

Invisalign ClinCheck can predict open gingival embrasures in adult extraction cases: a pilot study

Feng Guo^a; Chenxu Wang^a; Lei Han^b; Houxuan Li^c; Lang Lei^d; Li Mei^e

ABSTRACT

Objectives: To evaluate the accuracy of Invisalign ClinCheck in predicting open gingival embrasures (OGEs) and to identify predictors of OGEs in adult extraction cases.

Materials and Methods: Fifty-seven adult patients treated with Invisalign and four first premolar extractions were included in this retrospective study. OGEs were measured in maxillary and mandibular anterior regions using posttreatment intraoral photographs (actual OGEs) and the final step from the first treatment plan in ClinCheck (predicted OGEs). Prediction performance indicators including precision, sensitivity, specificity, false positive rate, false negative rate, and accuracy were evaluated at each tooth site. Predictors of OGEs (age, crowding, crown morphology, tooth movement, tooth site, treatment duration, and attachment design) were analyzed using binary logistic regression.

Results: Incidence of actual OGEs was like that of the ClinCheck predicted OGEs in the maxillary and mandibular anterior regions. The predictability of ClinCheck was satisfactory in both the maxilla and mandible, with accuracy rates of 94.0% and 86.0%, respectively. The most accurate prediction was for the maxillary central incisors, achieving a precision of 100% and an accuracy of 96.6%. The most significant predictors of OGEs included patient age at initial consultation, anterior crowding, tooth crown morphology, and type of tooth movement.

Conclusions: Invisalign ClinCheck predicted OGEs in adult patients treated with four premolar extractions. The accuracy of the prediction was satisfactory, 94% in the maxilla and 86% in the mandible, demonstrating great potential for clinical application. (*Angle Orthod.* 2025;95:389–396.)

KEY WORDS: Open gingival embrasures; Clear aligners; Invisalign; Extraction; ClinCheck

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INTRODUCTION

Open gingival embrasures (OGEs) are a common complication in adult patients undergoing orthodontic treatment. They are usually between the anterior teeth because of insufficient filling of interdental space by gingival papilla. They can cause food impaction and plaque retention, negatively affecting dental esthetics and periodontal health. About one-third of patients who have undergone orthodontic treatment develop OGEs. The incidence of OGEs between incisors has been reported to be 35% to 38% during clear aligner therapy (CAT) and 18% to 24% during fixed appliance treatment¹; however, these data were based on nonextraction cases.

The incidence of OGEs in extraction cases (four first premolar extractions) treated with fixed appliances was found to be as high as 53.3%.² The incidence of OGEs in CAT extraction cases, however, remains unclear. With advancement in CAT technology and the increasing demand for esthetics, CAT is increasingly used for more complex cases, including those requiring extraction.

While CAT offers several advantages over traditional fixed appliances,³⁻⁵ including improved esthetics, ease of maintain hygiene, greater acceptance by adult patients, and better periodontal health, drawbacks also exist. CAT could generate excessive initial stress, potentially leading to inferior periodontal condition.⁶ Additionally, clear aligners tend to encroach on the interdental space and hinder natural remodeling of the gingival papilla, potentially increasing the risk of OGEs.¹

Risk factors for OGEs often include patient age, severity of crowding, crown morphology, type of tooth movement, and duration of treatment.² It has been reported that approximately two-thirds of adult patients with crowded anterior teeth develop OGEs following orthodontic treatment.⁷ In addition, the design of attachments used in treatment has also been associated with OGEs.⁸ Authors of a recent in vivo study attempted to predict OGEs by evaluating alveolar bone height; however, the alveolar crest could not be assessed for crowded teeth, and the unpredictable nature of alveolar bone recession during orthodontic treatment added further complication.⁹ Accurate prediction of OGEs is of great clinical importance.

In 1997, Align Technology (Santa Clara, Calif) introduced the Invisalign system, a CAT technique that pioneered the clear aligner market. ClinCheck provides a visualization of the Invisalign treatment plan, tooth movement, and treatment outcomes.^{10,11} OGEs can sometimes be detected visually in ClinCheck, especially in the crowded cases treated with extractions; however, the reliability and accuracy of ClinCheck in predicting OGEs remain unclear.

