

Precision Diagnostic Reclassification of High-Grade Salivary Adenocarcinoma, NOS: A Systematic IHC Audit for Therapeutic Equity in a Regional Indian Cohort

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Abstract

Background: High-grade salivary gland carcinomas (SGCs) often lack architectural hallmarks, leading to their classification as "Adenocarcinoma, Not Otherwise Specified" (ANOS). This "wastebasket" category obscures actionable entities like Salivary Duct Carcinoma (SDC). In regional Indian centers, precision re-evaluation is vital for therapeutic equity. **Methods:** This retrospective immunohistochemical (IHC) audit of archival high-grade ANOS cases (n=5) from Odisha, India, using an AR, HER2, p63, CK5/6, CD117, and Ki67 panel integrated with 5th Edition WHO criteria. **Results:** All cases were reclassified as SDC (100% male; median age 62). Strong AR immunoreactivity served as the diagnostic pivot. We observed a strikingly high Ki67 index (80%) and uniform HER2 negativity. A biphasic staining pattern (basal marker positivity in non-ductal areas) suggested high-grade transformation or hybrid morphologies. **Conclusion:** Systematic IHC audits "unhide" SDC, facilitating a shift from palliative chemotherapy to targeted Androgen Deprivation Therapy, ensuring equitable access to precision oncology in regional registries.

Keywords: Salivary Duct Carcinoma; Adenocarcinoma, NOS; Androgen Receptor; Precision Oncology; Immunohistochemistry; India.

Introduction

The diagnostic landscape of salivary gland oncology is defined by an unparalleled degree of morphological complexity. Salivary gland tumors (SGTs) represent a highly heterogeneous group of neoplasms, with the most recent World Health Organization (WHO) classifications identifying fifteen benign and over twenty distinct malignant epithelial tumor types [1]. This diversity stems from the multifaceted nature of the salivary gland unit, consisting of acinar, ductal luminal, and abluminal myoepithelial or basal cells [1]. High-grade malignancies often lose these characteristic architectural cues, leading to a significant diagnostic challenge. Historically, tumors exhibiting overt malignancy—such as extreme cytological atypia

and high mitotic activity—but lacking specific lineage hallmarks were categorized under the "Adenocarcinoma, Not Otherwise Specified" (ANOS) "wastebasket" category [2].

The magnitude of this diagnostic ambiguity is significant: salivary gland carcinomas (SGCs) account for less than 5% of head and neck malignancies globally [3], yet ANOS has historically occupied a disproportionately large space in registries, accounting for 8% to 19% of cases [2]. In India, specifically in regions like Odisha and Manipur, SGCs constitute approximately 1.16% to 1.31% of total head and neck cancer diagnoses, with a significant portion requiring advanced diagnostic stratification to ensure clinical accuracy [4].

The clinical significance of differentiating Salivary Duct Carcinoma (SDC) from high-grade ANOS is critical. SDC is an

exceptionally aggressive subtype with a poor five-year survival rate of 40%–60% [5]. Unlike generic ANOS, SDC possesses "actionable" molecular targets, including the Androgen Receptor (AR) and HER2 overexpression, which serve as the foundations for personalized therapies such as Androgen Deprivation Therapy (ADT) and Trastuzumab [5,6]. Immunohistochemistry (IHC) has thus become an indispensable tool in evaluating cell differentiation and identifying these specific protein expressions [1]. SDC typically demonstrates a characteristic immunoprofile, being almost universally positive for AR (69%–100%) and frequently showing HER2 positivity (25%–90%) [5]. Morphologically, SDC resembles high-grade mammary ductal carcinoma and lacks myoepithelial differentiation, typically staining negative for basal markers like p63 and CK5/6 [7].

The framework of this study centers on the transition from descriptive morphology to precision oncology. While global trends suggest high reclassification rates of ANOS to SDC, this frequency remains undocumented within the specific demographic context of Odisha, India. Genetic and environmental factors in the Indian population may result in divergent phenotypic expressions, such as the unique HER2-negative/AR-positive profile or exceptionally high proliferative dynamics (Ki67).

