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Comparative evaluation of release of BPA and TEGDMA from four commercially available orthodontic adhesives – An In vitro study

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Abstract

Introduction: Orthodontic adhesives are widely used for bonding of brackets and lingual retention. A Review of literature shows that Bisphenol A (BPA) is an endocrine disruptor and also has adverse effects on human reproduction, development, metabolic disease, inflammation and gene expression whereas Triethylene Glycol Dimethacrylate (TEGDMA) is associated with allergies and cytotoxicity.

Aim: To evaluate and compare the relative amount of Bisphenol-A (BPA) and Triethylene Glycol Dimethacrylate (TEGDMA) release using Gas Chromatography coupled with Mass Spectrometry from four commercially available orthodontic adhesives used for fixed retention purpose.

Methodology: The study included minimum total sample size of 12 i.e 3 in each group.

GROUP A: 3 Samples of Enlight (Ormco)

GROUP B: 3 Samples of Transbond XT (3M UNITEK)

GROUP C: 3 Samples of Heliosit Orthodontic (Ivoclar)

GROUP D: 3 Samples of Orthocem (FGM)

For each sample of composite, 6 dots of composite representing the 6 anterior teeth were placed. Each dot was cured using a curing light (Coltolux LED) with a

wavelength of 450nm/470nm for 20 seconds. The cured sample was immediately immersed in Milli-Q Water. The immersion liquid was then lyophilized using Thermo Scientific Heto Powerdry LL1500 device and 100µL of Dichloromethane was added. The eluates were subjected to Gas Chromatography and Mass Spectrometry analysis and the relative amount of monomers of interest (BPA & TEGDMA) was calculated.

Result: It was observed that maximum relative amount of BPA released was by Heliocit Orthodontic Ivoclar (Group C) followed by Orthocem FGM (Group D) and then, by Enlight Ormco (Group A) and lastly by Transbond XT 3M (Group B) and the maximum relative amount of TEGDMA was released by Heliocit Orthodontic Ivoclar (Group C) followed by Orthocem FGM (Group D) and then by Transbond XT 3M (Group B) and lastly Enlight Ormco (Group A).

Conclusion: The results of this in-vitro study allowed us to substantiate a model of monomer release from an orthodontic bonded retainer and implies that all four orthodontic adhesives used in the study released BPA and TEGDMA. Clinicians must consider the adverse effects of these monomers and adhere to the clinical recommendations given to reduce BPA release. Also, increase the use BPA- Free Orthodontic Adhesives and chemically cured orthodontic adhesives whenever possible.

Keywords: Bonding, BPA, Cytotoxicity, TEGDMA

Introduction

Orthodontic retention is defined as maintaining teeth in optimal aesthetic and functional position after treatment.[1]

Fixed retainers are most commonly used in the orthodontic retention phase as they have several advantages, such as better aesthetics, no need for patient cooperation, effectiveness, and suitability for lifelong retention.[2]

These retainers are composed of metallic/braided wires which are bonded to enamel with a restorative resin composite or orthodontic flowable adhesives.[3]

The composite resins used in orthodontics consist of mineral fillers, resin matrix—generally bisphenol A diglycidyl ether dimethacrylate (Bis-GMA) synthesized from bisphenol A (BPA) and urethane—fluidifiers belonging to the triethylene glycol family, catalysts like camphroquinone, and many other additives.[4]

Polymers and their increasing presence in oral cavity have raised questions regarding the safety of the constituents of the resinous matrix. Orthodontic adhesives induce cytotoxicity, disturbing cell metabolism and inducing cell death, even up to 2 years after polymerization [8] and may cause inflammatory response in test animals.[6]

BPA is the most studied amongst the leached compounds and it was first developed in the 1890sas a synthetic estrogen.[7]

Its ability to bind and activate the estrogen receptor in humans makes it an endocrine disruptor and its effects are proven in animals and suspected in humans.[8]

Impurities left after resin synthesis, initially because of incomplete polymerization, and later because of resin degradation led to elution of BPA.[9]

