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LEVELS OF BISPHENOL A AND ANTIOXIDANTS IN SALIVA AFTER PLACEMENTOF RESIN BASED DENTAL SEALANT IN CHILDREN: AN IN VIVO STUDY

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Abstract:

Background: Resin based dental materials like composites and pit and fissure sealants have been used progressively for preventive and restorative purposes in pediatric dentistry. Nevertheless, the harmful systemic implications of these Bisphenol A (BPA) derivatives haven't been paid heed to. Owing to the growing apprehension with regard to the compounds that leach from resin based materials, it is very important to estimate the amount of monomers that leach out them. Other than being an endocrine disruptor, BPA is hypothesized to exert oxidative stress and cause an imbalance between the number of oxidants and antioxidants i.e. the Total Antioxidant Capacity (TAC). As the usage of BPA based dental materials is a growing public concern, as dentists it is critical to understand whether the use of BPA products pose a genuine exposure risk.

Objective: This study aims to observe the levels of BPA and total anti-oxidantcapacity (TAC)in saliva after placement of resin based pit and fissure sealants in children.

Methodology: 40 healthy children in the age group of 6 to 9 years were selected for the study and divided into two groups of 20 each by random sampling. Unstimulated saliva was collected before placement, immediately and 24 hours after placement of resin based sealants (Group A)and glass ionomer sealants (Group B). Concentration of BPA and TAC level in saliva were compared between the two groups using Enzyme Linked Immuno-Sorbent Assay (ELISA) andphotospectrometry respectively.

Results: Baseline BPA (ng/ml) concentrations in saliva were comparable in both Group A (0.39 ± 0.10) and Group B (0.40±0.11). However, exponentially higher BPA levels were seen immediately after placement of sealants (19.51±2.30) in Group A, when compared to Group B (0.42± 0.12). The mean BPA level decreased significantly after 24 hours of placement in Group A (1.42±0.39), although it was still significantly higher than Group B (0.43±0.14) and the baseline values for both the groups. The Baseline TAC (mM/l) levels in saliva were comparable in both, Group A (0.39±0.07) and Group B (0.38±0.05). A slight alteration was seen in the TAC levels immediately and 24 hours after placement, in both the Group A (0.40±0.08), (0.41±0.06) and Group B (0.39±0.06), (0.40±0.07). However, there was no significant difference in the TAC values before placement, after and 24 hours after placement in both thegroups.

Conclusion: Sources other than resin based materials contribute a baseline level of BPA in saliva. An exponential rise in the salivary BPA level was seen immediately after placement of resin based sealants. However, this level significantly dropped in 24 hours. In case of glass ionomer sealants, no change was seen immediately and 24 hours after the sealant placement. The TAC levels before, immediately and 24 hours after placement of resin and glass ionomer based sealants remained statistically insignificant.

Keywords: Bisphenol-A, Total antioxidant capacity, Saliva, Resin dental materials, Sealants

Introduction:

Starting in the late 1990s, scientists had sounded the alarm about BPA. There has been a scientific and journalistic controversy regarding its adverse effects in humans. BPA is one of the most mass-produced synthetic compounds manufactured and delivered around the world. Even though it is an acknowledged environmental estrogen, it is utilized across industries for production of routinely used consumer items such as polycarbonate plastic, the resin used for coating food and beverage cans and dental materials as well [1].

BPA can bind to estrogen receptors and is capable of acting as a hormonal disruptor and as an androgen antagonist. It has inflammatory characteristics and can generate liver damage by inducing oxidative stress [2,3].

However, the European Food Safety Authority (EFSA) in 2015, modified the proposed 'Tolerable daily intak' as 4µg/kg/day. In December 2017, BPA was reassigned as a chemical with solicitude (4). Resin based dental materials can lead to leaching of BPA and free monomers in saliva throughout polymerization and following placement [2]. The placement of resin based materials can potentially lead to an increase in the concentration BPA in saliva [5-11].

Saliva is loaded with antioxidants and is critical in keeping up oral wellbeing [12]. Saliva is a brilliant tool to supervise oral and systemic health as it mirrors the levels of body‟s multifarious markers, which help in identification of infectious, toxicological and hormonal diseases. One such biomarker is TAC, which helps in evaluation of oxidative stress [13]. With the advent of preventive dentistry, there is a surge in the use of preventive procedures like application of pit and fissure sealants and fluoride varnishes. Use of pit and fissure sealants has a primary and secondary prevention perspective to it, as sealing of the anatomic grooves can reduce caries risk and caries progression for non-cavitated carious lesions [14,15].

