

## Clinicopathological Study of CK19 Expression in Head and Neck Squamous Cell Carcinoma and Its Association with Tumor Grading: A Cross-Sectional Study.

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### Abstract

#### Introduction:

Head and neck squamous cell carcinoma (HNSCC) is a histologically heterogeneous group of tumors with diverse biological behavior. Cytokeratin 19 (CK19), part of the epithelial cytoskeletal network, may indicate tumor differentiation and progression. This cross-sectional study evaluated CK19 immunohistochemical expression in HNSCC and its correlation with histological grade using two grading systems.

#### Materials and Methods:

A cross-sectional study of 100 biopsy-confirmed HNSCC cases was conducted at the Department of Pathology, Pt. B.D. Sharma PGIMS, Rohtak. Tumors were graded using the WHO system and Anneroth's six-parameter system. CK19 expression was assessed by immunohistochemistry and scored 0-3 based on the percentage of stained tumor cells. Chi-square and Fisher's exact tests were used for statistical analysis.

#### Results:

Patients ranged from 28-86 years (mean  $57.16 \pm 11.70$  years) with a male predominance (88% male, 12% female). CK19 expression was positive in 69% of cases, rising with tumor grade: 41.4% in well-differentiated, 78.3% in moderately differentiated, and 84% in poorly differentiated carcinomas ( $p < 0.001$ ). Anneroth grading also showed a rising trend, though with inconsistencies in grade II/III classification. The highest CK19 expression (score 3) predominated in Anneroth grade III/IV tumors.

#### Conclusion:

CK19 expression is significantly associated with poor tumor differentiation in HNSCC, particularly by WHO grading, supporting its potential as a prognostic biomarker that may enhance grading accuracy and inform treatment strategies.

#### Recommendation:

CK19 immunohistochemistry should be considered an adjunct to routine histopathological grading in HNSCC, with larger, standardized, multicentric studies recommended to validate its prognostic reliability.

**Keywords:** cytokeratin 19 (CK19), Anneroth system, prognostic marker, immunohistochemistry, head and neck squamous cell carcinoma

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### Introduction

Head and neck squamous cell carcinoma (HNSCC) ranks as the sixth most prevalent cancer globally, comprising roughly 95% of all malignancies in the head and neck region.<sup>1</sup> It is a diverse group of tumors marked by uncontrolled proliferation of malignant epithelial cells occurring in sites such as the oral cavity, larynx, oropharynx, hypopharynx, nasal cavity, and paranasal sinuses.<sup>2</sup> Both global and regional data indicate that the majority of cases originate in the oral cavity or oropharynx. HNSCC is more frequently diagnosed in men, typically

between middle and late adulthood, although it can affect individuals of any age. Key risk factors include the independent use of tobacco (in various forms) and alcohol, while genetic susceptibility, immune status, environmental exposure, and occupational hazards also contribute to disease development.<sup>3</sup> Considerable research has been undertaken to identify biomarkers that can improve outcome prediction and support personalized treatment strategies in cancer care. In the context of HNSCC, the discovery of dependable tumor markers is crucial for early detection, prognostic evaluation, and guiding therapeutic decisions.

Among the emerging biomarkers, cytokeratin 19 (CK19) and its fragment CYFRA21-1 have shown promise as potential prognostic indicators.<sup>4</sup>

Cytokeratins are a family of intermediate filament proteins found in the cytoplasm of eukaryotic epithelial cells, playing a vital role in maintaining cellular structural integrity.<sup>5</sup> These water-insoluble proteins, typically ranging from 40 to 70 kDa in size, assemble into filaments 8-11 nm in diameter.<sup>6</sup> Based on their chemical properties and structural features, they are categorized into two groups: type I (acidic, CK9-CK20) and type II (neutral or basic, CK1-CK8).<sup>7</sup> Beyond structural support, cytokeratins are involved in cellular signaling, stress response, apoptosis, maintaining epithelial identity, and serve as indicators of cell proliferation.<sup>8</sup>

Among the various epithelial cytokeratins, CK19 is considered the least acidic. Structurally, it forms a heterotetramer composed of two type 1 and two type 2 keratin subunits, suggesting that its expression is regulated differently compared to other keratin genes. CK19 is present in simple epithelial tissues and in the basal layer of stratified squamous epithelium, where it is often associated with altered epithelial differentiation.<sup>4</sup>

Numerous tumor tissue biomarkers have been explored in HNSCC, with several gaining clinical validation as potential prognostic indicators. More than 70 markers have been studied so far. Among the most clinically relevant molecular markers are the epidermal growth factor receptor (EGFR), cyclin D1 (CCND1), excision repair cross-complementation group 1 (ERCC1), p16 protein expression, and the detection or status of human papillomavirus (HPV). Additionally, proteins such as Bcl-xL and Bcl-2, along with gene amplifications involving EMS1, FGFR1, and CCND1, have shown potential prognostic relevance in various clinical studies.<sup>2</sup>

Although CK19 immunoreactivity has been reported in HNSCC, its expression frequency and prognostic value continue to be debated. This study focuses on analyzing CK19 expression in these tumors via immunohistochemistry.