It also adversely affects human reproduction, development, metabolic disease, inflammation, and gene expression.[10] BPA is also known to alter immune responses.[11]

A review of the dental literature confirms the toxicity of the released monomers triethylene glycol dimethacrylate (TEGDMA) and indicates unavoidable concentrations of residual BPA.[12]

Architectural and ultrastructural changes in epithelial cells because of the penetration of uncured primers was found by Vande Vannet and Hanssens.[13]

Goldberg found the cytotoxic effects of TEGDMA and HEMA monomers for gingival cells and are most probably responsible for the allergies seen.[14]

Hence, this study aims to assess the monomer release using an invitro model of orthodontic retention and subjecting it to gas chromatography and mass spectrometry to measure the types and relative quantities of monomers (BPA and TEGDMA in particular) released by orthodontic adhesives used for fixed retentions.

To the best of our knowledge, this study is first of its kind where an attempt was made to compare the relative amount of BPA and TEGDMA release by GC-MS analysis from four commercially available orthodontic adhesives used for retention purposes (Enlight Ormco, Transbond XT 3M, Heliocit Ivoclar and Orthocem FGM).

Methodology

Sample size: Sample size estimation was done by using GPower software (version 3.0). Sample size was estimated for one way test.

A minimum total sample size of 12 was found to be sufficient for an alpha of 0.05, power of 95 %, 1.87 as effect size (assessed from a similar study). Thus, final sample size was found to be 12 i.e., 3 in each group.

F tests - ANCOVA: Fixed effects, main effects, and interactions

Analysis: A priori: Compute required sample size

Input: Effect size f = 2.65

α err prob = 0.05 Power

(1-β err prob) = 0.95

Numerator df = 10

Number of groups = 4

Number of covariates = 1

Output: Noncentrality parameter λ = 84.2700000

Critical F = 3.6365231

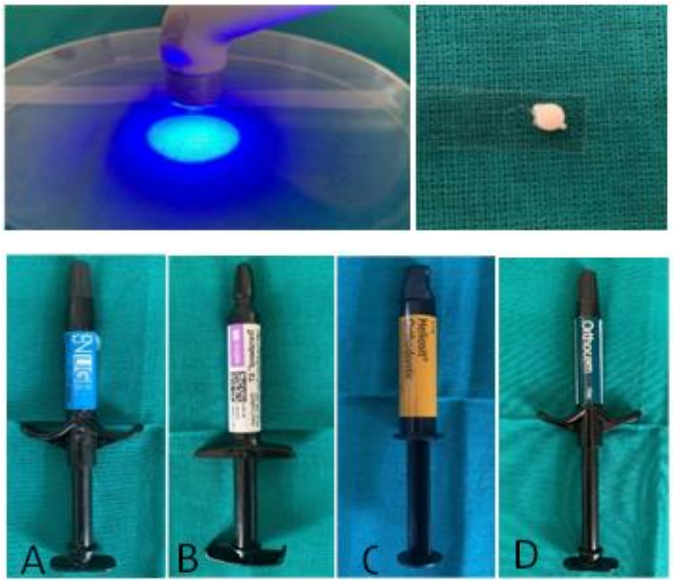
Denominator df = 7

Total sample size = 12 A

ctual power = 0.9763877

Grouping:

Four groups of the following orthodontic adhesives were made with 3 samples each (Figure 1):



GROUP A: 3 Samples of Enlight (Ormco)

GROUP B: 3 Samples of Transbond XT (3M UNITEK)

GROUP C: 3 Samples of Heliosit Orthodontic (Ivoclar)

GROUP D: 3 Samples of Orthocem (FGM)

Methodology

For each sample of composite, 6 dots of composite representing the 6 anterior teeth were placed on a mylar strip. To simulate the adhesive quantity applied to an orthodontic retainer, each dot composite was prepared using Calibrated Molds by Mini Mold Wire Bonder-L (Figure 2).

