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ORIGINAL ARTICLE

Longitudinal Changes in Masticatory Muscle Morphology and Elastographic Characteristics Following Restorative Treatment of Unilateral Dental Caries in Children: A Prospective Clinical Study

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ABSTRACT

Background: Unilateral dental caries may alter masticatory function by promoting asymmetric chewing; however, whether these functional changes are accompanied by morphological or elastographic changes in the masticatory muscles of children remains unclear.

Objectives: To evaluate longitudinal changes in the thickness of the masseter, temporalis and anterior digastric muscles and in the strain-elastographic characteristics of the masseter muscle before and after restorative treatment of unilateral dental caries in children.

Methods: In this prospective clinical study, 25 children aged 6–12 years with unilateral dental caries underwent ultrasonographic examinations at baseline and 3 and 6 months after restorative treatment. Caries status was assessed using the International Caries Detection and Assessment System II (ICDAS-II), Caries Assessment Spectrum and Treatment (CAST) and Decayed, Missing and Filled Teeth (DMFT/dmft) indices. Bilateral thicknesses of the masseter and temporalis muscles were measured at rest and during contraction, whereas anterior digastric thickness was assessed at rest. Masseter muscle elastographic characteristics were evaluated using strain elastography with semi-quantitative red, green and blue (RGB)-based pixel analysis.

Results: No significant longitudinal changes were observed in muscle thickness on either the carious or non-carious side (all $p > 0.05$). Likewise, no significant elastographic changes were detected on the carious side. On the non-carious side, significant longitudinal differences were observed in resting red ($p = 0.035$), contraction green ($p = 0.002$) and contraction blue ($p = 0.011$) RGB proportions. Pairwise analysis showed a significant change in contraction green proportion between baseline and 6 months (adjusted $p = 0.009$), with a moderate effect magnitude ($r = 0.592$).

Conclusions: Restorative treatment of unilateral dental caries was not associated with detectable short-term changes in masticatory muscle thickness. However, selected strain-elastographic characteristics of the masseter muscle changed over time

Abbreviations: CAST, Caries Assessment Spectrum and Treatment; DC/TMD, Diagnostic Criteria for Temporomandibular Disorders; DMFT/dmft, Decayed, Missing and Filled Teeth (in permanent/primary teeth); ICC, intraclass correlation coefficient; ICDAS-II, International Caries Detection and Assessment System II; IQR, interquartile range; MHz, megahertz; RGB, red, green and blue; ROI, region of interest; SD, standard deviation; SE, strain elastography; STROBE, Strengthening the Reporting of Observational Studies in Epidemiology; SWE, shear-wave elastography; TMD, temporomandibular disorders; TMJ, temporomandibular joint; USG, ultrasonography; W, Kendall's coefficient of concordance.

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despite stable muscle thickness. These findings suggest that strain elastography may provide information complementary to conventional ultrasonographic thickness assessment; however, the clinical significance of these changes requires further validation.

1 | Introduction

Dental caries is a biofilm-mediated, diet-modulated, multifactorial disease resulting from an imbalance between demineralization and remineralization processes in dental hard tissues [1]. If left untreated, it may lead to pain, infection, functional impairment and reduced quality of life [2]. According to the World Health Organization, dental caries remains one of the most prevalent chronic diseases in childhood, with particularly high and increasing prevalence in developing countries [3].

The impact of dental caries extends beyond local tooth destruction. Carious lesions, particularly those associated with pain or functional impairment in posterior teeth, may lead to loss of occlusal contact, reduced masticatory capacity and altered activity of the masseter and temporalis muscles [4, 5]. However, the functional consequences of dental caries vary according to lesion severity, pain and individual behavioural responses. Pain-related asymmetry may develop when dental pain during mastication promotes avoidance of the affected side and preferential chewing on the contralateral side [6]. In contrast, mechanically driven functional impairment may arise when carious destruction compromises tooth structure, occlusal contacts or the number of effective masticatory units, thereby reducing effective masticatory function even in the absence of overt pain [4, 5]. Although pain-related avoidance and compromised occlusal function may coexist, they represent distinct pathways through which unilateral dental caries may alter the distribution of masticatory function between sides. These consequences are particularly relevant during childhood because the masticatory system is still undergoing substantial functional and morphological development. Longitudinal studies have demonstrated that bite force and masseter muscle thickness change with growth and across stages of dentitional development, while craniofacial morphology is also associated with masseter muscle dimensions in growing individuals [7–9]. Accordingly, muscle characteristics observed in adults may not be directly generalizable to children, in whom age, dentitional maturation and craniofacial growth may influence both baseline muscle properties and their evolution over time. Moreover, skeletal muscle adaptation follows a continuum in which early responses may involve neuromuscular and biomechanical adjustments, whereas measurable structural changes may require more prolonged or intense functional loading [5, 10]. Therefore, functional and morphological responses to altered masticatory function may follow different temporal patterns in growing children.

Regardless of the predominant mechanism, unilateral caries-associated changes in chewing behaviour or occlusal function may produce unequal functional loading between the two sides of the masticatory system. Surface electromyographic studies have demonstrated that unilateral masticatory loading produces measurable asymmetries in muscle activity between sides, supporting the role of functional imbalance in the masticatory system [11]. Consistent with a mechanical contribution independent of overt pain, impaired masticatory performance has also been demonstrated in children with caries in whom dental pain was absent [12]. Available paediatric evidence further suggests partial functional recovery after restorative treatment: increases in bite force have been observed following restoration of carious teeth, including in children with unilateral caries [13] and a recent scoping review similarly identified treatment-associated recovery of masticatory function in paediatric populations [5]. These findings suggest that restoration of carious teeth may be accompanied by changes not only in local dental function but also in the functional environment to which the masticatory muscles are exposed during growth.

