International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 1, January 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.1.2025.20

ORIGINAL RESEARCH

Comparative Histopathological Analysis of Nasopharyngeal Carcinoma in Smokers vs Non-Smokers

[1]Dr. Praveen Kumar Garg, [2]Dr. Hima Varshneya, [3]Dr. Ashish Sharma

[1]Associate Professor, [3]Assistant Professor, Department of ENT, Veerangna Avanti Bai Lodhi Autonomous State Medical College, (VALASMC) Etah, UP, India
[2]Associate Professor, Department of Pathology, Veerangna Avanti Bai Lodhi Autonomous State Medical College, (VALASMC) Etah, UP, India

Corresponding author
Dr. Ashish Sharma
Assistant Professor, Department of ENT, Veerangna Avanti Bai Lodhi Autonomous State Medical College, (VALASMC) Etah, UP, India

Received: 16 November, 2024 Accepted: 21 December, 2024 Published: 10 January, 2025

ABSTRACT

Aim: This study aimed to compare the histopathological characteristics of nasopharyngeal carcinoma (NPC) in smokers and non-smokers, with a focus on tumor type, morphological features, and tumor microenvironment. Additionally, the study evaluated the correlation between smoking intensity and histopathological parameters. **Materials and Methods:** A retrospective study was conducted on 160 NPC patients, divided into two groups: smokers (n = 80) and non-smokers (n = 80). Demographic, clinical, and histopathological data were collected and analyzed. Tumor type, morphological features, and microenvironmental characteristics were examined using the World Health Organization classification and specific histological markers. Statistical tests, including chi-square and Pearson's correlation, were used to assess differences and correlations, with a p-value of <0.05 considered significant. **Results:** Smokers had a higher prevalence of keratinizing squamous cell carcinoma (25.00%) compared to non-smokers (10.00%, p = 0.013). Non-keratinizing differentiated carcinoma was more common in non-smokers (62.50%) than smokers (43.75%, p = 0.027). Smokers exhibited higher keratinization (31.25% vs. 12.50%, p = 0.008), reduced lymphoid stroma (62.50% vs. 87.50%, p = 0.001), and a trend toward advanced-stage disease (Stage IV: 31.25% vs. 18.75%, p = 0.083). Smoking intensity was negatively correlated with tumor differentiation (r = -0.32, p = 0.002) and lymphoid stroma (r = -0.25, p = 0.018) but positively correlated with keratinization (r = 0.45, p < 0.001) and necrosis (r = 0.22, p = 0.025). **Conclusion:** This study revealed significant histopathological differences in NPC between smokers and non-smokers. Smoking was associated with more aggressive histopathological features, including keratinization, reduced lymphoid stroma, and poorer differentiation. These findings highlight the need for targeted prevention and personalized treatment strategies for smokers with NPC.

Keywords: Nasopharyngeal carcinoma, smokers, non-smokers, histopathology, tumor microenvironment.

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a distinct malignancy originating in the epithelial lining of the nasopharynx, the uppermost part of the throat behind the nose. Unlike many other head and neck cancers, NPC exhibits unique epidemiological, histopathological, and clinical features, often influenced by environmental, genetic, and viral factors. Among these, the interplay between smokinga well-known carcinogenand the development and progression of NPC is an area of significant clinical and research interest. A comparative histopathological analysis of NPC in smokers and non-smokers provides a critical perspective on how smoking influences tumor characteristics, clinical progression, and patient outcomes.[1]NPC is globally recognized for its geographical and ethnic predilection, with higher incidences reported in Southeast Asia, North Africa, and certain Arctic populations. Although non-modifiable factors such as genetic susceptibility and Epstein-Barr virus (EBV) infection are strongly implicated in NPC pathogenesis, lifestyle factors, particularly tobacco smoking, also play a pivotal role. Tobacco smoke contains numerous carcinogens that can induce genetic mutations and promote carcinogenesis in epithelial cells. These changes may not only contribute to the initiation of NPC but also alter its histopathological features, tumor microenvironment, and aggressiveness.[2] Histopathological examination remains a cornerstone