The aims of this study were (1) to assess the predictability of OGEs with ClinCheck in adult patients treated with Invisalign and extraction of four first premolars and (2) to investigate the related predictors of OGE. The null hypothesis was that Invisalign ClinCheck could not predict OGEs in adult patients treated with four premolar extractions.

MATERIALS AND METHODS

Participants

This retrospective study was approved by the Ethics Committee (NJSH-2023NL-036). Participants were recruited from the Orthodontic Department, Nanjing Stomatological Hospital, Nanjing University, between December 2015 and June 2022. All participants signed informed consent.

Sample size calculation was performed using PASS software (version 21.0.3; NCSS, LLC, Kaysville, Utah, USA).

Sensitivity, the proportion of actual positives correctly predicted, influences the sample size needed to ensure adequate statistical power. An estimation of

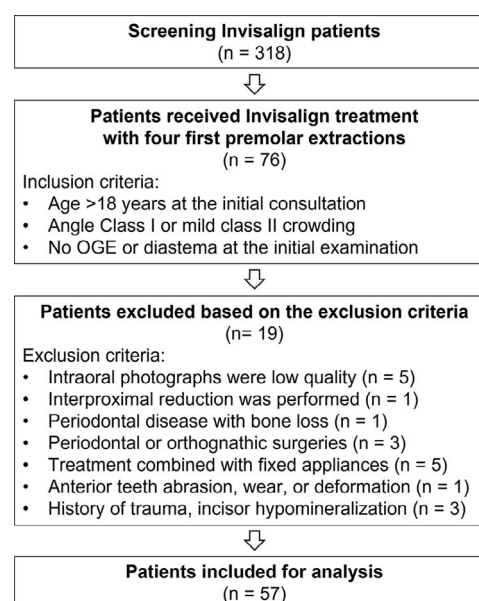


Figure 1. Flow diagram of the participant screening process.

prospective sensitivity was carried out in a pilot study involving 20 participants, based on the predictability of OGEs between upper central incisors, and the result showed a sensitivity of 86%. Sample size calculation indicated that a minimum of 55 participants was required to achieve a confidence level of 95% and a margin of error of 10%.

The study flow chart is shown in Figure 1. Inclusion criteria were (1) age over 18 years at the initial consultation; (2) angle Class I or mild Class II crowding malocclusion; (3) no OGEs or midline diastema at the initial examination; (4) completion of dual arch Invisalign treatment (Align Technology) with four first premolar extractions and wearing each aligner 22 h/d for 7 days. Exclusion criteria were (1) intraoral photographs of poor quality, with food debris or other obstruction in the proximal spaces of anterior teeth; (2) interproximal enamel reduction performed during treatment; (3) periodontal disease with bone loss exceeding one-quarter of the root and/or attachment loss over 2 mm; (4) requiring periodontal surgery, orthognathic surgery, or periodontally accelerated osteogenic orthodontics; (5) treatment combined with fixed appliances; (6) anterior tooth abrasion, wear, or deformation; and (7) history of orthodontic treatment, dental trauma, or incisor hypomineralization.

Measurement of OGEs

OGEs were measured in the maxillary and mandibular anterior regions using the intraoral photographs immediately after the removal of the aligners (actual OGEs) and the final step from the first treatment plan in ClinCheck (predicted OGEs). All measurements

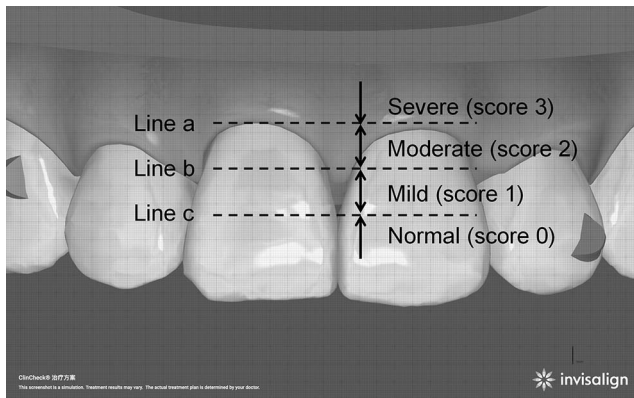


Figure 2. Classification of the severity of OGEs. Line (a) represents a tangent line passing through the highest gingival curvature of the crown; line (c) is parallel to line (a) and passes through the most cervical contact point; line (b) bisects the distance between lines (a) and (c).

were independently performed by three experienced specialists (two orthodontists and one periodontist), with any measurements that did not reach consensus being excluded after discussion.

