


Review

Efficiency and predictability of maxillary expansion with the Invisalign[®] system

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Abstract: The Invisalign[®] system aims to ensure aesthetics and achieve positive clinical results; however, its efficacy is still debatable. Along with lower incisor intrusion and upper molar distalization, arch expansion is one of the most predictable movements that may be achieved using clear aligners. This makes the system a conceivable method for the correction of crowding and posterior dentoalveolar crossbites. The aim of this study is to analyze the effectiveness and predictability of tooth expansion in permanent dentition with Invisalign[®] orthodontic aligners. A bibliographic search was carried out using the PubMed and EBSCOhost databases. Articles published between 2013 and 2023 and written in English were selected. Reporting of this review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. After a thorough review, 12 articles were included. In terms of efficiency, most studies showed an increase in all interdental measurements, with premolars showing the greatest expansion. On the other hand, predictability was weaker, as significant differences were found between the post-treatment models and the ClinCheck[®] software virtual model for some interdental measurements. We concluded that the Invisalign[®] system is effective in producing expansion movement, with reasonable predictability.

Keywords: tooth movement; Invisalign[®]; effectiveness; expansion; clear aligners

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Introduction

The orthodontic approach known as “clear align therapy” (CAT) uses thermoformed aligners to treat dental malocclusions. Since their introduction, Invisalign[®] aligners (Align Technology, Santa Clara, California) have gained recognition as an aesthetic alternative to traditional fixed orthodontic appliances. Orthodontic treatment using clear aligners provides a lot of benefits, including the elimination of aesthetic compromise, increased patient acceptance, easier cleaning, and improved control of in-office time. Additionally, it is less painful than a conventional fixed treatment and has a lower incidence of mucosal irritations, periodontal lesions, and enamel demineralization and abrasion [1-5]. As soon as they appeared in the market, they were the subject of investigations, several of which showed exceptional effectiveness in achieving tooth movements, such as intrusion, extrusion, distalization and expansion [5-7]. Arch expansion may be required to widen the dental arches, improve the transverse dimension of the smile, enhance the appearance, or as a mechanism to create space for crowding correction. Additionally, it can correct dentoalveolar posterior crossbites [8,9]. Mesiodistal tipping, according to a number of earlier investigations, is the most predictable movement, while tooth rotation and extrusion are the most difficult to execute. The precision of movements with clear aligners is reported to average 55% to 72% [10-12].

The mechanical characteristics of thermoplastic materials and their attachments design also affect how well teeth move. In the oral cavity, aligners are subjected to additional factors, such as elastic deformation, temperature, salivary enzymes and humidity, which may change their chemical and physical qualities [13-15]. Knowing how well the software predicts changes may enable the practitioner to foresee the need for overcorrection, minimizing additional aligners, mid-course adjustments, and treatment duration overall. Thus, the aim of this systematic review is to answer the following question: how effective and predictable are Invisalign® orthodontic aligners for tooth expansion movements?

Materials and Methods

Review guidelines

The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines were used to develop this systematic review. This study was accepted in the PROSPERO database (CRD42023430469).

Eligibility criteria

The guiding questions were defined according to the Population characteristics, Intervention type, Comparison parameters, Outcomes and Study design (PICOS) strategy, as presented in Table 1. Therefore, the following questions of this study were defined as (i) “Is the Invisalign® system effective for expansion movements?” and (ii) “Is the ClinCheck® software capable of predicting expansion movement?”. In this sense, the eligibility criteria for the studies to be included were defined accordingly with the PICOS strategy:

Table 1. PICOS strategy.

Population	Patients with completely permanent dentition and a need for orthodontic corrections.
Intervention	Orthodontic treatment with clear aligners.
Comparison	Comparison of pretreatment and posttreatment models (predicted tooth placement <i>versus</i> achieved tooth placement).
Outcomes	Effectiveness and predictability of tooth expansion with Invisalign® aligners.
Study design	Prospective and retrospective clinical studies, community trial, randomized clinical trial.

