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# Comparative Evaluation of BPA Release from Various Orthodontic Aligners – An In Vitro Study.

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**ABSTRACT**

**Objective:** This study was done to compare and evaluate the release of the Bisphenol A(BPA) from three different types of orthodontic thermoplastic aligners.

**Materials and Methods:** The materials used in the study were three different brands of thermoplastic aligners- Clear correct, Invisalign and V clear the samples were subjected to artificial saliva and the incubated artificial saliva was subjected to liquid chromatography and mass spectrometry to evaluate release of Bisphenol A (BPA).

**Results:** The amount of Bisphenol A released from Clear correct was 6.60 ng/ml, Invisalign was 9.59 ng/ml and V clear was 7.49 ng/ml. were within its normal threshold limit 4000ng/kg/bodyweight

**Conclusion:** All the samples used in the study have shown traces of Bisphenol A.even though it is advertised that the aligners are devoid of bisphenol A. The long-term use of thermoplastic aligners should be handled carefully. Cytotoxicity of aligners may lead to effect on the patient's overall general health.

**Keywords:** Bisphenol A(BPA),Clear correct aligners, Invisalign aligners, liquid chromatography and mass spectrometry, V clear aligners.

## I. INTRODUCTION

The Orthodontic thermoplastic aligners are considered as an aesthetic option for treatment of mild to moderate malocclusions. The materials used in the manufacturing of aligners are polypropylene,polycarbonate and thermoplastic polyurethanes. Each set of aligners are approximately worn 22 hours a day for a period of 14 days, the study conduct by **Katras et al. 2021**[1]shown that when aligners are exposed to hot and cold beverages, mechanical wear, and changes in pHof salivary enzymes which may lead to degradation and release of certain cytotoxic substances such as Bisphenol A, itis a synthetic compound released from polycarbonate present in the aligners.

According to Food and Drug Administration the normal safety levels for Bisphenol A is 4000ng/kg body weight/day.BPA is a precursor of Bisphenol A Glycidyl Methacrylate - BIS-GMA and Bisphenol A Di Methacrylate monomer-BIS-DMA, found in adhesive resins, composite resins, and aligners.
Bisphenol A is a chemical known to cause adverse health effects[7].

## II. MATERIALS AND METHODS

### 1)<u>Thermoplastic Aligners</u>

Sample of 30 pairs of new aligners obtained directly from manufacturer from various aligner companies were taken and divided into 3 groups.
Group 1 – 10 pairs of aligners from Invisalign, 10 maxillary aligners and 10 mandibular aligners.
Group 2 – 10 pairs of aligners from Clear correct, 10 maxillary aligners and 10 mandibular aligners.
Group 3 – 10 pairs of aligners from V clear, 10 maxillary aligners and 10 mandibular aligners.

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**2)Artificial saliva**

Brand name-WET MOUTH.

A sample of 10 aligner sets, 10 maxillary & 10 mandibular from 3 different aligner suppliers were selected, each aligner was cut into 3parts, 1 anterior part and 2 posterior parts to fit in the petri dishes and making the overall sample of 60 pieces of each manufacturer & they were colour coded with 3different colours Red, Blue, Yellow, and segregated as following groups.

Group A – 60 pieces of **Invisalign aligners,** red colour coded.

Group B – 60 pieces of **Clear correct aligners,** blue colour coded.

Group C – 60 pieces of **V clear aligners,** yellow colour coded.

The samples were sterilized with UV Steriliser for 15 min and this samples were transferred to air tight petri dishes with artificial saliva about 50ml added to this dish and this samples of aligners were incubated for 3 weeks at 37°C and this incubated artificial saliva were transferred to lab for testing for the release of Bisphenol A from the samples of aligners through liquid chromatography and mass spectrometry method.

**Figure1: Invisalign (Red)** **Figure2: Clear correct (Blue)** **Figure3:V clear (Yellow)**


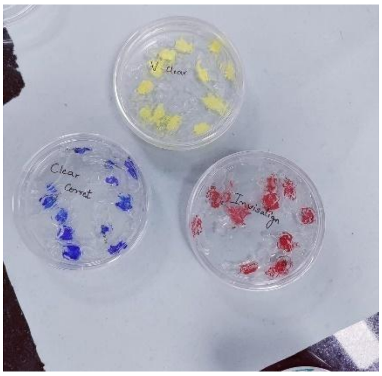



**Figure 4: Artificial saliva** **Figure 5: Samples in petri dishesFigure6: Samples in incubator at37°C**

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**Figure7: LC-MS chamber**

## III.STATISTICAL ANALYSIS

The data was statistically analyzed in statistical package for social sciences-SPSS software IBM corp 2013 version 22.0. ANOVA one way test was carried out for comparison of 3 different brand of aligners. Comparison between groups of aligners was done using Tukey's post hoc test.

