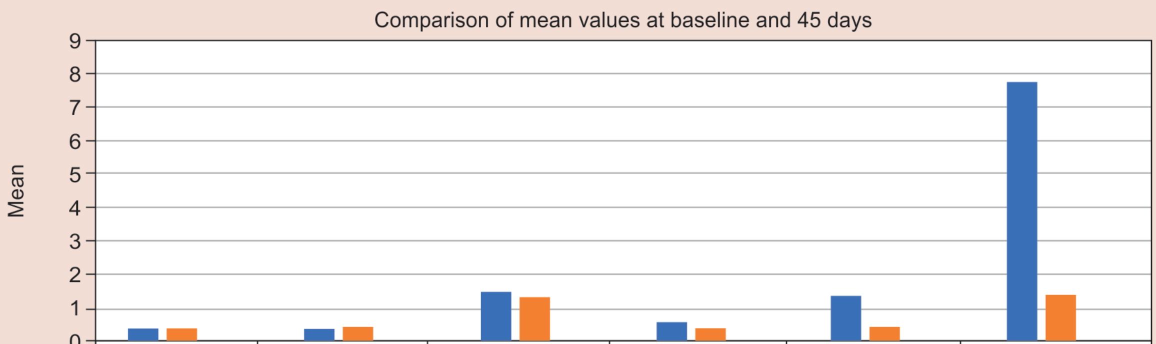
In group I, no statistically significant differences were detected among the variables assessed between the restored and control teeth at baseline ($p > 0.05$). However, at 45 days of follow-up, a statistically significant decrease was observed in the mean PI scores of zirconia crowns ($p < 0.05$) when compared to the control teeth. In group II, statistically, nonsignificant differences were observed among the variables assessed between the restored and control teeth at baseline ($p > 0.05$). At 45 days of follow-up, an increase in the PI scores ($p < 0.05$), GI score, and IL-6 levels ($p < 0.01$) were observed in SSCs when compared to their control teeth (Figs 6 and 7).



	PI Baseline	GI Baseline	IL-6 Baseline	PI 45th day	GI 45th day	IL-6 45th day
Zirconia crowns	0.423	0.462	1.4877	0.38	0.519	1.554
Control teeth	0.462	0.462	1.4635	0.5	0.442	1.566
p-value	0.619	1	0.555	0.021	0.34	0.98

Variables

Fig. 6: Comparison of PI scores, GI scores, and IL-6 levels between the teeth restored with preformed zirconia crowns and contralateral control teeth at baseline and 45 days of follow-up using Mann–Whitney *U* test



	PI Baseline	GI Baseline	IL-6 Baseline	PI 45th day	GI 45th day	IL-6 45th day
SSCs	0.3654	0.385	1.52969	0.5385	1.365	7.84654
Control teeth	0.3654	0.404	1.36569	0.3654	0.404	1.41215
p-value	1	0.839	0.158	0.021	0	0

Variables

Fig. 7: Comparison of PI scores, GI scores, and IL-6 levels between the teeth restored with preformed SSCs and respective contralateral control teeth at baseline and 45 days of follow-up using Mann–Whitney *U* Test

On time-dependent intragroup comparison, a decrease in mean PI scores was observed at 45 days of follow-up in zirconia crowns ($p < 0.05$) and a statistically significant increase in PI scores, GI scores, and IL-6 levels was observed in SSCs at 45 days of follow-up when compared to their respective baseline values. The control teeth of groups I and II at 45 days of follow-up showed statistically nonsignificant differences in their GI, PI, and IL-6 levels from baseline values (Figs 8 and 9).