Among the imaging modalities used to evaluate masticatory muscles, ultrasonography (USG) has gained increasing attention because it is non-invasive, free of ionizing radiation and capable of providing high-resolution soft-tissue imaging [14]. USG enables reliable assessment of muscle thickness, morphology and symmetry of superficial masticatory muscles at rest and during contraction [14]. These advantages are particularly relevant in paediatric populations, as examinations are rapid, well tolerated and require minimal patient cooperation, making USG especially suitable for longitudinal studies involving repeated measurements in children [15].

In recent years, advanced USG techniques such as strain elastography (SE) and shear-wave elastography (SWE) have expanded the assessment of tissue mechanical characteristics by providing qualitative, semi-quantitative or quantitative information, depending on the elastographic technique used. Elastography therefore complements conventional USG by providing additional information beyond morphological assessment alone [16, 17]. Paediatric-specific elastographic evidence remains scarce and available SWE data suggest that masseter stiffness values in children may differ from those reported in adult populations and may also vary according to biological factors such as sex, further supporting the need for paediatric-specific investigation of masticatory muscle elastographic characteristics [18]. Therefore, combining conventional USG with elastography provides complementary information on muscle morphology and elastographic characteristics, allowing these distinct dimensions of muscle assessment to be evaluated within the same longitudinal framework.

Despite these considerations, an important gap remains between what is known about caries-related functional impairment in children and whether and how masticatory muscle characteristics change after dental function is restored. Previous paediatric studies have primarily focused on functional outcomes such as bite force or masticatory performance, while imaging-based investigations of masticatory muscles in children have largely addressed normal development, craniofacial morphology, orthodontic treatment or reference elastographic characteristics rather than caries-related

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changes in masticatory muscle characteristics [7–9, 18]. This distinction is clinically important because, in growing children, changes related to treatment occur against a background of ongoing dentitional maturation, craniofacial growth and age-related muscle development. Consequently, determining whether morphological and elastographic characteristics of the masticatory muscles change over time after restoration of unilateral carious lesions may provide a more complete understanding of functional recovery than assessment of dental status or bite force alone. Although untreated dental caries has been shown to impair masticatory function and restorative treatment may improve functional measures in paediatric populations [5, 12, 13], longitudinal changes in the morphological and elastographic characteristics of the masticatory muscles following restorative treatment of unilateral dental caries remain insufficiently characterized, particularly when these changes are assessed using combined ultrasonographic and elastographic approaches.

Therefore, the aim of this prospective clinical study was to investigate changes in the morphological and elastographic characteristics of masticatory muscles in children aged 6–12 years with unilateral dental caries using USG and SE before restorative treatment and at 3- and 6-months follow-up. The null hypothesis was that no significant longitudinal changes would occur in the morphological or elastographic characteristics of the masticatory muscles on either side during the 6-months follow-up after restorative treatment.

2 | Methods

2.1 | Study Design and Ethical Considerations

This prospective clinical study was conducted at the Department of Pediatric Dentistry and the Ultrasonography Unit of the Department of Dentomaxillofacial Radiology, School of Dentistry, Çanakkale Onsekiz Mart University, Türkiye. The study was approved by the Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University (approval date: November 8, 2023; decision number: 2023–168). Written informed consent was obtained from the parents or legal guardians of all participants and verbal assent was obtained from the children. The study was conducted in accordance with the principles of the Declaration of Helsinki and is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.

2.2 | Study Population and Eligibility Criteria

Children aged 6–12 years who attended the Pediatric Dentistry Clinic for routine dental examination were consecutively screened for eligibility and enrolled in this prospective clinical study. All eligibility criteria were confirmed before the baseline ultrasonographic examination.

The inclusion criteria were: (1) age between 6 and 12 years; (2) presence of unilateral occlusal, occlusoproximal or proximal dentine caries; (3) body mass index within the age- and sex-specific normal range; (4) self-reported difficulty chewing on the carious side; (5) Class I molar relationship without dentofacial anomalies; (6) no history of orthodontic treatment or orthognathic surgery; (7) no previous restorative or endodontic treatment on the affected teeth; and (8) absence of missing teeth in the primary or permanent dentition.

The exclusion criteria were: (1) presence of bilateral dentine caries; (2) systemic or musculoskeletal disorders that could affect masticatory muscle function; (3) craniofacial developmental anomalies or non-Class I occlusal relationships; (4) clinical signs or symptoms suggestive of temporomandibular disorder (TMD) or parafunctional habits, including bruxism, muscle tenderness, pain or limitation of mandibular movement; (5) acute intraoral infection, inflammation, ulcerative lesions or painful periodontal conditions; (6) inability to complete the ultrasonographic examination because of insufficient cooperation; and (7) lack of written informed consent from a parent or legal guardian.

2.3 | Clinical Examination and Caries Assessment

A standardized clinical examination protocol was applied to all participants. Detailed medical and dental histories were obtained from parents or legal guardians. Extraoral examination included assessment of facial symmetry, facial profile, jaw relationships and temporomandibular joint (TMJ) function. TMJ evaluation comprised palpation for tenderness, detection of joint sounds (clicking or crepitation) and assessment of mandibular range of motion. The masseter and temporalis muscles were palpated to identify tenderness or pain on function and clinical signs of bruxism were assessed based on abnormal tooth wear, muscle tenderness and reported clenching or grinding habits.