The intervention—a systematic retrospective IHC audit—is expected to be effective because it applies high-resolution diagnostic criteria to archival cases that were limited by the technology of their time. By identifying "hidden" SDC cases, this study assumes that a significant subset of the local patient population was eligible for life-extending targeted therapies but remained unidentified due to generic labeling [9,10]. The primary aim of this investigation is to perform a systematic, retrospective IHC audit of archival high-grade ANOS cases (n=5) utilizing a comprehensive panel (AR, HER2, CK5/6, p63, CD117, and Ki67). By applying the 5th Edition WHO criteria, this project seeks to "unhide" SDC cases, quantify reclassification frequency, and ensure therapeutic equity for regional patients previously marginalized by descriptive diagnostic categories.

Methodology

Study Design and Case Selection

This retrospective study utilized clinical data and archival formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients diagnosed with poorly differentiated salivary gland carcinoma between 2023 and 2025. Specimens were sourced from the Department of Pathology at a tertiary care hospital in Odisha, India.

Inclusion and Exclusion Criteria

To ensure a homogenous study population, the following inclusion criteria were applied:

1. Histopathological diagnosis of poorly differentiated carcinoma confirmed by the Department of Pathology.
2. Availability of comprehensive clinical records and complete pathological data for each case.

Patients were excluded based on the following criteria:

1. Cases initially suspected of or diagnosed as SDC that were subsequently reclassified as other primary salivary gland tumors or non-salivary gland-derived malignancies upon specialist pathological review.
2. Incomplete clinical records or missing critical pathological data essential for formal analysis.

3. Malignant tumors determined to be metastatic to the salivary gland from distant primary sites.

Specimen Preparation and Histopathological Examination

Archival FFPE tissue blocks were sectioned and stained with hematoxylin and eosin (H&E) to facilitate morphological assessment and microscopic examination under optical microscopy.

Immunohistochemical Analysis

IHC was performed using a standardized streptavidin-biotin-peroxidase complex method for immunosurface labeling to further characterize the tumor phenotype. The panel of primary antibodies utilized included p63, CK5/6, S100, Androgen Receptor (AR), CD117, HER2, and Ki67.

Study Outcomes

The primary outcomes were defined as: (1) baseline patient demographics; (2) anatomic tumor location; (3) clinical presentation; and (4) pathological and immunophenotypic characteristics. Secondary outcomes included: (1) macroscopic tumor features; and (2) detailed microscopic histopathological findings.

Results

Patient Demographics and Clinical Presentation

From a cohort of 58 diagnosed cases, five were included in this study. The median age at presentation was 62 years (range: 49–69 years). While all patients presented with symptoms of a primary salivary gland mass, four (80%) reported a painless, gradually enlarging mass in the submandibular (Case 1) or parotid glands (Cases 2, 4, and 5). Notably, one patient (Case 3) presented with right-sided tongue numbness associated with a right submandibular gland mass.

Tumor Characteristics and Surgical Management

The anatomic distribution of the tumors was nearly equal between the parotid gland (n=3, 60%) and the submandibular gland (n=2, 40%). The maximum diameter of the tumors ranged from 2.6 cm to 4.7 cm, with a mean diameter of 3.7 cm. Surgical intervention was tailored to the clinical and radiological findings. Two patients (Cases 1 and 5) underwent tumor resection alone. The remaining three patients (Cases 2, 3, and 4) underwent tumor resection combined with neck lymph node dissection.

Histopathological Findings

Histopathological analysis confirmed a diagnosis of poorly differentiated carcinoma across all five cases. All tumors were found to be unifocal. Lymphovascular invasion (LVI) was identified in two cases (40%), while perineural invasion (PNI) was present in two cases (40%). Notably, all cases were negative for clear margins (C/M).

Quantitative assessment of tumor extension showed that Tumor Local Extension (TLE) values ranged from 0 to 35, with Case 3 showing the highest degree of local extension. Tumor Lymphatic Proliferation (TLP) was 0 in most cases (80%) except for Case 4, which had a value of 2. Regarding pathological staging according to the pTNM system, three cases were staged as pT2N0M0, one case was staged as pT3N0M0, and one case presented with more advanced regional involvement staged as pT3N2M0; all patients were negative for distant metastasis (M0) at the time of initial staging (**Table 1**).