To assess the predictability of ClinCheck on severity of OGEs, each site was also scored based on the Jemt index¹² (Figure 2). The severity of OGEs was scored by the position of the tip of the gingival papilla.

Predictors of OGEs

Predictors of OGEs were evaluated, including patient age, anterior crowding, crown morphology, treatment duration, type of tooth movement, and attachment design. The severity of anterior crowding between canines, which was defined as the difference between required length (the sum of maximum crown widths [CWs] of canines and incisors) and available length (arch length between mesial surface of bilateral first premolars), was measured on the pretreatment digital models.¹³

Crown morphology of the anterior teeth was determined by the ratio of CW and crown length (CL) based on the literature (Figure 3).¹⁴ Authors of a previous study confirmed the accuracy of ClinCheck for measuring tooth width.¹⁵ To minimize the measurement error caused by crowding, crown morphology was assessed using the final step of the treatment plan in ClinCheck.

Type of tooth movement of the anterior teeth was analyzed on the pretreatment and posttreatment lateral cephalograms using Image J (version 1.54g; National Institutes of Health, Bethesda, USA). The inclination and vertical and horizontal positions of maxillary and mandibular incisors were measured according to a previous study (Figure 4).¹⁶ Changes of these three parameters after orthodontic treatment were recorded as tipping, vertical, and horizontal movement.

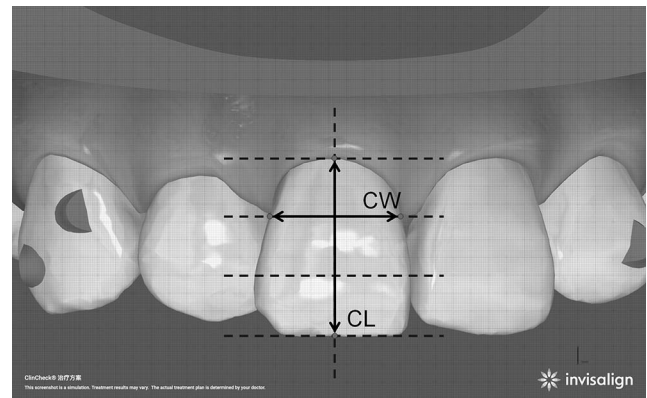


Figure 3. Measurement of tooth crown morphology. The crown was equally divided into three parts in the crown-root direction. The crown length (CL) refers to the distance from the gingival curvature to the middle of the incisal edge (or cusps of canines). The crown width (CW) refers to the width at the junction between the cervical 1/3 and middle 1/3 of the crown.

Buccal inclination, extrusion, and labial movement were denoted as positive values.

Invisalign attachment designs, including the location and number of attachments on the anterior teeth, were recorded for each participant according to the initial series of treatment in ClinCheck.

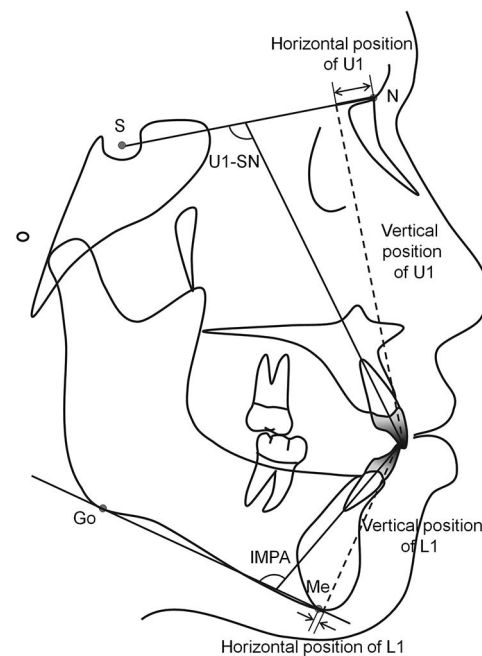


Figure 4. Measurement of tooth movement. U1-SN and IMPA represent inclination of maxillary and mandibular incisors, respectively. The displacement of the upper and lower incisors, both vertically and horizontally, was quantified parallel to the SN line and perpendicular to the mandibular plane, respectively.