Use of resin based pit and fissure sealants is favoured in children as glass ionomer sealants have lower retention rates and are 5 times more prone to lose retention from tooth surface as compared to resin based sealants after 2 to 3 years of follow up [16]. In the 6 to 8 years age group, 30% of the children have at least one pit and fissure sealant present. Since, exposure to dental sealants starts from a very early age, this is a matter of perturbation because children are at a higher risk of being adversely affected by being subjected to chemicals as compared to adults [17,18].

Thus, it is necessary to investigate and quantify the monomers leached from resin based. However not many studies in literature regarding the release of BPA due to resin based pit and fissure sealants in saliva in children are present. Hence the present study aimed to observe the levels of Bisphenol A and Total anti-oxidant capacity in saliva after placement of resin based pit and fissure sealants in children

Materials and Methods:

Ethical Clearance:

The present study was approved by the ethics committee of AB Shetty Memorial Institute of Dental Science (ABSM/EC/58/2018).

Study Population:

A total of 23 boys and 17 girls between the age of 6 to 9 years who reported to the Department of Pediatric and Preventive Dentistry, AB Shetty Dental College were selected for the study. The study subjects were divided into 2 groups of 20 children each by random

sampling. Informed consent was obtained from the parents/guardian of the children in English, Kannada or Malayalam, depending on their preferred language.

Inclusion Criterial:

 Age Group: 6-9 years

 Children in whom the occlusal surfaces of first permanent molars were indicated for pit and fissure sealant placement.

Exclusion criteria:

 Children with underlying systemic disease

 Children with with special needs.

 Uncooperative children.

 Children with well-established cavitated caries lesion.

 Individuals with pre-existing composite, glass ionomer cement or amalgam restorations.

A thorough dental examination was carried out following which, 40 children fulfilling the inclusion criteria were included in the study and randomly divided into Group A and Group B.

- In Group A (Experimental Group), resin pit and fissure sealant was placed in 20 children.
- In Group B (Control Group), glass ionomer pit and fissure sealant was placed in 20 children.

Protocols for sealant placement were followed as per manufacturer‟s instructions.

1. Assessment of occlusal surfaces was done using visible light and air to dry the tooth surface.

2. The tooth surface was cleaned with pumice and rubber cup to ensure pellicle removal. 38 Other methods used to clean the tooth surface prior to placing the sealant include air-polishing, hydrogen peroxide, and enameloplasty (19).

Isolation was done with the help of cotton rolls as adequate moisture isolation during resin sealant placement is the most critical step in sealant application (rubber dam isolation was not used as achieving adequate clamp retention might not always be possible in case of erupting permanent teeth). Since sealants do not bond to the tooth surface directly, conditioners and etchants are used to increase the surface area by creating microporosities and in turn increase their adhesiveness.39 Orthophosphoric acid and three salivary samples were collected from all the children:

1. Baseline unstimuated salivary samples were collected from subjects of both the groups. Oral prophylaxis and polishing with non-fluoridated paste was done. Resin and glass ionomer based sealants in Group A and Group B respectively.

2. Unstimulated salivary samples were collected immediately after the placement of sealants in both the groups.

3. After 24 hours, the children were recalled for collection of the final unstimulated salivary sample.

Following this, the samples were ana;lysed at Central Research Laboratory, KSHEMA for the evaluation of the BPA and TAC levels. In the laboratory, the samples were evaluated for:

Bisphenol A analysis was performed using ELISA method

10µl of samples were added to the testing sample wells and 40 µl of sample diluent was added to the same followed by which, 100µl of HRP conjugate reagent was added to the sample and the standard wells. The plate was covered with a seal plate membrane and it was mixed for 60 min at 37º incubation. Followed by this, wash solution was prepared. The seal membrane was gently removed, the liquid was drained. Each well was then filled with washing solution, kept for a minute and drained again. This was repeated 5 times. Followed by this, chromgen solution was added to each well and the plate was incubated for 15 min at 37º Celsius. Once the incubation was done, stop solution was added to the wells. 15 min after the stop solution is added, absorbance (OD) is measured for all the sample wells under 450nm. Followed by which, according to the standards concentration and the corresponding OD values, linear regression equation of the standard curve was calculated. Then, the concentration of BPA was measured in the samples.

Total Antioxidant Capacity using Photospectrometry (20).

