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ORIGINAL RESEARCH

EVALUATION OF SALIVARY TGF-B1 AS A GENETIC BIOMARKER IN SKELETAL CLASS III MALOCCLUSION WITH MANDIBULAR PROGNATHISM- A PILOT STUDY

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ABSTRACT

Background: Skeletal Class III malocclusion with mandibular prognathism presents diagnostic and treatment challenges due to its complex and variable growth patterns. Transforming Growth Factor Beta-1 (TGF-β1) is a key regulator of bone remodelling and craniofacial development, and salivary detection offers a non-invasive means of monitoring its activity.
Aim: To evaluate salivary TGF-β1 levels in adolescents with skeletal Class III malocclusion and mandibular prognathism, exploring its potential as a biological growth marker.
Ma**terials and Methods:**A cross-sectional study was conducted on adolescents clinically and cephalometrically diagnosed with skeletal Class III malocclusion due to mandibular prognathism. Unstimulated saliva samples were collected using the passive drool technique, processed, and stored at –80°C. TGF-β1 concentrations were quantified using an ELISA assay, with all samples analysed in duplicate.
Results: Salivary TGF-β1 concentrations showed substantial inter-individual variation, ranging from 595.2 ng/L to 2,376.0 ng/L. The mean value was 1,470.96 ng/L, with a median of 1,572.0 ng/L. The standard deviation of 593.30 ng/L indicated a wide spread of values, reflecting biological variability among participants.
Conclusion: Salivary TGF-β1 levels vary considerably among adolescents with skeletal Class III malocclusion and mandibular prognathism, likely reflecting individual differences in skeletal growth activity. Saliva-based testing may serve as a practical, non-invasive adjunct to conventional orthodontic diagnostics, potentially aiding in the prediction of growth trends and timing of interventions.

Keywords**:** Skeletal Class III malocclusion, mandibular prognathism, TGF-β1, salivary biomarkers, orthodontic growth prediction

INTRODUCTION

Craniofacial growth and development are complex biological processes shaped by the interaction of genetic, epigenetic, and environmental influences. Among the many dentofacial anomalies, skeletal Class III malocclusion remains one of the most challenging to fully understand, largely because of its varied causes and inconsistent clinical features. This malocclusion typically presents with mandibular prognathism, maxillary underdevelopment, or a combination of both, producing a concave facial profile and functional difficulties that often

require early corrective measures [1,2].

Within these patterns, mandibular prognathism is frequently the dominant feature of skeletal Class III and is well known for its strong familial occurrence, pointing to a possible hereditary basis [2,3]. Genetic factors in craniofacial form have been extensively explored, with numerous studies linking specific genes to mandibular size, positioning, and condylar growth behavior [3,4]. While cephalometric analysis remains useful for clinical categorisation, it provides little insight into the molecular and genetic processes driving these skeletal traits [5].

The recent integration of molecular biology into orthodontic research has expanded diagnostic possibilities. Of particular interest is Transforming Growth Factor Beta-1 (TGF-β1), a multifunctional cytokine with a central role in skeletal tissue turnover, cartilage formation, and bone matrix development [6]. TGF-β1 regulates osteoblast proliferation, stimulates extracellular matrix production, and supports the conversion of mesenchymal cells into bone-forming lineages — processes critical for mandibular growth [6,7]. Any imbalance in this pathway may alter mandibular structure and contribute to abnormal skeletal patterns such as prognathism [8,9].

TGF-β1 is expressed at several craniofacial sites, including the mandibular condyle, alveolar bone, and midface sutures, reflecting its importance in postnatal bone growth and structural adaptation to mechanical forces [6,9]. Experimental work has shown that increased TGF-β1 activity can enhance bone deposition, especially in regions under mechanical stress, reinforcing its possible role in the development of mandibular prognathism [10].

Traditionally, blood and tissue have been the primary sources for biomarker studies, but saliva has emerged as a practical, non-invasive alternative for molecular diagnostics. Saliva contains a diverse range of bioactive compounds — including growth factors, hormones, and cytokines — many of which closely parallel systemic physiological and pathological conditions [11]. It has been used successfully across medical disciplines and is gaining attention in orthodontics as a means of predicting growth changes and treatment responses [12].

Salivary biomarker testing offers particular advantages in young, growing patients, for whom invasive collection methods may be less acceptable. Elevated salivary TGF-β1 has previously been reported in periodontal and bone-related conditions, where it is linked to heightened osteoclastic and osteoblastic activity [13]. Despite these findings, its role in skeletal malocclusion, and specifically in cases with pronounced mandibular prognathism, remains largely unexplored.

Identifying the expression pattern of TGF-β1 in individuals with skeletal Class III malocclusion may shed light on its underlying genetic mechanisms and support the creation of targeted diagnostic and therapeutic approaches. Such molecular insights could complement conventional assessments, enabling orthodontists to better predict growth direction and determine the ideal timing for orthopedic or surgical treatment [13].