TMD-related signs and symptoms were screened using an age-adapted version of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I protocol for children and adolescents, based on previously proposed paediatric modifications [19–21]. The protocol was used solely as a standardized screening tool to identify participants requiring exclusion rather than to establish definitive TMD diagnoses. Before study initiation, the examiner responsible for these assessments was calibrated using repeated examinations of individuals not included in the study sample at a 2 week interval. Intra-examiner agreement was excellent (Cohen's $\kappa = 0.87$).

Intraoral examination was performed using a dental mirror and a blunt-ended WHO probe under adequate illumination. Occlusal relationships, intercuspation and molar relationships were clinically assessed. Caries severity was evaluated using the International Caries Detection and Assessment System II (ICDAS-II) [22], while the Caries Assessment Spectrum and Treatment (CAST) index was used to characterize the spectrum of caries progression [23]. Overall caries experience was recorded using the Decayed, Missing and Filled Teeth (DMFT/dmft) index [24]. All examinations were performed by a single calibrated examiner (M.D.).

Calibration for caries assessment was conducted 1 month before study initiation using 20 individuals who were not included in the final sample. Each participant was examined twice at a

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2 week interval, yielding Cohen's κ values ranging from 0.88 to 1.00 for the ICDAS-II, CAST and DMFT/dmft assessments, indicating excellent intra-examiner agreement. Radiographic examinations (panoramic, bitewing or periapical radiographs) were obtained only when clinically indicated to support the diagnosis.

2.4 | Ultrasonographic and Elastographic Imaging Protocol

All ultrasonographic examinations were performed by a single experienced dentomaxillofacial radiologist (H.E.) to minimize examiner-related variability. Imaging was performed using a Hitachi Arietta 65 USG system (Hitachi Medical Systems, Tokyo, Japan) equipped with a 5–13 MHz linear transducer. To ensure standardization, USG parameters, including gain, imaging depth and dynamic range, were kept constant throughout the study. For B-mode muscle-thickness measurements, a minimal and consistent amount of transducer pressure was applied using USG gel to avoid tissue compression and the probe was positioned perpendicular to the muscle fibres using the same anatomical landmarks at each examination.

Participants were examined in the supine position with the head slightly rotated to the contralateral side. After a standardized resting period of at least 5 min, measurements were obtained at rest (complete mandibular relaxation) and during maximum voluntary clenching. Three consecutive measurements were recorded for each condition and the mean value was used for statistical analysis.

Masseter muscle thickness was measured at the mid-belly immediately superior to the mandibular angle with the probe positioned parallel to the occlusal plane. Temporalis muscle thickness was measured above the zygomatic arch. Both muscles were evaluated at rest and during contraction. Anterior digastric muscle thickness was measured in the submandibular region at the level of the mandibular body at rest only because this muscle is not primarily activated during maximal clenching.

Masseter muscle elastographic characteristics were assessed using real-time SE. To minimize operator-dependent variability associated with free-hand compression, the transducer was maintained in continuous contact with the skin with minimal initial pre-compression and five consecutive gentle compression–relaxation cycles were applied with low and approximately uniform amplitude. The regularity and adequacy of compression were monitored in real time using the strain/compression quality indicator displayed by the elastography system. Excessive, insufficient or irregular compression was avoided and acquisitions not meeting these quality criteria were repeated. Elastographic acquisition was continued until a stable low-amplitude strain pattern and a visually consistent colour distribution within the masseter muscle were maintained over consecutive compression–relaxation cycles. The HI Strain algorithm was used to enhance the continuity and consistency of the elastographic display. Frames showing evident motion, loss of transducer contact, heterogeneous compression artefacts or unstable colour distribution were excluded. Three separate, technically acceptable elastographic acquisitions, each comprising five compression–relaxation cycles, were obtained for each side and measurement condition.

Elastographic images were exported for offline analysis. Semi-quantitative image analysis of the exported elastographic colour maps was performed using the web-based Image Colour Summarizer software (Martin Krzywinski, Canada). For each selected image, the region of interest (ROI) was confined to the masseter muscle belly, with the superficial and deep fascia, subcutaneous tissue and adjacent anatomical structures excluded. ROI placement was based on the same anatomical landmarks and was kept consistent across repeated examinations. The ROI was analysed to determine the relative percentages of red, green and blue (RGB) pixels, which were calculated as proportions of the total pixels within the selected ROI, providing a semi-quantitative assessment of elastographic colour distribution within the ROI. RGB percentages were calculated separately for each of the three technically acceptable acquisitions and the mean of the three measurements was used for statistical analysis.

Before study initiation, intra-examiner reliability of the ultrasonographic and elastographic measurements was assessed in 10 individuals who were not included in the final study sample. Each individual underwent two independent measurement sessions under identical acquisition conditions at a 2 weeks interval. Intraclass correlation coefficients (ICCs) ranged from 0.91 to 0.96 across muscle-thickness and elastographic measurements, indicating excellent intra-examiner reliability.

All measurements were performed bilaterally before restorative treatment (T0) and at 3 months (T1) and 6 months (T2) follow-up. The same anatomical reference points were used at each examination to ensure longitudinal consistency.

2.5 | Restorative Treatment

All carious teeth were restored with resin-based composite restorations by a single clinician (M.D.) following standardized clinical procedures. Baseline ultrasonographic examinations were completed on the same day before restorative treatment.

Local anaesthesia was administered using 4% articaine with 1:100000 epinephrine and rubber dam isolation was used in all cases. Cavity preparation was performed using high-speed diamond burs and carious dentine was removed with low-speed tungsten carbide burs and hand excavators following a minimally invasive approach.

