J M U H
Journal of Medicine and Urban Health

ORIGINAL ARTICLE

The Role of Mitotic Count and Ki-67 Index in Identifying Likely Benign Salivary Gland Tumors to Avoid Overtreatment

Pornpawee Chitrat, MD[1], Thirayost Nimmanon, MD, PhD[2], Parsinee Julimasart, MSc[1] Kulachet Wiwatwarayos MD, MA[1,2]

[1] *Institute of Pathology, Ministry of Public Health, Bangkok 10400, Thailand*

[2] *Department of Pathology, Phramongkutklao College of Medicine, Bangkok 10400, Thailand*

Corresponding author
Pornpawee Chitrat
pornpaweecht@gmail.com
Received 13 May 2025
Revised 30 September 2025
Accepted 30 October 2025
J Med Urban Health
2026;70(1):e7148
https://doi.org/10.62691/jmuh.2026.7148

ABSTRACT

Objective: This study evaluated and compared the utility of mitotic count and Ki-67 index in distinguishing benign and malignant salivary gland tumors. The primary objective was to identify optimal cut-off points for these proliferative indices to help guide diagnosis and avoid overtreatment in cases where definitive histomorphology is challenging, especially in small biopsy specimens.

Materials and Methods: A total of 88 salivary gland tumor specimens were evaluated, including 56 benign and 32 malignant cases. Mitotic count and Ki-67 index were quantified for all cases. The diagnostic performance of each marker was assessed by determining sensitivity, specificity, the area under the receiver operating characteristic curve and the Area Under the Curve (AUC).

Results: Benign tumors exhibited consistently low mitotic counts (average < 1) and Ki-67 indices (average < 2.00%). In contrast, malignant tumors showed significantly higher values. A mitotic count ≥ 2 and a Ki-67 index ≥ 5.00% were determined as optimal cut-off points. The Ki-67 index (AUC = 0.76) demonstrated a higher sensitivity (68.80%) than the mitotic count (50.00%), performing slightly better than the mitotic count (AUC = 0.73).

Conclusion: Diagnosing benign and malignant salivary gland tumors based on morphology alone in small biopsies can be challenging. A mitotic count ≥ 2 and a Ki-67 index ≥ 5.00% are linked with malignancy, and these proliferation markers serve as valuable adjuncts to improve diagnostic accuracy and guide appropriate patient management. This approach is particularly useful in preventing overtreatment.

Keywords: benign and malignant salivary gland tumors, Ki-67 index, mitotic count

INTRODUCTION

Salivary gland masses encompass a wide variety of tumor types, ranging from benign to malignant neoplasms. Tumors of the parotid gland are the most common, with an estimated annual incidence of 1-3 cases per 100,000 individuals. The majority of these are benign neoplasms (77.00-80.00%), with pleomorphic adenoma (PA) being the most frequent. Malignant salivary gland tumors (MSGTs) are relatively rare, with an incidence of approximately 1-2 cases per 100,000 per year, accounting for about 20.00-30.00% of all salivary gland tumors globally.[1-4] In certain Asian populations, including Thailand, the incidence may be slightly lower, with MSGTs representing only 2.00-3.00% of all head and neck neoplasms.[5]

Despite their lower incidence, MSGTs are associated with poorer clinical outcomes, including a higher risk of local recurrence and distant metastasis, necessitating aggressive treatment such as extensive surgical resection and adjuvant radiotherapy.[6] These interventions can significantly impair patient quality of life, particularly when complications such as facial nerve injury occur in parotid gland surgery.[7] Therefore, accurate and early diagnosis is essential for appropriate therapeutic management and optimizing clinical outcomes.

A significant diagnostic challenge in salivary gland pathology lies in the limited nature of small biopsy specimens. These procedures often obtain only a small and potentially unrepresentative portion of the lesion, which is problematic when distinguishing between benign and malignant tumors due to their overlapping morphological features. For instance, benign tumors like myoepithelioma and basal cell adenoma (BCA) can closely resemble their malignant counterparts, such as myoepithelial carcinoma (MC), basal cell adenocarcinoma (BCAC), epithelial-myoepithelial carcinoma (EMC), and adenoid cystic carcinoma (AdCC). Even salivary duct carcinoma (SDC) or carcinoma (Ca) ex PA in its intraductal phase may be difficult to distinguish from benign apocrine metaplasia in PA.