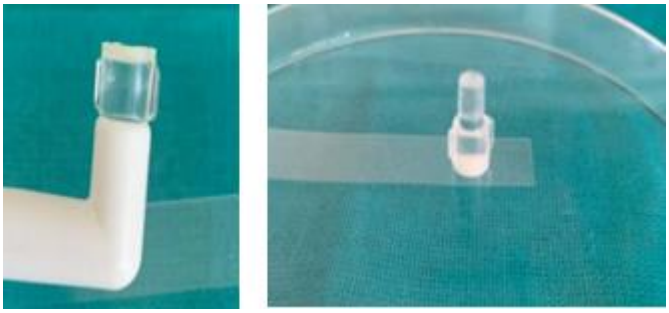


Figure 2: Composite loaded into mini mold wire bonder-L.

Each dot was cured using a curing light (Coltolux LED) with a wavelength of 450nm/470nm for 20 seconds (Figure 3). The distance between the curing light and the composite was reduced to a minimum of 1mm to prevent contact and mobilization of the mini mold while remaining as close as possible to the bond.

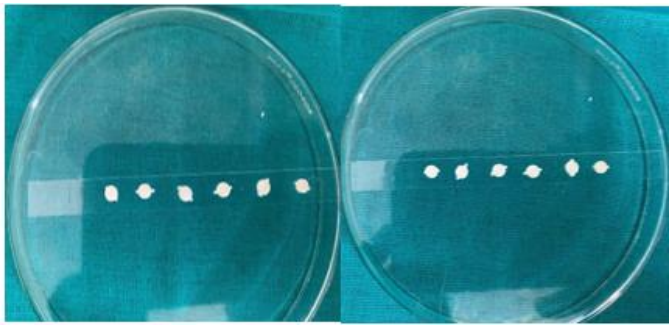


Figure 3: Curing Using Coltolux LED 450nm/470nm wavelength.

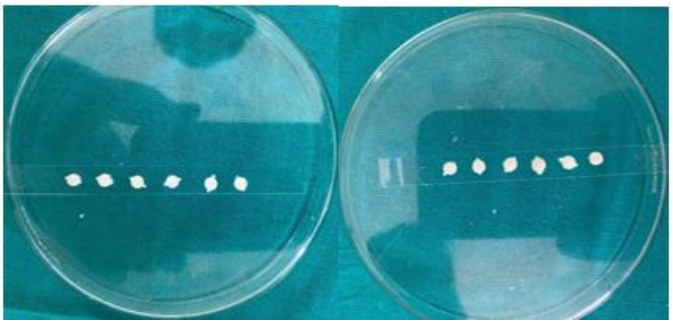


Figure 4: Cured Samples

The cured samples (Figure 4) were immediately immersed in Milli-Q Water (Surface area/Volume Ratio, $3cm^2/ml \pm 10\%$) for 24 hours at 37° C (Figure 5). The immersion liquid was then lyophilized using Thermo Scientific Heto Powerdry LL1500 device (Figure 6) and 100µL of Dichloromethane was added (Figure 7).

Figure 5: Sample preparation after adding Milli Q Water

Figure 6: Lyophiliser (Thermo Scientific, Heto Powerdry LL1500)

Figure 7: Pipette used for adding Milli Q water and Dichloromethane.

The eluates were subjected to Gas Chromatography and Mass Spectrometry:

Gas Chromatography was performed using Clarus 680 device (Figure 8) which employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 2 ml/min. The injector temperature was set at 250°C during the chromatographic run. The 1 and extract sample injected into the instrument the oven temperature was as follows: 40 °C (2 min); followed by 80 °C at the rate of 3 °C min^{-1} and 80 °C, where it was held for 1min and then followed by 150°C at the rate of 5°C min^{-1} ; it was held for 1 min, 30°C min to 280°C and then held for 5minutes.

Figure 8: Gas Chromatography and Mass Spectrometry (Clarus 680 and Clarus SQ 8C)

Mass Spectrometry was performed using Clarus SQ 8C DEVICE with Inlet line temperature 250 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da and the spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2014) library.