Table 1: Mean comparison among Invisalign, Clear Correct and V Clear

| Group | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|
| INVISALIGN | 9.33 | 10.01 | 9.59 | 0.37 |
| CLEAR CORRECT | 5.99 | 7.77 | 6.60 | 1.01 |
| V CLEAR | 7.28 | 7.81 | 7.49 | 0.28 |

**Statistical Analysis:** ANOVA one way test, Mean value in (ng/mL).

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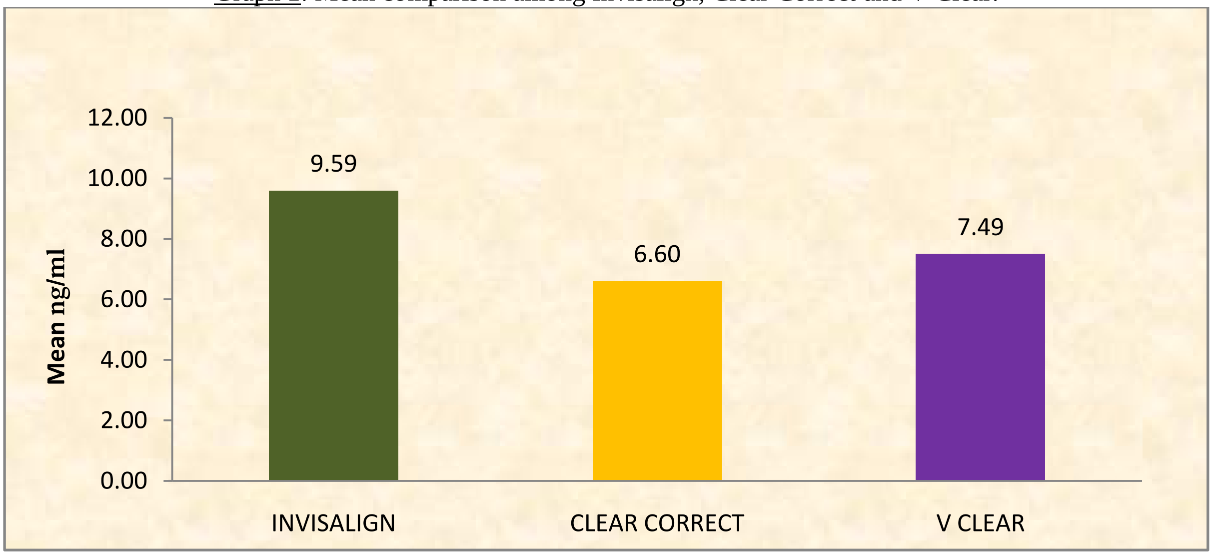
Table 2: Mean comparison in between Invisalign, Clear Correct and V Clear.