Although molecular diagnostics, such as the identification of HMGA2 or PLAG1 alterations, can be helpful, their routine application is often limited by cost, technical availability, and the absence of specific molecular signatures in many salivary gland tumors.[8] This has led to a growing interest in identifying adjunctive histopathologic markers that are both practical and informative in routine diagnostics.

The Ki-67 index is one such marker, commonly used as an indicator of cellular proliferation, as it is a nuclear protein expressed during all active phases of the cell cycle. Its expression is most closely associated with the mitotic phase. Due to its short half-life, the Ki-67 index provides a more accurate representation of active proliferation, minimizing residual staining once cells exit the proliferative cycle.[9] In parallel, mitotic count, which quantifies mitotic figures per 10 high-power fields or 2 mm^2, remains a standard but underutilized method for assessing proliferative activity.[10]

Despite the widespread use of the Ki-67 index in other tumor systems, comparative studies evaluating the mitotic count and Ki-67 index across various salivary gland tumors remain limited. Moreover, a universally accepted Ki-67 cut-off value to differentiate between benign and malignant salivary gland neoplasms is lacking. This study aimed to address these limitations by evaluating and comparing the Ki-67 index and mitotic count across a spectrum of benign and MSGTs to determine their average values, assess their diagnostic utility, and explore potential cut-off points. The ultimate goal is to enhance diagnostic accuracy in small biopsy specimens and support clinical decision-making, particularly in cases where definitive molecular testing is unavailable.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of the Institute of Pathology under the ethical approval number IOP-KM-R66-005, and the requirement for informed consent was waived due to the retrospective nature of the analysis.

Cases of benign and MSGTs were retrieved from formalin-fixed, paraffin-embedded (FFPE) tissue blocks archived at the Institute of Pathology, Ministry of Public Health, Bangkok, Thailand. All specimens were fixed in 10.00% neutral buffered formalin, processed, and embedded in paraffin. These specimens, including punch biopsies, needle biopsies, excisions, and surgical resections, were collected between 2017 and 2023. Due to the rarity of MSGTs, all 32 available malignant cases were included, consisting of 4 cases of Ca ex PA, 4 cases of low-grade Mucoepidermoid carcinoma (MEC), 4 cases of MC, 1 case of BCAC, 7 cases of AdCC, 3 cases of EMC, and 9 cases of SDC. For the benign group, a sample size was calculated using the formula for estimating the Finite Population Proportion to ensure statistical reliability.[11] The benign group included 36 cases of PA and 20 cases of BCA, totaling 56 benign salivary neoplasm cases. Clinical variables such as patient age, gland of origin, and disease duration were not used as selection criteria. These clinical factors were considered not to influence the histopathological evaluation or the interpretation of immunohistochemical (IHC) findings.

All specimens were sectioned, 4 micrometers thick, and stained with hematoxylin and eosin (H&E) as a routine process. Two pathologists independently reviewed all tumor sections to confirm the diagnosis in accordance with the 2024 WHO Classification of Head and Neck Tumors under a light microscope (Olympus BX53, Japan, field diameter 0.55 mm).

Mitotic count was evaluated in the most cellular regions of each tumor section, typically at the invasive tumor front, over 10 high-power fields or 2 mm^2, where mitotic activity is generally most prominent and has been associated with prognostic relevance.[12-14] Mitotic figures were identified and counted based on the criteria proposed by Van Diest et al.,[15-17] which define mitotic figures as cells exhibiting condensed chromatin, absence of a nuclear membrane, and no visible nucleolus. Structures such as apoptotic bodies, pyknotic nuclei, and hyperchromatic but non-mitotic nuclei were excluded. Counts were performed independently by a resident in anatomical pathology and a consultant pathologist. Both were blinded to the clinical and diagnostic information. The data were recorded based on 10 consecutive high-power fields (HPFs, 400x magnification) under a light microscope (Olympus BX53, Japan, field diameter 0.55 mm), starting from the field with the highest tumor presence. Any discrepancies were resolved through joint review and consensus.

