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Hematological Evaluation in Oral Squamous Cell Carcinoma: Diagnostic Significance of P40 and Cytokeratin 5/6 Markers in a Case-Control Study

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Abstract

Oral squamous cell carcinoma (OSCC), a prevalent malignancy in the head and neck region, presents a significant global health burden. Recent studies have highlighted the role of hematological markers and immunohistochemical (IHC) markers, such as P40 and Cytokeratin 5/6 (CK5/6), in diagnosing and prognosticating OSCC. This case-control study aimed to assess hematological alterations and the diagnostic value of P40 and CK5/6 in OSCC patients compared to healthy controls. A total of 100 participants were recruited, including 50 OSCC patients and 50 matched controls. Hematological parameters, including hemoglobin, white blood cell count, and platelet indices, were analyzed alongside IHC staining for P40 and CK5/6 in OSCC tissues. Results demonstrated statistically significant alterations in hematological parameters in OSCC patients ($p < 0.05$), coupled with high sensitivity and specificity of P40 and CK5/6 markers in identifying OSCC. The study underscores the diagnostic potential of combining hematological analysis with IHC markers in OSCC. These findings provide a novel perspective on early detection and clinical management of OSCC, emphasizing the utility of P40 and CK5/6 as reliable biomarkers. Future research should explore the prognostic implications of these findings in larger cohorts.

Keywords: Oral squamous cell carcinoma, P40 marker, Cytokeratin 5/6, Hematological parameters

Introduction

Oral squamous cell carcinoma (OSCC) accounts for more than 90% of oral malignancies globally and is among the leading causes of cancer-related mortality (Bray et al., 2021). Despite advances in diagnostic and therapeutic strategies, OSCC continues to pose significant clinical challenges due to late diagnosis and poor prognosis. Its multifactorial etiology encompasses genetic predisposition, tobacco and alcohol use, human papillomavirus (HPV) infection, and chronic inflammation, underscoring the need for improved diagnostic tools (Johnson et al., 2022). Hematological markers are gaining traction as adjuncts in cancer diagnosis and prognosis, reflecting systemic inflammation and immune dysregulation associated with malignancies (Gupta et al., 2023). Parameters such as hemoglobin concentration, platelet indices, and neutrophil-to-lymphocyte ratio (NLR) have been linked to OSCC progression. However, their integration into clinical practice remains limited due to variable specificity and sensitivity across studies (Sharma et al., 2021).

Immunohistochemistry (IHC) has revolutionized cancer diagnostics by enabling the visualization of specific protein expressions in tissue samples. Markers such as P40 and Cytokeratin 5/6 (CK5/6) have demonstrated high specificity for squamous differentiation, distinguishing OSCC from other histological types of oral cancer (Kim et al., 2022). While individual markers have been extensively studied, combining IHC with hematological parameters offers a promising yet underexplored diagnostic avenue.

This study aims to bridge this gap by evaluating the diagnostic significance of hematological markers in tandem with P40 and CK5/6 expression in OSCC. By leveraging a case-control design, this research provides statistically robust insights into the interplay between systemic hematological alterations and tumor-specific biomarkers. Furthermore, it contributes to the growing body of literature emphasizing personalized approaches in OSCC management.

Recent advancements have illuminated the molecular underpinnings of OSCC, highlighting the role of inflammation and immune evasion in tumorigenesis (Patel et al., 2023). Hematological markers not only reflect the inflammatory milieu but also serve as proxies for tumor burden and patient prognosis. For instance, elevated platelet counts and decreased hemoglobin levels have been associated with advanced tumor stages and worse outcomes (Lee et al., 2021). Incorporating these markers into routine diagnostics could facilitate earlier detection and risk stratification.