Therefore, this study aims to evaluate salivary TGF-β1 levels in adolescents diagnosed with skeletal Class III malocclusion, specifically those with mandibular prognathism. By recognising specific expression profiles, we aim to explore TGF-β1's potential as a dependable, non-invasive biomarker for predicting skeletal growth patterns and informing early orthodontic planning.

MATERIALS AND METHODS

Study Design and Ethical Considerations

This pilot study was conducted following a cross-sectional design to investigate the levels of salivary Transforming Growth Factor Beta 1 (TGF-β1) in individuals diagnosed with skeletal Class III malocclusion exhibiting mandibular prognathism. Prior to sample collection, ethical clearance was obtained from the institutional review board. Informed consent was secured from all participants or their guardians after thoroughly explaining the study purpose and procedures.

Participant Selection

A total of ten adolescent individuals were recruited from an orthodontic outpatient unit based on clinical and radiographic confirmation of skeletal Class III malocclusion. All selected participants presented with a prominent mandibular growth pattern and a negative ANB angle suggestive of prognathism. Inclusion criteria comprised growing individuals aged between 12 and 17 years, with no history of orthodontic or orthopedic intervention. Subjects with systemic illnesses, syndromic craniofacial anomalies, acute oral infections, or salivary gland dysfunction were excluded to eliminate confounding influences on salivary biomarker expression.

Saliva Sample Collection Protocol

Saliva collection was carried out under controlled conditions to ensure sample consistency and accuracy. Unstimulated whole saliva was collected using the passive drool technique. Participants were asked to refrain from eating, drinking, or performing oral hygiene

procedures for at least 90 minutes before sample collection. To maintain diurnal consistency, all samples were obtained in the morning hours between 9:00 AM and 11:00 AM in a quiet room with the participant seated upright, head tilted slightly forward, and instructed to allow saliva to pool naturally before expelling it into a sterile collection tube. A minimum volume of 2 mL was collected per subject.

Immediately after collection, samples were placed on ice and transported to the laboratory for preliminary processing. They were centrifuged at 3000 revolutions per minute (rpm) for 20 minutes at 4°C to separate the clear supernatant from debris and cellular components. The resulting supernatant was carefully aliquoted and stored at –80°C until biochemical analysis was performed.

Quantification of TGF-β1 Using ELISA

The concentration of salivary TGF-β1 was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (BT Lab® Human TGF-β1, Cat. No. E0134Hu), which operates on the sandwich ELISA principle. The assay utilized pre-coated wells with anti-human TGF-β1 antibodies, enabling specific binding of the cytokine present in the sample.

All reagents and samples were equilibrated to room temperature prior to assay initiation. The assay began with the preparation of a standard curve using serial dilutions of the provided TGF-β1 standard solution to achieve concentrations of 150, 300, 600, 1200, and 2400 ng/L. Each standard and saliva sample was assayed in duplicate to ensure intra-assay reliability.

For the test wells, 40 µL of each saliva sample was pipetted into the appropriate wells, followed by the addition of 10 µL of biotin-conjugated anti-TGF-β1 antibody. Subsequently, 50 µL of streptavidin–horseradish peroxidase (HRP) conjugate was added to facilitate signal amplification. The microplate was sealed and incubated at 37°C for 60 minutes in a humidified chamber.

Following incubation, the wells were washed five times using a pre-diluted wash buffer to remove unbound reagents. A 1:1 mixture of substrate solutions A and B was then dispensed (50 µL each), and the plate was incubated in the dark at 37°C for 10 minutes to allow color development. The enzymatic reaction was terminated by adding 50 µL of stop solution to each well, changing the color from blue to yellow.

The optical density (OD) of each well was immediately recorded using a microplate reader set to a wavelength of 450 nm. The TGF-β1 concentration for each sample was extrapolated from the standard calibration curve using regression analysis. Only data from assays with intra-assay coefficient of variation (CV) values under 10% were accepted to ensure assay precision.

RESULTS

The present study aimed to evaluate salivary concentrations of Transforming Growth Factor Beta 1 (TGF-β1) in adolescents diagnosed with skeletal Class III malocclusion characterized by mandibular prognathism. Quantitative analysis of the cytokine was performed using an ELISA-based approach, with concentrations inferred from the standard curve constructed using known values ranging from 0 to 2400 ng/L.