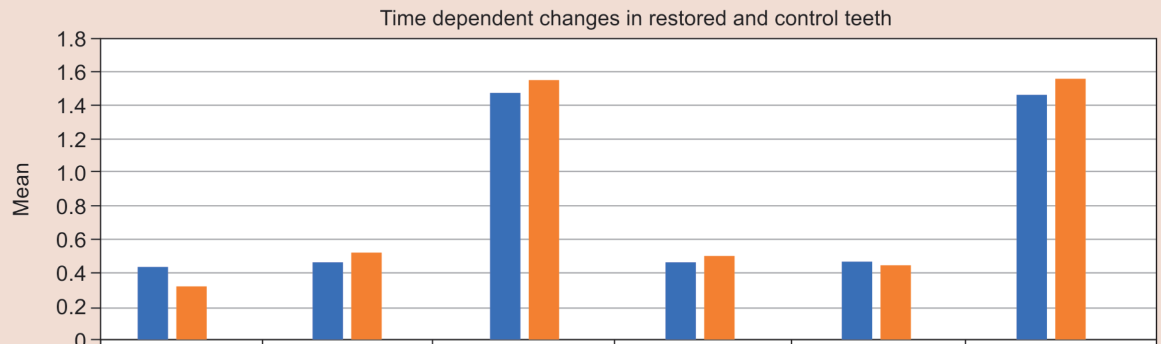
On intergroup comparison, baseline values of PI, GI, and IL-6 levels between the restored teeth of groups I and II was statistically nonsignificant. However, at 45 days of follow-up, zirconia crowns performed superiorly with a lower mean score of all the variables when compared to the SSCs ($p < 0.01$) (Table 1). On the other hand, statistically nonsignificant differences were observed in the mean values of the variables between the control teeth of groups I and II at baseline and at 45 days of follow-up ($p > 0.05$).

In Vitro Analysis

Stainless steel crowns showed a higher CFU/mL compared to the preformed zirconia crowns and the difference in their mean value was statistically highly significant ($p < 0.01$) as shown in Table 2.

Discussion

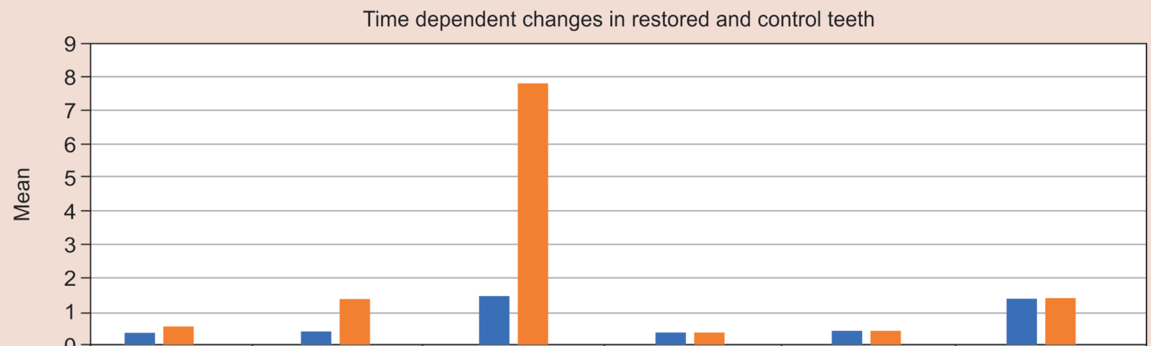
Dental crowns are frequently incorporated into rehabilitation practices, but their efficacy in the oral cavity, particularly in primary dentition, remains ambiguous. With prolonged and close interaction with gingival and oral mucosa, these materials may create a conducive environment for microbial adhesion. This can contribute to challenges such as plaque accumulation, gingival inflammation, and the onset of secondary caries, posing risks to gingival and periodontal health, especially in instances of suboptimal oral hygiene practice.[5,17,18] SSCs and zirconia crowns are



	PI Restored teeth	GI Restored teeth	IL-6 Restored teeth	PI Control teeth	GI Control teeth	IL-6 Control
Baseline	0.423	0.462	1.48769	0.462	0.462	1.46346
45th day	0.308	0.519	1.55408	0.5	0.442	1.56592
p-value	0.014	0.083	0.055	0.414	0.317	0.221

