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Tooth loss in patients undergoing long-term maintenance at a private dental clinic in Japan: A retrospective study

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Abstract

Background: Tooth loss is generally considered the final outcome of oral disease. This retrospective study was performed to identify risk factors for tooth loss in patients undergoing long-term maintenance therapy.

Methods: We surveyed 1145 adult patients who underwent maintenance therapy for ≥5 years after they had undergone active treatment from January 2015 to December 2016 and established a baseline status. The study variables were patient compliance, sex, number of teeth lost, cause of tooth loss, age at start of maintenance, number of remaining teeth at start of maintenance, duration of maintenance, smoking status, use of salivary secretion inhibitors, and diabetes mellitus. Additionally, 57 patients who did not undergo maintenance therapy were surveyed to examine and compare the effects of maintenance therapy. Statistical analyses were performed to assess the correlation of each variable with tooth loss.

Results: The average number of teeth lost under maintenance therapy was 0.07/year. Significantly fewer teeth were lost in the maintenance than non-maintenance group. Most of the teeth lost were non-vital teeth, and the most common cause of tooth loss was tooth fracture. Patient age, number of remaining teeth at start of maintenance, use of salivary secretion inhibitors, and diabetes mellitus were related to tooth loss.

Conclusions: To the best of our knowledge, this is first large-scale study of tooth loss in patients undergoing long-term maintenance therapy within a general dental clinic. Our findings demonstrate that starting maintenance therapy when patients are younger and possess more teeth may prevent future tooth loss.

Key words: Compliance, maintenance, patient age, remaining teeth, tooth loss, private

dental clinic

Background

Dental studies have shown that dental caries and periodontal disease are the most common pathological conditions affecting the oral cavity. More specifically, several studies have shown that the most common cause of tooth loss (TL) is dental caries, followed by periodontal disease.[1-5] Thus, control of dental caries and periodontal disease is critical for prevention of TL.

A major etiological factor underlying the pathogenesis of dental caries and periodontal disease is the formation of a biofilm on the tooth surface. Removal of dental plaque is critical in preventing dental caries and periodontal disease. Axelsson and Lindhe[6] and Axelsson et al.[7] found that regular maintenance therapy reduced the incidence of dental caries and periodontal disease, significantly lowering the risk of TL. Other studies have also shown decreased TL with regular maintenance therapy.[8-29] Axelsson et al.[7] reported that the most frequent cause of TL in patients undergoing maintenance therapy was root fractures. Other studies showed that the most frequent cause of TL, even with maintenance therapy, was periodontal disease.[30,31] Various factors, such as age, smoking, diabetes mellitus (DM), and others, are also related to TL, and the risk of TL increases with the presence of additional non-vital teeth (those that have undergone root canal treatment).[30,32-34] Despite reports that various factors affect TL, many of these studies were conducted under special circumstances, such as at a university hospital or within a specialist clinic, where patient demographics were limited. Additionally, few studies on the risk factors for TL have utilized large sample sizes. Furthermore, differences in patients' immunological and genetic backgrounds, cultural factors, and socioeconomic features may affect the risk of TL.

Therefore, to verify these prior findings derived from specific or small patient

70 populations, we conducted a retrospective analysis in a private general clinic at a primary
71 medical institution in Japan. More than 11,000 local residents utilize this clinic for their
72 family dentistry needs. We investigated the effectiveness of long-term maintenance on TL
73 as well as the various causes of TL (both with and without maintenance) to identify
74 optimal prevention strategies. Using this approach, we aimed to determine the most
75 convenient and effective methods for preventing TL in this population and thus improve
76 the clinical dental care of our patients.
77
78 **Methods**
79 The present survey was conducted at a private dental clinic (Kawahara Dental Clinic) in
80 Mima, Tokushima, Japan. The population of Mima is approximately 30,000. This dental
81 clinic was selected because it is a general dental clinic and not a university hospital or
82 specialist clinic. Dental examinations were performed and associated records were
83 obtained by one of the authors (H.K., a general dentist) with the assistance of several
84 trained dental hygienists.
85 Before active treatment, all patients received education regarding oral disease, dental
86 caries, and strategies to control the risk of periodontal disease. They also underwent
87 individual risk assessments. Cariogram computer software (D Bratthall, Malmo,
88 Sweden)[35] was used for caries risk assessment, and the Oral Health Information Suite
89 (PreViser Corp., Concord, NH, USA)[36] was used for periodontal disease risk assessment.
90 Following active treatment, including restorative, endodontic, and periodontal therapy,
91 the patients underwent reevaluation of clinical parameters (probing depth, bleeding on
92 probing, plaque index, and number of teeth present). The periodontal therapy involved
93 oral hygiene enhancement, scaling and root planing, and/or surgical therapy according to