Overview of Data Distribution

All ten participants successfully provided unstimulated saliva samples, and the ELISA analysis yielded definitive optical density (OD) readings across the group. These OD values were translated into actual TGF-β1 concentrations using the linear region of the assay's standard curve, demonstrating reliable sensitivity within the tested range. Figure 1

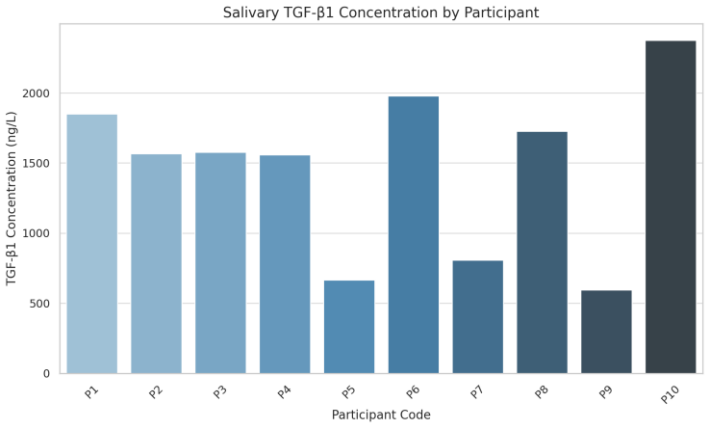

Figure 1. Salivary TGF-β1 Concentration of the Participants-This bar graph displays the TGF-β1 concentration for each participant individually. Each bar corresponds to a single subject, highlighting inter-individual differences in salivary expression levels.

The calculated salivary TGF-β1 concentrations exhibited marked variability among participants, with values spanning from **595.2 ng/L** to **2376.0 ng/L**. This dispersion underscores the biological heterogeneity in cytokine expression, even among individuals presenting w

ith a similar skeletal malocclusion phenotype.

A summary of the data is presented in **Table 1**, which outlines the OD values along with the corresponding TGF-β1 concentrations for each anonymized participant.

Table 1. Salivary OD and Corresponding TGF-β1 Concentrations (ng/L)

Participant Code	OD Value	TGF-β1 Concentration (ng/L)
P1	0.771	1850.4
P2	0.653	1567.2
P3	0.657	1576.8
P4	0.650	1560.0
P5	0.278	667.2
P6	0.825	1980.0
P7	0.337	808.8
P8	0.720	1728.0
P9	0.248	595.2
P10	0.990	2376.0

This table presents for each participant, their corresponding optical density (O D) readings obtained through ELISA, and the calculated TGF-β1 concentrations in nanograms per liter (ng/L).

TABLE 2. This table details the measured salivary TGF-β1 concentrations (ng/L) for each participant, the deviation of each value from the calculated mean, and the squared deviation values. These calculations form the basis for determining the variance and standard deviation of the dataset.

Participant Code	TGF-β1 Concentration (ng/L)	Deviation from Mean	Squared Deviation
P1	1850.4	379.44	143,974.71
P2	1567.2	96.24	9,262.14
P3	1576.8	105.84	11,202.11
P4	1560.0	89.04	7,928.12
P5	667.2	-803.76	646,030.14
P6	1980.0	509.04	259,121.72
P7	808.8	-662.16	438,455.87
P8	1728.0	257.04	66,069.56
P9	595.2	-875.76	766,955.58
P10	2376.0	905.04	819,097.40

Descriptive Statistics and Data Characteristics

The mean TGF-β1 concentration among the study population was calculated to be 1470.96 ng/L, indicating a moderately elevated baseline across the sample. The median value, which represents the central tendency unaffected by extremes, was 1572.0 ng/L, slightly above the mean—suggesting a mild left skew in the dataset. The range of concentrations extended from a minimum of 595.2 ng/L to a maximum of 2376.0 ng/L, reflecting a broad biological variation.

The standard deviation was 593.30 ng/L, denoting considerable spread from the mean. Such dispersion likely corresponds to differences in intrinsic genetic signaling, metabolic activity, and possibly, individual growth stages.Table 2

Expression Trends Among Participants

Notably, three participants exhibited very high TGF-β1 levels exceeding 1800 ng/L, suggesting a potential upregulation of osteogenic or remodeling activity. One participant (P10) demonstrated a peak concentration of 2376.0 ng/L, nearing the upper detection range of the assay kit. In contrast, the lowest cytokine levels were observed in P9 (595.2 ng/L) and P5 (667.2 ng/L), indicating suppressed or baseline-level activity of TGF-β1 within the oral environment.

Participants with concentrations between 1500–1800 ng/L represented the mid-range of the expression spectrum and constituted the largest subgroup, pointing toward a common cytokine activity profile in skeletal Class III individuals with active mandibular remodeling.

These observed trends may suggest differential cytokine regulation within a seemingly homogenous clinical phenotype, emphasizing the potential of salivary TGF-β1 as a stratifying biomarker for skeletal maturity, remodeling rate, or future growth direction.

DISCUSSION

The present study examined salivary TGF-β1 levels in adolescents with skeletal Class III malocclusion and mandibular prognathism, revealing a wide variation among individuals. Such variability suggests that TGF-β1 is highly dynamic, likely reflecting the differences in tissue growth activity, cellular signalling, and developmental stage unique to each patient.