## Materials and Methods

### Study design

This was a cross-sectional, observational study designed to evaluate the immunohistochemical expression of CK19 in histologically confirmed cases of head and neck squamous cell carcinoma (HNSCC) and to examine its association with tumor grade. As a cross-sectional design, all clinical, demographic, and immunohistochemical data were collected and assessed at a single point in time for each case, with no follow-up, allowing description of the prevalence and distribution of CK19 expression across grade categories at the time of diagnosis.

### Study setting

The study was conducted in the Department of Pathology, Pt. B.D. Sharma Postgraduate Institute of Medical Sciences (PGIMS), Rohtak, Haryana, India, a tertiary care teaching hospital providing comprehensive histopathology, cytopathology, hematopathology, and immunohistochemistry services to a large referral population from Haryana and neighbouring states. The study included biopsy-confirmed HNSCC cases collected over a period of 2 years from 2020 to 2022 from specimens received from Otorhinolaryngology, Surgical Oncology, and Radiotherapy departments.

### Participants

Cases were identified through review of the pathology archive and accession registers of the Department of Pathology; the study was record- and specimen-based, and participants were not directly examined or interviewed, nor were any additional clinical procedures performed for study purposes. Formalin-fixed, paraffin-embedded tissue blocks and the corresponding clinical request forms/case records were retrieved for each eligible case, and demographic and clinical details were extracted from hospital records.

### Inclusion criteria

(i) patients with a histopathologically confirmed diagnosis of squamous cell carcinoma of the head and neck region (oral cavity, oropharynx, larynx, hypopharynx, nasal cavity, or paranasal sinuses) on incisional or excisional biopsy submitted to the Department of Pathology during the study period; (ii) availability of adequate formalin-fixed, paraffin-embedded tissue for both routine histopathological grading and CK19 immunohistochemical staining; and (iii) availability of the relevant demographic and clinical information (age, sex, smoking history, presenting symptom, and tumor site) in the hospital records.

### Exclusion criteria

(i) cases with insufficient or exhausted tissue precluding immunohistochemical analysis; (ii) patients who had received neoadjuvant chemotherapy or radiotherapy before biopsy, since prior treatment can alter tumor morphology and marker expression; (iii) recurrent tumors; (iv) non-squamous or metastatic malignancies of the head and neck region; and (v) cases with incomplete clinical records precluding assessment of the study variables.

### Variables

The primary outcome variable was CK19 immunohistochemical expression, assessed both as a binary status (positive/negative) and as a semi-quantitative expression score (0-3). The principal explanatory variable

was tumor grade, assessed independently by two systems: the WHO grade (well, moderately, or poorly differentiated squamous cell carcinoma) and Anneroth's multifactorial grade (grades I-IV, derived from six morphological parameters: keratinization, nuclear pleomorphism, mitotic count, pattern of invasion, stage of invasion, and lymphoplasmacytic infiltrate). Additional variables recorded were age, sex, smoking history, presenting symptom, and anatomical site of the tumor.

### Data sources

For each case, formalin-fixed, paraffin-embedded tissue sections were stained for CK19 by immunohistochemistry, and cytoplasmic and/or membranous staining within tumor cells was assessed by light microscopy; a minimum of 500 tumor cells were counted per case to determine the percentage of positively stained cells, from which an expression score of 0 (negative, 0%), 1 (weak, 1-25%), 2 (moderate, 26-50%), or 3 (strong, >50%) was assigned.<sup>11</sup> Histological grade was assessed on hematoxylin- and eosin-stained sections using the WHO system (well, moderately, poorly differentiated) and Anneroth's six-parameter multifactorial system, with the total score classifying each tumor into grade I-IV (Table 1). Demographic and clinical

data (age, sex, smoking history, presenting symptom, and tumor site) were abstracted from case records and the structured proforma completed at the time of specimen submission.

### Analysis

Data were entered and analysed using SPSS software, version 24 (IBM Corp., Armonk, NY, USA). Categorical variables (grade categories, CK19 expression status/score, sex, smoking status) were summarised as frequencies and percentages, and the continuous variable age was summarised as mean  $\pm$  standard deviation. Associations between CK19 expression (status and score) and WHO grade or Anneroth grade were assessed using the Chi-square test or Fisher's exact test, as appropriate to expected cell counts. A two-sided p-value of less than 0.05 was considered statistically significant.

### Ethical Approval

The study was approved by the Institutional Ethics Committee of Pt. B.D. Sharma PGIMS, Rohtak.