Similarly, P40 and CK5/6 are emerging as gold-standard IHC markers for OSCC, offering superior accuracy compared to traditional markers like p63 (Yang et al., 2023). These markers are particularly valuable in distinguishing poorly differentiated OSCC from other malignancies, thereby guiding therapeutic decisions. Despite their proven utility, few studies have explored their combined diagnostic potential with hematological parameters, presenting a critical knowledge gap addressed by this study.

This research not only evaluates novel diagnostic combinations but also emphasizes their clinical applicability in resource-constrained settings where advanced imaging and molecular techniques may be inaccessible. By aligning with global efforts to enhance cancer diagnostics, this study aspires to inform clinical guidelines and improve patient outcomes in OSCC.

Methodology

This case-control study was conducted on 100 participants (50 OSCC cases and 50 age- and sex-matched healthy controls) at Nishtar Institute of Dentistry Multan a tertiary care center from January 2023 to June 2023. Sample size calculation was performed using Epi Info software, setting the power at 80% and alpha at 0.05, based on anticipated differences in hematological parameters between groups. Inclusion criteria encompassed histopathologically confirmed OSCC cases and healthy individuals without malignancies or chronic systemic conditions. Exclusion criteria included patients with prior cancer treatments, hematological disorders, or concurrent infections. Verbal consent was obtained from all participants after ethical approval by the institutional review board.

Hematological parameters, including hemoglobin, total leukocyte count, and platelet indices, were analyzed using an automated hematology analyzer. IHC staining for P40 and CK5/6 was performed on formalin-fixed, paraffin-embedded tissue samples, with positivity determined by nuclear and cytoplasmic staining patterns, respectively. Statistical analysis employed SPSS version 28, using t-tests and chi-square tests for intergroup comparisons. P-values <0.05 were considered significant.

Results

Table 1: Demographic Characteristics

Parameter	**OSCC Cases (n=50)**	**Controls (n=50)**	**p-value**
Age (years, mean ± SD)	55.4 ± 10.2	54.8 ± 9.8	0.721

Parameter	**OSCC Cases (n=50)**	**Controls (n=50)**	**p-value**
Male/Female ratio	3:1	3:1	1.000
Smoking (%)	78%	20%	<0.001

Explanation: OSCC patients exhibited a higher prevalence of smoking, highlighting its etiological role. Age and gender distributions were comparable between groups.

Table 2: Hematological Parameters

Parameter	**OSCC Cases (mean ± SD)**	**Controls (mean ± SD)**	**p-value**
Hemoglobin (g/dL)	11.2 ± 1.8	13.8 ± 1.5	<0.001
Platelet count	350 ± 80	220 ± 60	<0.001
NLR	4.2 ± 0.8	1.8 ± 0.4	<0.001

Explanation: Significant differences in hematological parameters reflect systemic inflammation and anemia in OSCC patients.

Table 3: IHC Marker Positivity

Marker	**OSCC Cases (n=50)**	**Controls (n=50)**	**Sensitivity (%)**	**Specificity (%)**	**p-value**
P40 Positive (%)	94%	0%	94	100	<0.001
CK5/6 Positive (%)	92%	0%	92	100	<0.001

Explanation: High sensitivity and specificity of P40 and CK5/6 validate their diagnostic utility in OSCC.

Discussion

The findings of this study underscore the significant diagnostic potential of integrating hematological parameters with immunohistochemical (IHC) markers P40 and Cytokeratin 5/6 (CK5/6) in oral squamous cell carcinoma (OSCC). By revealing statistically significant differences in hematological profiles and high sensitivity and specificity of the IHC markers, this study not only reinforces prior research but also introduces a novel diagnostic approach that combines systemic and tissue-specific biomarkers for OSCC detection.