Table 1. Key Performance Indicators for Evaluating the Predictability of Open Gingival Embrasures (OGEs) with ClinCheck

Performance Indicator	Definition	Formula
True positive (TP)	Cases where ClinCheck correctly predicts the occurrence of OGEs	—
False positive (FP)	Cases where ClinCheck incorrectly predicts the occurrence of OGEs	—
True negative (TN)	Cases where ClinCheck correctly predicts the absence of OGEs	—
False negative (FN)	Cases where ClinCheck incorrectly predicts the absence of OGEs	—
Precision	The proportion of actual positives among cases predicted as positive	TP/(TP + FP)
Sensitivity	The proportion of actual positives that were predicted as positives	TP/(TP + FN)
Specificity	The proportion of actual negatives that were predicted as negatives	TN/(TN + FP)
False positive rate	The proportion of actual negatives that were predicted as positives	FP/(FP + TN)
False negative rate	The proportion of actual positives that were predicted as negatives	FN/(FN + TP)
Accuracy	The proportion of correctly predicted ones out of all cases	(TP + TN)/(TP + TN + FP + FN)

Interrater and Intrarater Reliability

Analysis of a random selection of 20 participants was conducted as a pilot study and to determine sample size. The same measurements were repeated after a 2-week interval. Interclass and intraclass correlation coefficients were calculated to be 0.93 and 0.98, respectively, indicating excellent reliability of the measurements.

Statistical Analysis

Statistical analyses were performed with SPSS (version 23; IBM, Armonk, NY) and R (version 4.1.1; R Foundation for Statistical Computing, Vienna, Austria). A χ^2 test was used to compare between predicted OGEs (ClinCheck) and actual OGEs (posttreatment photographs). Confusion matrix heatmaps were plotted to explore the discrepancy between predicted OGEs and actual OGEs at each site. Predictability of OGEs with ClinCheck was quantified through key performance indicators, including precision (the proportion of actual positives among cases predicted as positive), sensitivity, specificity, false positive rate (FPR), false negative rate (FNR), and accuracy (Table 1). The predictors of

OGEs including patient age, anterior crowding, crown morphology, type of tooth movement, tooth site, treatment duration, and attachment design were then analyzed using binary logistic regression, with the occurrence of OGEs as the outcome variable. A P value $< .05$ was considered statistically significant.

RESULTS

A total of 57 adult patients (7 male, 50 female; mean age = 25.5 ± 4.8 years; mean treatment duration = 30.2 ± 3.8 months) were included in the study. The actual incidence of mild OGEs (maxilla = $17.2\% \pm 1.5\%$; mandible = $45.3\% \pm 1.9\%$) and the ClinCheck predicted incidence of mild OGEs (maxilla = $16.5\% \pm 2.0\%$; mandible = $53.3\% \pm 4.4\%$) was similar ($P > .24$ for all; Table 2; Figures 5 and 6). The actual incidence of moderate OGEs (maxilla = $0.4\% \pm 0.1\%$; mandible = $7.1\% \pm 4.3\%$) was also like the ClinCheck predicted incidence of moderate OGEs (maxilla = $0.4\% \pm 0.1\%$; mandible = $6.7\% \pm 5.9\%$; $P > .87$ for all). No severe OGEs were observed either in the maxilla or the mandible (Table 2). Discrepancy of the OGE scores was evaluated through confusion matrix heatmaps, in which all sites

Table 2. Incidence of Open Gingival Embrasures (OGEs) Was Similar Between the Actual OGEs (Measured in Posttreatment Photographs) and the Predicted OGEs (Measured in ClinCheck; $P > .24$ for All)