For the evaluation of the Ki-67 proliferative index, staining was performed using a mouse monoclonal antibody against Ki-67 (clone MIB-1; Dako, Agilent Technologies, USA) at a dilution of 1:300. IHC analysis was performed on 4-μm-thick FFPE tissue sections using the Leica Bond automated staining system with Bond Polymer Refine Detection. Heat-induced epitope retrieval was carried out using an ethylenediaminetetraacetic acid-based buffer for 25 minutes, followed by a peroxide block for 5 minutes. Sections were incubated with the primary antibody for 40 minutes, followed by treatment with a post-primary reagent (10 minutes) and polymer (10 minutes). Detection was completed using 3,3'-diaminobenzidine chromogen for 3 minutes, and all slides were counterstained with hematoxylin for 15 minutes to visualize nuclear morphology. Positive Ki-67 expression was indicated by a brown nuclear signal. The index was calculated as the percentage of positively stained tumor nuclei relative to the total number of tumor nuclei counted.[18] Representative areas demonstrating the highest tumor cellularity ("hotspot" regions) were selected under low-power magnification. Within these areas, 1,000 tumor cell nuclei were manually counted at high-power magnification (400×). The counts were performed independently by a resident in anatomical pathology and a consultant pathologist, blinded to diagnostic information. Any discrepancies between observers were resolved by consensus to ensure consistency and minimize observer bias.

Statistical analyses were conducted using IBM SPSS Statistics 26 software, with a significance threshold at p-value ≤ 0.05. The diagnostic utility of the Ki-67 index and mitotic count was evaluated using ROC curve analysis, a non-parametric statistical method used to assess discriminatory performance between benign and malignant neoplasms.

The diagnostic performance was quantified using the AUC, which is the summary metric derived from the ROC curve and represents the probability that the classifier will correctly rank a randomly chosen malignant case higher than a randomly chosen benign case. AUC results were interpreted as follows: AUC ≥ 0.90 (Excellent), 0.80 ≤ AUC < 0.90 (Good), 0.70 ≤ AUC < 0.8 (Fair), 0.60 ≤ AUC < 0.7 (Poor) and 0.50 ≤ AUC < 0.60 (Fail). AUC values greater than 0.50 were considered meaningful, with 0.80 or higher generally considered acceptable. Graphs were generated using Program R.

RESULTS

This study analyzed the average mitotic count and Ki-67 index across various salivary gland tumor types. The benign group included 36 cases of PA and 20 cases of BCA. The malignant group comprised 4 cases each of Ca ex PA, low-grade MEC, and MC, 1 case of BCAC, 7 cases of AdCC, 3 cases of EMC, and 9 cases of SDC. As shown in **Table 1**, benign neoplasms, including PA and BCA, showed consistently low Ki-67 indices (0.47% and 1.70%, respectively) and low mitotic counts (0.44 and 0.55, respectively). In contrast, malignant tumors demonstrated significantly higher proliferative activity. Among the malignant tumors, SDC had the highest average Ki-67 index (14.38%) and mitotic count (12.13), followed by Ca ex PA (Ki-67: 12.50%, mitotic count: 7.00). Notably, low-grade MEC showed a high average Ki-67 index (10.00%) but a low mitotic count (0.25). Representative photomicrographs illustrating the typical histomorphology and proliferative activity of a benign and a malignant tumor in this cohort are provided in **Figure 1**.

To evaluate the diagnostic utility of the Ki-67 index and mitotic count, a ROC curve was constructed. A mitotic count of ≥ 2 yielded a sensitivity of 50.00 % and a specificity of 91.10 % for distinguishing malignant from benign tumors. A Ki-67 index of ≥ 5.00% demonstrated a higher sensitivity of 68.80% and a

Table 1 Average Values of the Mitotic Count and Ki-67 Index Classified by Tumor Type

Tumor Types	Average Ki-67 Index (%)	Average of Mitotic Count (per 10 HPF)
Pleomorphic adenoma	0.47	0.44
Basal cell adenoma	1.70	0.55
Myoepithelial carcinoma	5.00	2.75
Basal cell adenocarcinoma	5.00	2.00
Adenoid cystic carcinoma	5.71	2.14
Epithelial-myoepithelial carcinoma	8.33	7.33
Mucoepidermoid carcinoma, low grade	10.00	0.25
Carcinoma ex pleomorphic adenoma	12.50	7.00
Salivary duct carcinoma	14.38	12.13