A self-etch universal adhesive (Clearfil Universal Bond; Kuraray Noritake Dental Inc., Tokyo, Japan) was applied according to the manufacturer's instructions and light-cured using an LED curing unit (D-Light Pro; GC Corporation, Tokyo, Japan). Restorations were completed with a resin composite (Clearfil Majesty Aesthetic; Kuraray Noritake Dental Inc., Tokyo, Japan) placed in increments not exceeding 2 mm. Final contouring, occlusal adjustment and polishing were performed using Sof-Lex discs (3 M ESPE, St. Paul, MN, USA). Participants and their

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parents received standardized oral hygiene instructions and were scheduled for clinical and ultrasonographic follow-up examinations at 3 and 6 months.

2.6 | Outcome Measures

The primary outcomes were longitudinal changes in the morphological and elastographic characteristics of the masseter muscle on both the carious and non-carious sides following restorative treatment. Morphological changes were assessed by ultrasonographic measurements of muscle thickness, whereas elastographic characteristics were evaluated using SE with semi-quantitative RGB colour distribution analysis. Measurements were obtained at baseline (T0), 3 months after restorative treatment (T1) and 6 months after treatment (T2). Secondary outcomes included longitudinal changes in temporalis and anterior digastric muscle thickness measured at the same time points.

2.7 | Sample Size Calculation

An a priori sample size calculation was performed using G*Power software (version 3.1; Heinrich Heine University, Düsseldorf, Germany). In the absence of directly comparable longitudinal paediatric studies evaluating ultrasonographic or elastographic changes in the masticatory muscles following restorative treatment of unilateral dental caries, the calculation was based on longitudinal data reported by Serra et al. [25], who evaluated changes in masticatory function following dental rehabilitation in children aged 6–9 years. In that study, maximum bite force increased from 302 ± 61 N before rehabilitation to 345 ± 43 N at the 6 months follow-up ($p < 0.0001$). Using the minimum standardized paired effect corresponding conservatively to the reported significance level (Cohen's $d_z = 1.02$), a two-sided α level of 0.05 and a statistical power of 98%, the minimum required sample size was calculated as 18 participants. To account for an anticipated 20% loss to follow-up, the required recruitment sample was increased to 23 participants.

2.8 | Statistical Analysis

The normality of continuous variables was assessed using the Shapiro–Wilk test. As the data were not normally distributed, nonparametric statistical methods were applied. Descriptive statistics are presented as mean ± standard deviation (SD), median and interquartile range (IQR) for continuous variables and as frequencies and percentages for categorical variables. Comparisons of continuous variables across independent groups were performed using the Mann–Whitney *U* test or the Kruskal–Wallis *H* test, as appropriate. Longitudinal differences across the three examination time points (T0, T1 and T2) were evaluated using the Friedman test, followed by post hoc Wilcoxon signed-rank tests with Bonferroni correction for pairwise comparisons. Paired comparisons between the carious and non-carious sides at each time point were performed using the Wilcoxon signed-rank test. Effect sizes were calculated for all inferential comparisons. For Friedman tests, Kendall's coefficient of concordance (W) was calculated as $\chi^2/[N(k-1)]$, where N represents the number of participants and k the number of repeated measurements. For Wilcoxon signed-rank tests, effect size r was calculated as $|Z|/\sqrt{N}$, where N represents the number of paired observations. In accordance with recently proposed dentistry-specific effect-size recommendations, r values of 0.20–<0.40, 0.40–<0.70 and ≥0.70 were interpreted as small, moderate and large effects, respectively [26]. A two-sided p-value <0.05 was considered statistically significant. For post hoc Wilcoxon comparisons following a significant Friedman test, statistical significance was evaluated using the Bonferroni-adjusted threshold of $p < 0.0167$ (0.05/3). All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Chicago, IL, USA).

3 | Results

A total of 25 children (16 girls and 9 boys; mean age: 9.28 ± 1.62 years) were included in the study. No participant presented with ICDAS-II Codes 0–2. The most prevalent ICDAS-II category was Code 4 (distinct shadow of dentine; 44%), followed by Code 5 (distinct cavity with visible dentine; 32%) and Code 6 (extensive cavity with visible dentine; 16%). CAST index results similarly indicated a predominance of advanced caries lesions, classified as Code 4 with 56% and 28% as Code 5. Mean DMFT and dmft values were 0.76 ± 0.93 and 1.12 ± 1.27, respectively (Table 1). No statistically significant differences in muscle thickness or elastographic parameters were observed among groups stratified by ICDAS-II and CAST indices ($p > 0.05$).

Comparisons between the carious and non-carious sides were performed using the Wilcoxon signed-rank test. No statistically

TABLE 1 | Demographic and clinical characteristics of the study group.

Variable	Category	*N*	%
Sex	Male	9	36
	Female	16	64
ICDAS-II	Code 3	2	8
	Code 4	11	44
	Code 5	8	32
	Code 6	4	16
CAST	Code 2	1	4
	Code 4	14	56
	Code 5	7	28
	Code 6	2	8
	Code 7	1	4
Variable	Mean		SD
Age (years)	9.28		1.62
DMFT	0.76		0.93
dmft	1.12		1.27

Abbreviations: CAST, Caries Assessment Spectrum and Treatment; DMFT/dmft, Decayed, Missing and Filled Teeth index for permanent/primary dentition; ICDAS-II, International Caries Detection and Assessment System II; N, number; SD, standard deviation.

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significant differences were observed in the thickness of the masseter, temporalis or anterior digastric muscles between sides at any of the three time points (T0, T1 and T2) (all $p \geq 0.109$; $r = 0.007–0.327$). Similarly, masseter muscle RGB proportions did not differ significantly between the carious and non-carious sides across any measurement condition or time point (all $p \geq 0.060$; $r = 0.013–0.377$), with effect sizes remaining below the threshold for a moderate effect.