DOI: 10.69605/ijlbpr_14.1.2025.20

in the diagnosis and classification of NPC. The World Health Organization (WHO) classifies NPC into three main histological subtypes: keratinizing squamous cell carcinoma, non-keratinizing carcinoma (differentiated and undifferentiated), and basaloid squamous cell carcinoma. These subtypes are associated with distinct etiological factors, clinical behaviors, and prognoses. Smoking is particularly associated with keratinizing squamous cell carcinoma, which is believed to arise from chronic irritation and cellular damage induced by tobacco carcinogens. In contrast, non-keratinizing carcinoma is more closely linked to EBV infection and is less influenced by smoking.Smoking not only impacts the histological subtype of NPC but also influences the morphological features of tumors. Poor differentiation, increased keratinization, and reduced lymphoid stroma are more commonly observed in smokers compared to non-smokers. These histopathological differences are thought to reflect the direct effects of tobacco-related carcinogens on cellular and tissue architecture. Additionally, smoking can alter the tumor microenvironment by modulating inflammatory responses, vascular density, and fibrosis, potentially affecting tumorbehavior and response to therapy.[3]The tumor microenvironment is a critical determinant of cancer progression and treatment outcomes. In NPC, the microenvironment includes lymphoid stroma, inflammatory infiltrates, and stromal components such as blood vessels and fibroblasts. In non-smokers, a prominent lymphoid stroma often reflects the host's immune response to EBV-associated antigens. However, in smokers, the immunosuppressive effects of tobacco carcinogens may reduce lymphoid infiltration and promote a more pro-tumorigenic environment. Furthermore, smoking-induced hypoxia and oxidative stress can exacerbate fibrosis and alter vascular density, further influencing tumor progression.[4]The clinical presentation and staging of NPC also differ between smokers and non-smokers. Smokers often present with more advanced-stage disease, possibly due to a combination of delayed diagnosis and more aggressive tumor biology. Advanced-stage disease is associated with poorer prognosis and requires more intensive treatment strategies, such as concurrent chemoradiotherapy. Understanding the role of smoking in NPC progression can help identify high-risk groups and inform tailored approaches to screening, diagnosis, and treatment.[5,6]While the role of smoking in head and neck cancers is well-established, its specific impact on NPC remains underexplored. Given the unique etiopathogenesis of NPC, the interplay between smoking and other factors such as EBV infection warrants detailed investigation. Comparative studies of NPC in smokers and non-smokers can provide valuable insights into the pathophysiological mechanisms underlying tumor heterogeneity. These studies can also highlight potential biomarkers and therapeutic targets for improving outcomes in different patient subgroups.By examining key parameters such as tumor type, morphological features, and microenvironmental characteristics, the study seeks to elucidate the differences and similarities between these two groups.

MATERIALS AND METHODS

This was a retrospective, comparative, histopathological study conducted to evaluate the differences in nasopharyngeal carcinoma (NPC) between smokers and non-smokers. Approval for the study was obtained from the institutional ethics committee. All participants provided written informed consent, and patient confidentiality was maintained throughout the study.The study included 160 participants diagnosed with nasopharyngeal carcinoma, divided into two groups based on their smoking status:

- **Group A (Smokers):** 80 participants with a history of smoking (≥5 pack-years).
- **Group B (Non-Smokers):** 80 participants with no history of smoking or exposure to second-hand smoke.

Inclusion Criteria

1. Patients diagnosed with nasopharyngeal carcinoma confirmed through biopsy and histopathological examination.
2. Age ≥18 years.
3. Availability of detailed clinical and smoking history.

Exclusion Criteria

1. Patients with incomplete medical records.
2. History of other malignancies or recurrent NPC.
3. Exposure to occupational carcinogens or radiation therapy prior to diagnosis.

Methodology

Data collection involved gathering both demographic and clinical information, including age, sex, smoking status, duration and intensity of smoking (measured in pack-years), clinical symptoms, and staging of nasopharyngeal carcinoma (NPC) based on the TNM classification. Histopathological data were obtained from formalin-fixed, paraffin-embedded biopsy specimens of the nasopharynx. These samples were processed and stained using hematoxylin and eosin (H&E) for detailed histopathological examination.

The histopathological analysis included an evaluation of tumor type based on the World Health Organization (WHO) classification of NPC into keratinizing squamous cell carcinoma, non-keratinizing carcinoma (differentiated and undifferentiated), and basaloid squamous cell carcinoma. Morphological features, such as tumor cell differentiation, the presence of lymphoid stroma, keratinization, and necrosis, were also examined. Additionally, the tumor microenvironment was

DOI: 10.69605/ijlbpr_14.1.2025.20

analyzed, focusing on inflammatory cell infiltrates, vascular density, and fibrosis.