Inclusion criteria

1. Articles published between 2013 and March 2023;
2. Articles written in the English language;
3. Availability: complete articles that refer to the topic and are not restricted;
4. Prospective/retrospective clinical studies, community trials, randomized clinical trials;
5. Articles whose study mentions patients with completely permanent dentition.

Exclusion criteria

1. Summary does not fit the subject under study;
2. Articles without free full text available;
3. Articles whose reading did not provide useful information;
4. Case reports, systematic review articles, theses and dissertations;
5. Articles written in languages other than English.

Search strategy

Electronic literature searches were carried out in the PubMed and EBSCOhost databases. Articles written in English and published between 2013 and 2023 were selected.

The following keywords and MeSH terms were employed in the search strategy: *((clear aligners) AND (tooth movement)) OR ((expansion) AND (Invisalign®) AND effectiveness)*.

Data related to the search strategy are shown in Table 2.

Selection of articles and data collection

The search terms previously highlighted were used to perform an advanced search. Duplicates were manually removed. The titles and abstracts of the potentially relevant articles underwent a preliminary analysis to determine whether they met the purposes of the study. The clinical trials that met the inclusion criteria were completely reviewed and their eligibility was evaluated. Finally, the relevant data was collected and arranged in Table 3.

Table 2. Search strategy.

Databases	Keywords	Articles found	Selected articles
PubMed and EBSCOhost	<i>((clear aligners) AND (tooth movement)) OR ((expansion) AND (Invisalign®) AND effectiveness)</i>	549	12

Quality assessment of data

The ROBINS-I tool was used to assess the methodological quality of the studies, as all the studies approved in this analysis were non-randomized. Two authors (AR and AO) independently evaluated the quality of the selected articles based on seven bias domains: confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, selection of the reported results, and overall bias. Two studies, [16] and [17], were considered to have a minimal risk of bias, making them comparable to well-conducted randomized trials. Most of the studies ([18-26]) had a moderate risk of bias. Only one study, [27], had a serious risk of bias. The results are presented in Table 4.

Results

Selection of articles

The bibliographic search identified a total of 549 articles. After duplicates were eliminated, 540 articles remained, which, after reading the titles and abstracts, were reduced to 53, 18 of which were excluded for not meeting the inclusion criteria. At this stage, 35 articles were individually reviewed to assess the quality and type of each study. From these 35 articles, 23 were excluded for not providing pertinent information. Finally, 12 studies were included. These data are illustrated in Fig. 1.

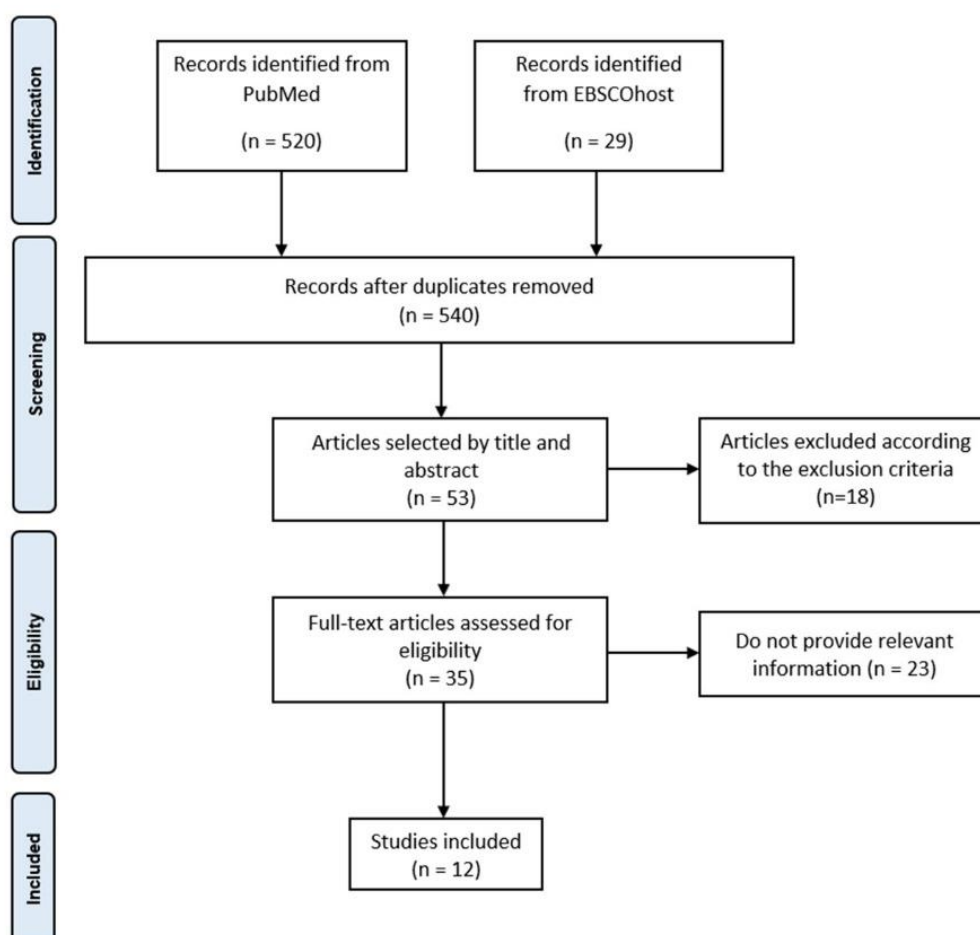
**Figure 1.** Selection of articles.