Abbreviation: HPF, high-power field

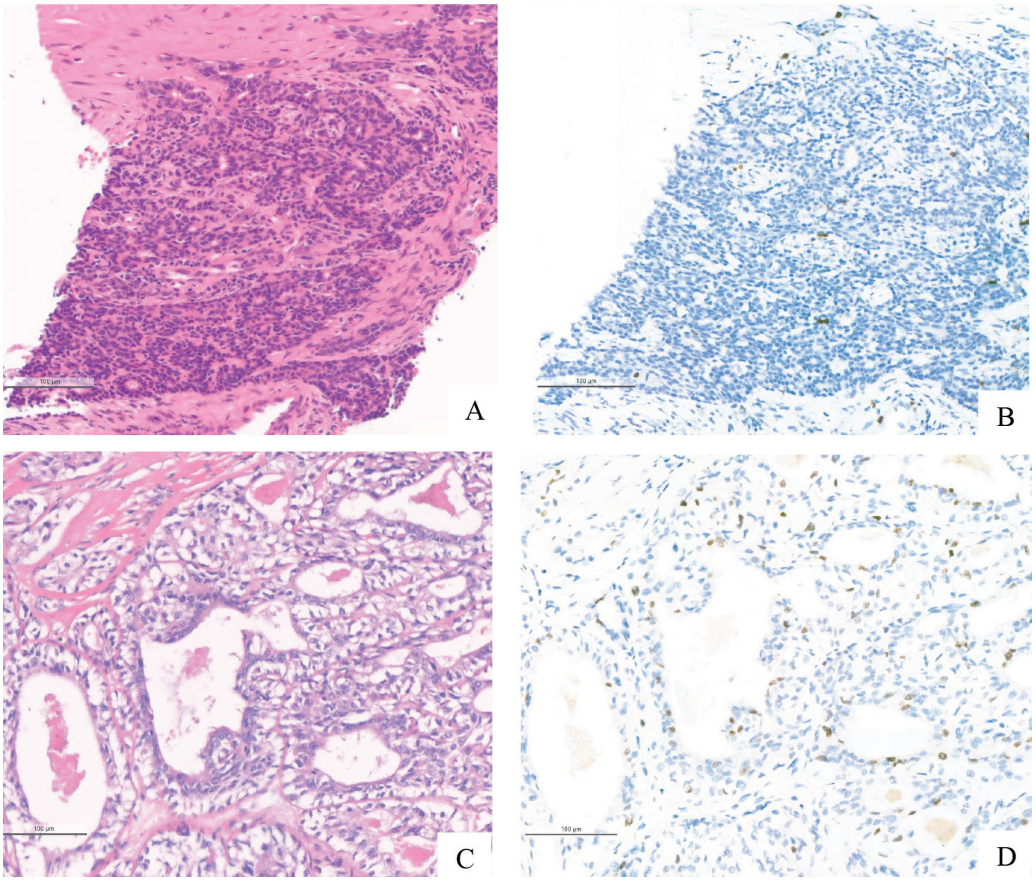

Figure 1 Histological features and the Ki-67 proliferative index, representative of the benign and malignant salivary gland tumors. (A, B) Basal cell adenoma (benign). (A) Hematoxylin and eosin stain shows a biphasic tumor morphology with no mitotic figures identified. (B) Ki-67 immunohistochemistry reveals a low proliferative index of approximately 1.00%. (C, D) Epithelial-myoepithelial carcinoma (malignant). (C) Hematoxylin and eosin stain shows a biphasic malignant tumor; no typical mitotic figures are present in this field. (D) Ki-67 immunohistochemistry demonstrates a significantly higher proliferative index of approximately 14.00%. All images were captured at 20x magnification. Scale bar represents 100 μm.