Table 1: Clinicopathological Characteristics and Diagnostic Profiles of Primary Salivary Gland Tumors: A Case Series of Five Patients

Case	Gender	Age (years)	Region	Symptom	Maximum diameter of the tumor(cm)	Surgical approach	TF	LVI	PNI	C/M	TLE	TLP	pTNM	AR/Her-2/neu	Ki67 index
1	Male	58	Left submandibular gland	Painless mass gradually enlarges	2.6	Tumor resection	Unifocal	Present	Absent	Negative	0	0	pT2N0M0	AR+ ve/ Her-2 -ve	80%
2	Male	69	Right parotid gland	Painless mass gradually enlarges	3.1	Tumor resection and lymph node dissection	Unifocal	Absent	Absent	Negative	13	0	pT2N0M0	AR+ ve/ Her-2 -ve	80%
3	Male	67	Right submandibular gland	Numbness of the right tongue	4.7	Tumor resection and lymph node dissection	Unifocal	Absent	Absent	Negative	35	0	pT3N0M0	AR+ ve/ Her-2 -ve	80%
4	Male	49	Right parotid gland	Painless mass gradually enlarges	3.8	Tumor resection and lymph node dissection	Unifocal	Present	Present	Negative	27	2	pT3N2M0	AR+ ve/ Her-2 -ve	80%
5	Male	62	Left parotid gland	Painless mass gradually enlarges	4.3	Tumor resection	Unifocal	Absent	Present	Negative	29	0	pT2N0M0	AR+ ve/ Her-2 -ve	80%

Immunohistochemical (IHC) profiling revealed a complex staining pattern, particularly highlighting the distinction between ductal

structures and other tumor areas. The findings are summarized in **Table 2**.

Table 2: Immunohistochemical Expression Profile

IHC Marker	Result
P63	Immunoreactive in part of tumor not showing ductal structures; Non-immunoreactive in ductal structures
CK5/6	Immunoreactive in part of tumor not showing ductal structures; Non-immunoreactive in ductal structures
S100	Non-immunoreactive in the tumor
AR	Immunoreactive strongly in ductal structures; Non-immunoreactive in other parts of the tumor
CD117	Non-immunoreactive in tumor
Her2	Non-immunoreactive in tumor
Ki67	Immunoreactive in 80% of tumor cells in both areas

Microscopically, the tumors exhibited a biphasic architectural distribution. The ductal components demonstrated strong, diffuse nuclear immunoreactivity for AR and were negative for basal markers. Conversely, the non-ductal areas, which showed a solid growth pattern with overt cytological atypia, were non-

immunoreactive for AR but displayed positivity for p63 and CK5/6. A high proliferative index was maintained throughout the lesion, with Ki67 immunoreactivity observed in 80% of tumor cells across both morphological components.