TurboMass Ver.6.1 software was used for the analysis. The relative amount of monomers of interest was calculated using the chromatogram with the formula:

Relative Amount = Area of Peak of BPA or TEGDMA/Total Area* 100

The calculated relative amount of monomers of interest will be compared with that of the other composite Samples.

Results

The data was analysed by SPSS (21.0 version). Shapiro Wilk test was used to check which all variables were following normal distribution. Data was normally distributed; therefore, bivariate analyses were performed using the parametric tests i.e., One way ANOVA test followed by Tukey's test for post hoc comparison. Level of statistical significance was set at p-value less than 0.05.

Significant difference was seen in the relative amount of BPA released when compared among four study groups as $p<0.05$. Maximum Relative amount of BPA was found to be released in Group C and least was seen in Group B [Table1 and Graph 1].

Group A and Group B showed no significant difference in BPA release. However, BPA was significantly more released in Group C and Group D compared to Group A. Additionally, Group C exhibited a significantly higher release of BPA compared to Group D. [Table 2 and Graph 2].

Significant difference was seen in the relative amount of TEGDMA released when compared among four study groups as $p<0.05$. Maximum Relative amount of TEGDMA was found to be released in Group C and least was seen in Group A [Table and Graph 3].

No significant difference was seen in the relative amount of TEGDMA released in Group A and Group B. Relative amount of TEGDMA was found to be released

significantly more in Group C and Group D as compared to Group A. Relative amount of TEGDMA was found to be released significantly more in Group C and Group D as compared to Group B. Relative amount of TEGDMA was found to be released significantly more in Group C as compared to Group D [Table 4 and Figure 4].

Table 1: Inter-Group Comparison of relative amount of BPA released.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A*	3	.733	.0577	.0333	.590	.877	.7	.8
Group B†	3	.567	.1528	.0882	.187	.946	.4	.7
Group C‡	3	9.767	.1528	.0882	9.387	10.146	9.6	9.9
Group D‡	3	8.433	.2082	.1202	7.916	8.950	8.2	8.6
Total	12	4.875	4.4426	1.2825	2.052	7.698	.4	9.9

*Group A (Enlight Ormco), †Group B (Transbond XT 3M), ‡Group C (Heliocit Ivoclar), ‡Group D (Orthocem FGM)

Graph 1: Inter-Group Comparison of relative amount of BPA released

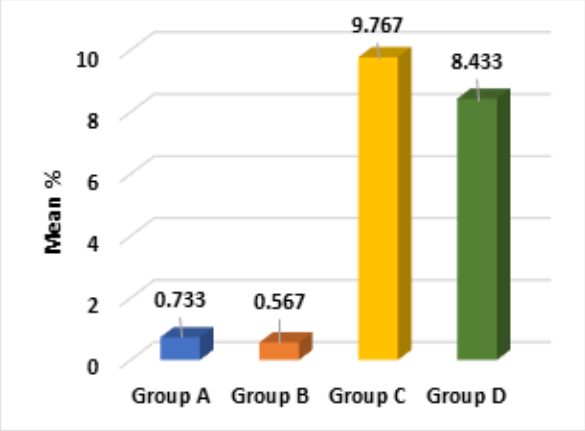
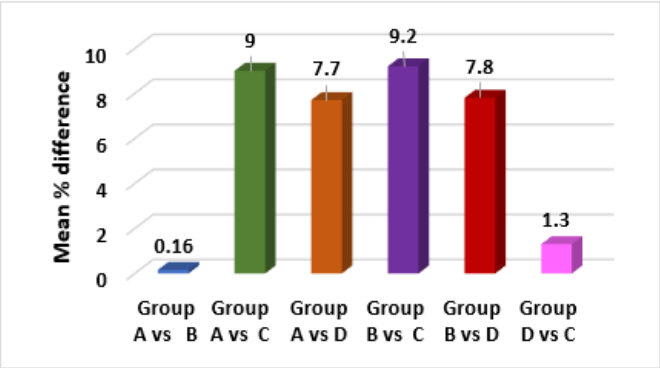


Table 2: Post hoc pairwise comparison for relative amount of BPA released.