Table 3. Data and outcomes from articles.

Author and year of publication	Study Design	Objective	Number of participants	Measurement	Outcomes
Houle JP <i>et al.</i> (2017) [19]	Retrospective study	To evaluate the predictability of arch expansion using Invisalign® CA.	$n = 64$ ClinCheck® and STL files.	<ul style="list-style-type: none"> Linear values of both arches C, PMs, and 1st Ms at lingual gingival margins and cusp tips. 	<ul style="list-style-type: none"> For every maxillary measurement, $p < 0,05$ between ClinCheck® and T1. $p > 0,05$ for measurements at the cusp tips of the lower arch teeth. Predictability in upper arch: 72.8% Predictability in lower arch: 87.7%
Solano-Mendoza B <i>et al.</i> (2017) [26]	Retrospective study	To validate a new method for quantifying the predictability of expansion with the Invisalign® CA.	$n = 116$ 3D models and ClinCheck®.	<ul style="list-style-type: none"> Linear values of upper arch widths for C, PMs, and 1st palatal gingival margins and cusp tips, C depth, arch depth, 1st M rotation, and 1st M inclination. T0 and T1. 	<ul style="list-style-type: none"> The amount of expansion programmed by the software is not predictable. $p > 0,05$ between the 3D model and ClinCheck® at T1 (except 1st M cuspid width and arch depth).
Charalampakis O <i>et al.</i> (2018) [25]	Retrospective study	To evaluate the efficacy of tooth movements with the Invisalign® system.	$n = 20$ ClinCheck®, digital models and 3D image analysis software.	<ul style="list-style-type: none"> TP and T1 models were superimposed on the initial ones. 	<ul style="list-style-type: none"> IPM expansion was accurate for premolars, but the average amounts of planned expansion were very low.
Zhou N <i>et al.</i> (2020) [20]	Retrospective study	To evaluate the efficacy and movement pattern of upper arch expansion using the Invisalign® system.	$n = 20$ Digital models and CBCT.	<ul style="list-style-type: none"> T0 and after completing the expansion phase. 	<ul style="list-style-type: none"> $p < 0,05$ between the expected and actual expansion amounts. IC crown: $79.75 \pm 15.23\%$ 1st IPM crown: $76.1 \pm 18.32\%$ 2nd IPM crown: $73.27 \pm 19.91\%$ 1st IM crown: $68.31 \pm 24.41\%$
Morales-Burruezo I <i>et al.</i> (2020) [27]	Retrospective study	To evaluate the efficiency and predictability of the Invisalign® system for arch expansion.	$n = 114$ ClinCheck® and RulerSwift for Mac OS X.	<ul style="list-style-type: none"> T0, T1 and TP (limited to the 1st treatment phase). Linear values of upper C, PMs, 1st and 2nd Ms, and angular dimension widths for 1st Ms. 	<ul style="list-style-type: none"> Widths underwent significant advances because of treatment. Cs predictability: 74.8% 1st PMs and 2nd PMs predictability: 80.3% and 81% 1st Ms predictability: 79.1% 2nd Ms predictability: 65.2%
Lione R <i>et al.</i> (2021) [17]	Prospective study	To investigate tooth movements during arch expansion with CA treatment.	$n = 28$ ClinCheck® and STL files.	<ul style="list-style-type: none"> Linear values of upper C, PMs, 1st and 2nd Ms at buccal cusp tips. T0, T1 and TP. 	<ul style="list-style-type: none"> $p < 0,05$ for all measurements (except for upper 2nd M). Greatest increase in maxillary width: 1st and 2nd PM. Poor predictability for maxillary C ($p < 0,05$).