Polyacrlic acid was used in case of resin based sealants and glass ionomer sealants respectively. Acid removal was done and the surface was air dried. Resin based sealants were placed in Group A and light cured for polymerization whereas glass ionomer sealants were placed in Group B. Preparation of TAC reagent is done before starting the procedure. 100 µl of the sample was pipetted into a clean test tube. 5% Trichloroacetic acid (TCA) was added to precipitate the proteins in the sample. 100 µl of the clear supernatant was then transferred to another test tube and 1ml of TAC reagent was added to it. Following which, the mixture was incubated in a water bath for 90 min at 90º Celsius. After the mixture was cooled, the optical density was read using photospectrometry at 695nm. The concentration of the total antioxidants was then obtained by plotting the absorbance of the test against standard graph and the concentrations were expresses in mM/l.

Statistical Analysis:

Statistical analysis was carried out using SPSS (Statistical Package for Social Sciences) Version 24.0 (IBMCorporation, Chicago, USA). Descriptive and analytical statistics were done. The data is represented in mean, median and standard deviation. The normality of continuous data was analysed by Shapiro-Wilk test. As the data did not followed normal distribution, non-parametric tests were used to analyse the data. The Mann-Whitney U test is a nonparametric test that allows two groups to be compared without making the assumption that values are normally distributed. The p value <0.05 is considered to be statistically significant.

Results:

A total of 40 subjects fulfilling the inclusion criteria were included in the study and randomly divided into Group A and Group B. The mean age of the study population was 7.85±1.13 in Group A and 7.75±1.06 in Group B. There were 11 (55.0%) males and 9 (45.0%) females in Group A and 12 (60.0%) males and 8 (40.0%) females in Group B **(Table 1).**

Table 1: Demographic summary of the study population:

Groups	**Case** (n=20)	**Control** (n=20)
Age (years)	**Mean** ± **SD**	**Mean** ±**SD**
	7.85 ±1.13	7.75 ±1.06
Sex	**N (%)**	**N (%)**
Male	11 (55.0)	12 (60.0)
Female	9 (45.0)	(40.0)

Baseline Bisphenol A concentrations in saliva were found to be 0.39±0.10 ng/ml in Group A and 0.40±0.11 ng/ml in Group B respectively. Baseline Total antioxidant capacity levels in saliva were found to be 0.39±0.07 mM/l in Group A and 0.38±0.05 mM/l in Group B respectively **(Table 2).**

Table 2: Baseline Levels of Bisphenol-A And Total Anti-Oxidant Capacity

Timeline	**Group A** (n=20)	**Group B** (n=20)
	Mean ±SD	Mean±SD
Baseline BPA levels (ng/ml)	0.39±0.10	0.40 ±0.11
Baseline TAC levels (mM/l)	0.39±0.07	0.38±0.05

The Bisphenol A levels in Group A, immediately and 24 hours after placement of resin based sealants were found to be 19.51±2.30 ng/ml and 1.42±0.39 ng/ml respectively. The Total antioxidant capacity levels immediately and 24 hours after placement of resin based sealants were found to be 0.40±0.08 mM/l and 0.41±0.06 mM/l respectively **(Table 3)**

Table 3: Levels of Bisphenol-A And Total Anti-Oxidant Capacity In Saliva Immediately and 24 hours After Placement of Resin Based Pit And Fissure Sealants

Parameters	Timeline	Mean±SD
Bisphinol A (ng/ml)	Immediately after placement	19.51±2.30
	24hr after placement	1.42 ±0.39
TAC(mM/l)	Immediately after placement	0.40±0.08
	24hr after placement	0.42±0.06

The Bisphenol A levels in Group B, immediately and 24 hours after placement of glass ionomer based sealants were found to be 0.42±0.12 ng/ml and 0.43±0.14 ng/ml respectively. The Total antioxidant capacity levels immediately and 24 hours after placement of glass ionomer based sealants were found to be 0.39±0.06 mM/l and 0.40±0.07 mM/l respectively**. (Table 4)**

Table 4: Levels Of Bisphenol-A And Total Anti-Oxidant Capacity In Saliva Immediately And 24 Hours After Placement Of Glass Ionomer Based Pit And Fissure Sealants

Parameters	**Timeline**	**Mean±SD**
Bisphinol-A (ng/ml)	Immediately after placement	0.42±0.12
	24hr after placement	0.43±0.14
TAC mM/l	Immediately after placement	0.39±0.06

	24hr after placement	0.40±0.07

The level of BPA in saliva between the two groups was compared. The baseline level of BPA was comparable in Group A (0.39±0.10 ng/ml) and Group B (0.40±0.11ng/ml). There was no statistically significantdifference at baseline (**p=986**) between the two groups. The level of BPA immediately after sealant placement was higher in GroupA (19.51±2.30 ng/ml) than Group B (0.42± 0.12ng/ml). The levels showed a statistically significant difference (**p<0.001**) between Group Aand Group B. The level of BPA 24 hours after placement of pit and fissure sealant was higher in Group A (1.42±0.39 ng/ml) than Group B(0.43±0.14 ng/ml). The levels showed a statistically significant difference (**p=0.002**) betweenGroup A and Group B **(Table 5 and Figure 1)**