Tooth Site	Actual OGEs, No. (%)				Predicted OGEs, No. (%)				P Value
	Normal	Mild	Moderate	Severe	Normal	Mild	Moderate	Severe	
Maxilla									
13-12	47 (82.5)	10 (17.5)	0	0	46 (80.7)	11 (19.3)	0	0	0.81
12-11	47 (82.5)	9 (15.8)	1 (1.8)	0	47 (82.5)	10 (17.5)	0	0	1.00
11-21	47 (82.5)	10 (17.5)	0	0	49 (86.0)	8 (14.0)	0	0	0.61
21-22	46 (80.7)	11 (19.3)	0	0	48 (84.2)	9 (15.8)	0	0	0.62
22-23	48 (84.2)	9 (15.8)	0	0	47 (82.5)	9 (15.8)	1 (1.8)	0	0.80
Total	235 (82.5)	49 (17.2)	1 (0.4)	0	237 (83.2)	47 (16.5)	1 (0.4)	0	0.82
Mandible									
43-42	29 (50.9)	26 (45.6)	2 (3.5)	0	27 (47.4)	28 (49.1)	2 (3.5)	0	0.71
42-41	25 (43.9)	27 (47.4)	5 (8.8)	0	19 (33.3)	33 (57.9)	5 (8.8)	0	0.25
41-31	24 (42.1)	26 (45.6)	7 (12.3)	0	18 (31.6)	30 (52.6)	9 (15.8)	0	0.24
31-32	26 (45.6)	26 (45.6)	5 (8.8)	0	21 (36.8)	33 (57.9)	3 (5.3)	0	0.34
32-33	32 (56.1)	24 (42.1)	1 (1.8)	0	29 (50.9)	28 (49.1)	0	0	0.57
Total	136 (47.7)	129 (45.3)	20 (7.1)	0	114 (40.0)	152 (53.3)	19 (6.7)	0	0.70

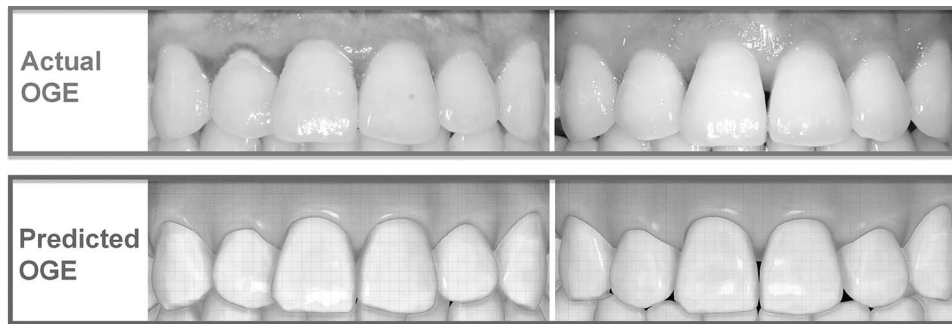


Figure 5. Example of the actual and ClinCheck predicted OGEs.

predicted with Score 2 indeed developed mild to moderate OGEs in the posttreatment photographs (Figure 7).

The performance of prediction (precision, sensitivity, specificity, FPR, FNR, and accuracy) varied across different tooth sites in the maxilla and mandible (Table 3). In general, the predictability was satisfactory in both maxilla and mandible, with accuracy rates of 94.0% and 86.0%, respectively. The most accurate prediction was for maxillary central incisors, achieving precision of 100% and accuracy of 96.6%. Precision and sensitivity in the maxilla (81.3% and 79.6%) were slightly lower than those in the mandible (81.9% and 94.0%); specificity and accuracy in the maxilla (96.2% and 94.0%) were significantly greater than those in the mandible (77.2% and 86.0%). Prediction in the maxilla showed a low FPR (3.8%) and a high FNR (20.4%). Prediction in the mandible exhibited a high FPR (22.8%) and a low FNR (6.0%), indicating that ClinCheck may underestimate the incidence of OGEs in the maxilla and overestimate it in the mandible.

Most participants exhibited mild crowding in the maxilla (70.2%), while fewer exhibited crowding in the mandible (59.6%). Attachment designs primarily targeted the canines in the anterior region (Table 4).