No statistically significant changes were observed in the thickness of the masseter, temporalis or anterior digastric muscles across the three measurement periods (T0, T1 and T2), either at rest or during contraction, on both the carious and non-carious sides (Friedman tests; all $p \geq 0.073$; Kendall's W = 0.011–0.104) (Table 2). Masseter muscle thickness during contraction showed minimal variation across time points on both sides. On the non-carious side, mean thickness was 9.38 ± 1.27 mm (median: 9.50; IQR: 1.10) at T0 and 9.50 ± 1.56 mm (median: 9.60; IQR: 2.00) at T2 ($p = 0.759$; Kendall's W = 0.011). Similarly, on the carious side, mean thickness was 9.44 ± 1.31 mm (median: 9.30; IQR: 1.60) at T0 and 9.81 ± 1.58 mm (median: 10.00; IQR: 1.60) at T2 ($p = 0.332$; Kendall's W = 0.044). Temporalis muscle thickness demonstrated a comparable pattern, with no statistically significant temporal changes on either side ($p \geq 0.073$; Kendall's W = 0.021–0.104). Anterior digastric muscle thickness, measured at rest only, also remained stable across all time points on both sides ($p \geq 0.120$; Kendall's W = 0.057–0.085).

On the non-carious side, statistically significant time-dependent changes were observed in several elastographic parameters (Table 3). During contraction, green RGB proportions differed significantly across the three time points (Friedman $p = 0.002$; Kendall's W = 0.245), with mean values decreasing from 49.29% ± 8.80% at T0 to 45.27% ± 9.65% at T1 and 42.81% ± 6.48% at T2. Post hoc Wilcoxon signed-rank tests with Bonferroni correction identified a significant difference between T0 and T2 (adjusted $p = 0.009$; $r = 0.592$, moderate effect). Blue RGB proportions during contraction also differed significantly over time (Friedman $p = 0.011$; Kendall's W = 0.179), increasing descriptively from 29.09% ± 7.05% at T0 to 31.96% ± 9.32% at T1 and 32.21% ± 7.20% at T2. At rest, red RGB proportions showed an overall temporal difference (Friedman $p = 0.035$; Kendall's W = 0.134), with the highest mean value observed at T1 (25.60% ± 7.19%). These temporal changes in RGB proportions are illustrated in Figure 1A–C. In contrast, no statistically significant changes were observed in any RGB proportion on the carious side, either during contraction or at rest (Friedman tests; all $p \geq 0.135$; Kendall's W = 0.003–0.080) (Table 4).

4 | Discussion

The present prospective clinical study investigated longitudinal changes in the morphological and elastographic characteristics of the masticatory muscles following restorative treatment of unilateral dental caries in children. The findings revealed a dissociation between morphological stability and temporal variation in selected strain-elastographic characteristics of the masseter muscle. Although the principal post hoc elastographic

TABLE 2 | Muscle thickness measurements (mm) across time points on the non-carious and carious sides.

Muscle	Side/State	T0				T1				T2					
		Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR	p^a	Kendall's W
Masseter	NCS, Contr.	9.38	1.27	9.50	1.10	9.54	1.31	9.70	1.20	9.50	1.56	9.60	2.00	0.759	0.011
	NCS, Rest.	6.52	1.15	6.40	1.40	6.34	1.26	6.40	1.90	6.44	1.03	6.30	1.20	0.502	0.028
	CS, Contr.	9.44	1.31	9.30	1.60	9.38	1.62	9.90	1.40	9.81	1.58	10.00	1.60	0.332	0.044
	CS, Rest.	6.53	1.15	6.60	1.10	6.38	1.38	6.20	1.40	6.67	1.11	6.70	1.30	0.388	0.038
Temporalis	NCS, Contr.	6.91	1.32	6.90	1.30	7.22	0.94	7.30	1.10	7.25	0.94	7.30	1.20	0.597	0.021
	NCS, Rest.	6.20	1.23	6.30	1.10	6.50	0.82	6.20	1.00	6.49	0.75	6.30	0.80	0.435	0.033
	CS, Contr.	6.81	0.96	6.80	0.90	7.20	0.89	7.20	1.00	7.24	1.16	7.00	1.60	0.102	0.091
	CS, Rest.	6.10	0.95	6.20	0.70	6.40	0.69	6.40	0.90	6.33	0.91	6.20	1.40	0.073	0.104
Anterior Digastric	NCS, Rest.	4.74	0.67	4.60	1.00	4.50	0.77	4.50	1.00	4.70	0.63	4.90	1.00	0.239	0.057
	CS, Rest.	4.57	0.71	4.50	0.80	4.50	0.62	4.30	1.00	4.64	0.72	4.60	0.70	0.120	0.085

Note: Kendall's W represents the effect size for the Friedman test.
Abbreviations: Contr., contraction; CS, carious side; IQR, interquartile range; NCS, non-carious side; Rest., resting; SD, standard deviation; T0, before restorative treatment; T1, 3 months after treatment; T2, 6 months after treatment.
[a]Friedman test.

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TABLE 3 | Masseter muscle strain elastography (RGB analysis) on the non-carious side across time points.