Table 5: Inter Group Comparison of Bisphenol A

Timeline	**Group A** (n=20)	**Group B** (n=20)	**P-value**
	Mean±S.D.	Mean ±S.D.	
Before placement	0.39 ±0.10ng/ml	0.40±0.11ng/ml	0.986
Immediately after placement	19.51±2.30ng/ml	0.42±0.12ng/ml	<0.001**
24 hours after placement	1.42 ±0.39ng/ml	0.43±0.14ng/ml	0.002**

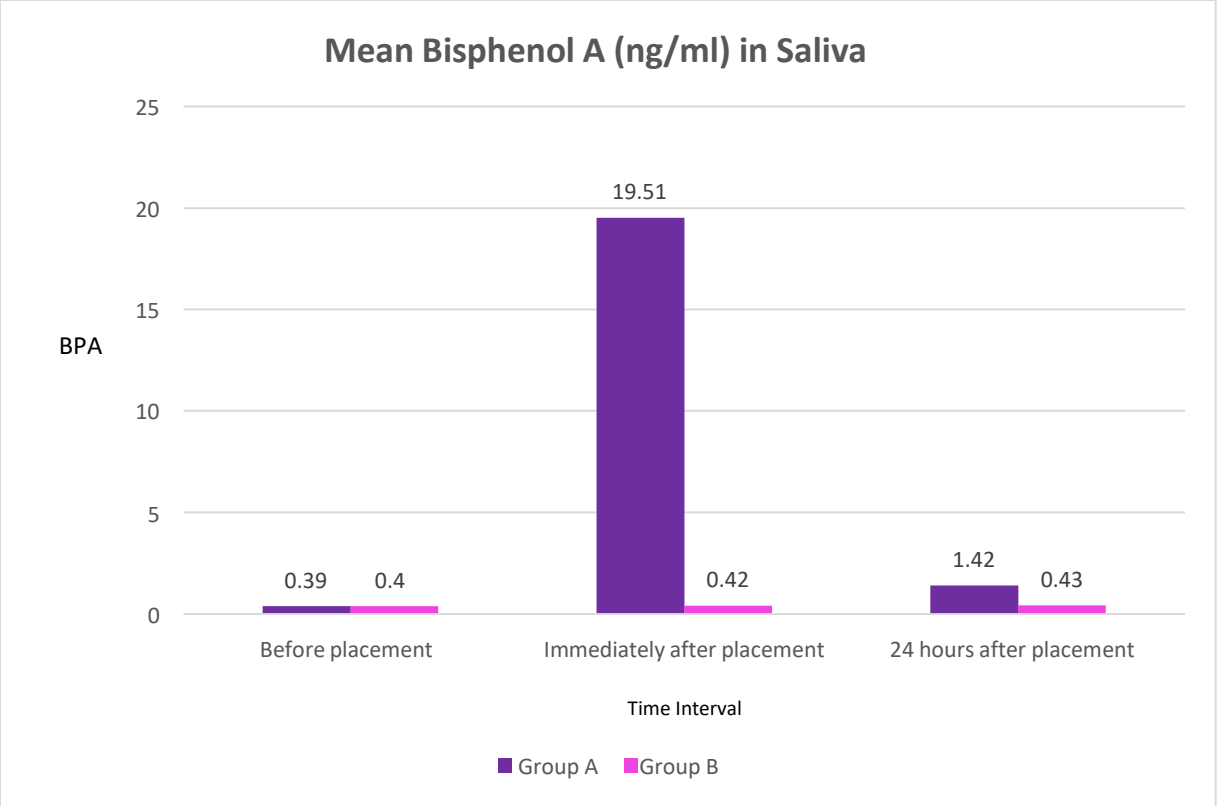

Figure 1: Comparison of level of Bisphenol A (ng/ml) in saliva between the two groups

The intra-group comparison of the level of BPA in saliva of Group A was done:
The level of BPA increased from baseline (0.39 ±0.10 ng/ml) to immediately after placement of sealant (19.51±2.30ng/ml) and then reduced at 24 hours after placement (1.42±0.39ng/ml). Statistically significant (**p<0.001**) increment was seen in the levels of BPA All the three comparisons had significant (**p<0.001**) mean differences in the level of BPA. **(Table 6)**