Riede U <i>et al.</i> (2021) [18]	Retrospective study	To investigate the precision of CA in achieving expansion or contraction of the maxilla and occlusal contacts.	$n = 30$ ClinCheck® and STL files.	<ul style="list-style-type: none"> T0 model, a scan-based CC model, a T1 clinical model, and a CC model of treatment outcome as simulated. Linear values of upper C, PMs, and 1st Ms at palatal gingival margins and buccal cusp tips. 	<ul style="list-style-type: none"> Effectiveness: 45% Not better with SmartTrack® than with the previously used Ex30® material. $p < 0,05$ for precision of transfer from the casts to TP and between the simulated and clinical outcomes.
Vidal-Bernárdez ML <i>et al.</i> (2021) [22]	Retrospective study	To evaluate the performance of upper and lower orthodontic expansion with the Invisalign® system.	$n = 167$ ClinCheck® and digital models.	<ul style="list-style-type: none"> ModT1, ModT2 and the final CC. Linear values of both arches C, PMs, and 1st Ms at lingual gingival margins and cusp tips. 	<ul style="list-style-type: none"> The efficiency of arch expansion showed a statistically significant difference. Predictability at the coronal level: 98-100% Predictability at the gingival level: 85-90%
D'Antò V <i>et al.</i> (2022) [21]	Prospective study	To investigate the predictability of CA therapy.	$n = 70$ ClinCheck® and STL files.	<ul style="list-style-type: none"> Torque, tip, and rotation. T0, at the end of stage 15 and at TP for stage 15. 	<ul style="list-style-type: none"> $p > 0,05$ between TP and T1 movement for all the assessed movements. Largest hyper-performance: torque of the 2nd M ($+2.3^\circ \pm 3.1^\circ$). Greatest under-performance: tip of the 1st M ($-2.3^\circ \pm 3.3^\circ$).
D'Antò V <i>et al.</i> (2023) [23]	Retrospective study	To investigate the predictability of dentoalveolar expansion and molar inclination with CA, at the first set of aligners.	$n = 30$ STL files, Geomagic Control X and a 3D metrology software.	<ul style="list-style-type: none"> Linear values of both arches C, PMs, and 1st Ms at lingual gingival margins and cusp tips. T0, T1 and TP. 	<ul style="list-style-type: none"> Total mandibular accuracy: 64% and 67% at the cusp level, and 59% at the gingival level. Total maxillary accuracy: 67% and 71% at the cusp level, and 60% at the gingival level.

Galluccio G <i>et al.</i> (2023) [24]	Retrospective study	To evaluate the maxillary arch transverse expansion using the Invisalign® CA system.	$n = 28$ ClinCheck® and STL files.	<ul style="list-style-type: none"> T0, T1 and final virtual models by CC. Linear values of upper C, PMs, and 1st Ms at palatal gingival margins and buccal cusp tips. 	<ul style="list-style-type: none"> $p > 0,05$ in predictability for vestibular measurements. $p < 0,05$ in predictability for gingival measurements. Overall accuracy of the expansion: 70%.
Castroflorio T <i>et al.</i> (2023) [16]	Prospective study	To evaluate the predictability of tooth movement with the Invisalign® system.	$n = 79$ ClinCheck®, STL files and Geomagic Qualify®.	<ul style="list-style-type: none"> T1 digital models and final virtual models. Angulation, inclination, rotation, mesio-distal movement, vertical movement, and buccal/lingual movement. 	<ul style="list-style-type: none"> The lack of correction was significant for all movements (except for the rotation of upper 1st M). The prescribed OTM, the group of teeth and movement, the frequency of aligner change, and the use of attachment influence the outcome.