94 the individual patients' needs. The reassessment of clinical parameters was used as the
95 baseline (BL) for each patient. The duration of maintenance was measured from the BL.
96 All patients were required to meet the following five criteria established by Miyamoto et
97 al.[37] before the end of active treatment and entry into periodontal maintenance therapy:
98 1) <10% of sites with bleeding on probing, 2) an overall plaque score of <15%, 3) <10%
99 of sites with a probing depth of ≥4 mm, 4) no defective restorations, and 5) no active
100 dental caries. The maintenance period and the contents of maintenance were determined
101 with reference to the study by Miyamoto et al.[37] Evaluation of the maintenance interval
102 was conducted by a dental hygienist at 3 or 6 months and was based on the patient's
103 periodontal tissue condition. Patients with a periodontal pocket of ≥4 mm at the time of
104 the reevaluation visit were assigned to a 3-month maintenance regimen. The maintenance
105 interval was not changed for any patient during the study.
106 After reevaluation, all patients underwent maintenance, which consisted of a full-mouth
107 clinical examination, supragingival scaling and polishing, and subgingival debridement
108 using Gracey curettes, an ultrasonic scaler, rubber cups, and abrasive paste. Oral hygiene
109 instructions and application of fluoride were provided according to the patients'
110 individual needs. When a need for treatment was determined during maintenance, the
111 dentist (H.K.) provided the appropriate treatment (tooth extraction, restoration, prosthetic
112 rehabilitation, and/or endodontic treatment).
113 The cause of any TL was determined by the dentist (H.K.) during extraction. These
114 causes included dental caries, periodontal disease, root fracture, and others (e.g., an apical
115 lesion, trauma, tooth transposition, or tooth extraction for convenience). The extraction
116 of wisdom and deciduous teeth was excluded from the analysis. Tooth extraction for
117 which the patient's consent was not obtained before the BL was also excluded. Patient

118 compliance during maintenance was divided into the following two categories according
119 to the classification established by Miyamoto et al.[37] Regular: the following two
120 requirements are met; 1) attending ≥70% of expected maintenance visits, 2) the interval
121 between visits during maintenance does not exceed a maximum of 2 years. Irregular: the
122 criteria for regular compliance have not been met, but continued maintenance visits were
123 carried out.
124
125 *Sample collection*
126 A total of 3907 patients visited the Kawahara Dental Clinic from 2015 to 2016; 2458 of
127 the 3907 patients were adult patients (age of >18 years at the end of 2016). Of the 2458
128 adult patients, 1943 patients (maintenance patients) visited the clinic for maintenance
129 after establishment of their BL. In addition, 106 patients declined continuous maintenance
130 after establishing their BL and visited the clinic only because of problems and not for
131 maintenance (non-maintenance patients). An additional 409 patients were undergoing
132 active treatment.
133 In 1145 of the maintenance patients, ≥5 years had elapsed since their BL was established
134 at the end of 2016 (MP5 group). Similarly, 57 non-maintenance patients were seen for ≥5
135 years after establishing their BL (NMP5 group). Patients who received implant treatment
136 were excluded.
137
138 *Data collection*
139 Data on the following 12 characteristics were collected in the MP5 group: compliance,
140 sex, age at BL, number of remaining teeth (RT) at BL, years from BL, number of teeth
141 lost by various causes (dental caries, periodontal disease, root fracture, others), smoking

status (yes/no) (patients who quit smoking during maintenance were included), use of salivary secretion inhibitors (SSIs) (yes/no) (antidepressants, anxiolytics, diuretics, antihypertensives, antiarrhythmic drugs, and other drugs that inhibit salivary secretion), and DM (yes/no) (whether treatment has been done for DM). The smoking status, use of SSIs, and DM were recorded at BL. We collected data on the following four characteristics in the NMP5 group: years from BL, age at BL, number of RT at BL, and number of teeth lost since BL.