Group	Mean Difference	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Group A* vs B†	.1667	.1247	-.233	.566
Group A* vs C‡	-9.0333	.1247	-9.433	-8.634
Group A* vs D‡	-7.7000	.1247	-8.099	-7.301
Group B† vs C‡	-9.2000	.1247	-9.599	-8.801
Group B† vs D‡	-7.8667	.1247	-8.266	-7.467
Group D‡ vs C‡	1.3333	.1247	.934	1.733

Graph 2: Post hoc pairwise comparison for relative amount of BPA released.

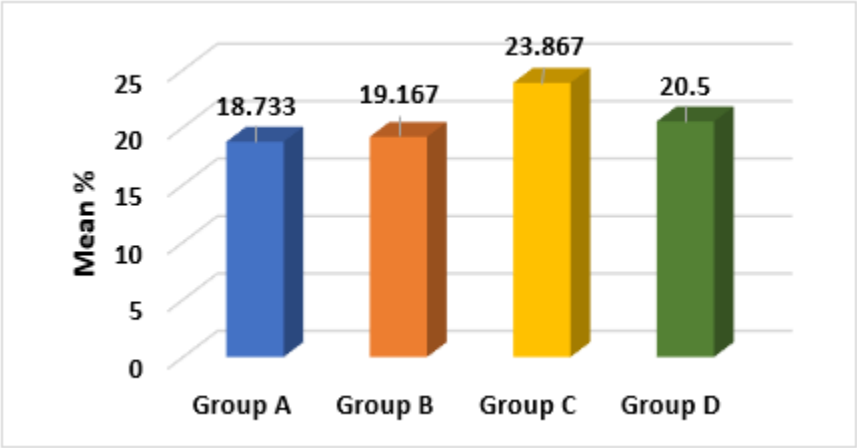


*Group A (Enlight Ormco), †Group B (Transbond XT 3M), ǂGroup C (Heliocit Ivoclar), ᶠGroup D (Orthocem FGM)

Table 3: Inter-Group Comparison for relative amount of TEGDMA released.

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A*	3	18.733	.2082	.1202	18.216	19.250	18.5	18.9
Group B†	3	19.167	.0577	.0333	19.023	19.310	19.1	19.2
Group Cǂ	3	23.867	.3055	.1764	23.108	24.626	23.6	24.2
Group Dᶠ	3	20.500	.2646	.1528	19.843	21.157	20.2	20.7
Total	12	20.567	2.1120	.6097	19.225	21.909	18.5	24.2

Graph 3: Inter-Group Comparison for relative amount of TEGDMA released.



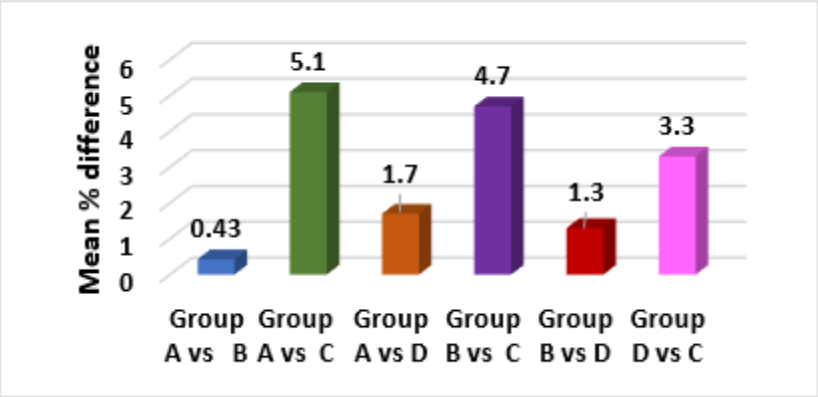
*Group A (Enlight Ormco), †Group B (Transbond XT 3M), ǂGroup C (Heliocit Ivoclar), ᶠGroup D (Orthocem FGM)

Table 4: Post hoc pairwise comparison for relative amount of TEGDMA released.