C, canine; CA, clear aligners; CBCT, cone beam computer tomography; CC, ClinCheck®; CT, clinical trial; IC, intercanine; IM, intermolars; IPM, interpremolars; M, molar; ModT1, digital models at the beginning of treatment; ModT2, digital models at the end of treatment; n , number of participants; OTM, orthodontic tooth movement; PM, premolar; TP, virtually planned; T0, pre-treatment; T1, end result achieved.

Table 4. Assessment of risk of bias for expansion outcome using the ROBINS-I Tool.

Author and year of publication	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result
Houle JP <i>et al.</i> (2017) [19]	L	M	M	L	L	L	L
Solano-Mendoza B <i>et al.</i> (2017) [26]	L	L	L	L	M	M	L
Charalampakis O <i>et al.</i> (2018) [25]	L	L	L	L	M	L	L
Zhou N <i>et al.</i> (2020) [20]	M	M	M	L	L	M	L
Morales-Burruezo I <i>et al.</i> (2020) [27]	L	M	M	S	M	S	L
Lione R <i>et al.</i> (2021) [17]	L	L	L	L	L	L	L
Riede U <i>et al.</i> (2021) [18]	M	M	M	L	M	M	L
Vidal-Bernárdez ML <i>et al.</i> (2021) [22]	M	L	M	M	M	M	M
D'Antò V <i>et al.</i> (2022) [21]	L	M	M	M	L	M	L
D'Antò V <i>et al.</i> (2023) [23]	L	M	M	M	L	M	L
Galluccio G <i>et al.</i> (2023) [24]	L	L	M	L	L	L	L
Castroflorio T <i>et al.</i> (2023) [16]	L	L	L	L	L	L	L

L: low risk of bias; M: moderate risk of bias; S: serious risk of bias.

Discussion

Dentoalveolar crossbite can be corrected, crowding can be solved, and arch form can be modified with arch expansion, which can be performed at the canine, premolar and molar levels, or differentiated by maintaining a stable sector. According to several previous studies that assessed the expansion of dental arches, the width of the arch should only be increased by a maximum of 2 to 3 mm per quadrant in order to reduce the danger of harming the periodontium and, more importantly, inducing gingival recession [24,28,29]. Despite the defended efficacy of the treatment, there is still disagreement among experts about its true therapeutic potency. Therefore, the aim of this study is to investigate the effectiveness and predictability of expanding the maxillary and mandibular arches using Invisalign® clear aligners as an orthodontic appliance.

Kravitz *et al.*, in 2009, was the first team to conduct a prospective clinical study on the effectiveness of tooth movement with the Invisalign® clear aligners. They observed that transverse expansion was not fully accurate. The mean accuracy of tooth movement in the anterior region was 40.5%, which is significantly less precise than the values reported in later investigations. This could be due to the development and improvement of the system [7].

In 2016, Solano-Mendoza *et al.* investigated the predictability of the expansion in the upper arch, with the same system, through gingival and cusp widths. Comparing post-treatment virtual models with clinical models, they found a lack of precision for all width measurements [26].

In 2017, Houle *et al.* evaluated the predictability of arch expansion with the Invisalign® system and found that transverse changes in the maxillary arch had 72.8% overall accuracy, and that the mandibular arch had an overall accuracy of 87.7%, thus concluding that the system would be predictable [19].