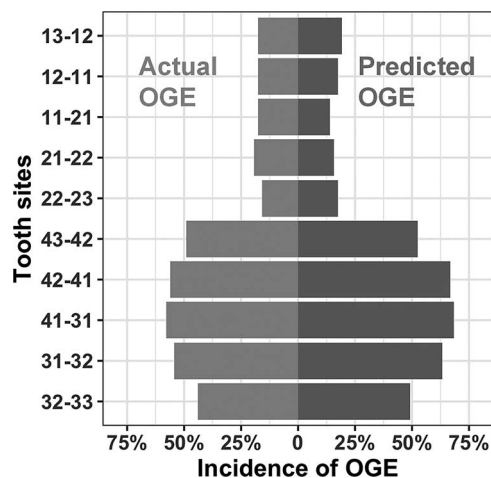


Figure 6. Pyramid chart of the actual and ClinCheck predicted OGEs.

Binary logistic regression analyses suggested that the most significant predictors of OGEs included patient age at initial consultation, anterior crowding, tooth crown morphology, and type of tooth movement (Table 5). Tooth site, treatment duration, and attachment design did not show significance as predictors of OGEs in ClinCheck.

DISCUSSION

OGEs are a common complication in adult patients with crowded anterior teeth following orthodontic treatment. We were the first to explore the predictability of OGEs using Invisalign ClinCheck. Results indicated that ClinCheck could predict OGEs in the anterior teeth with satisfactory accuracy of 94% in the maxilla and 86% in the mandible. Key predictors of OGEs in adult extraction cases included patient age at initial consultation, degree of anterior crowding, tooth crown morphology, and type of tooth movement.

Accurately predicting OGEs is critically important in clinical practice. For instance, during treatment planning, clearly communicating and predicting risk for OGE using ClinCheck can help patients better understand expected treatment outcomes, thus reducing potential disappointment and dissatisfaction caused by OGEs. In addition, predicting OGEs could also enable planning of interproximal enamel reduction or axial tooth movement to minimize the negative impact of OGEs on dental esthetics.^{8,17} Early prediction of OGEs often highlights the need for a multidisciplinary approach involving orthodontics and periodontology, such as bone grafting, gingival surgery, and periodontal maintenance therapy.¹⁸ Minimizing OGEs during orthodontic treatment can reduce the challenge for subsequent restorative treatment since OGEs often increase the complexity of prosthodontic procedures.¹⁹

Patient factors can affect the development of OGEs. For example, patient age was found to significantly impact the development of OGEs, as observed in both the present study and previous research.^{13,20} In addition, alveolar bone undergoes dynamic remodeling and

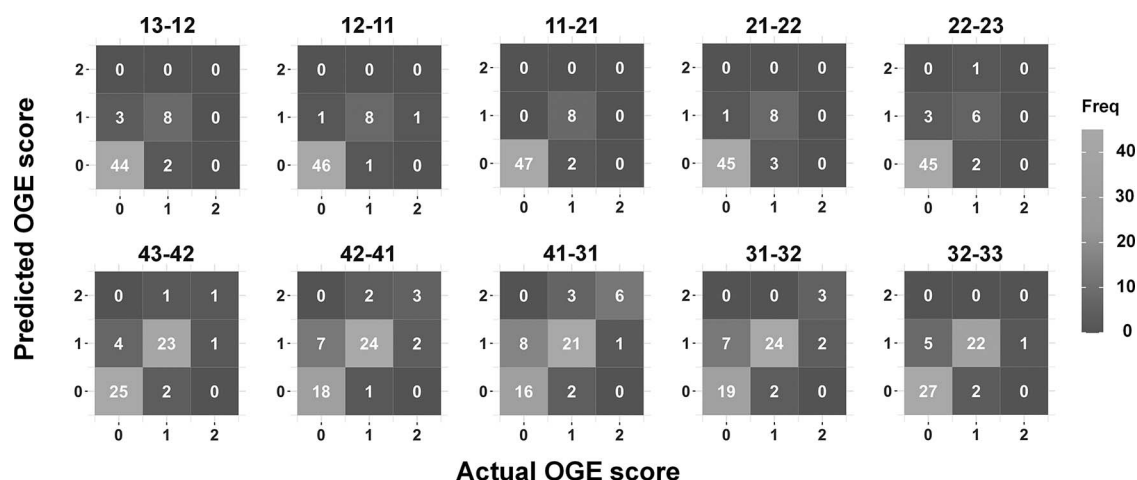


Figure 7. Confusion matrix of the actual and ClinCheck predicted OGE scores at different tooth sites.