Table 6: Intra-Group Comparison Of Bisphenol-A In The Resin Based Pit AndFissure Sealant Group

Timeline	N	Mean±SD	P-value
Before placement	20	0.39±0.10ng/ml	<0.001
Immediately after placement	20	19.51±2.30ng/ml	
Immediately after placement	20	19.51±2.30ng/ml	<0.001
24 hours after placement	20	1.42±0.39ng/ml	
Before placement	20	0.39±0.10ng/ml	<0.001
24 hours after placement	20	1.42±0.39ng/ml	

INTRA-GROUP COMPARISON OF BISPHENOL-A IN THE GLASS IONOMERBASED PIT AND FISSURE SEALANT GROUP:

The intra-group comparison of the level of BPA in saliva of Group B was done: The level of BPA in baseline (0.40±0.11 ng/ml) was comparable to the level seen immediately (0.42±0.12ng/ml) and 24 hours after placement (0.43±0.14ng/ml) of sealant. No Statistically significant (**p<0.001**) difference was seen in the levels of BPA**:** Between baseline and immediately after placement of sealant, Between immediately and 24 hours after placement of sealant and Between baseline and 24 hours after placement of sealant. All the three comparisons had did not show any significant mean differences(**p>0.05**) in the level of BPA **(table 7 and Figure 2).**

Table 7: Intra-Group Comparison Of Bisphenol-A In The Glass Ionomer Based Pit And Fissure Sealant Group

Timeline	N	Mean ±SD	P-value
Before placement	20	0.40 ±0.11ng/ml	0.103
Immediately after placement	20	0.42±0.12ng/ml	
Immediately after placement	20	0.42±0.12ng/ml	0.707
24 hours after placement	20	0.43±0.14ng/ml	
Before placement	20	0.40±0.11ng/ml	0.066
24 hours after placement	20	0.43±0.14ng/ml	

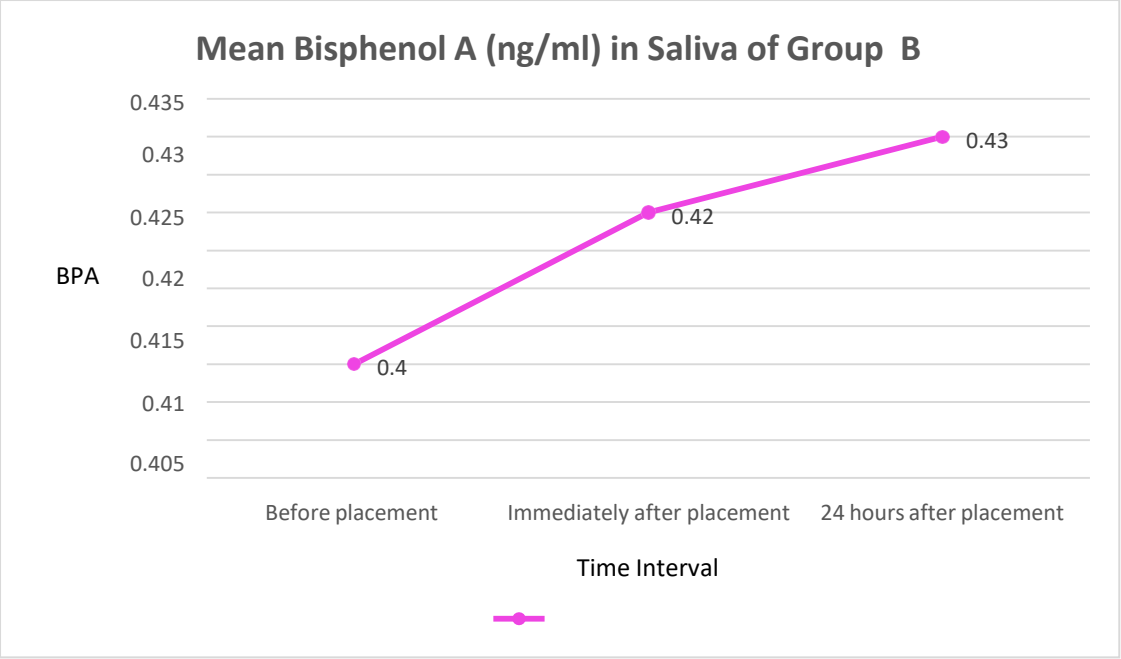

Figure 2: Intragroup comparison of level of Bisphenol A (ng/ml) in Group B

INTER GROUP COMPARISON OF TOTAL ANTI-OXIDANT CAPACITY:

The level of TAC in saliva between the two groups was compared. The baseline level of TAC was comparable in Group A (0.39±0.07mM/l) and Group B (0.38±0.05mM/l). There was no

statistically significant difference at baseline (**p=0.608**) between the two groups.
The level of TAC immediately after sealant placement was comparable in Group A (0.40±0.08mM/l) and Group B (0.39±0.06mM/l). There was no statistically significant difference (**p=0.564**) between the TAC levels immediately after sealant placement in the two groups.