**Table 1: Anneroth's Multifactorial Grading System<sup>9,10</sup>**

| Morphological Parameter      | Score 1                                   | Score 2                                | Score 3                                                             | Score 4                                                         |
|------------------------------|-------------------------------------------|----------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------|
| Keratinization degree        | >50% of cells keratinized                 | 20-50% keratinized                     | 5-20% keratinized                                                   | 0-5% keratinized                                                |
| Nuclear pleomorphism         | Little                                    | Moderately abundant                    | Abundant                                                            | Extreme                                                         |
| Number of mitoses/hpf        | 0-1                                       | 2-3                                    | 4-5                                                                 | >5                                                              |
| Pattern of invasion          | Pushing, well-delineated borders          | Infiltrating solid cords/bands/strands | Small groups/cords of cells                                         | Marked, widespread dissemination in small groups                |
| Stage of invasion            | Carcinoma in situ / questionable invasion | Distinct invasion, lamina propria only | Invasion below lamina propria, adjacent to muscle/glands/periosteum | Extensive deep invasion replacing stroma, infiltrating jaw bone |
| Lymphoplasmacytic infiltrate | Marked                                    | Moderate                               | Slight                                                              | None                                                            |

*Note. Total score classifies tumor grade as follows: Grade I = 5-10; Grade II = 11-15; Grade III = 16-20; Grade IV = 20+.*

### Results

#### Participant flow

During the study period, 128 cases of histopathologically suspected head and neck squamous cell carcinoma (HNSCC) were identified and assessed for eligibility. Of

these, 28 cases were excluded: 12 had inadequate tissue for immunohistochemical evaluation, 8 had incomplete clinical or pathological records, and 8 had received prior treatment before tissue evaluation. Thus, 100 biopsy-confirmed HNSCC cases met the predefined inclusion criteria and were included in the final analysis (Figure 1).

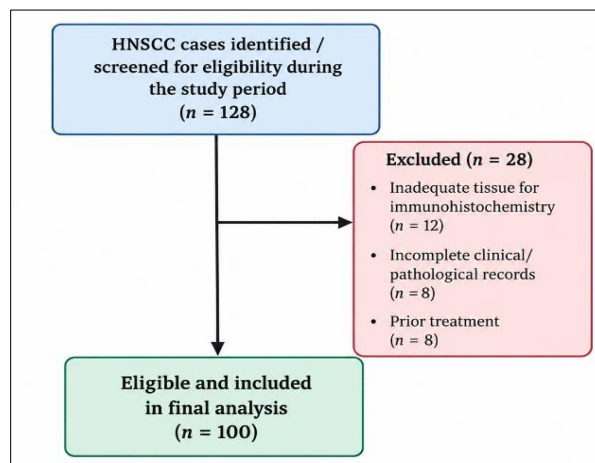


**Figure 1: Participant flow diagram**

### Demographic and clinicopathological findings

Patient ages ranged from 28 to 86 years, with a mean age of  $57.16 \pm 11.70$  years; the 51-70 year age group comprised the largest proportion of cases (59%). A clear male predominance was noted, with 88% of patients being male and 12% female. Smoking history was present in 71% of patients. The most frequently reported symptom was difficulty swallowing (45%), followed by voice changes (21%); other symptoms included throat pain, chewing and breathing difficulties, and oral ulceration. The tongue was

the most commonly involved site (35%), followed by the larynx (21%).

Using Anneroth's multifactorial grading system, the largest proportion of the 100 cases were grade II (46%), followed by grade III (40%), grade I (10%), and grade IV (4%). By WHO grading, 29 (29%) cases were well differentiated SCC (WDSCC), 46 (46%) were moderately differentiated SCC (MDSCC), and 25 (25%) were poorly differentiated SCC (PDSCC). CK19 immunohistochemical staining was positive in 69% of cases and negative in 31%; by expression score, 31 (31%) cases scored 0, 17 (17%) scored 1, 14 (14%) scored 2, and 38 (38%) scored 3 (Table 2).

**Table 2: Clinicopathological Parameters of the Study Population (N = 100)**

| Parameter             | Category      | Frequency (%)           |
|-----------------------|---------------|-------------------------|
| Age                   | Mean $\pm$ SD | $57.16 \pm 11.70$ years |
| Sex                   | Male          | 88 (88%)                |
|                       | Female        | 12 (12%)                |
| Smoking history       | Smoker        | 71 (71%)                |
|                       | Non-smoker    | 29 (29%)                |
| WHO grade             | WDSCC         | 29 (29%)                |
|                       | MDSCC         | 46 (46%)                |
|                       | PDSCC         | 25 (25%)                |
| Anneroth's grade      | Grade I       | 10 (10%)                |
|                       | Grade II      | 46 (46%)                |
|                       | Grade III     | 40 (40%)                |
|                       | Grade IV      | 4 (4%)                  |
| CK19 expression       | Negative      | 31 (31%)                |
|                       | Positive      | 69 (69%)                |
| CK19 expression score | 0             | 31 (31%)                |
|                       | 1             | 17 (17%)                |
|                       | 2             | 14 (14%)                |
|                       | 3             | 38 (38%)                |