Parameter	State	T0				T1				T2					
		Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR	p^a	Kendall's W
Red (%)	Contr.	21.60	6.43	22.80	4.30	23.38	3.51	23.80	4.90	24.39	4.20	24.50	5.00	0.145	0.077
	Rest.	21.38	7.28	21.70	6.50	25.60	7.19	25.10	7.80	23.58	5.36	22.60	6.50	**0.035**	0.134
Green (%)	Contr.	49.29	8.80	47.70	5.60	45.27	9.65	44.30	13.30	42.81	6.48	40.60	6.80	**0.002**	0.245
	Rest.	58.32	12.45	62.50	18.90	54.71	9.35	55.30	13.40	58.07	9.29	58.60	14.10	0.289	0.050
Blue (%)	Contr.	29.09	7.05	28.20	7.00	31.96	9.32	32.40	14.30	32.21	7.20	32.60	9.30	**0.011**	0.179
	Rest.	20.38	10.97	15.80	16.70	20.02	9.70	16.70	16.10	19.02	8.32	16.60	12.50	0.607	0.020

Note: Bold values indicate statistical significance. Kendall's W represents the effect size for the Friedman test. For parameters with a significant overall time effect, post hoc Wilcoxon signed-rank tests were performed with Bonferroni correction. Only the T0–T2 comparison for green RGB proportion during contraction remained significant after correction (adjusted $p = 0.009$; $r = 0.592$, moderate effect).

Abbreviations: Contr., contraction; IQR, interquartile range; Rest., resting; SD, standard deviation; T0, before restorative treatment; T1, 3 months after treatment; T2, 6 months after treatment.

[a]Friedman test.

finding was of moderate magnitude, the overall pattern does not establish that functional adaptation occurred as a direct consequence of restorative treatment. Rather, it suggests that elastographic characteristics may vary over time even in the absence of detectable alterations in muscle thickness over the relatively short follow-up period. Accordingly, the null hypothesis was partially rejected, as temporal changes were identified in selected strain-elastographic parameters but not in muscle thickness.

USG offers several advantages for longitudinal evaluation of masticatory muscles in children because it is non-invasive, free of ionizing radiation and provides reliable assessment of superficial muscle morphology [15]. When combined with elastography, conventional ultrasonographic evaluation can be complemented by assessment of tissue mechanical characteristics, allowing simultaneous investigation of muscle morphology and mechanical behaviour [16, 27]. This multimodal approach was considered particularly appropriate for the present longitudinal study because it enabled repeated examinations without radiation exposure while providing information beyond muscle thickness alone.

The additional use of SE provided important complementary information beyond conventional ultrasonographic thickness measurements. Although no significant morphological changes were detected during follow-up, elastography identified time-dependent changes in selected RGB proportions of the masseter muscle. The coexistence of stable muscle thickness and changes in elastographic characteristics may indicate that these measures capture different aspects of muscle behaviour; however, it should not be interpreted as evidence that mechanical adaptation necessarily precedes structural remodelling. Previous studies have demonstrated that elastographic measurements of the masticatory muscles are sensitive to differences in muscle state and functional loading [16, 28]. Furthermore, Olchowy et al. [18] established reproducible reference stiffness values for the paediatric masseter muscle using SWE, supporting the feasibility of elastographic assessment in children. However, SWE-derived quantitative stiffness measurements are not directly equivalent to the semi-quantitative RGB distributions obtained using SE in the present study. The RGB-based pixel analysis used here enabled semi-quantitative evaluation of elastographic colour distribution without additional imaging procedures. Nevertheless, SE-derived RGB distributions may be influenced by acquisition conditions, externally applied compression and ROI definition; therefore, careful standardization is required and some degree of measurement-related variability should be considered when interpreting longitudinal changes [16].

In the present study, masseter muscle thickness remained unchanged on both the carious and non-carious sides throughout the follow-up period. This finding indicates that the functional disturbance associated with unilateral dental caries and its subsequent treatment were not accompanied by detectable short-term changes in masseter morphology. In growing children, however, masseter muscle thickness is influenced not only by functional loading but also by ongoing craniofacial development and individual morphological characteristics. Kiliaridis et al. [29] demonstrated associations between masseter thickness, facial morphology and dental arch dimensions, whereas