Like Houle *et al.*, in 2018, Charalampakis *et al.* did not detect major differences between planned and executed movements in the horizontal plane, again suggesting that the software was capable of accurately predicting the expansion movement. They also observed that the maxillary intercanine width represented the highest discrepancy between what was planned and what was really accomplished. These authors argue that this can happen because the upper canines have the longest roots and the crown has a conical shape with a few undercuts to improve aligner retention. Expansion in the premolar area was effectively accurate, but the mean amount of planned expansion was only 1.76 mm for the lower premolars and 1.49 mm for the upper premolars [25].

More recently, in 2020, Zhou *et al.* performed the first investigation in which 3D tomographic images (cone beam computer tomography, CBCT) were used to assess changes in upper arch expansion caused by the Invisalign® system. The results of this study showed that Invisalign® aligners can be effective, as the results obtained were 79.75% at the canine cuspid level, 76.10% and 73.27% at the first and second premolar level, respectively, and 68.31% at the first molar level. These authors also evaluated the efficiency of the expansion by bodily movement in the maxillary first molar and reported that the root-to-crown expansion movement ratio was approximately 2:5 [20].

Also in 2020, Morales-Burruezo *et al.* evaluated the performance of the Invisalign® system in producing expansion in the upper arch. Regarding the first objective, determining the effectiveness of expansion movement, the data showed that clear aligners are a useful method to achieve transverse expansion since the outcomes revealed a greater or lesser extent of a rise in all tooth widths. As percentages, the predictability was 74.8% at the canine level, 80.3% at the first premolar, 81% at the second premolar, 79.1% at the first molar and 65.2% at the second molar, which was not considered in our study. Some studies have assessed the expansion at the gingiva level; however, since the gum is removed from the digital model during the ClinCheck® treatment planning process, before the software computes all the parameters and procedures, and only at the end is organized virtually without applying any specific criteria, these outcomes cannot be consistently accurate and will differ among ClinCheck® results [27].

Vidal-Bernárdez *et al.*, one year later, in 2021, evaluated the efficacy and predictability of arch expansion using Invisalign® clear aligners. They observed statistically significant effects on the cusp and gingival widths in both arches. Their results showed a predictability of 99.26% for the mandibular arch at the cuspid level in canines, of 100% in first premolars, of 100.38% in second premolars, and of 99.78% in first molars. The predictability achieved for the upper arch at the cuspid level was 98.35% for the canines, 99.36% for the first premolars, 100.58% for the second premolars, and 98.32% for the first molars [22].

Also in 2021, Lione *et al.* evaluated tooth movements during upper arch expansion. With the exception of the maxillary second molars, statistically significant differences were detected in all measurements. The greatest increase in the upper arch width was detected in the maxillary first and second premolars: +3.5 mm and +3.8 mm, respectively. They claim that this occurred as a result of the premolars' straight-line positioning. The predictability of the ClinCheck® software was assessed after the end of the treatment. For the linear measurements, statistically significant differences were detected only for the intercanine width, with a minor discrepancy (1.6 mm) between the predicted and achieved movements, reflecting the low predictability between the ClinCheck® virtual animation and the observed treatment results for this measurement [17].

In 2023, Galluccio *et al.* evaluated the maxilla and found that the percentage of expansion achieved was lower than that predicted in all measurements. The overall clinical accuracy of the expansion treatment was 70.88%. The greatest difference between what was achieved and what was planned occurred at the level of the width of the molars' buccal cusps (1.05 mm). The greater expansion was verified at the level

of the first interpremolar width (2.7 mm) [24]. Also in 2023, D'Antò *et al.* evaluated the predictability of dentoalveolar expansion with the clear aligners. According to their results, the lower arch had an overall accuracy of 64%, while the upper arch had an accuracy of 67%. As in the aforementioned study, the cusps of the maxillary and mandibular first premolars experienced the greatest expansion (2.42 mm and 1.72 mm, respectively) [23].

Some authors identified an observed trend in the maxillary arch, showing that the accuracy of the virtual tool decreases when moving from anterior to posterior. This phenomenon was referred to as a “drawbridge expansion model” by Lione *et al.* and may be mainly due to differences in cortical bone thickness, loss of aligner fit in the posterior sections during the displacement of the anterior teeth, the higher occlusal load, the greater resistance of soft tissue from the cheeks in the posterior region, and the greater resistance of the multirooted tooth to orthodontic movement. Another possibility is that the mechanical behavior of the aligners' distal part is similar to the one that happens with conventional fixed appliances. The force exerted by an arch wire's end decreases as interbracket distance and wire flexibility rise: consequently, lower forces might be discharged, leading to less accurate movement [16,17,19,20,23].