*Note. SD = standard deviation; WDSCC = well differentiated squamous cell carcinoma; MDSCC = moderately differentiated squamous cell carcinoma; PDSCC = poorly differentiated squamous cell carcinoma.*

All cases of WDSCC were classified as Anneroth grade I or II, with none falling into grade III/IV. Most MDSCC cases were distributed almost equally between grade II and III, while 88% of PDSCC cases fell into Anneroth grade III or IV (Figure 1-4).

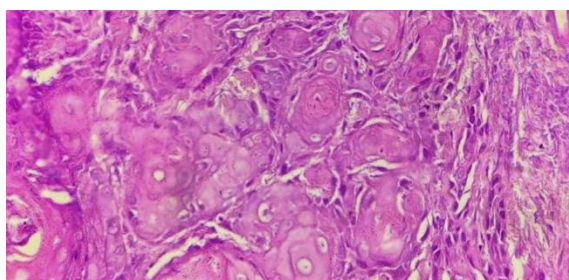


FIG.1. HNSCC: Well differentiated squamous cell carcinoma (H&E, 400X)

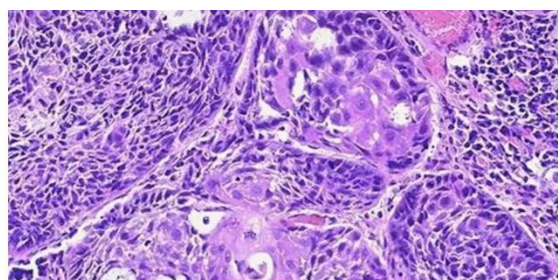


FIG.2. HNSCC: Moderately differentiated squamous cell carcinoma (H&E, 400X)

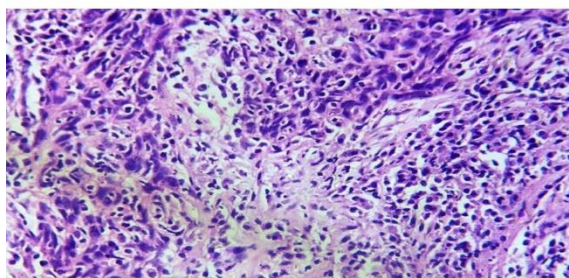


FIG.3. HNSCC: Poorly differentiated squamous cell carcinoma (H&E, 100X)

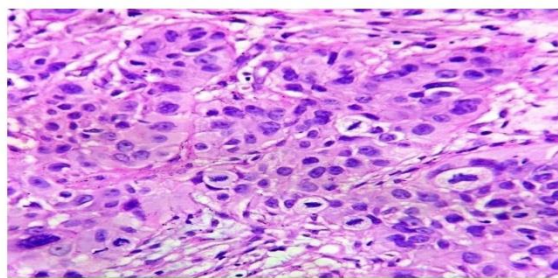


FIG.4. HNSCC: Prominent mitotic activity (Anneroth's score 4) (H&E, 400X)

Fisher's exact test was used to explore the association between Anneroth's grade and WHO grade. There was a significant difference between groups in the distribution of WHO grade ( $\chi^2 = 58.620$ ,  $p < 0.001$ ); the Anneroth grading system mainly stratified the MDSCC group into two subgroups (Table 3).

**Table 3: Correlation of Anneroth's Grading with WHO Grading System**

| Anneroth's Grade | WDSCC (n = 29) | MDSCC (n = 46) | PDSCC (n = 25) | $\chi^2 / p$                     |
|------------------|----------------|----------------|----------------|----------------------------------|
| I (n = 10)       | 9 (31.0%)      | 1 (2.1%)       | 0 (0%)         | $\chi^2 = 58.620$<br>$p < 0.001$ |
| II (n = 46)      | 20 (69.0%)     | 23 (50.0%)     | 3 (12.0%)      |                                  |
| III (n = 40)     | 0 (0%)         | 22 (47.9%)     | 18 (72.0%)     |                                  |
| IV (n = 4)       | 0 (0%)         | 0 (0%)         | 4 (16.0%)      |                                  |

**Note.** Fisher's exact test. WDSCC = well differentiated squamous cell carcinoma; MDSCC = moderately differentiated squamous cell carcinoma; PDSCC = poorly differentiated squamous cell carcinoma.

The CK19 IHC positivity rate increased from Anneroth grade I to grade III, while positivity fell slightly in grade IV compared with grade III (75% vs. 92.5%); this difference was not statistically significant and may reflect the small

number of grade IV cases (Figure 5-8). Fisher's exact test showed a significant difference between groups in the distribution of CK19 expression score across Anneroth grade ( $\chi^2 = 34.626$ ,  $p < 0.001$ ) (Table 4).