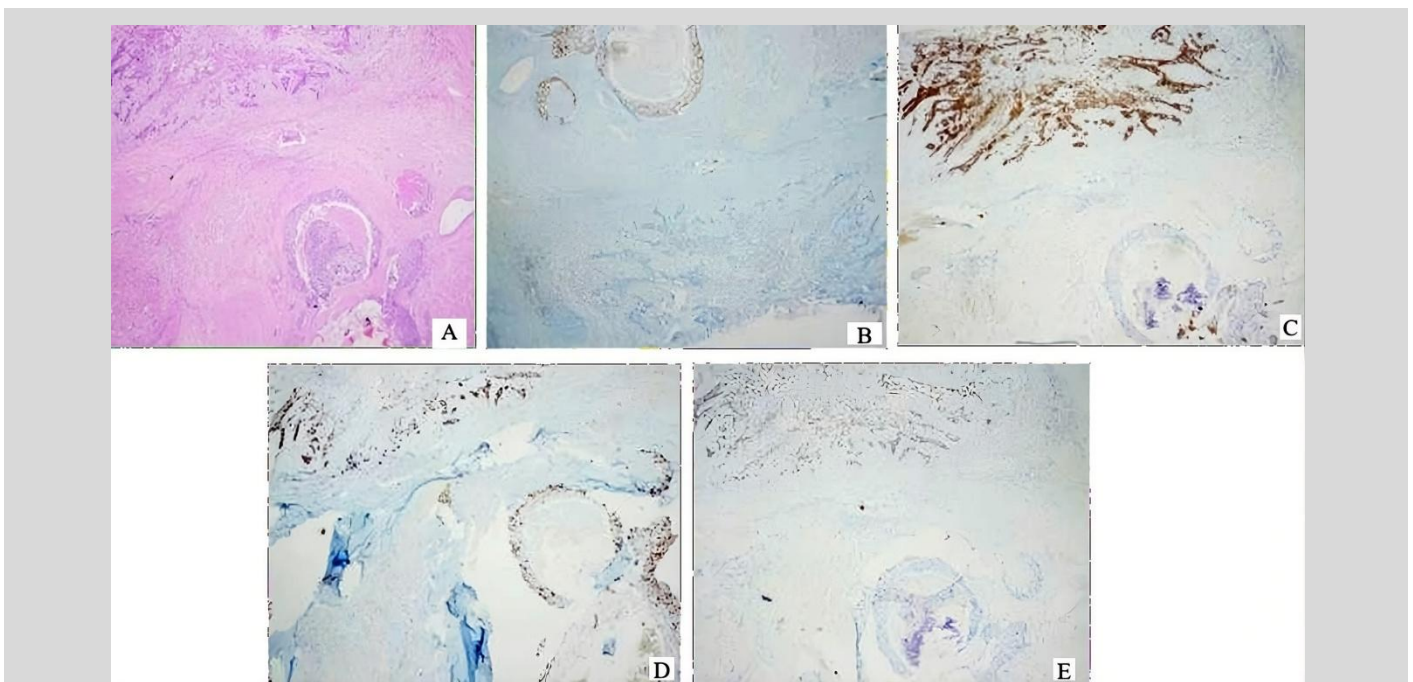


Figure 1: Representative histopathological and immunohistochemical features of high-grade salivary adenocarcinoma. (A) Hematoxylin and Eosin (H&E, 10x) section showing poorly differentiated carcinoma with solid growth and overt cytological atypia. (B) Intense, diffuse nuclear immunoreactivity for Androgen Receptor (AR) within ductal structures, with loss of expression in solid/undifferentiated components (IHC, 10x). (C) CK5/6 expression localized to the non-ductal basaloid components, highlighting the biphasic architectural distribution (IHC, 10 xs). (D) High proliferative dynamic demonstrated by a Ki67 labeling index of approximately 80%, involving both ductal and solid tumor areas (IHC, 10x). (E) p63 immunoreactivity restricted to the abluminal/basaloid cells in non-ductal areas, further confirming the diagnostic segregation from classic purely luminal ductal carcinoma (IHC, 10x).

Discussion

The diagnostic landscape of salivary gland oncology in Odisha is characterized by profound morphological complexity. High-grade malignancies in this region often lack the architectural cues necessary for traditional classification, leading to their historical relegation to the ANOS "wastebasket" category [10,11]. The primary finding of this investigation is the successful reclassification of these archival ANOS cases as SDC through the systematic application of a contemporary IHC panel. In direct alignment with our specific aims, strong AR immunoreactivity within ductal structures served as the definitive diagnostic pivot [2,12]. This confirms our study's rationale: a significant portion of the high-grade salivary tumors in regional Indian centers obscures aggressive,

molecularly actionable entities that remain unidentified under purely descriptive microscopy.

Clinical and Histopathological Dynamics

Our findings in this tertiary care cohort align with global demographic trends, showing a median age of 62 years and a 100% male predominance—a classic hallmark of SDC [13]. The significant involvement of the submandibular gland (40%) and the presence of PNI in 40% of cases underscore the regional aggressiveness of these high-grade adenocarcinomas [14,15]. Histopathological indicators, specifically the presence of LVI, high T-stage (T3), and an exceptionally high Ki67 index of 80%, confirm a biological profile of intense aggression [16,17].