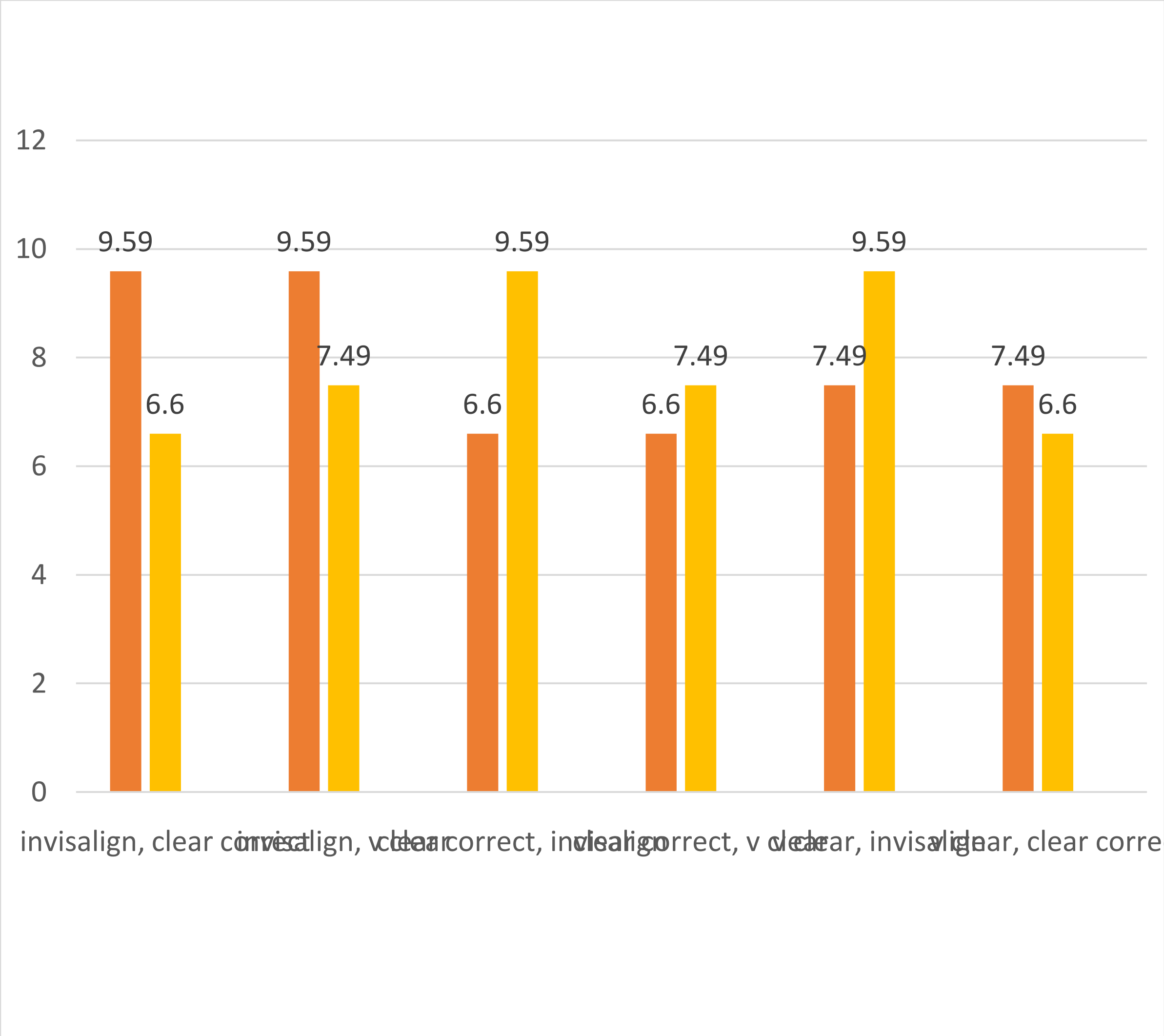
| Multiple Comparisons | | | |
|---|---|---|---|
| **(I) GROUP** | **(J) GROUP** | **Mean Difference (I-J)** | **P value** |
| **INVISALIGN** | **CLEAR CORRECT** | **2.99** | **0.003** |
| | **V CLEAR** | **2.10** | **0.016** |
| **CLEAR CORRECT** | **INVISALIGN** | **-2.99** | **0.003** |
| | **V CLEAR** | **-0.89** | 0.282 |
| **V CLEAR** | **INVISALIGN** | **-2.10** | **0.016** |
| | **CLEAR CORRECT** | **0.89** | 0.282 |

**Statistical Analysis:** Tukey's post hoc test. **S:** Statistically significant if P<0.05;
NS: Not significant.

Graph 1: Mean comparison among Invisalign, Clear Correct and V Clear.



Graph 2: Mean comparison in between Invisalign, Clear Correct and V Clear.




## IV. RESULTS

The mean salivary BPA levelsrelease from the samples were Invisalign 9.59ng/ml, Clear correct 6.60ng/ml and V clear 7.49ng/ml. The mean BPA levels among groups statistical significance difference was found between Invisalign and Clear correct, Invisalign and V clear, whereas non-significant statistical difference was found between Clear correct and V clear.

## V. DISCUSSION

Bisphenol- A is a byproduct of polycarbonate present in aligners. the safety limits of BPA 4000ng/kg body weight/day, beyond this limit it may be toxic to person using aligners and may cause some adverse effects[2].

As patient usually wears each set of aligners for 22 hours a day for at least 14 days, and, in most of the comprehensive cases there is requirement of more than 60 sets of aligners for a minimum duration of 1 year, due to long term use in the oral cavity these aligners are exposed to hot and cold beverages, change in pH of the saliva etc so, aligners may degrade and release BPA[1].

Since most of orthodontic patients are young adults and a small number of patients are pregnant women whose fetus growth is influenced by environmental factors. Constant exposure to BPA lead to endocrine disruption that may be harmful to the patient and result in numerous health problems**Taylor JA, Vom Saal** et al. 2001[6].

The safety levels of BPA value were currently determined as 50000ng/kg/day in United States, the estimated safe amount and has been lowered by European food safety authority to 4000ng/kg body weight/day based on new evidence and approach[1].