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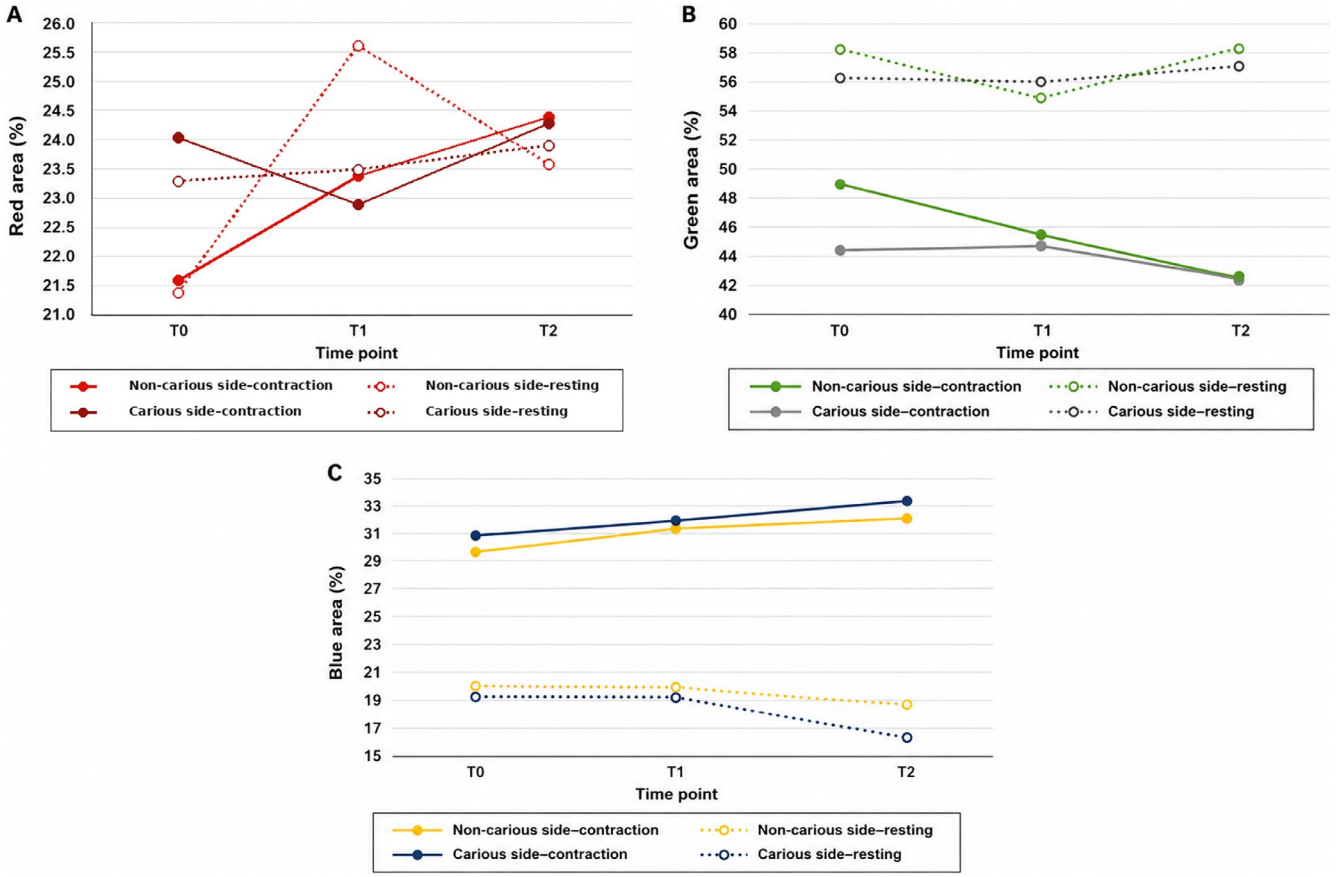


FIGURE 1 | Changes in the RGB elastographic parameters of the masseter muscle during follow-up. (A) Red area (%), (B) Green area (%) and (C) Blue area (%) on the carious and non-carious sides at rest and during maximum voluntary contraction. (T0, before restorative treatment; T1, 3 months after treatment; T2, 6 months after treatment).

Papagiannopoulos et al. [30] reported age-related differences in paediatric masseter thickness. More recently, longitudinal evidence from untreated growing children demonstrated an increase in masseter thickness over approximately 1 year, further emphasizing the contribution of normal growth to temporal changes in muscle morphology [9]. Accordingly, developmental and inter-individual variability should be considered when interpreting longitudinal muscle-thickness measurements in paediatric populations. The stability of masseter thickness in the present study should therefore not be interpreted either as evidence against altered muscle behaviour or, conversely, as indirect evidence that adaptation occurred at another biological level.

Temporalis and anterior digastric muscle thicknesses remained stable throughout the follow-up period. Lee et al. [31] similarly reported limited changes in temporalis muscle thickness in response to altered functional conditions, indicating that morphological responses may differ among the masticatory muscles. The absence of detectable changes in the temporalis and anterior digastric muscles in the present study should therefore be interpreted cautiously, particularly given their distinct functional roles and the potential influence of growth-related variability on muscle morphology in children. Moreover, stability in muscle thickness does not exclude changes in muscle activation or recruitment, which cannot be determined from morphological USG alone.

In contrast to the stability of muscle thickness, longitudinal variation was observed in selected strain-elastographic characteristics of the masseter muscle on the non-carious side. The principal post hoc elastographic finding was of moderate magnitude, indicating that the observed difference was not merely of negligible magnitude despite its statistical detectability. Nevertheless, the direction of individual RGB components should be interpreted cautiously. SE provides a semi-quantitative representation of tissue deformation rather than an absolute measurement of muscle stiffness and changes in RGB proportions therefore cannot be directly equated with corresponding changes in stiffness or force transmission. Moreover, because the RGB components represent relative proportions within the elastogram, changes in individual colour components should not be regarded as independent mechanical responses. Previous elastographic studies have demonstrated differences in masseter mechanical characteristics across functional and clinical conditions; however, methodological heterogeneity limits direct comparison across techniques and studies [32]. Importantly, SE relies on tissue deformation induced by manual compression and is inherently more dependent on operator technique than SWE, with reproducibility potentially influenced by the acquisition procedure [33]. Accordingly, the present RGB findings are compatible with temporal variation in masseter elastographic characteristics but do not establish a specific biomechanical mechanism or demonstrate that elastographic changes necessarily precede morphological remodelling.

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TABLE 4 | Masseter muscle strain elastography (RGB analysis) on the carious side across time points.

Parameter	State	T0				T1				T2					
		Mean	SD	Median	IQR	Mean	SD	Median	IQR	Mean	SD	Median	IQR	p^a	Kendall's W
Red (%)	Contr.	24.03	4.44	23.70	4.90	22.89	5.74	22.70	3.40	24.27	4.62	23.20	4.70	0.155	0.075
	Rest.	23.28	6.70	23.40	6.10	23.48	5.97	23.30	6.60	23.90	7.22	23.20	10.80	0.932	0.003
Green (%)	Contr.	44.76	8.64	43.00	7.00	45.25	10.71	44.80	13.80	42.44	8.36	41.40	9.20	0.529	0.025
	Rest.	56.53	11.60	58.40	11.50	56.31	7.53	56.60	10.90	58.22	8.73	59.40	12.80	0.825	0.008
Blue (%)	Contr.	31.20	7.82	32.80	8.30	31.96	11.92	31.10	12.60	33.49	10.23	35.10	16.60	0.227	0.059
	Rest.	20.15	10.96	18.00	13.70	20.11	7.23	19.70	10.20	16.68	5.06	17.80	4.60	0.135	0.080

Note: Kendall's W represents the effect size for the Friedman test.
Abbreviations: Contr., contraction; IQR, interquartile range; Rest., resting; SD, standard deviation; T0, before restorative treatment; T1, 3 months after treatment; T2, 6 months after treatment.
[a]Friedman test.