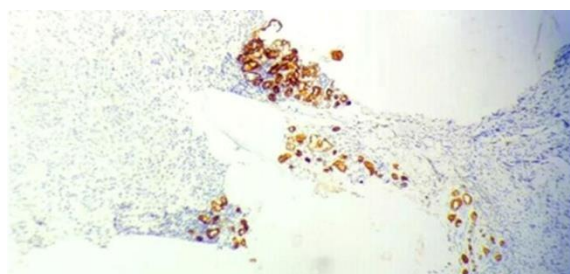


FIG.5. HNSCC: CK 19 negative (score 0) with normal salivary gland acini as internal control (IHC, 100X)

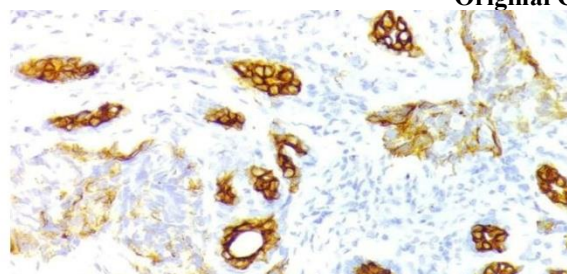


FIG.6. HNSCC: CK 19 positive; score 1 (IHC, 400X)

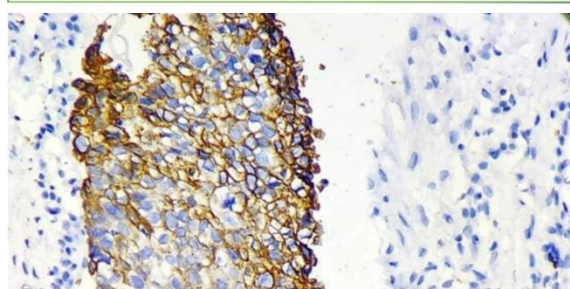


FIG.7. HNSCC: CK 19 positive; score 2 (IHC, 400X)

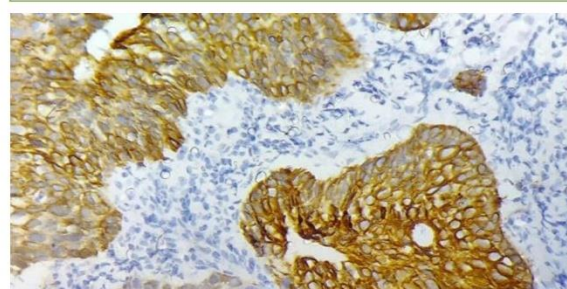


FIG.8. HNSCC: CK 19 IHC positive; score 3 (IHC, 400X)

**Table 4: Correlation of Anneroth's Grading with CK19 IHC Expression Score**

| Anneroth's Grade | Score 0    | Score 1    | Score 2   | Score 3    | $\chi^2 / p$                     |
|------------------|------------|------------|-----------|------------|----------------------------------|
| I (n = 10)       | 7 (70%)    | 0 (0%)     | 3 (30%)   | 0 (0%)     | $\chi^2 = 34.626$<br>$p < 0.001$ |
| II (n = 46)      | 20 (43.4%) | 10 (21.8%) | 6 (13.0%) | 10 (21.8%) |                                  |
| III (n = 40)     | 3 (7.5%)   | 7 (17.5%)  | 5 (12.5%) | 25 (62.5%) |                                  |
| IV (n = 4)       | 1 (25%)    | 0 (0%)     | 0 (0%)    | 3 (75%)    |                                  |

Note. Fisher's exact test. Scores 0 = negative; 1-3 = positive (weak, moderate, strong).

Of 29 WDSCC cases, 12 (41.4%) showed positive CK19 expression, and 17 (58.6%) were negative. Of 46 MDSCC cases, 36 (78.3%) were positive, and 10 (21.7%) were negative. Of 25 PDSCC cases, 21 (84%) were positive, and

4 (16%) were negative (Table 5). Fisher's exact test showed a significant difference between groups in the distribution of CK19 expression score across WHO grade ( $\chi^2 = 21.995$ ,  $p < 0.001$ ).

**Table 5: Correlation of WHO Grading of Tumor with CK19 IHC Expression Score**

| WHO Grade      | Score 0    | Score 1   | Score 2   | Score 3    | $\chi^2 / p$                     |
|----------------|------------|-----------|-----------|------------|----------------------------------|
| WDSCC (n = 29) | 17 (58.6%) | 6 (20.7%) | 4 (13.8%) | 2 (6.9%)   | $\chi^2 = 21.995$<br>$p < 0.001$ |
| MDSCC (n = 46) | 10 (21.7%) | 7 (15.2%) | 5 (10.9%) | 24 (52.2%) |                                  |
| PDSCC (n = 25) | 4 (16%)    | 4 (16%)   | 5 (20%)   | 12 (48%)   |                                  |

Note. Fisher's exact test. WDSCC = well differentiated squamous cell carcinoma; MDSCC = moderately differentiated squamous cell carcinoma; PDSCC = poorly differentiated squamous cell carcinoma.