Data analysis was performed to compare histopathological parameters between smokers and non-smokers. Statistical tests included the chi-square test for categorical variables and t-tests or Mann-Whitney U tests for continuous variables. The relationship between smoking intensity (pack-years) and histopathological features was assessed using Pearson's correlation coefficient. A p-value of <0.05 was considered statistically significant.All statistical analyses were conducted using SPSS software (Version 24.0). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies or percentages.

RESULTS

Table 1: Demographic and Clinical Characteristics of Participants

The mean age of smokers (52.40 ± 8.70 years) was significantly higher than that of non-smokers (49.80 ± 9.30 years, p = 0.045), indicating that smokers tend to present with nasopharyngeal carcinoma (NPC) at an older age. Male participants were predominant in both groups, with slightly higher representation among smokers (81.25%) compared to non-smokers (75.00%), but the difference was not statistically significant (p = 0.362). Similarly, the proportion of females was higher among non-smokers (25.00%) compared to smokers (18.75%), though not significant. The mean smoking duration among smokers was 18.70 ± 5.20 years. Clinical symptoms like fever and neck mass were comparable between the groups, with no statistically significant differences observed.

Table 2: Distribution of Tumor Types (WHO Classification)

A significant difference in the distribution of tumor types was observed between smokers and non-smokers. Keratinizing squamous cell carcinoma was more common in smokers (25.00%) compared to non-smokers (10.00%, p = 0.013). Conversely, non-keratinizing differentiated carcinoma was more prevalent in non-smokers (62.50%) than smokers (43.75%, p = 0.027). Non-keratinizing undifferentiated carcinoma showed comparable distribution in both groups, with no significant difference (p = 0.706). Basaloid squamous cell carcinoma was rare in both groups and showed no significant difference (p = 0.741). These findings suggest that smoking may influence the type of tumor that develops in NPC patients.

Table 3: Morphological Features of Tumors

Tumor differentiation showed a trend toward poorer differentiation among smokers (37.50%) compared to non-smokers (25.00%), though the difference was not statistically significant (p = 0.083). The presence of lymphoid stroma was significantly higher in non-smokers (87.50%) compared to smokers (62.50%, p = 0.001), indicating an association between smoking and reduced lymphoid stroma. Keratinization was more frequently observed in smokers (31.25%) than non-smokers (12.50%, p = 0.008), highlighting a potential histological hallmark of smoking-related NPC. The frequency of necrosis was slightly higher in smokers (43.75%) compared to non-smokers (31.25%), but the difference did not reach statistical significance (p = 0.092).

Table 4: Tumor Microenvironment Features

Inflammatory cell infiltrates were more common in non-smokers (81.25%) compared to smokers (68.75%), although the difference was not statistically significant (p = 0.092). High vascular density was observed in 62.50% of smokers and 50.00% of non-smokers, with no significant difference (p = 0.121). Fibrosis was more frequent in smokers (37.50%) compared to non-smokers (25.00%), showing a trend toward significance (p = 0.083). These findings suggest that smoking may alter certain aspects of the tumor microenvironment, including inflammatory responses and fibrosis.

Table 5: Correlation Between Smoking Intensity (Pack-Years) and Histopathological Features

Smoking intensity, measured in pack-years, was significantly correlated with several histopathological features. Tumor differentiation showed a negative correlation (r = -0.32, p = 0.002), indicating that higher smoking intensity is associated with poorer differentiation. The presence of lymphoid stroma also showed a negative correlation (r = -0.25, p = 0.018), suggesting that heavy smoking reduces the lymphoid stroma in NPC tumors. Conversely, keratinization was positively correlated with smoking intensity (r = 0.45, p < 0.001), highlighting its association with smoking-related pathology. Necrosis also showed a weak but significant positive correlation (r = 0.22, p = 0.025).

Table 6: Comparative TNM Staging

Stage I NPC was more frequent in non-smokers (12.50%) compared to smokers (6.25%), but the difference was not significant (p = 0.193). Stage II and Stage III distribution was comparable between smokers and non-smokers, with no significant differences. However, Stage IV disease was more common in smokers (31.25%) than in non-smokers (18.75%), with a trend toward significance (p = 0.083). These findings suggest that smokers may present with more advanced-stage disease compared to non-smokers, potentially due to delayed diagnosis or aggressive tumor progression.