Statistical analyses

All data for the MP5 and NMP5 groups were gathered and organized into a table. We examined significant differences in the presence or absence of TL in the MP5 and NMP5 groups by a logistic regression analysis approach.

Among patients in the MP5 group, the Steel–Dwass test was performed to compare the average age at BL, years from BL, and number of RT at BL according to number of teeth lost. Next, among patients in the MP5 group, logistic regression analysis was performed to determine the relationship between the presence of TL and the following factors: compliance (regular or irregular), sex (male or female), age at BL (≤49, 50–59, or ≥60 years), number of RT at BL (28–24, 23–20, or ≤19 teeth), smoking status (yes or no), use of SSIs (yes or no), and DM (yes or no).

All statistical analyses were performed with JMP version 14 (SAS Institute, Cary, NC, USA), and $P<0.05$ indicated statistical significance. The statistical analysis was not verified by an independent statistician.

Results

166 All data of the patients in the MP5 group (n = 1145) and NMP5 group (n = 57) were
167 selected from patient records obtained from the general dentist (H.K.) at Kawahara Dental
168 Clinic. The average number of teeth lost per year was 0.07 in the MP5 group and 0.22 in
169 the NMP5 group (Table 1). The average number of teeth lost per year was approximately
170 three times as high in the NMP5 group as in the MP5 group. Logistic regression analyses
171 revealed a significant difference in number of teeth lost since BL between the MP5 and
172 NMP5 groups (Table 1).

173

174 **Table 1.** Comparison of MP5 and NMP5 groups

	Sample size	AGE (age)	YEAE (years)	RT (teeth number)	TL (teeth number)	Average number of TL/year (teeth number)	Logistic regression analyses	
	n	Mean ±SD	Mean ±SD	Mean ±SD	n	n	Odds ratio (95% CI)	P-value
MP5	1145	46.8±15.9	9.0±2.5	23.9±5.5	748	0.07	reference	
NMP5	57	46.9±21.5	8.4±2.5	21.7±7.4	107	0.22	2.41 (1.41–4.11)	0.0013

175 Abbreviations: SD, standard deviation; AGE, age at baseline; RT, remaining teeth at
176 baseline; YEAR, years from baseline; NMP5, patients not undergoing maintenance for
177 ≥5 years from baseline; MP5, patients undergoing maintenance for ≥5 years from
178 baseline; TL, tooth loss; CI, confidence interval.

179

180 The total number of teeth lost in the MP5 group was 748, and the most common cause
181 was root fracture (60% of all cases of TL). Most root fractures occurred in teeth that had
182 undergone root canal treatment and were fitted with casting posts and screw posts. In
183 most cases of TL due to caries, the caries were below the margin of the prosthesis in root
184 canal-treated teeth. Most cases of TL due to periodontal disease were caused by a
185 worsening of the furcation lesion. In addition, 90% of teeth lost were non-vital teeth
186 (Table 2).
187
188 **Table 2.** Tooth loss by cause and vital versus non-vital tooth status in the MP5 group

TL Cause	Number of vital teeth	Number of non-vital teeth	Total number of teeth (%)
Dental caries	9	161	170 (23%)
Periodontal disease	39	48	87 (12%)
Root fracture	3	446	449 (60%)
Other	21	21	42 (5%)
Total (%)	72 (10%)	676 (90%)	

189 Abbreviations: MP5, patients undergoing maintenance for ≥5 years from baseline; TL,
190 tooth loss.
191
192 As age at BL increased in the MP5 group, the number of RT at BL decreased and TL
193 increased. Additionally, TL due to dental caries, periodontal disease, and root fractures

194 increased as age at BL increased (Table 3).

195

196 **Table 3.** Characteristics of patients in the MP5 group, by age

Age group of AGE (age)	Number of patients(n)	Average of YEAR (year)	Average of RT (teeth number)	Average number of TL (teeth number)	Average number of TL/ year (teeth number)	Average number of TL per year by cause (teeth number)			
						Dental caries	Periodontal disease	Root fracture	Other
≤29	173	9.32	27.67	0.115	0.012	0.001	0	0.004	0.006
30-39	219	8.62	27.04	0.228	0.026	0.002	0.001	0.018	0.004
40-49	207	9.34	25.36	0.541	0.057	0.009	0.003	0.041	0.002
50-59	260	8.90	22.93	0.796	0.089	0.022	0.009	0.053	0.003
≥60	286	9.31	19.28	1.255	0.134	0.034	0.020	0.075	0.005

197 Abbreviations: AGE, age at baseline; RT, remaining teeth at baseline; YEAR, years from
198 baseline; MP5, patients undergoing maintenance for ≥5 years from baseline; TL, tooth
199 loss.