### Comparison of study findings with previous literature

To contextualise the present findings, demographic, grade-distribution, and CK19 expression data from this study were tabulated alongside comparable published series (Tables 6-10); these comparisons are interpreted in the Discussion.

**Table 6: Comparison of Demographic Profile, Grade Distribution and CK19 Positivity with Previous Studies**

| Study                                  | Mean Age               | Male: Female | WDSCC      | MDSCC      | PDSCC      | CK19 Positivity |
|----------------------------------------|------------------------|--------------|------------|------------|------------|-----------------|
| Zhong et al. <sup>11</sup> (n = 33)    | 58.8 y                 | 28:5         | 51.2%      | 36.7%      | 12.1%      | 90.9%           |
| Fillies et al. <sup>14</sup> (n = 192) | 59 y                   | 154:38       | 22.9%      | 39.1%      | 38%        | 40.6%           |
| Babiker et al. <sup>15</sup> (n = 100) | 56 y                   | 76:24        | 22%        | 34%        | 44%        | 58%             |
| Coelho et al. <sup>16</sup> (n = 67)   | 55 y                   | 57:10        | 44.5%      | 47.8%      | 7.8%       | 11.9%           |
| Santoro et al. <sup>17</sup> (n = 38)  | 64.83 y                | 27:11        | 13.1%      | 47.4%      | 49.5%      | 40.7%           |
| <b>Present study (n = 100)</b>         | <b>57.16 ± 11.70 y</b> | <b>88:12</b> | <b>29%</b> | <b>46%</b> | <b>25%</b> | <b>69%</b>      |

*Note. WDSCC = well-differentiated squamous cell carcinoma; MDSCC = moderately differentiated squamous cell carcinoma; PDSCC = poorly differentiated squamous cell carcinoma.*

**Table 7: Comparison of Anneroth Grade Distribution with Previous Studies**

| Study                                | Grade I         | Grade II        | Grade III       | Grade IV      |
|--------------------------------------|-----------------|-----------------|-----------------|---------------|
| Akhter et al. <sup>18</sup> (n = 50) | 26 (52%)        | 23 (46%)        | 1 (2%)          | None          |
| Doshi et al. <sup>19</sup> (n = 57)  | 30 (52.6%)      | 25 (43.9%)      | 2 (3.5%)        | None          |
| <b>Present study (n = 100)</b>       | <b>10 (10%)</b> | <b>46 (46%)</b> | <b>40 (40%)</b> | <b>4 (4%)</b> |

**Table 8: Comparison of CK19-Positive Cases by WHO Grade with Previous Studies**

| Study                           | WDSCC                | MDSCC                | PDSCC              |
|---------------------------------|----------------------|----------------------|--------------------|
| Zhong et al. <sup>11</sup>      | 14/17 (82.4%)        | 12/12 (100%)         | 4/4 (100%)         |
| Safadi et al. <sup>20</sup>     | 14/22 (63.4%)        | 7/10 (70%)           | 3/4 (75%)          |
| Babiker et al. <sup>15</sup>    | 10/22 (45%)          | 16/34 (47%)          | 30/44 (68%)        |
| Coelho et al. <sup>16</sup>     | 3/30 (10%)           | 4/32 (12.5%)         | 1/5 (20%)          |
| Ernst et al. <sup>4</sup>       | 4/19 (21.1%)         | 29/87 (33.3%)        | 8/23 (34.8%)       |
| Bombeccari et al. <sup>13</sup> | 5/13 (38.4%)         | 5/5 (100%)           | None               |
| <b>Present study</b>            | <b>12/29 (41.4%)</b> | <b>36/46 (78.3%)</b> | <b>21/25 (84%)</b> |

**Table 9: Comparison of CK19 IHC Score Distribution with Previous Studies**

| Study (n)                            | Score 0    | Score 1    | Score 2    | Score 3    |
|--------------------------------------|------------|------------|------------|------------|
| Zhong et al. <sup>11</sup> (33)      | 9.1%       | 30.3%      | 36.4%      | 24.2%      |
| Babiker et al. <sup>15</sup> (100)   | 42%        | 17%        | 20%        | 21%        |
| Santoro et al. <sup>17</sup> (38)    | 23.7%      | 7.9%       | 7.9%       | 60.5%      |
| Bombeccari et al. <sup>13</sup> (18) | 44.4%      | 27.8%      | 27.8%      | None       |
| <b>Present study (100)</b>           | <b>31%</b> | <b>17%</b> | <b>14%</b> | <b>38%</b> |