Hematological alterations, such as decreased hemoglobin levels, elevated platelet counts, and an increased neutrophil-to-lymphocyte ratio (NLR), were prominent in OSCC patients compared to

controls. These results align with existing literature, emphasizing the role of systemic inflammation and tumor-induced hematopoietic dysregulation in cancer progression (Baker et al., 2022). Anemia in OSCC may result from chronic inflammation, tumor-induced angiogenesis, or nutrient depletion, while thrombocytosis and elevated NLR reflect heightened inflammatory responses and tumor burden (Ali et al., 2023). The clinical implications of these markers extend beyond diagnosis to prognosis, as studies have associated these alterations with advanced tumor stages and poor survival outcomes (Wu et al., 2022).

The high diagnostic accuracy of P40 and CK5/6, as demonstrated in this study, corroborates their established role in squamous cell carcinoma diagnostics. P40, a p63 isoform, has emerged as a superior marker due to its exclusive expression in squamous differentiation and absence in non-squamous tissues (Rajesh et al., 2022). Similarly, CK5/6, a basal cell cytokeratin, has proven invaluable in distinguishing OSCC from other oral malignancies. The combined sensitivity and specificity of these markers in this study (94% and 92%, respectively) surpasses many previously reported values, reflecting methodological robustness and the significance of these markers in real-world diagnostic settings (Chen et al., 2022).

Combining hematological parameters with IHC markers offers a multifaceted approach to OSCC diagnosis. Hematological markers provide insights into the systemic effects of the tumor, while IHC markers confirm tissue-specific malignancy. This complementary diagnostic model is particularly advantageous in resource-limited settings, where reliance on single diagnostic modalities may compromise accuracy (Singh et al., 2023). For instance, the integration of NLR and P40 positivity in high-risk populations could enable earlier detection and intervention, potentially improving patient outcomes.

Several studies have highlighted the prognostic implications of hematological markers in cancer. Elevated NLR, for example, has been linked to poorer overall survival and disease-free survival in OSCC (Zhang et al., 2023). Similarly, thrombocytosis has been identified as a marker of aggressive tumor biology, correlating with increased metastatic potential and treatment resistance (Adams et al., 2021). The incorporation of these markers into diagnostic workflows not only aids in early detection but also facilitates risk stratification and personalized treatment planning.

Despite these strengths, certain limitations must be acknowledged. The study's case-control design, while suitable for evaluating diagnostic markers, precludes assessment of longitudinal changes in hematological parameters and IHC marker expression. Future prospective studies

should explore the dynamic relationship between systemic inflammation and tumor progression, potentially unveiling novel prognostic markers or therapeutic targets (Matsumura et al., 2023).

Another consideration is the potential variability in IHC marker expression due to technical factors, such as tissue fixation and staining protocols. While this study employed standardized methods to minimize bias, further multicenter validation is warranted to ensure reproducibility and generalizability of findings (Lu et al., 2024). Additionally, while hematological markers are readily accessible and cost-effective, their specificity for OSCC remains limited, necessitating their integration with highly specific markers like P40 and CK5/6 for optimal diagnostic accuracy (Elango et al., 2021.).

The clinical applicability of this study lies in its potential to bridge the gap between basic research and clinical practice. By demonstrating the diagnostic synergy of hematological and IHC markers, it provides a scalable model for OSCC detection, particularly in high-prevalence regions. Moreover, the findings align with contemporary trends in oncology, which emphasize the importance of biomarker-driven approaches for early cancer detection and personalized care (Yin et al., 2022).

In conclusion, this study advances the diagnostic paradigm for OSCC by combining systemic and tissue-specific markers, thereby addressing critical gaps in early detection and risk stratification. The significant associations between hematological parameters and IHC markers underscore their utility as complementary diagnostic tools. Future research should aim to validate these findings in larger, more diverse cohorts and explore their integration into clinical guidelines, ultimately enhancing diagnostic precision and patient outcomes.

Conclusion

This study highlights the combined diagnostic potential of hematological parameters and IHC markers (P40 and CK5/6) in OSCC. It addresses gaps in early detection strategies, paving the way for more integrative diagnostic approaches. Future research should focus on larger cohorts and explore prognostic implications.

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