The consistently high Ki67 index of 80% across both architectural components further underscores an aggressive, unified proliferative dynamic, regardless of the divergent phenotypic differentiation observed. While our cohort size (n=5) reflects the rarity of these malignancies, the 100% reclassification rate identifies a significant diagnostic gap within regional registries. This extreme proliferative rate (significantly exceeding the global SDC median of 37–55%) suggests that while AR-positivity provides a rationale for ADT, the biological aggression may necessitate a combined chemohormonal approach. In such high-proliferative variants, ADT may serve as an effective backbone to reduce tumor burden, but the rapid cell turnover indicates that traditional cytotoxic agents or emerging AR-pathway inhibitors (enzalutamide) might be required in tandem to counteract the inherent risk of rapid disease progression.

Immunophenotypic Interpretation and Therapeutic Impact

The systematic IHC audit proved transformative for clinical management. The identification of AR-positive SDC phenotypes shifts the local standard of care from generic oncology toward precision medicine. Unlike generic ANOS, AR-positive SDC is eligible for ADT using agents such as bicalutamide [18]. Interestingly, the uniform HER2 negativity observed in this cohort contrasts with the 25%–90% positivity rates reported in Western literature [15,19]. This divergence necessitates a therapeutic pivot in regional management, placing a heavier reliance on hormonal strategies and aggressive surgical intervention.

The observed biphasic staining pattern—characterized by the localization of basal markers (p63, CK5/6) to non-ductal components and strong AR expression restricted to ductal structures—was instrumental in this reclassification. This unique immunophenotype suggests that the regional SDC variants in this cohort may not represent "classic" purely luminal carcinomas. Instead, the segregation of basal and luminal markers indicates a possible origin from high-grade transformation (HGT) of a pre-existing low-grade entity, such as epithelial-myoepithelial carcinoma (EMC), or the presence of a hybrid tumor morphology. Morphologically, the non-ductal areas exhibited a solid growth pattern with significant cytological atypia, yet retained a basaloid/myoepithelial-like protein expression signature.

Strategic Implications and Limitations

Transitioning to routine IHC for high-grade salivary gland carcinomas involves increased laboratory costs; however, the opportunity cost of failing to test is the continued use of ineffective treatments. In the context of the Indian population, implementing the 5th Edition WHO criteria is essential to minimize the "NOS" category [4,8,14].

Beyond the immediate clinical implications, the systematic IHC audit utilized here serves as a reproducible diagnostic framework. By demonstrating that a targeted panel can effectively "empty" the ANOS wastebasket, we propose this protocol as a scalable model for other regional oncology centers across the Indian subcontinent. Implementing such a standardized re-evaluation process is a critical step toward minimizing descriptive "NOS" labeling, refining cancer registry data, and ensuring that patients in resource-limited settings are not excluded from the benefits of modern precision oncology.

Conclusion

This investigation underscores that immunohistochemically driven re-evaluation is essential to transcend the limitations of descriptive

morphology in salivary gland oncology. By deconstructing the generic "Adenocarcinoma, NOS" category, we identified a high prevalence of AR-positive SDC within this regional cohort, highlighting a critical opportunity to transition from palliative chemotherapy to targeted ADT. Despite unique local variations like uniform HER2 negativity, the clinical mandate is clear: integrating contemporary WHO criteria and routine IHC profiling into regional tertiary care is fundamental for delivering precision oncology and improving outcomes in these aggressive malignancies.

Declaration

Funding statement

Nil

Conflict of Interest

Nil

IEC Approval

The study was performed according to ethical parameters, after receiving the IEC approval letter from AHRCC, Cuttack, Odisha, India.

Consent to participate and consent for publication

Taken

Availability of data and materials (ADM):

On request to Corresponding author

Authors' contributions

Bagmi Mishra, Pushpanjali Behera, Anup Kumar Pramanik, Subrat Kumar Samantara, Subhransu Kumar Hota, Sashibhusan Dash: Concepts, Design, Data acquisition, Definition of intellectual content, Literature search, Manuscript preparation, Manuscript editing, Manuscript review.

Bagmi Mishra, Pushpanjali Behera, Anup Kumar Pramanik & Sashibhusan Dash: Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review.

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Nil

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