**Table 10: Comparison of CK19 IHC Score by WHO Grade with Previous Studies**

| Study                           | WHO Grade             | Score 0      | Score 1      | Score 2      | Score 3      |
|---------------------------------|-----------------------|--------------|--------------|--------------|--------------|
| Zhong et al. <sup>11</sup>      | WDSCC (n = 17)        | 17.6%        | 41.2%        | 35.3%        | 5.9%         |
|                                 | MDSCC (n = 12)        | 0%           | 16.7%        | 50%          | 33.3%        |
|                                 | PDSCC (n = 4)         | None         | 25%          | None         | 75%          |
| Bombeccari et al. <sup>13</sup> | WDSCC (n = 13)        | 61.5%        | 30.7%        | 7.8%         | None         |
|                                 | MDSCC (n = 5)         | None         | 20%          | 80%          | None         |
|                                 | PDSCC (n = 0)         | None         | None         | None         | None         |
| <b>Present study</b>            | <b>WDSCC (n = 29)</b> | <b>58.6%</b> | <b>20.7%</b> | <b>13.8%</b> | <b>6.9%</b>  |
|                                 | <b>MDSCC (n = 46)</b> | <b>21.7%</b> | <b>15.2%</b> | <b>10.9%</b> | <b>52.2%</b> |
|                                 | <b>PDSCC (n = 25)</b> | <b>16%</b>   | <b>16%</b>   | <b>20%</b>   | <b>48%</b>   |

*Note. WDSCC = well-differentiated squamous cell carcinoma; MDSCC = moderately differentiated squamous cell carcinoma; PDSCC = poorly differentiated squamous cell carcinoma.*

## Discussion

### Demographic profile.

The mean age ( $57.16 \pm 11.70$  years), male predominance (88%), and high smoking prevalence (71%) observed here match the pattern typically reported for HNSCC; the male predominance is plausibly related to higher tobacco use among men in this population. Smoking prevalence was broadly concordant with Ernst et al.<sup>4</sup> (63.5% smokers,  $n = 129$ ).

### Symptoms and site.

Difficulty swallowing (45%) and voice change (21%) were the leading complaints, mirroring the principal symptoms reported by Sanderson et al.<sup>12</sup> The tongue was the commonest site (35%), consistent with Bombeccari et al.<sup>13</sup>, likely reflecting its greater exposure to tobacco- and alcohol-related carcinogens.

### WHO grade distribution.

29% of tumors were well, 46% moderately, and 25% poorly differentiated. Grading by microscopic features is inherently subjective and prone to interobserver variability; the frequent default to the moderately-differentiated category, also seen in other series, underscores the need for more standardized, objective grading criteria.

### Overall CK19 positivity.

CK19 positivity (69%) fell within the wide 11.9-90.9% range reported previously (Table 6) — higher than Fillies et al.<sup>14</sup> (40.6%), Babiker et al.<sup>15</sup> (58%), Coelho et al.<sup>16</sup> (11.9%), and Santoro et al.<sup>17</sup> (40.7%), but lower than Zhong et al.<sup>11</sup> (90.9%), likely reflecting differences in antibody clones, staining protocols, thresholds, and case-mix rather than true biological differences.

### Anneroth grade distribution.

Grade II predominated (46%), consistent with Akhter et al.<sup>18</sup> and Doshi et al.<sup>19</sup> (Table 7), though this study had a markedly higher proportion of grade III tumors, possibly reflecting referral-population case-mix or interobserver variation in applying the six Anneroth parameters.

### CK19 by WHO grade.

CK19 positivity rose with WHO grade — 41.4% (WDSCC), 78.3% (MDSCC), 84% (PDSCC),  $p < 0.001$  — paralleling the upward trend reported by Zhong et al.<sup>11</sup>, Safadi et al.<sup>20</sup>, Babiker et al.<sup>15</sup>, Coelho et al.<sup>16</sup>, Ernst et al.<sup>4</sup>, and Bombeccari et al.<sup>13</sup> (Table 8), although several of these did not reach significance, possibly owing to smaller samples.

### CK19 score distribution.

Scores were 31% negative, 17% weak, 14% moderate, and 38% strong. Comparison with Zhong et al.<sup>11</sup>, Babiker et al.<sup>15</sup>, Santoro et al.<sup>17</sup>, and Bombeccari et al.<sup>13</sup> (Table 9) showed no consistent cross-study pattern, likely reflecting differences in grade case-mix, sample size, and IHC protocol between centers.

### CK19 score by WHO grade.

CK19 score correlated significantly with WHO grade ( $p < 0.001$ ). A clear gradient was seen in WDSCC and PDSCC, but MDSCC showed an unexpectedly high proportion of score-3 cases (52.2%), also seen to a lesser extent in Zhong et al.<sup>11</sup> (Table 10) — likely reflecting MDSCC heterogeneity, consistent with its split into two Anneroth subgroups above.