200

201 The relationships between the study variables (compliance, sex, smoking, SSI use, and
202 DM control) and TL in the MP5 group are shown in Table 4. Compliance and smoking
203 were not significantly related to TL; however, SSI use and DM were significantly
204 associated with TL. All patients with DM (yes) in this study were well controlled
205 (hemoglobin A1c <7%, National Glycohemoglobin Standardization Program). In this
206 study, many patients used two or more drugs for SSIs. Patients using multiple SSIs had

more TL. There was no significant difference in TL between men and women in this study (Table 4).

[Table 4 here]

The results of the analysis according to number of teeth lost are shown in Table 5. Within the MP5 group, the following classification of the number of teeth lost was established: TL0 group, 0 teeth lost; TL1 group, 1 tooth lost; TL2 group, 2 teeth lost; TL3 group, 3 teeth lost; TL4 group, 4 teeth lost; and TL5 group, ≥5 teeth lost. The results of the Steel–Dwass test were as follows. Age at BL was significantly different between TL0 and TL1 ($P < 0.0001$). However, age at BL was not significantly different between TL1 and TL2 ($P = 0.347$), TL2 and TL3 ($P = 1.0$), TL3 and TL4 ($P = 0.141$), or TL4 and TL5 ($P = 1.0$). Similar results were obtained for the number of RT at BL (TL0 vs. TL1: $P < 0.0001$, TL1 vs. TL2: $P = 0.405$, TL2 vs. TL3: $P = 0.970$, TL3 vs. TL4: $P = 0.266$, and TL4 vs. TL5: $P = 0.937$). However, for years from BL, there was no significant difference between TL0 and TL1 ($P = 0.142$), TL1 and TL2 ($P = 0.502$), TL2 and TL3 ($P = 0.443$), TL3 and TL4 ($P = 0.934$), or TL4 and TL5 ($P = 0.687$).

[Table 5 here]

The results of the logistic regression analyses for age and RT at BL according to number of teeth lost in the MP5 group are shown in Table 6. For age at BL, the presence or absence of TL was significantly different between patients who were ≤49 years old and those who were 50–59 years old, and between patients who were age ≤49 years and those age ≥60

231 years. There was also a significant difference between patients age 50–59 years and those

232 age ≥60 years (P = 0.0038, odds ratio = 1.65). As for the number of RT at BL, we observed

233 a significant difference between patients who had 28–24 teeth and those with 23–20 teeth,

234 and between patients who had 28–24 teeth and those with ≤19 teeth. However, there was

235 no significant difference between patients with 23–20 teeth and those with ≤19 teeth (P =

236 0.99).

237

238 **Table 6.** Logistic regression analysis results for age and RT with TL in the MP5 group

TL Cause		Patients (number)	TL count (%)	Odds ratio (95% CI)	P-value
AGE (years)	≤49	599	182 (24.3%)	Reference	
	50-59	260	207 (27.6%)	2.62(1.90-3.60)	<.0001
	≥60	286	359 (48.0%)	4.32(3.18-5.87)	<.0001
RT (number of teeth)	28-24	819	320 (42.7%)	Reference	
	23-20	125	159 (21.2%)	3.30(2.25-4.85	<.0001
	≤19	201	269 (35.9%)	3.29(2.39-4.53)	<.0001

239 Abbreviations: CI, confidence interval; AGE, age at baseline; RT, number of remaining

240 teeth at baseline; MP5, patients undergoing maintenance for ≥5 years from baseline; TL,

241 tooth loss.

242 **Discussion**

243 This retrospective study was performed to investigate TL in 1145 patients undergoing
244 long-term maintenance therapy at a private dental clinic in Japan. These findings provide
245 valuable insights into the factors that affect TL in people visiting general dental clinics. A
246 better understanding of these factors is important to prevent TL in future.