The occurrence of longitudinal elastographic changes on the non-carious side may be compatible with altered asymmetric muscle use associated with the unilateral oral condition, although this interpretation remains hypothetical. The self-reported difficulty chewing on the carious side before treatment may plausibly have been accompanied by reduced use of that side; however, chewing-side preference and redistribution of masticatory loading were not objectively assessed in the present study. Furthermore, the absence of significant side-to-side differences precludes concluding that the carious and non-carious sides followed distinctly different adaptive trajectories. An alternative explanation is that the observed elastographic pattern reflects changes in neuromuscular recruitment or coordination rather than a direct mechanical response to altered occlusal loading. Supporting the distinction between muscle function and morphology, Andrade et al. reported asymmetric masseter electromyographic activity in children with unilateral posterior crossbite despite the absence of corresponding differences in ultrasonographic muscle thickness [34]. Temporal variation in SE-derived RGB distributions may therefore reflect a combination of functional, neuromotor, developmental and measurement-related influences rather than a single treatment-induced adaptive mechanism. The temporal variation in resting red proportion should likewise be interpreted cautiously, as attributing this pattern to changes in basal muscle tone or protective muscle activity would be speculative in the absence of concurrent objective assessment of muscle activation.

The principal strengths of this study include its prospective longitudinal design with repeated intra-individual assessments, which reduced the influence of inter-individual biological variability and the combined use of USG, SE and RGB-based image analysis to evaluate morphological and elastographic characteristics of the masticatory muscles. Furthermore, all ultrasonographic and elastographic examinations were performed by a single experienced examiner using the same equipment and a standardized imaging protocol, including controlled compression procedures and repeated acquisitions, enhancing measurement consistency.

Several limitations should be considered. Ultrasonographic and elastographic assessments remain operator-dependent despite standardized acquisition procedures. In addition, the RGB-based analysis of SE images provides a semi-quantitative assessment of relative elastographic colour distribution rather than an absolute measurement of tissue stiffness and the lack of paediatric reference values specifically for SE-derived RGB distributions limits direct clinical interpretation. Although an a priori sample size calculation was performed, the relatively small sample size and 6 months follow-up may not have been sufficient to detect subtle or longer-term changes in masticatory muscle characteristics. Importantly, difficulty chewing on the carious side was based on the children's self-report and was not corroborated by an objective assessment of masticatory function. Subjective assessments of chewing function may not necessarily correspond to objectively measured masticatory performance [35] and the use of a self-reported criterion for participant eligibility may therefore have introduced potential selection bias and may limit the generalizability of the findings to the broader population of

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children with unilateral caries, particularly those who do not report chewing difficulty. Furthermore, the absence of an objective functional measure prevented direct evaluation of whether longitudinal changes in muscle characteristics were associated with changes in masticatory function following restorative treatment. The absence of an untreated comparison group also limits causal attribution of the observed temporal changes specifically to restorative treatment. Finally, ongoing craniofacial growth and the relatively homogeneous caries severity of the study population may also have influenced the magnitude of the observed longitudinal changes.

Despite these limitations, the present study provides novel longitudinal evidence that selected elastographic characteristics of the masseter muscle may change over time even in the absence of measurable morphological changes. Rather than establishing early functional adaptation, these findings suggest that SE may provide complementary information beyond conventional ultrasonographic assessment of muscle thickness. Future longitudinal studies integrating elastography with objective functional assessments, such as bite force, masticatory performance and surface electromyography, are warranted to further clarify whether these elastographic changes correspond to alterations in masticatory function or neuromuscular activity and to determine their clinical significance.

5 | Conclusion

This prospective clinical study found no detectable short-term changes in the thickness of the masseter, temporalis or anterior digastric muscles of children during a 6 months follow-up after restorative treatment of unilateral dental caries. In contrast, strain elastography identified temporal variation in selected elastographic characteristics of the masseter muscle on the non-carious side, with the principal post hoc finding showing a moderate effect magnitude.

These findings indicate that longitudinal changes in elastographic characteristics may occur despite stable muscle thickness. However, the observed changes should not be interpreted as evidence of treatment-induced functional adaptation or as demonstrating that biomechanical changes precede morphological remodelling. Although strain elastography may provide information complementary to conventional ultrasonographic thickness assessment, its clinical significance in this context remains uncertain and further validation is required before its routine clinical implementation can be considered.

Author Contributions

Mesude Demiroğlu: conceptualization, methodology, investigation, formal analysis, writing – original draft, data curation. **Hakan Eren:** conceptualization, data curation, formal analysis, visualization, writing – review and editing, supervision, methodology, investigation, software, validation. **Berkant Sezer:** conceptualization, data curation, formal analysis, visualization, writing – original draft, writing – review and editing, project administration, supervision, investigation, methodology.

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The authors have nothing to report.

Ethics Statement

The study protocol was approved by the Clinical Research Ethics Committee of Çanakkale Onsekiz Mart University (approval date: November 8, 2023; decision number: 2023–168). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Consent

Written informed consent was obtained from the parents or legal guardians of all participants and verbal assent was obtained from the children.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

For transparency, the peer review documents associated with this article are available at https://doi.org/10.1111/joor.70362.

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