RESEARCH ARTICLE

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Deciphering the Predictive Utility of N-telopeptide (NTx) and C-telopeptide (CTx) for Early Bony Invasion in Oral Squamous Cell Carcinoma (OSCC)

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Abstract

Background: Early bony invasion in oral squamous cell carcinoma (OSCC) often remains undetected by conventional CT imaging. Bone turnover markers like N-telopeptide (NTx) and C-telopeptide (CTx) may serve as sensitive biomarkers for subclinical bone involvement. **Objective:** To evaluate the potential of serum NTx and CTx levels as diagnostic markers for early bony invasion in OSCC patients whose CT scans showed no radiological evidence of bone involvement. **Methods:** A prospective observational study was conducted on 50 OSCC patients with negative CT reports for bone invasion. Serum NTx and CTx levels were measured using ELISA. Based on biomarker levels, patients were categorized into two groups: Group A (elevated NTx and/or CTx) and Group B (normal levels). Statistical comparisons, ROC curve analysis, and logistic regression were employed to assess the diagnostic potential of these biomarkers. **Results:** Group A (n = 25) showed significantly elevated levels of serum NTx (mean ± SD: 20.4 ± 4.1 nM BCE) and CTx (860 ± 130 ng/L) compared to Group B (NTx: 11.5 ± 2.9 nM BCE; CTx: 540 ± 110 ng/L), with $p < 0.001$. ROC analysis (receiver operating characteristic) revealed AUCs (area under the curve) of 0.902 for NTx and 0.928 for CTx, suggesting excellent diagnostic accuracy. Logistic regression confirmed serum NTx and CTx as independent predictors of probable microinvasion. A subgroup analysis based on tumor histopathology revealed NTx to be more sensitive in moderately differentiated tumors, while CTx was more sensitive in poorly differentiated tumors. **Conclusion:** Elevated serum levels of NTx and CTx in OSCC patients with negative CT scans may indicate early bony microinvasion. Incorporating biomarker screening into diagnostic protocols could enhance treatment planning and reduce recurrence.

Keywords: Bone biomarker- N-telopeptide- C-telopeptide- Early invasion

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Introduction

Oral squamous cell carcinoma (OSCC) is a prevalent malignancy, particularly in developing countries. A crucial prognostic factor in OSCC is bone invasion, which influences surgical margins, treatment decisions, and overall prognosis. Traditionally, bone involvement is assessed using imaging modalities such as computed tomography (CT), which, although effective, may fail to detect subtle or early microinvasion [1]. This limitation poses a risk of under treatment, potentially leading to recurrence.

Bone resorption markers such as N-telopeptide of type I collagen (NTx) and C-telopeptide (CTx) are degradation products of bone matrix and reflect osteoclastic activity [2]. Elevated serum or urinary levels of these telopeptides are indicative of increased bone turnover and have been explored in various metabolic bone disorders and malignancies with skeletal involvement [3]. In prostate [4] and breast [5] cancer, for instance, these markers have demonstrated utility in detecting bone metastasis even before radiological changes appear. These bone turnover markers are predictors of mortality risk in cancer patients with bone metastasis but yet the idealistic behavior of N-telopeptide and C-telopeptide in predicting bony invasion in the initial stages of oral cancer is poorly recognized, despite their significant potential utility [6].

This study explores the hypothesis that elevated levels of these biomarkers in OSCC patients with negative CT scans may reflect subclinical bone invasion, prompting earlier intervention and pursues to contribute for the development of more valuable diagnostic surgical and prognostic strategies. The findings of this study have the potential to shift the rationale of the management of oral cancer with negative CT findings for bony involvement, ultimately improving patient outcomes and quality of life.