247 Previous studies on tooth maintenance used small sample sizes or only included patients
248 from specific environments, such as those in university hospitals or specialized clinics.
249 However, the clinic in the present study was a general dental clinic visited by many local
250 residents. The study population was large, and there was no significant bias in the sample
251 size of each age group (maximum, n = 286; minimum, n = 173). The average number of
252 years from BL for each age group at BL was also very similar (maximum, 9.32 years;
253 minimum, 8.62 years). The 1145 patients who were included in this study lost 748 teeth
254 out of a total of 27,737 teeth during maintenance therapy that lasted for an average of
255 9.09 years. The average number of teeth lost per year was only 0.07. Previous studies
256 have reported an average of 0.1 to 0.2 teeth lost per year.[38]

257 In this study, the MP5 group showed significantly less TL than the NMP5 group. The
258 average age at BL and average years from BL were very similar between the two groups,
259 but the number of patients in the NMP5 group was small (n = 57). The average number
260 of teeth lost per year was 0.22 in the NMP5 group (about three times the number in the
261 MP5 group). The difference between the MP5 and NMP5 groups was statistically
262 significant. This result closely resembles the results of the study by Costa et al.[29] In their
263 study, the average number of teeth lost per year was 0.12 in the regular compliers and
264 0.36 in the irregular compliers (about three times the number among regular complies).
265 Their study also suggested a beneficial effect of maintenance on TL. In addition, their

266 study was conducted in a general dental clinic, demonstrating the broad effectiveness of
267 maintenance for TL.

268 In the present study, root fractures were the most common cause of TL, followed by
269 caries and periodontal disease. These results are similar to those from a study in a general
270 dental clinic by Axelsson et al.[7] In another study showing that the primary cause of TL
271 was periodontal disease, data samples were obtained from three periodontists' private
272 offices.[30] In another study, patient data were obtained after treatment by a periodontist.[31]
273 In these studies, most patients were considered to be at high risk of periodontal disease.
274 In the present study, the status of periodontal disease at BL was as follows: healthy or
275 gingivitis, n = 316 (27.6%); stage I periodontitis, n = 436 (38.1%); stage II periodontitis,
276 n = 273 (23.8%); and stage III and IV periodontitis, n = 120 (10.5%). Most patients were
277 healthy or had gingivitis, stage I periodontitis, or stage II periodontitis. The fact that few
278 patients had stage III and IV periodontitis may explain why the rate of TL due to
279 periodontal disease was low and why TL during maintenance decreased. Therefore, it is
280 difficult to compare our results with those of other studies reporting periodontal treatment
281 and maintenance in patients with severe periodontal disease.

282 In our study, 90% of teeth lost were non-vital teeth. This may be explained by a previous
283 study in which root canal treatment had a significant effect on TL.[33] Root canal treatment
284 may also reportedly affect the deterioration of furcation lesions in molars.[33] One study
285 showed that as the number of non-vital teeth increased, TL due to root fractures and caries
286 increased.[34] Therefore, a decrease in the number of non-vital teeth may reduce TL due to
287 caries, periodontal disease, and root fractures.

288 Interestingly, we found no significant differences in the effect of smoking on TL under
289 maintenance. Smoking is, however, a clear risk factor for periodontal disease.[39] In one

290 study by Costa et al.,[29] smokers exhibited significantly more TL than nonsmokers ($P <$
291 0.05, odds ratio = 4.22). While the average age of smokers in our study was 45.3 years,
292 most smokers in the study by Costa et al.[29] were >55 years of age. Although the number
293 of RT at BL was very similar between the two groups in our study, age might have
294 increased the risk of TL in the study by Costa et al.[29]. Notably, smoking is a risk factor
295 for periodontal disease but not a direct pathogenic factor. Despite our findings, we will
296 continue to educate patients on the risk of TL and periodontal disease caused by smoking
297 and offer them smoking cessation guidance.

298 The relationship between DM and periodontal disease is well known.[39] Patients with
299 DM lose significantly more teeth than those without DM. In the present study, the odds
300 ratio was 2.78 with regard to DM. It was 2.73 in the above-mentioned study by Costa et
301 al.[29] and 2.64 in a study by Al-Shammari et al.[40]; these results are very similar to ours.