Group	Mean Difference	Std. Errazor	95% Confidence Interval	
			Lower Bound	Upper Bound
Group A* vs B†	-.4333	.1871	-1.032	.166
Group A* vs Cǂ	-5.1333	.1871	-5.732	-4.534

Group A* vs D[Ŧ]	-1.7667	.1871	-2.366	-1.168
Group B† vs C[ǂ]	-4.7000	.1871	-5.299	-4.101
Group B† vs D[Ŧ]	-1.3333	.1871	-1.932	-.734
Group D[Ŧ] vs C[ǂ]	3.3667	.1871	2.768	3.966

Graph 4: Post hoc pairwise comparison for relative amount of TEGDMA released.



*Group A (Enlight Ormco), †Group B (Transbond XT 3M), ǂGroup C (Heliocit Ivoclar), Ŧ Group D (Orthocem FGM)

Discussion

For an ideal dental resin composite, all monomers should be converted to polymers after polymerization. However, a complete polymerization is hard to achieve in clinical treatment. Moreover, crosslinked resins can swell in organic solvent under oral conditions.[15] Organic solvents penetrate into the matrix, make the interpenetrating polymer network swollen, facilitating the release of un-polymerized leachable monomers and finally cause them to diffuse out.[16] Therefore, it is hypothesized that a denser network by larger filler size and weight in resin composite is favourable for inhibiting monomer diffusion.

Known as the foremost leachable compound of resin composite, TEGDMA can be eluted by water in quantities significantly exceeding the half-maximum effect concentration (EC50).[17] The effect of TEGDMA on gingival fibroblasts and other cells has been studied in much research. Results show that, TEGDMA can easily penetrate HeLa S3 cell membrane,[18] disturb cell homeostasis and cause cell death by ways of cellular apoptosis, suppressed cell proliferation or mineralization[19] in a concentration and time dependent manner.

Accumulating evidence powerfully indicate that TEGDMA increases the formation and accumulation of reactive oxygen species (ROS) in gingival and pulpal fibroblasts,[20] while ROS is considered to be a critical factor inducing immediate adverse cellular reactions to resin monomers. Gerzina TM [21] in 1996 examined the monomer diffusion from the bonding resins in combination with the resin composites and it was seen that in TEGDMA-containing bonding resin when combined with TEGDMA-containing resin composite; TEGDMA leach out through dentine was more. It was concluded that the bonding resins assessed paved way for monomer, to enter into the pulp space and did not inhibit the monomer movement from resin composites to the pulp.

On the other hand, BPA – a chemical component used for manufacturing of polycarbonate plastics and epoxy resins. There has been an increase in exposure to this

compound because of the use of orthodontic adhesives in day-to-day practice. When ingested, biologically active BPA gets conjugated in liver and is rapidly metabolized and excreted through bile or urine. It has a half-life of approximately 5.3 hours.[22]

Even though BPA is rapidly excreted, many clinical studies have found detectable amount of Beta glucuronidase in humans that plays a role in deconjugation of the ingested BPA. This results in the bioaccumulation of BPA after exposure. It has also been found that at low concentrations, BPA is lipophilic and hence gets attached to lipocytes accumulating in the tissues. A relationship between blood levels of BPA and body fat in women has also been reported.[23] Due to this bioaccumulation, BPA can exert many potential risks such as altered rate of growth and sexual maturation, testosterone reduction, feminization in boys, defective reproductive organ function, fertility problems, altered immune function, and improper neural activity.[24]

BPA released from dental biomaterials was first reported by Olea et al [25] in 1966 where in they had found out that 20µL of saliva of patients who had undergone fissure sealing with Bis-GMA containing resin had 90 to 931µg of BPA levels. In 1982, Thompson, Miller and Bowles[26] with the use of UV spectroscopy, reported 80-90% loss of residual monomers and diluents from orthodontic composites, when immersed in water and ethanol for 48 hours.

Interpretation of results

On inter-group comparison of BPA release, it was found that Heliocit Ivoclar Orthodontic Adhesive (Group C) released highest relative amount of BPA i.e., 9.767% ± 0.1528 compared to other three Orthodontic Adhesive Groups. Although it was found that all orthodontic adhesives used in this study released BPA with the least relative amount of release by Transbond XT Orthodontic Adhesive (Group B) i.e., 0.567% ± 0.1528.