However, a prevailing concern regarding BPA exposure is its ability to bind to the estrogenic

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receptors ERα and ERβ, through which this endocrine-active substance may affect humans at doses above the presumably safe tolerable daily intake i.e., 4000 ng/kg/day **Taylor JA, Vom Saal** et al. 2001[6].

Multi-system health effects are being linked to BPA, there is evidence of transcription, proliferation, and differentiation in related genes in response to low dose BPA. In oral keratinocytes, which are the first targets of BPA by oral intake showed absorption of BPA, which shows that BPA can diffuse through oral mucosal tissues **Taylor JA, Vom Saal** et al. 2001[6].Adverse effects, such as cancer cell proliferation is linked with BPA doses above the reference dose[1].

Study done by **Deling Fan et al. 2022[8]**on exposure of preschool aged children to highly concerned bisphenol analogue shows BPA and BPFas the predominant types of BPs, although BPA and its analogues (except for BPAF and BPP) were detected in urine samples of preschool age children's. Furthermore, elevated human urinary BPA levels have been associated with peripheral arterial disease, cardiovascular disease, diabetes, abnormal liver enzyme levels and obesity in children and adolescents**Taylor JA, Vom Saal** et al. 2001[6].

A study conducted by **Katras et al. 2021[1]**on Invisalign aligners incubated in artificial saliva observed the mean amount of bisphenol A release was about 3.5ng/mL after 10 days. The result of this study relates accordance to the present study.

A study conducted by **Esra et al. 2023[2]**on 5 different brands of aligners like Duran Essix cp, Smart track, Zendura A, Taglus premium and Zendura Flx, they incubated the aligners in saline for 8 weeks, showed traces of bisphenol A release. The results of this study relate accordance to the present study

A study conducted by **Al hendi et al.2022[3]**on leaching of bisphenol A from Invisalign aligners in ethanol solution showed that no traces bisphenol A release. The result of the study was contrast to the present study.

A study conducted by **Matthew et al…2013 [4]**on different retainers like thermo formed biocryl and fixed retainer with Transband Xt, they incubated the retainers in artificial saliva for 2weeks, showed traces of bisphenol A release. The results of this study relate accordance to the present study.

A study conducted by **Raghavan et al..2016[5]**to evaluate and compare the bisphenol A levels in saliva of patients using vacuum formed retainer and Hawley's retainer showed traces of bisphenol A release.The results of this study relate accordance to the present study.

The clinical significance of Bisphenol A release in different types of aligners is a topic of concern. BPA is a chemical that can be found in certain plastics, including those used in the production of dental aligners. These clinical implications arise because BPA is known to have endocrine-disrupting properties, which means it may interfere with hormonal activity in the body and causing many adverse effects. Patients and practitioners should be aware of these concerns and may choose aligners that are labeled as BPA-free or opt for alternative materials if they have specific worries about BPA exposure. Safety measures to overcome the release of Bisphenol A is by soaking them in deionized water or demineralized water, before wearing according to **Katras et al. 2021[1]** this could significantly decrease the BPA release.

The limitations of the present study were as this was conducted in in-vitro conditions by using artificial saliva this may not perfectly replicate the complexity of natural saliva. As natural saliva has variations in composition and flow rates that could impact BPA release differently. Incubating aligners in a static environment may not mimic the dynamic conditions of the oral cavity during activities such as chewing and swallowing according to **Katras et al. 2021[1]**Dynamiccondition could influence BPA release differently. Variations in temperature and pH within the oral cavity could influence BPA release. Hence, in in-vivo conditions different results may be observed**.**Moreover,BPA has many analogues like BPB, BPF, BPP, BPS, BPZ, BPAF AND BPAP research on BPA may give results differently and results of other analogue may vary**Deling fan et al. 2022 [8].**

Various Further research can be done like, Research on long-term study can be done as the aligners are worn for 15 to 18 months, other types of toxic materials. may release from the thermoplastic aligners. Research on Method of manufacturing like thermoforming and 3D printing may have effects on the release of Bisphenol A.Research on Effect of various beverages on Bisphenol A release from the orthodontic thermoplastic aligners. And further research on long term effect of BPA release from orthodontic appliance like thermoplastic retainers, thermoplastic aligners and fixed retainers causing cytotoxicity affecting the general health of the patient.

## VI. CONCLUSION

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Traces of Bisphenol A was found in all the three different types of orthodontic thermoplastic aligners in artificial saliva.

The mean amount of Bisphenol A release was lesser than threshold limit 4000ng/kg bodyweight.

Although the aligners are advertised as Bisphenol A free. the clinician should be cautious with the long-term usage of thermoplastic aligner.

The cumulative effect of Bisphenol A can cause cytotoxicity and can cause other adverse effects on the general health of the patient.

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