Variables

Fig. 8: Intragroup mean comparison of time-dependent changes observed in the PI scores, GI scores, and IL-6 levels from baseline to 45 days of follow-up in teeth restored with preformed zirconia crowns and control teeth using Wilcoxon signed-rank test



	PI Restored teeth	GI Restored teeth	IL-6 Restored teeth	PI Control teeth	GI Control teeth	IL-6 Control
Baseline	0.365	0.385	1.52969	0.365	0.404	1.36569
45th day	0.538	1.365	7.84654	0.365	0.404	1.41215
p-value	0.011	0.003	0.001	1	1	0.345

Variables

Fig. 9: Intragroup mean comparison of time-dependent changes observed in the PI scores, GI scores, and IL-6 levels from baseline to 45 days of follow-up in teeth restored with SSCs and Control teeth using Wilcoxon signed-rank test

Table 1: Intergroup comparison of mean values of the variables assessed between children receiving zirconia crowns and SSCs at 45 days of follow-up using Mann–Whitney *U* test

Parameters assessed	*Groups*	*Mean*	*Standard Deviation*	*Median*	*Mean difference*	*Mann–Whitney U value*	*p-value*
PI scores 45 days (restored teeth)	1	0.308	0.1096	0.25	−0.2308	28.000	0.001**
	2	0.538	0.2002	0.5			
PI scores 45 days (control teeth)	1	0.500	0.2282	0.5	0.1346	56.500	0.118^{NS}
	2	0.365	0.1651	0.25			
GI scores 45 days (restored teeth)	1	0.519	0.2156	0.5	−0.8462	18.000	0.001**
	2	1.365	0.5166	1.5			
GI scores 45 days (control teeth)	1	0.442	0.1813	0.5	0.0385	75.000	0.592^{NS}
	2	0.404	0.1626	0.5			
IL-6 levels 45 days (restored teeth)	1	1.5541	0.23725	1.593	−5.43846	3.000	0.000**
	2	7.8465	4.44092	8.06			
IL-6 levels 45 days (control teeth)	1	1.56592	0.217840	1.573	−0.003385	51.500	0.091^{NS}
	2	1.41215	0.244711	1.373			

*, statistically significant difference ($p < 0.05$); **, statistically highly significant difference ($p < 0.01$); GI, gingival index; IL, interleukin; NS, nonsignificant difference ($p > 0.05$); PI, plaque index

Table 2: Intergroup comparison of the CFU/mL between preformed zirconia crowns and SSCs using independent *t*-test

Groups	*N*	*Mean*	*Standard error mean*	*Standard deviation*	*T-value*	*p-value*
CFU/mL on preformed zirconia crowns	13	$1.96*10^8$	$1.466*10^7$	$5.286*10^7$	−3.376	0.003**
CFU/mL on SSCs	13	$2.76*10^8$	$1.844*10^7$	$6.650*10^7$		

*, statistically significant difference ($p < 0.05$); **, statistically highly significant difference ($p < 0.01$)

widely used in pediatric patients, and hence, these full-coverage restorations were considered in the present study.

Since 1950, SSCs have remained the predominant choice for full coverage restorations in primary and young permanent teeth. Due to its durability, efficiency, longevity, cost-effectiveness, and reliability, it is often considered restoration of choice, or the "gold standard," among all pediatric crowns.[4] However, children and parents frequently raise the metallic appearance as an issue.