Most studies have found that, despite body movement, the increase in transverse diameters is primarily caused by crown tipping, which requires root displacement. This finding has an important significance in guiding the application of Invisalign® aligners [17,19,20,24].

Thus, in order to increase the efficiency of bodily expansion, Zhou *et al.* suggested introducing negative crown torque into ClinCheck® to improve physical translation movement rather than tipping. Although Lione *et al.* included this torque in their study, they still noticed buccal tipping of all teeth. Therefore, it was proposed that the magnitude of expansion for each aligner should be reduced, and compensating torque should be added. Root displacement is still regarded as unpredictable, despite the fact that clinicians can utilize aids such as attachments to augment system rigidity and counterweight moments to manage it [17,20].

Riede *et al.*, as well as Zhou *et al.*, found a negative relationship between the amounts of planned expansion and bodily expansion: the more movement planned, the less predictable it was [18,20]. In contrast, Houle *et al.* and Lione *et al.* argue that predictability will not necessarily improve if the rate of expansion is reduced. It could be worthwhile to reevaluate this in the near future, as knowing the limitations of the device helps to reduce errors [17,19].

More studies are necessary since the Invisalign® system is constantly evolving in terms of software, materials, and auxiliary devices. Castroflorio *et al.* stated that certain biomechanical parameters, such as the geometry of the attachments and auxiliaries used during treatment, were a limiting factor of the predictability of orthodontic tooth movement. They also emphasized the significance of the professional's experience with the Invisalign® technique in obtaining good treatment outcomes [16]. With this, we can justify the various divergences between authors. Additionally, because of variations in clinical protocols, treatment plans, sample sizes, data collection and processing methodologies, among other factors, it is very challenging to draw absolute comparisons between the current study and previous ones and even between several previously conducted clinical studies.

Clinical relevance

According to reports, a significant proportion of patients using Invisalign® aligners (70% to 80%) would require an intermediate correction or additional aligners (AA), which suggests that the accuracy of ClinCheck® software treatment planning is low. This can be due to the practitioner's inexperience with the technique, the software, or a lack of patient compliance [7]. Planning overexpansion in the software could be an alternative that would help to reduce the need for intermediate AA. The transverse relationship of the teeth can be improved with the help of aids such as crossbite elastics [30]. Studies on the efficacy of Invisalign® clear aligners would help to improve the treatment outcome, reducing treatment time. Knowing how effective this system is in creating dentoalveolar expansion allows professionals to offer more aesthetic solutions to patients seeking orthodontic treatment.

Limitations of the study

This study confirmed that there are differences between authors' assessments of various dental groups. This constitutes a major limitation in the comparison of results. In order to guard against this, the standardization criteria utilized in the candidate recruitment and selection process, as well as the process of measuring the interdental widths, must be repeatable, objective, and described in future studies [30].

Conclusions

The results of the study allowed us to conclude that: (1) clear aligners are an effective tool in producing expansion movement; (2) predictability was reasonable for arch expansion; (3) the expansion follows a decreasing gradient moving posteriorly, mainly in the maxilla; (4) the expansion obtained is mostly dentoalveolar and achieved by crown tipping.

Thus, we can conclude that the Invisalign® system is effective in producing the expansion movement, since the intended amount of expansion was achieved in the majority of the studies, with expansion being most effective in the premolar area. The authors draw conflicting conclusions on predictability, but overall

it was reasonable. Further investigation is needed to better understand the mechanisms that influence the expansion movement and its long-term stability.

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Author Contributions

ASR planned the overall design and conception of the work, acquired, analyzed and interpreted the data, and drafted the present manuscript. MG substantially revised the manuscript, ACO revised the manuscript, TP conceived and designed the work, drafted and substantially revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

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