 https://doi.org/10.62691/jmuh.2026.7148

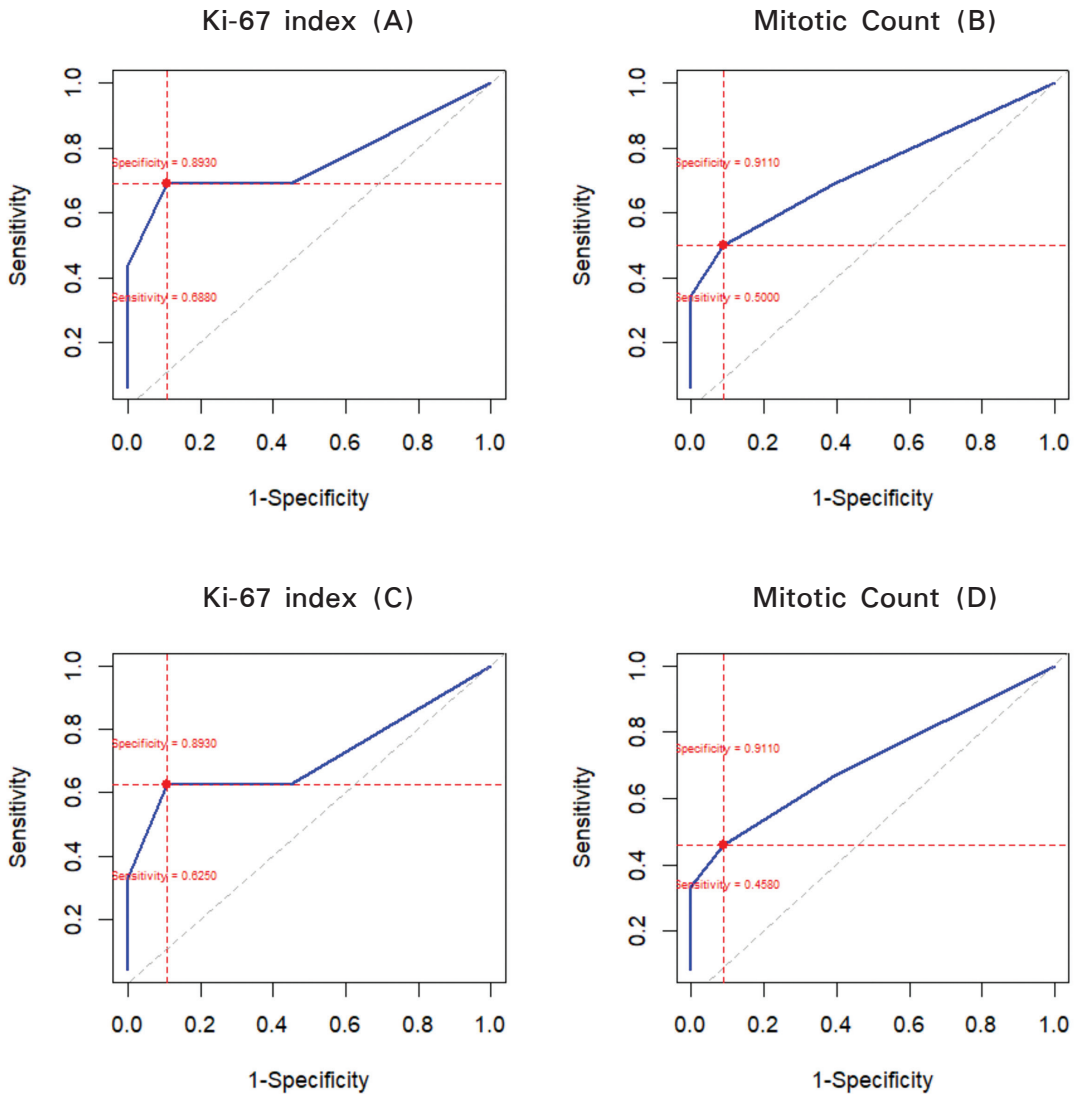

Figure 2 Receiver operating characteristic curves for the Ki-67 index (A, C) and mitotic count (B, D). Panels (A) and (B) represent analyses including salivary duct carcinoma, whereas panels (C) and (D) represent analyses excluding salivary duct carcinoma.

specificity of 89.30%. As presented in Table 2 and Figure 2, the Ki-67 index consistently maintained a higher sensitivity than the mitotic count at comparable false positive rates, suggesting it may offer greater utility in identifying malignant tumors.

For the Ki-67 index, the AUC was 0.76 (95% CI: 0.64-0.88), while the mitotic count showed a slightly lower AUC of 0.73 (95% CI: 0.61-0.85). When SDC cases were excluded, both markers demonstrated an AUC of 0.71. The AUC values for both markers were above 0.70, which is considered fair diagnostic performance, confirming their relevance in a broad spectrum of MSGTs.