Earlier experimental work has shown that even modest changes in TGF-β1 activity can have significant biological effects. Hall and colleagues [14], using a mouse model with targeted overexpression of TGF-β1 in salivary glands, found that excessive production of this cytokine led to fibrosis, disrupted gland architecture, and reduced saliva output. Although our participants did not have pathological salivary changes, these findings underline how sensitive glandular tissues are to variations in TGF-β1 levels.

Fibrotic changes in salivary glands have been further reviewed by Andraščíková et al. [15], who described TGF-β as a major driver of excessive extracellular matrix production, leading to stiffness and loss of normal function. In the context of craniofacial growth, similar signalling could influence connective tissues and bone surrounding the mandible, subtly modifying growth patterns.

TGF-β1 signalling is not uniform across all cell types. Muñoz Forti and co-workers [16] highlighted that epithelial cells, fibroblasts, immune cells, and vascular cells each respond differently to TGF-β cues, leading to diverse functional outcomes. This cell-specific variability may explain why, in our study, certain individuals showed much higher salivary TGF-β1 values than others despite having the same clinical diagnosis.

The influence of TGF-β1 begins early in life. Jaskoll and Melnick [17] demonstrated that excessive TGF-β1 in developing mouse salivary glands reduced normal branching patterns, affecting organ structure. Although our research involved adolescents rather than developing embryos, the same principle of growth modulation by TGF-β1 likely applies to jaw and facial development.

Recent research has explored how this pathway might be modulated for therapeutic purposes. Zhang et al. [18] reported that extracellular vesicles derived from saliva could reduce TGF-β-driven fibrosis in experimental models, suggesting a possible future strategy for controlling unwanted tissue changes. Lee et al. [19] and Kim et al. [20] also found that blocking TGF-β receptor signalling in cultured salivary gland progenitor cells altered their differentiation patterns — an approach that might one day help guide tissue regeneration.

TGF-β1's role extends beyond glandular tissue to skeletal biology. Tang and colleagues [22] discovered that TGF-β1 released during bone resorption attracts bone marrow–derived mesenchymal stem cells to the site, where they differentiate into bone-forming cells. This process couples bone breakdown with new bone formation — a mechanism that could directly influence jaw growth in Class III malocclusion.

Further evidence of TGF-β1's involvement in craniofacial growth comes from studies by Ueki et al. [23], who found that periosteum — a key tissue in bone growth — responds strongly to TGF-β signalling during mandibular

distraction osteogenesis in rabbits. Similarly, Massagué [24] detailed how TGF-β interacts with multiple molecular pathways, adjusting its function according to the surrounding cellular environment.

In skeletal repair and development, Liu et al. [25] and Tuli et al. [26] reported that TGF-β1 works in concert with other growth factors such as BMP-2 to promote the production of bone-specific proteins and cartilage differentiation, further emphasising its central role in shaping skeletal structures.

Finally, Kaufman and Lamster [27] reviewed the clinical value of saliva as a diagnostic fluid, stressing that it reflects systemic physiological and pathological states in a way that is both reliable and non-invasive. This supports the practical advantage of using salivary TGF-β1 testing in orthodontics, particularly for younger patients where blood sampling or repeated imaging is less desirable.

Taken together, these findings suggest that salivary TGF-β1 is more than just a passive reflection of systemic processes — it may actively mirror and possibly influence craniofacial growth activity. The variability we observed could correspond to different phases of mandibular growth or responses to mechanical forces in the masticatory system. Longitudinal studies are now needed to track these levels over time and clarify their potential as a predictive tool in orthodontic diagnosis and treatment planning.

CONCLUSION

This study demonstrated that salivary TGF-β1 levels in adolescents with skeletal Class III malocclusion and mandibular prognathism show marked individual variation. These differences likely reflect unique biological rhythms of growth, bone remodelling, and tissue adaptation occurring in each patient. Those with higher concentrations may be experiencing more active skeletal changes, while lower levels could indicate a period of relative stability.

The non-invasive nature of saliva collection makes it particularly suitable for growing patients, offering a simple and well-tolerated method to monitor biological markers over time. If validated by larger and longitudinal studies, salivary TGF-β1 could become a valuable addition to conventional orthodontic assessment tools, helping clinicians to anticipate growth direction, choose optimal treatment timing, and personalise intervention strategies.Our findings highlight the potential of combining molecular diagnostics with traditional orthodontic evaluation, paving the way for more precise, biology-driven treatment planning in skeletal Class III malocclusion.

DECLARATIONS

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Competing Interests:

The authors have no competing interests to declare.

Ethical Approval:

The study was approved by the appropriate ethics committee and conducted according to relevant guidelines and regulations.

Informed Consent:

Not applicable.

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