### CK19 score by Anneroth grade.

Score-0 negativity was highest in Anneroth grade I (70%) and fell with increasing grade as higher scores became more frequent ( $\chi^2 = 34.626$ ,  $p < 0.001$ ). No comparable published data on this specific correlation were identified.

### Generalizability

As a single-center, hospital-based cross-sectional study conducted at a tertiary referral institute in northern India, the demographic profile of this cohort, particularly the high prevalence of smoking and marked male predominance, reflects the regional referral population and may not generalize directly to populations with different tobacco-use patterns, sex distributions, or HPV-driven disease profiles, where CK19 expression frequency and its grade correlation could differ. The immunohistochemical scoring and grading methods used are standard and broadly reproducible across pathology laboratories, so the observed association between CK19 expression and tumor grade is plausibly generalizable in direction, if not necessarily in exact magnitude, to other HNSCC populations, particularly in comparable South Asian referral settings with similar risk-factor exposure.

### Conclusion

Some interesting observations were made during this study of CK19 expression in head and neck squamous cell carcinoma. The WHO grading system, which involves considerable subjectivity, warrants reassessment and stratification to increase its prognostic significance and its relation to disease staging. Anneroth's system, based on six parameters, is one attempt to reduce this subjectivity, although it too is not foolproof, since the individual parameters assessed also carry an element of subjectivity. The moderately differentiated group appears to be a

heterogeneous group that could be stratified into at least two subtypes in the present study. Notably, CK19 IHC staining correlated well with the grades defined by WHO criteria, more so than with those defined by the Anneroth grading system, which showed occasional peaks that could not be explained. CK19 IHC expression may therefore be considered one parameter correlating with tumor differentiation/grade. Further larger, more elaborate studies are needed to establish the role of CK19 IHC staining as a reliable prognostic marker.

### Limitations

This study has several limitations. It was a single-center, cross-sectional study, which limits generalizability and precludes assessment of CK19 expression in relation to treatment response or survival outcomes. Data were largely extracted from archival records rather than direct patient assessment, which may introduce documentation-related bias for variables such as smoking history and presenting symptoms. The number of cases in Anneroth grade IV ( $n = 4$ ) was small, limiting the precision of estimates in this subgroup and likely contributing to the non-significant CK19 positivity difference observed between grade III and grade IV. Both the WHO and Anneroth grading systems carry an inherent element of interobserver subjectivity that was not formally assessed (e.g., no interobserver concordance/kappa statistic was calculated). Finally, CK19 was assessed using a single antibody clone and scoring protocol, and direct comparability with other published series is limited by variation in antibody selection, staining methods, and scoring thresholds across studies.

### Recommendations

Based on these findings, the following are recommended: (1) larger, multicentric, prospective studies with standardized CK19 immunohistochemical protocols and scoring criteria are needed to validate CK19 as a reliable prognostic biomarker in HNSCC; (2) future studies should assess interobserver concordance for both WHO and Anneroth grading systems to better quantify and address subjectivity in tumor grading; (3) the moderately differentiated SCC category may benefit from further stratification, given the heterogeneity observed in this and other studies; and (4) studies incorporating clinical follow-up and survival outcomes are needed to determine whether CK19 expression independently predicts prognosis and could inform treatment decision-making in HNSCC.

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immunohistochemical staining, and record retrieval during this study.

### List of Abbreviations

HNSCC — Head and Neck Squamous Cell Carcinoma; CK19 — Cytokeratin 19; IHC — Immunohistochemistry; WHO — World Health Organization; WDSCC — Well Differentiated Squamous Cell Carcinoma; MDSCC — Moderately Differentiated Squamous Cell Carcinoma; PDSCC — Poorly Differentiated Squamous Cell Carcinoma; SPSS — Statistical Package for the Social Sciences; HPV — Human Papillomavirus; EGFR — Epidermal Growth Factor Receptor; CCND1 — Cyclin D1; ERCC1 — Excision Repair Cross-Complementation Group 1; hpf — High-Power Field; SD — Standard Deviation; CYFRA21-1 — Cytokeratin 19 Fragment.

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### Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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### Authors' Contributions

Dr. Akhil Kumar — Conceptualization of the study, study design, methodology, literature review, supervision of data collection, interpretation of findings, formal analysis, validation, manuscript preparation. Dr. Vibhuti Thukral — Methodology, literature review, supervision of data collection, interpretation of findings, manuscript preparation. Dr. Rajnish Kalra — Conceptualization of the study, study design, methodology, literature review, supervision of data collection, interpretation of findings, formal analysis, validation.

### Conflicts of Interest

The authors declare that there are no known or potential conflicts of interest.

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