Materials and Methods

Study Design and Participants: A prospective,

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observational study was conducted in the Cranio-Maxillo-Facial surgery unit of Indira Gandhi Institute of Medical Sciences in collaboration with Department of Biochemistry, from January 2024 to January 2025. Fifty patients with histologically confirmed OSCC and no radiological evidence of bone invasion on CECT were enrolled. Histopathologically confirmed OSCC cases with negative CECT report for bony invasion were included in this study and this choice was made to specifically evaluate markers for potential micro-invasion. Patients with overt CT-documented bone invasion were excluded along with patient's with prior history of skeletal metastasis, metabolic bone diseases, chronic renal or hepatic disease and current bisphosphonate or steroid therapy.

Grouping: Patients were divided into Group A (n = 25) with elevated serum NTx and/or CTx levels beyond standard reference range and Group B (n = 25) with normal serum NTx and CTx levels

Biochemical Analysis: Serum samples were collected and stored at -80°C. NTx and CTx were measured using enzyme-linked immunosorbent assay (ELISA). Serum levels of N-telopeptide (NTx) and C-telopeptide (CTx) were measured using commercially available ELISA kits according to the manufacturer's protocol. Both assays were based on the competitive enzyme-linked immunosorbent assay principle. Reference values were based on manufacturer-provided cut-offs.

Outcome definitions: The primary outcome analyzed in this study was elevated bone-turnover marker level (NTx or CTx above the ROC-derived threshold), therefore elevated markers are interpreted in this manuscript as potential indicators of micro-invasion that require prospective confirmation.

Statistical Analysis: Data were analyzed using SPSS v25. Independent t-tests compared biomarker levels between groups. Receiver Operating Characteristic (ROC) curves assessed diagnostic accuracy. Logistic regression evaluated the predictive value of biomarkers. $p < 0.05$ was considered statistically significant.

Results

Fifty OSCC patients with no radiologic evidence of bony invasion on CT were enrolled and categorized into two groups based on serum biomarker levels. Group A (n = 25) comprised patients with elevated NTx and/or CTx levels, while Group B (n = 25) had normal biomarker levels. Baseline demographic data showed no statistically significant differences between the groups in terms of age or gender distribution (Table 1).

Both NTx and CTx levels were significantly higher in Group A compared to Group B (Table 2). Figure 1A shows the box plot comparison of serum NTx levels between the two groups. The interquartile range is significantly shifted upward in Group A, indicating consistently elevated bone resorption. Figure 1B similarly depicts the distribution of serum CTx, with Group A showing higher values and less overlap with Group B, further supporting the hypothesis of subclinical bone involvement.

Receiver Operating Characteristic (ROC) curves were constructed to evaluate the diagnostic accuracy of NTx and CTx in detecting possible microinvasion Table 3. Figure 2 displays the ROC curves for both biomarkers. The steep curves and high AUC values indicate excellent diagnostic capability. CTx performed marginally better than NTx,

Table 1. Patient Demographics and Clinical Characteristics

Variable	Group A (n = 25)	Group B (n = 25)	p value
Age (mean ± SD)	57.2 ± 8.5	55.6 ± 9.8	0.48
Gender (M:F)	15:10	14:11	0.78

Table 2. Biomarker Levels in Study Groups

Biomarker	Group A (Mean ± SD)	Group B (Mean ± SD)	p value
NTx (nM BCE)	20.4 ± 4.1	11.5 ± 2.9	<0.001
CTx (ng/L)	860 ± 130	540 ± 110	<0.001