exhibits a decrease in elasticity and flexibility with age.²¹ The potential for regeneration also declines with age. Researchers have shown that the gingival papilla decreases by 0.012 mm/y as individuals grow older.²⁰ It was also observed that adults who had extraction of four premolars tended to experience greater alveolar bone loss than adolescents, which may potentially increase the risk of OGEs.²² The severity of anterior crowding was also found to be associated with an increased risk of OGEs in the current study, which was consistent with previous findings.^{2,7} Another influencing factor of OGEs is periodontal phenotype. Authors of a previous study reported that individuals with thick and wide gingiva had better periodontal health than those with thin and narrow gingiva.²³ Patients with thick-scalloped and thick-flat periodontal biotypes may at a lower risk of developing OGEs.

Treatment factors can also influence the development of OGEs. Although clear aligners are convenient for oral cleaning due to being removable, aligners may

promote accumulation of plaque around the marginal gingiva.²⁴ Clear aligners tend to encroach upon the interdental space and hinder the natural remodeling of gingival papilla, potentially increasing the risk of OGEs. Authors of future studies could consider including other periodontal parameters, such as gingival index and bleeding on probing, to better predict OGEs. Tooth extraction may elevate the risk of OGEs since every 1.0 mm of anterior tooth retraction can lead to an approximately 0.4 mm reduction in palatal alveolar bone height during CAT treatment.²⁵ Researchers have suggested that, when the distance between the interproximal contact point and the bone crest exceeded 5 mm, the incidence of OGEs increased to over 2%.²⁶ Additionally, no significant correlation was observed between OGEs and attachment design, possibly due to the lack of detailed categorization of attachment shapes.⁸

ClinCheck demonstrated a satisfactory ability to predict OGEs in both the maxilla and mandible in this

Table 3. Performance of Prediction (%) of ClinCheck at Different Tooth Sites in the Maxilla and Mandible^a

Tooth sites	Precision	Sensitivity	Specificity	FPR	FNR	Accuracy
Maxilla						
13-12	72.7	80.0	93.6	6.4	20.0	91.2
12-11	90.0	90.0	97.9	2.1	10.0	96.4
11-21	100.0	80.0	100.0	0.0	20.0	96.6
21-22	88.9	72.7	97.8	2.2	27.3	96.4
22-23	60.0	75.0	91.8	8.2	25.0	89.5
Average	81.3	79.6	96.2	3.8	20.4	94.0
Mandible						
43-42	86.7	92.9	86.2	13.8	7.1	89.5
42-41	81.6	96.9	72.0	28.0	3.1	86.0
41-31	79.5	93.9	66.7	33.3	6.1	82.5
31-32	80.6	93.5	73.1	26.9	6.5	84.2
32-33	82.1	92.0	84.4	15.6	8.0	87.7
Average	81.9	94.0	77.2	22.8	6.0	86.0

^a FPR indicates false positive rate; FNR, false negative rate.

Table 4. Measurement of Risk Factors for Maxilla and Mandible

	Maxilla		Mandible	
Anterior crowding (mm)	2.02 (0.62, 4.45)		3.79 ± 2.63	
0–4 mm, No. (%)	40 (70.18)		34 (59.65)	
4–8 mm, No. (%)	12 (21.05)		20 (35.09)	
>8 mm, No. (%)	5 (8.77)		3 (5.26)	
Type of tooth movement				
Tipping movement (°)	–15.22 ± 6.98		–10.81 ± 5.53	
Vertical movement (mm)	–0.33 ± 1.24		–1.83 ± 1.40	
Horizontal movement (mm)	–5.88 ± 2.45		–4.09 ± 1.89	
Crown morphology (%)	13 76.79 ± 10.10	43 68.39 ± 9.07		
	12 80.66 ± 12.99	42 64.12 ± 8.27		
	11 80.05 ± 11.42	41 58.16 ± 8.14		
	21 79.64 ± 11.19	31 56.92 ± 7.79		
	22 79.62 ± 13.42	32 62.94 ± 8.17		
	23 76.28 ± 9.96	33 69.09 ± 8.48		
Attachments, No. (%)	13 57 (100.00)	43 57 (100.00)		
	12 18 (31.58)	42 2 (3.51)		
	11 2 (3.51)	41 1 (1.75)		
	21 3 (5.26)	31 1 (1.75)		
	22 30 (52.63)	32 2 (3.51)		
	23 56 (98.25)	33 56 (98.25)		
Treatment duration (mo)	30.24 ± 3.80			