The level of TAC 24 hours after sealant placement was comparable in Group A (0.41±0.06mM/l) and Group B (0.40±0.07mM/l). There was no statistically significant difference (**p=0.558**) between the TAC levels 24 hours after sealant placement in the two groups. **(Table 8)**

Table 8: Inter group comparison of Total anti-oxidant capacity

Timeline	**Case** (n=20)	**Control** (n=20)	**P-value**
	Mean±SD	Mean±SD	
Before placement	0.39±0.07mM/l	0.38 ±0.05mM/l	0.608
Immediately after placement	0.40 ±0.08mM/l	0.39±0.06mM/l	0.564
24 hours after placement	0.41±0.06mM/l	0.40±0.07mM/l	0.558

INTRA-GROUP COMPARISON OF TOTAL ANTI-OXIDANT CAPACITY IN THERESIN BASED PIT AND FISSURE SEALANT GROUP

The intra-group comparison of the level of TAC in saliva of Group A was done: The level of TAC in baseline (0.39±0.07 ng/ml) was comparable to the level seen immediately (0.40± 0.08ng/ml) and 24 hours after placement (0.41±0.06ng/ml) of sealant in Group A. No Statistically significant (**p<0.001**) difference was seen in the levels of TAC: Between baseline and immediately after placement of sealant, Between immediately and 24 hours after placement of sealant and Between baseline and 24 hours after placement of sealant (Table 9).

Table 9: Intra-Group Comparison Of Total Anti-Oxidant Capacity In The Resin BasedPit And Fissure Sealant Group

Timeline	N	Mean±SD	P-value
Before placement	20	0.39±0.07mM/l	0.091
Immediately after placement	20	0.40±0.08mM/l	
Immediately after placement	20	0.40±0.08mM/l	0.066
24 hours after placement	20	0.41±0.06mM/l	
Before placement	20	0.39±0.07mM/l	0.069
24 hours after placement	20	0.41±0.06mM/l	

INTRA-GROUP COMPARISON OF TOTAL ANTI-OXIDANT CAPACITY IN THEGLASS IONOMER BASED PIT AND FISSURE SEALANT GROUP:

The intra-group comparison of the level of TAC in saliva of Group B was done. The level of TAC in baseline (0.38±0.05 ng/ml) was comparable to the level seen immediately (0.39±0.06ng/ml) and 24 hours after placement (0.40±0.07ng/ml) of sealant. No Statistically significant (**p<0.001**) difference was seen in the levels of TAC: Between baseline and immediately after placement of sealant, Between immediately and 24 hours after placement of sealant and Between baseline and 24 hours after placement of sealant **(Table 10 , Figure 3).**

Table 10: Intra-Group Comparison Of Total Anti-Oxidant Capacity In The Glass IonomerBased Pit And Fissure Sealant Group

Timeline	N	Mean ±SD	P-value[#]
Before placement	20	0.38±0.05mM/l	0.148
Immediately after placement	20	0.39±0.06mM/l	
Immediately after placement	20	0.39±0.06mM/l	0.624
24 hours after placement	20	0.40±0.07mM/l	
Before placement	20	0.38±0.05mM/l	0.078
24 hours after placement	20	0.40±0.07mM/l	