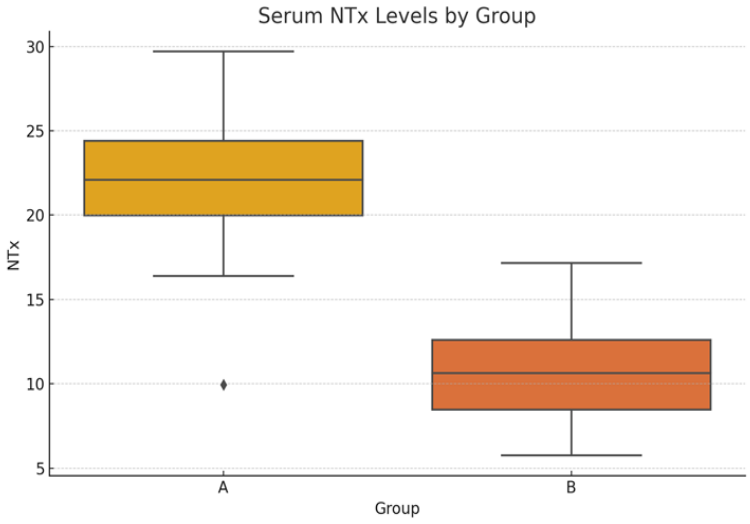

Figure 1A. Box plot Comparison of Serum NTx Levels between the Two Groups

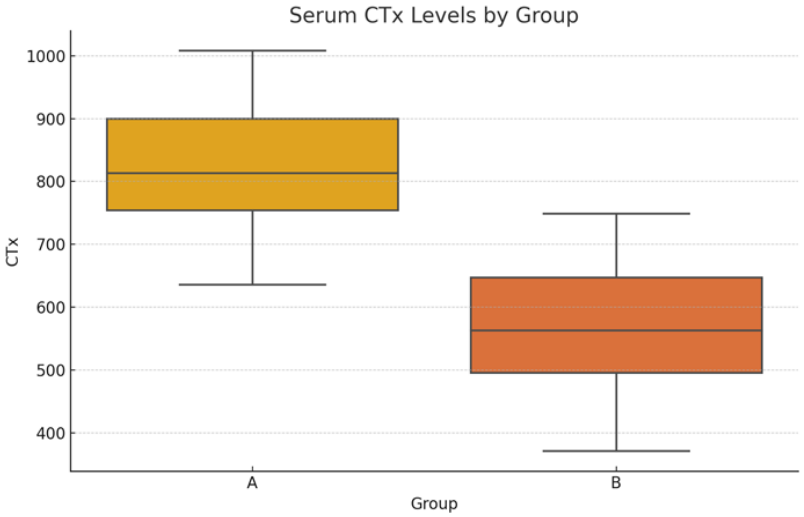

Figure 1B. Box Plot Comparison of Serum CTx Levels between the Two Groups

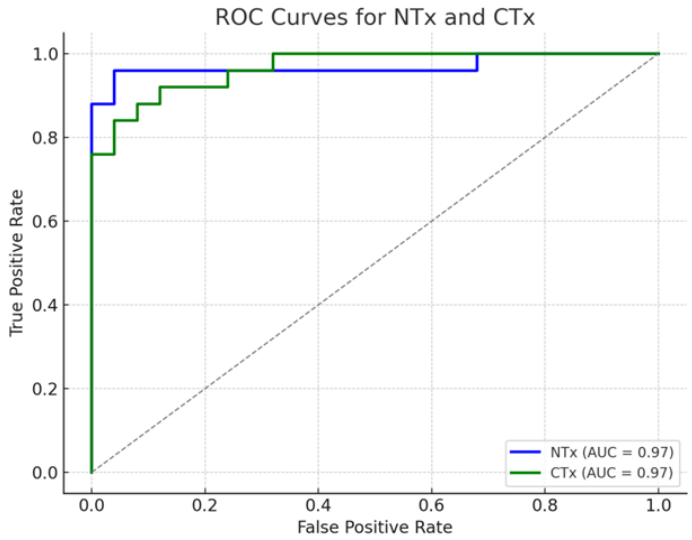

Figure 2. ROC Curves Comparing Diagnostic Performance of NTx and CTx

Table 3. ROC Analysis with AUC (Area under Curve), Optimal Cut off, Sensitivity & Specificity of NTx and CTx

Biomarker	AUC	Cut off	Sensitivity	Specificity
NTx	0.902	15.8 nM BCE(Nanomolar bone collagen equivalents)	88%	84%
CTx	0.928	655 ng/L(Nanograms per liter)	92%	80%

though both markers were highly accurate.