The recently developed prefabricated zirconia crowns address the esthetic issue. These crowns are completely bio-inert, have a smooth, glazed and polished surface, and have superior corrosion resistance. Despite being acknowledged as a restorative material in permanent dentition for an extended period, the utilization of zirconia crowns in primary dentition only commenced in 2008.[19]

In the *in vivo* clinical and immunological analysis, SSCs crowns showed a statistically significant increase in their mean PI scores, GI scores, and IL-6 levels at 45 days of follow-up when compared to their baseline values and the preformed zirconia crowns (Fig. 9 and Table 1). A remarkable observation in the present study was the significantly better PI scores of the teeth restored with zirconia crowns when compared to their baseline values and the control teeth after 45 days of follow-up ($p < 0.05$) (Figs 6 and 8).

It could be attributed to its smooth surface and minimal susceptibility to plaque accumulation.[20] The results are in congruence with a study done by Taran and Kaya in 2018 where the authors found that zirconia crowns showed better PI and GI scores compared to the control teeth.[21]

On the other hand, SSCs necessitate manipulation *via* cutting, crimping, and trimming to ensure accurate marginal fit, thereby introducing surface defects and roughness. These imperfections promote plaque buildup and serve as ideal sites for microbial attachment. Moreover, this manipulation process may induce distortion in the metal substructure of metal crowns or result in the release of metal ions upon contact with marginal gingiva, a concern absent in zirconia crowns.[22,23] Similar studies were done by Sharaf and Farsi in 2004 and Beldüz Kara and Yilmaz in 2014 where the authors found that primary molars restored with SSCs were associated with gingivitis.[24,25] Another investigation conducted by Ozen et al. in 2014 revealed that metal margins exhibited greater gingival inflammation in comparison to zirconia crowns.[26] In contrast, Durr et al. in 1982 reported no difference in the level of gingivitis around teeth that were restored with SSCs and the control teeth.[27]

In the *in vitro* microbial analysis, the colony counts of *L. casei* were significantly higher ($p < 0.01$) in SSCs when compared to the preformed zirconia crowns (Table 2). The outcome corroborates a study carried out by Mathew et al. in 2020, which indicated a notably higher adhesion of *S. mutans* to SSCs compared to zirconia crowns.[28]

Scheuerman et al. asserted that the irregularities of polymeric surfaces encourage bacterial adherence and facilitate the formation of biofilm deposits.[29] Myers et al. noted that plaque formation occurs readily on the surface of SSCs irrespective of the polishing techniques employed. This phenomenon may be attributed to physicochemical interactions involving electrostatic and van der Waals forces between the restoration surface and microorganisms.[30] The properties of SSCs, including surface roughness and energy, are influential factors in microbial growth. Greater surface area and roughness correlate with increased bacterial adhesion. In contrast, zirconia exhibits a highly smooth surface, reducing both surface roughness and energy, thereby inhibiting microbial adhesion.[31,32]

These could be the probable reasons for higher bacterial adhesion onto the surfaces of the SSCs in the present study.

Based on the findings obtained from the present study, it is evident that zirconia crowns outperformed SSCs across all evaluated criteria. Consequently, zirconia crowns emerge as a viable alternative to SSCs for primary teeth, offering advantages in terms of esthetics and superior periodontal health.

Conclusion

The result of the present study suggests that:

- Preformed zirconia crowns are superior to SSCs pertaining to the maintenance of periodontal health post cementation.
- Preformed zirconia crowns harbored lesser bacterial colony growth on their surfaces when compared to the SSCs.

Thus, the present study suggests the use of preformed zirconia crowns over SSCs in pediatric dental patients indicated for full coverage restorations.

Clinical Significance of the Study

There is a lack of research on the comparison of immunological and microbiological changes caused by full coverage restorations in the gingival health of pediatric dental patients. Thus, the present study enabled us to clinically, immunologically, and microbiologically assess the effect of SSCs and preformed zirconia crowns on the gingival health of an individual.

Limitations

- The present study had a very short follow-up period and a small sample size all of which can lead to biased results.
- A part of the study was done in vitro due to which a correlation between the inflammatory marker, IL-6, and *L. casei* could not be established.
- The present study did not comparatively assess the physical properties of SSCs and preformed zirconia crowns.

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