DISCUSSION

This study aimed to evaluate the use of mitotic count and Ki-67 index as supplementary diagnostic tools for differentiating between benign and MSGTs, especially in cases where small biopsy specimens present a diagnostic challenge due to overlapping features. The results indicate that both markers have significant value in this regard. The findings showed a clear distinction in proliferation rates between benign and malignant tumors. Benign tumors like PA and BCA consistently showed low mitotic counts and Ki-67 indices. In contrast, malignant tumors such as SDC, Ca ex PA, and MEC exhibited much higher values for

Table 2 Sensitivity and Specificity for Mitotic Count and Ki-67 Index

Marker	Sensitivity (%)	Specificity (%)	AUC
Overall (including SDC)			
Mitotic count (≥ 2)	50.00	91.10	0.73
Ki-67 index (≥ 5.00%)	68.80	89.30	0.76
Overall (excluding SDC)			
Mitotic count (≥ 2)	45.80	91.10	0.71
Ki-67 index (≥ 5.00%)	62.50	89.30	0.71

Abbreviations: AUC, area under the curve; SDC, salivary duct carcinoma

these indices. These results are consistent with findings from a previous report in the literature[19-20].

A key finding was the establishment of optimal cut-off points: a mitotic count of ≥ 2 and a Ki-67 index of ≥ 5.00%. When a tumor's proliferation markers meet or exceed these thresholds, the likelihood of it being benign is significantly reduced. This is particularly important for differentiating benign tumors like myoepithelioma and BCA from their malignant counterparts, such as MC and BCAC. The study found the Ki-67 index to be a slightly more sensitive marker than the mitotic count. This was supported by ROC curve analysis, a non-parametric method that graphically illustrates a test's diagnostic ability by plotting sensitivity against the false positive rate. The Ki-67 index yielded a slightly better AUC—a summary measure of overall diagnostic performance where 1.0 is a perfect test—of 0.76 (95% CI: 0.64–0.88), compared to 0.73 (95% CI: 0.61–0.85) for the mitotic count. Since an AUC value greater than 0.70 is considered a fair diagnostic performance, this confirms that both markers are relevant for a wide range of MSGTs. The use of this non-parametric statistical method was appropriate for this study, as the data on mitotic counts and Ki-67 indices were not normally distributed.

While these markers are valuable, they should not be used as the sole basis for diagnosis. For example, some malignant tumors like MEC can have high Ki-67 indices but low mitotic counts. This highlights the need to interpret these findings in conjunction with other histomorphological features, as well as with clinical data and imaging studies. The study's results showed that both markers, when used together with morphological features, can significantly enhance diagnostic accuracy.

The study was also limited by its sample size, particularly the rarity of MSGTs, which led to a smaller number of malignant cases (32 cases). Despite this, the study provides valuable insights into the utility of these markers and supports their integration into routine diagnostic practice to improve patient care and prevent overtreatment.

CONCLUSION

Based on the study's results, a mitotic count of ≥ 2 and a Ki-67 index of ≥ 5.00% are strongly linked with MSGTs. When a tumor meets or exceeds these thresholds, the probability of it being benign decreases significantly. Therefore, these proliferation markers, especially when used with morphology, clinical findings, and imaging studies, can significantly improve diagnostic accuracy, particularly in small biopsy specimens where morphology alone can be misleading.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgments

We would like to thank the Institute of Pathology, Department of Medical Services, Ministry of Public Health. No additional funding resource was involved.

Author Contributions

Conceptualization: K.W.
Data curation: P.C., K.W.
Formal analysis: P.C., P.J.
Funding acquisition: -
Investigation: P.C.

Methodology: T.N., K.W.

Project administration: P.C., K.W.

Resources: T.N., K.W.

Software: T.N., K.W.

Supervision: T.N., K.W.

Validation: T.N., K.W.

Visualization: P.C., P.J.

Writing – original draft preparation: P.C., K.W.

Writing – review & editing: T.N., K.W.

Data Availability Statement

The data analyzed in this study are stored at the Institute of Pathology, Ministry of Public Health, Thailand. Due to patient confidentiality and institutional regulations, the data are not publicly available. However, researchers may request access from the corresponding author, subject to approval from the Institute of Pathology's Ethics Committee.