study; however, it may underestimate the incidence of OGEs in the maxilla while overestimating it in the mandible. This discrepancy could be attributed to generally lower periodontal health of the mandibular anterior teeth than the maxillary anterior teeth.²⁷ Additionally, the narrower crowns of mandibular anterior teeth increase the risk of gingivitis relative to the wider maxillary anterior teeth.²⁸ In addition, proximity of the sublingual gland duct orifice in the mandible may contribute to increased accumulation of plaque and calculus, further exacerbating gingivitis.

Limitations existed in the current study. With 57 participants and numerous variables and inferential tests, in this study, we may have had an increased type I error rate. Typically, a sample size 5 to 10 times the number of variables is needed for robust results. Additionally, logistic regression may not have yielded reliable outcomes. Due to its retrospective nature, in this study, we did not assess gingival biotype, probing depth, or gingival index. Additional series needed more than 70% patients receiving Invisalign treatment.²⁹ ClinCheck predicted OGEs were based on the final step of the initial treatment plan, which may not have accounted for changes occurring during refinements, potentially leading to discrepancies. Long-term follow-up would be beneficial for the study, as oral hygiene and OGEs may undergo slight changes over time after debonding. The test/validation procedures, including participant selection, were missing from the experimental design. As a result, this study functioned more as a pilot or exploratory analysis, requiring more extensive data collection and further investigation. Future research could be enhanced with larger sample sizes and volumetric analysis of OGEs. Using artificial intelligence to develop more accurate and comprehensive models for predicting OGEs and validating predictive models with independent datasets could help ensure consistent performance across various malocclusions and patient conditions.

CONCLUSIONS

- Invisalign ClinCheck predicted OGEs in adult patients treated with four premolar extractions.
- Accuracy of the prediction was satisfactory, at 94% in the maxilla and 86% in the mandible, demonstrating great potential for clinical application.

Table 5. Predictors of OGEs Analyzed With Binary Logistic Regression^a

Variables	Maxilla			Mandible		
	OR	95% CI	P Value	OR	95% CI	P Value
Patient's age (y)	1.16	1.08, 1.26	< .001***	1.08	1.01, 1.15	.021*
Anterior crowding (mm)	1.41	1.20, 1.65	< .001***	1.40	1.23, 1.60	< .001***
Crown morphology						
Mesial tooth shape (W/L)	0.93	0.88, 0.97	.002**	0.99	0.95, 1.03	.457
Distal tooth shape (W/L)	0.97	0.93, 1.02	.249	0.96	0.92, 1.00	.031*
Type of tooth movement						
Tipping movement (°)	1.08	0.98, 1.19	.147	0.92	0.87, 0.98	.013*
Vertical movement (mm)	1.47	1.08, 2.01	.016*	0.95	0.78, 1.16	.625
Horizontal movement (mm)	0.66	0.51, 0.85	.001**	1.00	0.84, 1.19	.989
Tooth sites						
3-2 vs 1-1	0.40	0.10, 1.64	.202	1.79	0.45, 7.10	.411
2-1 vs 1-1	0.88	0.31, 2.50	.816	1.14	0.53, 2.47	.734
Treatment duration (mo)	1.04	0.94, 1.15	.445	1.05	0.98, 1.13	.191

^a OGE indicates open gingival embrasures; CI, confidence interval; and OR, odds ratio; * $P < .05$ was considered significant, shown in bold; ** $P < .01$; *** $P < .001$.

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