On Post hoc analysis of BPA release, there was no significant difference in the relative amount of BPA released between Group A (Enlight Ormco) and Group B (Transbond XT 3M) whereas Group C (Heliocit Ivoclar) and Group D (Orthocem FGM) released significantly higher relative amount of BPA when compared to Group A (Enlight Ormco) and Group B (Transbond XT 3M) as p value is <0.05. On comparison with Group D (Orthocem FGM), the relative amount of BPA released was found to be significantly high in Group C (Heliocit Ivoclar) as p value is <0.05.

On inter-group comparison of TEGDMA release, it was found that Heliocit Ivoclar Orthodontic Adhesive (Group C) released highest relative amount of TEGDMA i.e., 23.867% ± 0.3055 compared to other three Orthodontic Adhesive Groups. Although it was found that all orthodontic adhesives used in this study released TEGDMA with the least relative amount of release by Transbond XT Orthodontic Adhesive (Group B) i.e., 18.733% ± 0.2082.

On Post hoc analysis of TEGDMA release, there was no significant difference in the relative amount of TEGDMA released between Group A (Enlight Ormco) and Group B (Transbond XT 3M) whereas Group C (Heliocit Ivoclar) and Group D (Orthocem FGM) released significantly higher relative amount of TEGDMA when compared to Group A (Enlight Ormco) and Group B (Transbond XT 3M) as p value is <0.05. On comparison with Group D (Orthocem FGM), the relative amount of TEGDMA released was found to be significantly high in Group C (Heliocit Ivoclar) as p value is <0.05.

Reasons for these differences in the BPA and TEGDMA released at different time interval for different

orthodontic adhesives may be attributed to variations in the manufacturing and in the degree of material degradation. But the exact cause is unknown. However, further studies are required for quantification of the monomers to accurately assess the cause of higher amount of BPA and TEGDMA release.

From this study conducted, results show that BPA and TEGDMA was released from all the four groups assessed.

In Group A: Enlight Ormco, relative amount of BPA and TEGDMA released are 0.733% ± 0.577 and 18.733% ± 0.2082 at 24 hours of duration in Milli Q Water after curing.

In Group B: Transbond XT 3M, relative amount of BPA and TEGDMA released are 0.567% ± 0.1528 and 19.167 ± 0.577 at 24 hours of duration in Milli Q Water after curing.

In Group D: Orthocem FGM, relative amount of BPA and TEGDMA released are 20.500% ± 0.2646 at 24 hours of duration in Milli Q Water after curing.

By arranging the groups in decreasing order of relative amount of BPA released, Heliocit Ivoclar occupies the first position followed by Orthocem FGM followed by Enlight Ormco and lastly Transbond XT 3M.

Also, by arranging the groups in decreasing order of relative amount of TEGDMA released, Heliocit Ivoclar occupies the first position followed by Orthocem FGM followed by Transbond XT 3M and lastly Enlight Ormco.

This is the first study to involve four different commercially available orthodontic adhesives to analyse and compare the relative amount of BPA and TEGDMA released in Milli Q Water after 24 hours using Gas Chromatography coupled with Mass Spectrometry (GC-MS).

Conclusion

With the result of the present study, it could be inferred that orthodontic adhesives used for retention purposes release BPA and TEGDMA even after curing.

The results of this in-vitro study allowed us to substantiate a model of monomer release from an orthodontic bonded retainer and implies that all four orthodontic adhesives used in the study released BPA and TEGDMA. Clinicians must consider the adverse effects of these monomers and adhere to the clinical recommendations given to reduce BPA release. Also, increase the use BPA- Free Orthodontic Adhesives and chemically cured orthodontic adhesives whenever possible.

However, this study must be extended in large scale for longer duration and also, a unique in vivo method must be followed for assessing the BPA and TEGDMA leached solely due to orthodontic adhesives to identify the potential toxic effects caused only by the orthodontic adhesives.

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