REFERENCES

1. Bishop JA, Thompson LDR, Wakely PE, Weinreb I. Tumors of the salivary glands. Arlington: ARP Press; 2021.
2. Boukheris H, Curtis RE, Land CE, Dores GM. Incidence of carcinoma of the major salivary glands according to the WHO classification, 1992 to 2006: a population-based study in the United States. Cancer Epidemiol Biomarkers Prev 2009;18(11):2899-906. doi: 10.1158/1055-9965.EPI-09-0638.
3. Speight PM, Barrett AW. Salivary gland tumours. Oral Dis 2002;8(5):229-40. doi: 10.1034/j.1601-0825.2002.02870.x.
4. WHO classification of tumours. Head and neck tumours. 5th ed. Lyon: International Agency for Research on Cancer; 2024.
5. Mahomed Y, Meer S. Primary epithelial minor salivary gland tumors in South Africa: a 20-year review. Head Neck Pathol 2020;14(3):715-23. doi: 10.1007/s12105-019-01111-4.
6. Khuhaprema T, Srivatanakul P, Attasara P, Sriplung. H, Wiangnon S, Sumitsawan Y. Cancer in Thailand. Bangkok: National Cancer Institute; 2010.
7. Bell RB, Dierks EJ, Homer L, Potter BE. Management and outcome of patients with malignant salivary gland tumors. J Oral Maxillofac Surg 2005;63(7):917-28. doi: 10.1016/j.joms.2005.03.006.
8. Dulguerov P, Marchal F, Lehmann W. Postparotidectomy facial nerve paralysis: possible etiologic factors and results with routine facial nerve monitoring. Laryngoscope 1999;109(5):754-62. doi: 10.1097/00005537-199905000-00014.
9. Voz ML, Aström AK, Kas K, Mark J, Stenman G, Van de Ven WJ. The recurrent translocation t(5;8)(p13;q12) in pleomorphic adenomas results in upregulation of PLAG1 gene expression under control of the LIFR promoter. Oncogene 1998;16(11):1409-16. doi: 10.1038/sj.onc.1201660.
10. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984;133(4):1710-5. doi: 10.4049/jimmunol.133.4.1710.
11. Yamane T. Statistics, An Introductory Analysis. 2nd ed. New York: Harper and Row; 1967.
12. Ellis PS, Whitehead R. Mitosis counting–a need for reappraisal. Hum Pathol 1981;12(1):3-4. doi: 10.1016/s0046-8177(81)80235-3.
13. Ibrahim A, Lashen A, Toss M, Mihai R, Rakha E. Assessment of mitotic activity in breast cancer: revisited in the digital pathology era. J Clin Pathol 2022;75(6):365-72. doi: 10.1136/jclinpath-2021-207742.
14. Meuten DJ, Moore FM, George JW. Mitotic count and the field of view area: time to standardize. Vet Pathol 2016;53(1):7-9. doi: 10.1177/0300985815593349.
15. Scolyer RA, Thompson JF. Mitotic rate in melanoma should be recorded as the number of mitoses per mm^2 (not per high power field): surgeons tell your pathologists! Am J Surg 2013;206(1):142-3. doi: 10.1016/j.amjsurg.2012.11.012.
16. van Diest PJ, van der Wall E, Baak JP. Prognostic value of proliferation in invasive breast cancer: a review. J Clin Pathol 2004;57(7):675-81. doi: 10.1136/jcp.2003.010777.
17. van Diest PJ, Baak JP, Matze-Cok P, Wisse-Brekelmans EC, van Galen CM, Kurver PH, et al. Reproducibility of mitosis counting in 2,469 breast cancer specimens: results from the Multicenter Morphometric Mammary Carcinoma Project. Hum Pathol 1992;23(6):603-7. doi: 10.1016/0046-8177(92)90313-r.
18. Biesterfeld S. Methodische Aspekte bei der standardisierten Beurteilung der mitotischen Aktivität von Tumorgeweben [Methodologic aspects of a standardized evaluation of mitotic activity in tumor tissues]. Pathologe 1997;18(6):439-44. doi: 10.1007/s002920050239.
19. Kazanceva A, Groma V, Smane L, Kornevs E, Teibe U. Proliferative potential in benign mixed salivary gland tumors and its value in primary and recurrent neoplasms. Stomatologija 2011;13(2):35-41.
20. Suzzi MV, Alessi A, Bertarelli C, Cancellieri A, Procaccio L, Dall'olio D, et al. Prognostic relevance of cell proliferation in major salivary gland carcinomas. Acta Otorhinolaryngol Ital 2005;25(3):161-8.