302 Previous studies have also shown significant associations between TL and systemic
303 diseases such as hypertension, heart disease, cerebrovascular disease, rheumatoid arthritis,
304 and asthma.[4,30] Although the relationship between the etiology of the disease and TL is
305 clear with regard to DM, this relationship remains unclear for many other diseases. The
306 adverse effect of thirst is reportedly associated with the therapeutic drugs used for many
307 diseases.[41] However, no reports have described the relationship between SSI use and TL.
308 The present study showed a significant association between SSI use and TL. Based on
309 this finding, the significant relationship between various systemic diseases and TL is not
310 due to the disease etiology and may be increased by the use of SSIs.

311 In this study, 1145 patients lost 748 teeth during an average of 9.09 years of maintenance.
312 However, 766 patients lost no teeth. TL was biased in some patients. In total, 179 (15.6%)
313 patients in the TL2, TL3, TL4, and TL5 groups lost 548 teeth (73.2%). Table 5 shows the

age at BL, years from BL, and number of RT at BL in each TL group. Age at BL was significantly different between TL0 and TL1. There were no significant differences between TL1 and TL2, between TL2 and TL3, between TL3 and TL4, or between TL4 and TL5. However, age at BL was very similar between TL2 and TL3 and between TL4 and TL5 (P = 1). Therefore, age at BL may have been divided into the TL0 group, TL1 group, TL2/TL3 group, and TL4/TL5 group. Similar results were obtained for the number of RT at BL. However, the number of years from BL was not significantly different between the consecutive groups (TL0, TL1, TL2, TL3, TL4, and TL5). The number of years from BL may not be associated with TL.

For age and RT at BL, logistic analysis was performed by classifying the patients based on the average value in each TL group and the results of the Steel–Dwass test (Table 6). Age at BL was significantly different between ages ≤49 and 50–59 years, between ≤49 and ≥60 years, and between ages 50–59 years and ≥60 years (P = 0.0038, odds ratio = 1.65). TL reportedly increases with age, and our results were similar.[42,43] The number of RT at BL was also significantly different between 28–24 and 23–20 RT and between 28–24 and ≤19 RT, but not between 23–20 and ≤19 RT (P = 0.99). These findings suggest that TL was less likely to occur if the number of RT at BL was ≥24 than ≤23. Interestingly, the number of RT around the age of 50 years was about 24. Perhaps the increase in TL with age may be due to a decrease in the number of RT. These results suggest that if the number of RT is ≥24, there is a high possibility that TL can be prevented during maintenance. One explanation for the increase in TL as the number of RT decreases may be the use of fixed or removable dentures. We excluded patients treated with implants from this study. Within the MP5 group, the use of dentures increased as the number of RT decreased. Few patients with ≥24 RT used removable dentures. Several studies have

338 revealed significant loss of denture abutments.[42-44] Another explanation may be that
339 patients with a low number of RT have received more restorative prosthetic treatment in
340 the past and have more non-vital teeth. Some reports have also described significant loss
341 of non-vital teeth.[33]

342 **Conclusion**

343 We found that significantly fewer teeth were lost per year in patients who underwent
344 maintenance than in those who did not. Most lost teeth were non-vital teeth, and tooth
345 fracture was the most common cause of TL. We also found that statistically significant
346 risk factors for TL were patient age at the start of maintenance, number of remaining teeth
347 at the start of maintenance, use of SSIs, and DM. Our results will be of great interest to
348 dentists involved in long-term maintenance care of patients in a general practice. This
349 study suggests that maintenance from a younger age in patients with fewer non-vital teeth
350 and more remaining teeth can help to reduce TL. Additionally, DM and the use of SSIs
351 can affect TL.

352

353 **Abbreviations**

354 TL: Tooth loss

355 DM: Diabetes mellitus

356 BL: Baseline

357 MP5: Maintenance patients for ≥5 years from baseline

358 NMP5: Non-maintenance patients for ≥5 years from baseline

359 RT: Remaining teeth

360 SSIs: Salivary secretion inhibitors

361

Declarations

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Review Committee of Tokushima University Hospital (Approval number: 2674). In this study, the patients' right to privacy protection was respected; additionally, written informed consent was obtained from all patients. This research was conducted in full accordance with the Declaration of Helsinki established by the World Medical Association.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no competing interests.

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