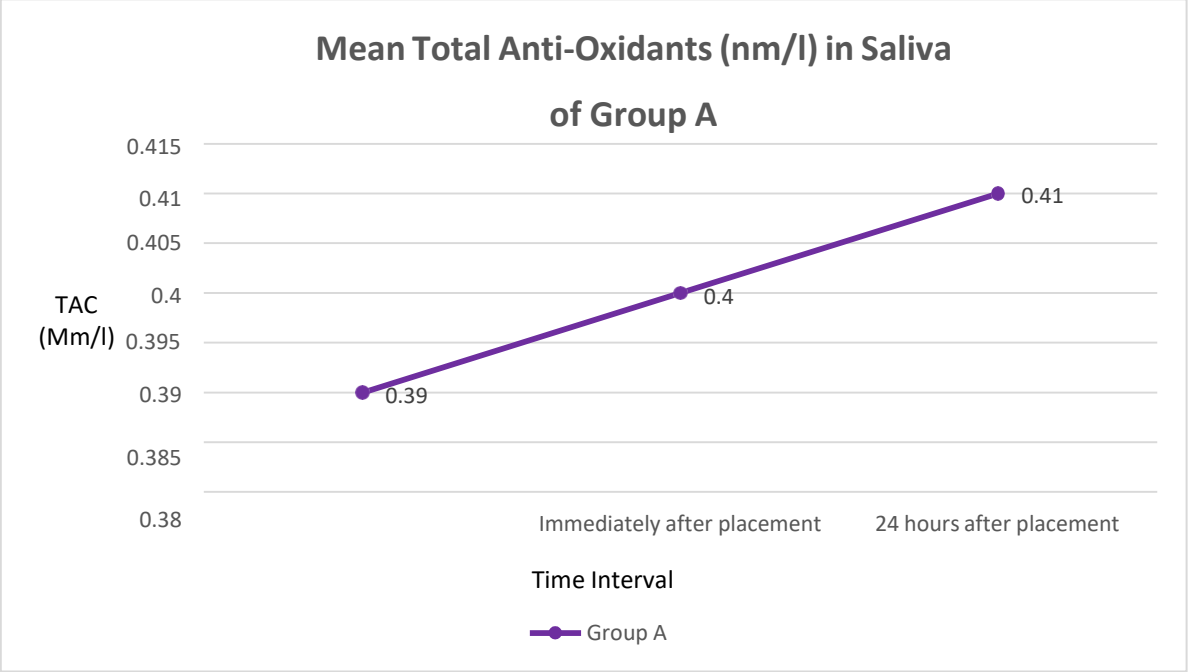

Figure 3: Intragroup comparison of Total anti-oxidant capacity (mM/l) in saliva inGroup A

Discussion:

In 1891, BPA was first unified by an acid catalyzed chemical reaction of phenol and acetone. This synthetic chemical is an Endocrine active substance (EAS) and is capable of impacting the well being of humans. (21-23). Despite this, BPA is used for the mass production of several popular consumer products like food and beverage packaging, detergents, cars, including composite resin based dental materials. Even though, the pure form of BPA is not used for fabrication of dental resin, its derivatives are used routinely for the same (24).

Although, dental resins are chemically stable, they can lead to release of BPA and free monomers in saliva during polymerization and following placement due to degradation.6 These derivatives pass into the saliva, approximate the mucosa and by the virtue of being cytotoxic in nature, are capable of gingival marginal retraction and alteration in pulp tissue by entering the dentinal tubules. This can also lead to formation of secondary caries, as these unbound monomers can promote bacterial

multiplication. (25)

In children and young adults, pit and fissure type of caries represent about 90%of the caries in permanent posterior teeth and 44% of the caries in primary teeth. This can be attributed to the presence of deep grooves on the occlusal surface that are capable of entrapment of microbes and debris, thus escalatingthe susceptibility to occlusal caries.

With the advent of preventive dentistry, children are exposed to dental sealants ata very young age.The present study was conducted to investigate whether resin based pit and fissure sealants account for BPA release in saliva. 6 to 9 year old children, without any pre-existing dental restorations and deep grooves and fissures on the occlusal surface of their first permanent molars, were chosen for the study. The risk of developing caries is the highest during the first few years after the eruption of the tooth, especially in cases of children affected with Early Childhood Caries as these children have an increased risk for caries development in the permanent dentition. Also, permanent molars are positionedin a rearward region in the child's oral cavity and can compromise his or her capacity to cleanse and eliminate food debris from these areas. This leads to an elevated occurrence rate of occlusal pit and fissure caries in permanent first molars (26).

It was found that the baseline levels of BPA were comparable in both Group A (0.39±0.10ng/ml) and Group B (0.40±0.11ng/ml). Both the groups had a baseline amount of BPA in their saliva. This implies that there are other sourcesthat lead to a constant BPA exposure. It has been stated that breast milk and feeding bottles are the primary sources of BPA exposures in infants, however as the child grows, the resin used in food and beverage packaging becomes the predominant source. The mean BPA baseline values were in accordance to the baseline values estimated by the p[revious studies. The presence of BPA in the samples from both the groups validates the fact that use of plastic products leads to a detectable amount of BPA exposure. (11,13)

The TAC levels were found to be comparable in both Group A (0.39±0.07mM/l) and

Group B (0.38±0.05mM/l). There is a baseline TAC level present in saliva at all times, this can be attributed to the fact that antioxidants serve as the first lineof defence against free radicals, which are capable of inducing oxidative stress. Pathologic conditions, biochemical processes like metabolism, respiration,aerobic reactions and immune functions in the body, radiation, pollutants in the environment etc can all lead to oxidative stress (27).