In Group A, 72% had both markers elevated, 16% had isolated NTx elevation, and 12% had isolated CTx elevation. This suggests a synergistic role of both markers in detecting bone turnover activity associated with early invasion. Figure 3 illustrates the percentage of patients in each group with elevated NTx, CTx, or both Binary logistic regression was performed to assess the predictive value of elevated biomarkers NTx showed a stronger association with potential microinvasion with an Odds ratio of 1.19[(95% CI: 1.10–1.30), $p < 0.001$], in comparison to CTx with an Odds ratio of 1.01[(95% CI: 1.00–1.01), $p < 0.01$] (Table 4).

An exploratory subgroup analysis based on tumor histopathology revealed variations in biomarker elevation relative to tumor differentiation. Moderately differentiated OSCC cases (n = 22) demonstrated the highest mean of NTx levels (21.8 ± 3.9 nM BCE), followed by poorly

Table 4. Logistic Regression Showing Odds Ratios for Biomarkers

Biomarker	Odds ratio(OR)	Interpretation
NTx	1.19	Increased odds of bony invasion
CTx	1.01	No significant association (neutral)

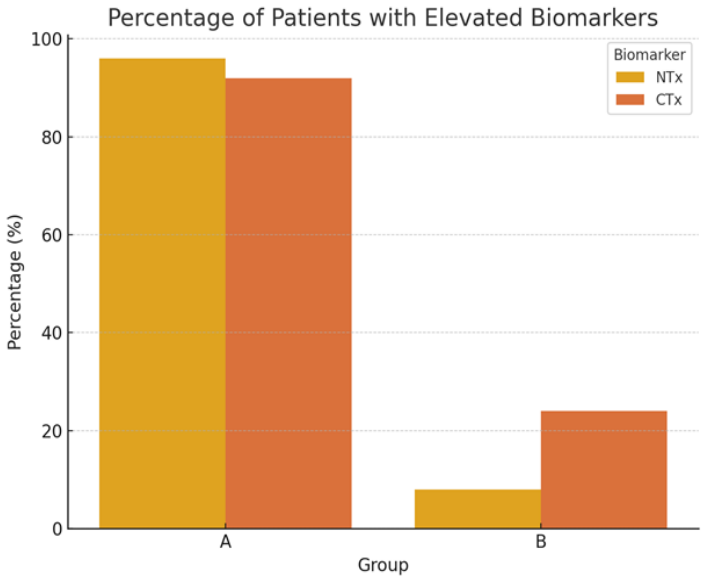

Figure 3. Percentage of Patients in Each Group with Elevated Biomarkers

differentiated OSCC (20.1 ± 3.5 nM BCE) and well-differentiated OSCC (17.2 ± 2.8 nM BCE), whereas In contrast, CTx levels were more markedly elevated in poorly differentiated OSCC (890 ± 115 ng/L), compared to moderately differentiated (865 ± 135 ng/L) and well-differentiated types (805 ± 105 ng/L) (Figure 4).

Discussion

Despite the absence of CT-detectable bony invasion, a substantial proportion of OSCC patients demonstrated elevated levels of serum NTx and CTx. These findings suggest a possible occurrence of microinvasion, undetectable by conventional imaging. Elevated NTx and CTx are indicative of active bone turnover and resorption, potentially triggered by early tumor infiltration into cortical or medullary bone. Some of recent studies like meta-analysis by Li et al. (2023) evaluated the diagnostic and prognostic value of N-telopeptide (NTx), a bone resorption marker, in detecting bone metastasis across various human cancers. The study analyzed data from multiple clinical studies and found that elevated NTx levels were significantly associated with the presence of bone metastasis. Additionally, higher NTx levels correlated with poorer prognosis, suggesting its potential role in predicting disease progression. The authors concluded that NTx could serve as a useful non-invasive biomarker for both diagnosing and monitoring bone metastasis in cancer patients. Consequently, elevated NTx/CTx in our cohort should be regarded as a signal of possible bone involvement rather than proof of metastasis. Future prospective studies that include paired advanced imaging or histological sampling will be required to establish how well these biomarkers predict confirmed