The BPA level was exponentially high immediately after placement of sealants in Group A (19.51±2.30ng/ml). This can be attributed to the fact that inadequate curing and unpolymerized resins lead to elution of free monomers up to 24 hours after placement of the resin based sealants. Intake of dietary fluids, erosion and degeneration of resin restorative material due to mechanical, thermal or chemical influences can also induce release of BPA from the sealants.Furthermore, consumption of food that leads to a temperature change of over 60º Celsius inthe oral cavity can lead to formation of micro cracks in these restorations, thereby leading to deterioration and leaching out of monomers. Thus, this confirms the hypothesis that BPA can be found in saliva, in much higher levels than the baseline after placement of resin based sealants as within the considered samples, the BPA values were significant. These values obtained in this studyare significantly lower than that stated by Sasaki *et al.*, Joskow *et al.*,(11,13). The dissimilarities in the composition of resin based materials tested, the dimensions and number of surfaces restored, sampling procedures, assessment time intervals, sensitivity and detection limitsof the different methods used for analysis vary between studies. These factors may account for diverging results immediately and 24 hours after the placement of the resin based sealants.

The BPA levels decreased significantly 24 hours after sealant placement in Group A (1.42±0.39). Several factors like consumption of water, gargling with tepid water can significantly reduce the unpolymerized present on the surface of the sealant thereby, decreasing the concentration of BPA in saliva. (9). Once ingested, the active form of BPA i.e. the uncongugated BPA, gets rapidly conjugated in the liver, followed by which it gets excreted via bile or urine. The half life is approximately 5.3 hours, which explains the exponential decrease of levels of BPA 24 hours after placement. The

speedy excretion of BPA is the reason as to why its use has been declared safe some public health authorities. However, in the present study isolation was done with the help of cotton rolls in order to maintain uniformity in all the subjects, as rubber dam clamps may not be retentive during early stages of tooth eruption.

The TAC levels recorded immediately and 24 hours after placement of sealantsin Group A were (0.40±0.08) and (0.41±0.06) respectively. Ramezani *et al.,* found elevated levels of TAC in the resin based group as compared to the amalgam group, however the restoration were placed at least one and a half month before the evaluation was done (20). In the present study, the TAC values were assessed before, immediately after and 24 hoursafter placement, thus a longer follow up could have lead to varied time related results. The mean TAC levels immediately and 24 hours after placement of sealants in Group B did not change significantly and were found to be (0.39±0.06) and (0.40±0.07) respectively. Doakar et al compared the oxidative stress before and after placement of amalgam, resin, glass ionomer cements and found that amalgam lead to the maximum amount of oxidative stress. Resin and glass ionomer cements showed a no significant difference in the TAC levels (28).

In this study, even though there was a sudden surge in the salivary BPA levels immediately and after placement of resin based sealants, these levels were still significantly lower than the tolerable daily intake of BPA given by EFSA, which is 4µg/kg/day.(4) Even without the placement of any dental material, a baseline level of BPA was detectable in the salivary samples. Food has been customarily held accountable for most of the BPA ingestion, however in a study where the urinary BPA level was evaluated in adults after fasting, the results still showed presence of BPA, thus concluding that other polycarbonate materials lead to a reasonable amount of BPA exposure.61 Additionally, studies state that BPA exposure can happen not just through the gastrointestinal tract, but through respiratory and dermal tract as well.62

BPA, a xenoestrogen, modifies the biological activity of estrogen, imitates the phenolic A ring of 17- β-estradiol thus, causing changes in both the neuroendocrine axis and the hypothalamic-pituitary-ovarian-uterine axis. (12) Animal studies have shown that BPA blocks the activity of androgens and has inflammatory characteristics. Oral delivery of leached products from resin materials lead to reproductive toxicity and reduced fertility in both the male and female mice. (2).

Even after taking the limitations of this study in account, the results of this study show

that resin based dental materials are contribute to the overall BPA exposure. Thus, minimizing the amount of BPA leached in saliva by adequately curing the sealant, rinsing after application and polishing of the sealant surface. Furthermore, using composites without BPA and using glass ionomerpit and fissure sealants in growing children should be considered.

Conclusion:

Even after taking the limitations of this study in account, the results of this study show that resin based dental materials are contribute to the overall BPA exposure. Thus, minimizing the amount of BPA leached in saliva by adequately curing the sealant, rinsing after application and polishing of the sealant surface. Furthermore, using composites without BPA and using glass ionomerpit and fissure sealants in growing children should be considered.

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Conflict of Interest:
None

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