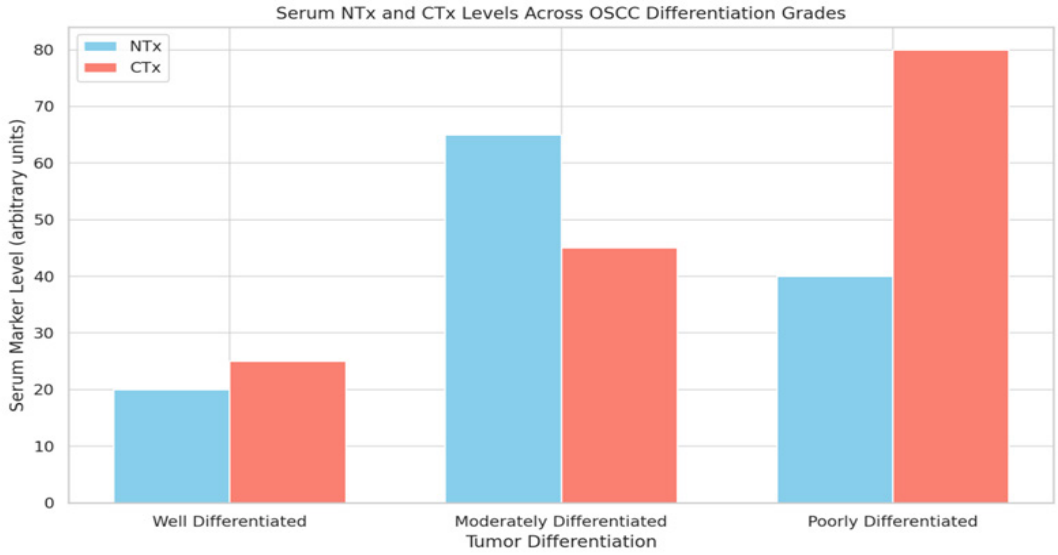

Figure 4. Differential Expression of Serum NTx and CTx Across Histological Grades of OSCC

bony invasion.

Clézardin et al. [7] provide a comprehensive review of the mechanisms underlying bone metastasis, particularly focusing on how cancer cells interact with the bone microenvironment to promote osteolysis or osteogenesis. Furthermore, it discusses a wide range of biomarkers like telopeptides both diagnostic and prognostic, that reflect bone turnover and tumor activity. The review emphasizes the importance of integrating mechanistic insights with biomarker development to improve the diagnosis, monitoring, and treatment of bone metastasis. Similarly Iuliani et al. [8] described both established and emerging biomarkers that can predict the development of bone metastasis in cancer patients and emphasized the potential of combining multiple biomarkers mainly NTx and CTx to improve early detection and risk assessment.

In another recent study by Gentile et al. [9] , authors have highlighted the importance of identifying biomarkers like NTx and CTx, capable of stratifying patients by their risk of developing bone metastasis and found that it is essential for establishing personalized diagnostic and therapeutic approaches, ideally at the earliest stages of disease. In this context, the emergence of "omics" technologies has accelerated the discovery of potential biomarkers associated with osteotropism, including dysregulated genes, proteins, and micro RNAs. Equivalently, Song et al. [10] focused on key circulating biomarkers mainly NTx and CTx that have potential utility in the diagnosis and therapeutic monitoring of bone metastasis. The article highlights how these non-invasive markers can provide real-time insights into bone remodeling dynamics and tumor–bone interactions, aiding in early detection, prognosis, and treatment response.

Many other research supports this hypothesis as indicated by studies like, Coleman et al. [11] , Yang et al. [12] , Jiang et al. [13], Bhadresha et al. [14] and Kanak et al. [15] emphasized the utility of bone resorption markers in malignancies with skeletal metastasis. Similarly, Galliera [16] highlighted their application in cancer-induced bone disease and how bone markers have improved greatly in terms of sensitivity and specificity and could be useful for an early diagnosis of bone metastasis.

More specifically, Elaasser et al. [3] presented a comprehensive review of bone metastasis that emphasized the dynamic role of both imaging techniques and biomarker analysis like collagen telopeptides in primitive detection of bone metastasis. Supporting our study, a recent research paper by Liu et al. [17], underscores the critical role of osteoclasts in bone metastasis and highlights several biomarkers—from collagen degradation products (CTX, NTX) and osteoclast activity markers (TRACP 5b) to matrix proteins and ncRNAs that may serve for diagnosis, monitoring, and therapeutic targeting.

In our study, ROC curve analysis demonstrated high AUC values for both biomarkers, and logistic regression confirmed their predictive value. These findings align with prior literature in prostate and breast cancers where bone markers preceded radiologic evidence. In OSCC, this could translate into earlier surgical planning, potentially involving segmental resection, even if CT appears normal.

A subgroup analysis further revealed that serum NTx was particularly elevated in moderately differentiated OSCC, potentially reflecting balanced tumor-induced osteoclastic activity that is robust yet structured. Conversely, CTx was highest in poorly differentiated OSCC, consistent with the aggressive nature and greater bone turnover seen in high-grade malignancies. This pattern supports the hypothesis that NTx may serve as a marker of early and organized bone degradation, while CTx could indicate more advanced or chaotic osteolysis associated with poorly differentiated tumors. This histological distinction aligns with earlier work by Orita et al. [18], who reported that bone marker expression can vary with tumor grade in head and neck cancers. Tandon [19] has highlighted the need for integrating histological profiling with biomarker levels may thus refine prognostication and tailor surgical strategies more precisely.

In our study, Logistic regression analysis revealed that NTx had an odds ratio of approximately 1.19, indicating a positive association with early bony invasion in oral cancer. This suggests that higher NTx levels may be linked to increased odds of bone involvement, even when conventional imaging such as CT scans shows no evidence of invasion. In contrast, CTx showed an odds ratio close to 1.01, implying minimal or no predictive value in this context. These findings support the potential utility of NTx as a more reliable biomarker for detecting early or micro invasive bone changes that may not be radiologically evident.

However, our study may have limitations due to single-center design and limited sample size which also calls for caution in generalizing results. Because we restricted enrolment to CT-negative patients, the study population represents a narrower clinical spectrum and may not reflect marker performance in patients with more advanced or radiographically evident bone invasion. This spectrum bias limits the generalizability of sensitivity/specificity estimates to broader clinical populations; subsequent studies should include a full spectrum of disease (including CT-positive patients and healthy controls) and perform external validation.

Future research should include multicentric trials with intraoperative bone margin histology and long-term follow-up to assess recurrence and survival outcomes.

Hence to conclude, serum NTx and CTx are promising non-invasive biomarkers for detecting early bony invasion in OSCC patients with negative CECT findings. Incorporating these markers into diagnostic workflows may aid in optimizing treatment and improving patient outcome.

Author Contribution Statement

Priyankar Singh: Lead Researcher, Patient Recruitment, Data Collection, Manuscript Drafting. Nimmi Singh: Data Analysis, Statistical Interpretation, Tables & Tures. Ravi Shekhar: ELISA Testing, Lab Coordination, Biomarker Data Management. Rekha Kumari: ELISA Testing, Lab Coordination, Biomarker Data Management. Swati Singh: Supervision, Conceptual Oversight, Final Approval.

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None.

Ethical clearance

Ethical clearance was obtained from Institute's ethical committee of Indira Gandhi Institute of Medical Sciences, Patna

Conflict of interest

None.

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