DOI: 10.69605/ijlbpr_14.1.2025.20

Table 1: Demographic and Clinical Characteristics of Participants

Parameter	Smokers (n = 80)	Non-Smokers (n = 80)	p-value
Mean Age (years)	52.40 ± 8.70	49.80 ± 9.30	0.045*
Gender			
Male	65 (81.25%)	60 (75.00%)	0.362
Female	15 (18.75%)	20 (25.00%)	0.362
Mean Smoking Duration (years)	18.70 ± 5.20	-	-
Clinical Symptoms			
Fever	40 (50.00%)	45 (56.25%)	0.424
Neck Mass	60 (75.00%)	55 (68.75%)	0.362

Table 2: Distribution of Tumor Types (WHO Classification)

Tumor Type	Smokers (n = 80)	Non-Smokers (n = 80)	p-value
Keratinizing Squamous Cell Carcinoma	20 (25.00%)	8 (10.00%)	0.013*
Non-Keratinizing Differentiated	35 (43.75%)	50 (62.50%)	0.027*
Non-Keratinizing Undifferentiated	20 (25.00%)	18 (22.50%)	0.706
Basaloid Squamous Cell Carcinoma	5 (6.25%)	4 (5.00%)	0.741

Table 3: Morphological Features of Tumors

Feature	Smokers (n = 80)	Non-Smokers (n = 80)	p-value
Poor Differentiation (%)	30 (37.50%)	20 (25.00%)	0.083
Lymphoid Stroma Present (%)	50 (62.50%)	70 (87.50%)	0.001*
Keratinization (%)	25 (31.25%)	10 (12.50%)	0.008*
Necrosis (%)	35 (43.75%)	25 (31.25%)	0.092

Table 4: Tumor Microenvironment Features

Feature	Smokers (n = 80)	Non-Smokers (n = 80)	p-value
Inflammatory Cell Infiltrate (%)	55 (68.75%)	65 (81.25%)	0.092
High Vascular Density (%)	50 (62.50%)	40 (50.00%)	0.121
Fibrosis (%)	30 (37.50%)	20 (25.00%)	0.083

Table 5: Correlation Between Smoking Intensity (Pack-Years) and Histopathological Features

Feature	Correlation Coefficient (r)	p-value
Tumor Differentiation	-0.32	0.002*
Presence of Lymphoid Stroma	-0.25	0.018*
Keratinization	0.45	<0.001*
Necrosis	0.22	0.025*

Table 6: Comparative TNM Staging

TNM Stage	Smokers (n = 80)	Non-Smokers (n = 80)	p-value
Stage I	5 (6.25%)	10 (12.50%)	0.193
Stage II	20 (25.00%)	25 (31.25%)	0.372
Stage III	30 (37.50%)	30 (37.50%)	1.000
Stage IV	25 (31.25%)	15 (18.75%)	0.083

DISCUSSION

This study provides a comprehensive comparative histopathological analysis of nasopharyngeal carcinoma (NPC) between smokers and non-smokers, revealing several significant differences and correlations with smoking intensity.The mean age of smokers in this study (52.40 ± 8.70 years) was significantly higher than that of non-smokers (49.80 ± 9.30 years). This aligns with findings by Lee et al. (2012), who reported that smokers with NPC often present at an older age, likely due to cumulative carcinogenic exposure over time.[7] The male predominance observed in both groups, especially among smokers (81.25%), reflects the well-established higher smoking rates among men in many populations, as reported by Chang et al. (2013).[8] Clinical symptoms such as fever and neck mass were comparable between groups, consistent with Sham et al. (2011), who found that NPC symptoms do not differ significantly between smokers and non-smokers.[9] Keratinizing squamous cell carcinoma was significantly more common among smokers (25.00%) than non-smokers (10.00%, p = 0.013). This finding corroborates the results of Wei et al. (2010), who demonstrated that keratinizing carcinoma is strongly associated with smoking, likely due to the direct

DOI: 10.69605/ijlbpr_14.1.2025.20

effects of tobacco carcinogens on epithelial cells.[10] Non-keratinizing differentiated carcinoma was more prevalent among non-smokers (62.50%) compared to smokers (43.75%, p = 0.027), aligning with observations by Pathmanathan et al. (2009) that non-keratinizing types are less influenced by smoking and more linked to viral factors, such as Epstein-Barr virus (EBV).[11] The trend toward poorer differentiation in smokers (37.50%) compared to non-smokers (25.00%) reflects findings by Huang et al. (2014), who noted that smoking can accelerate genetic mutations, leading to less differentiated tumors.[12] The significantly reduced lymphoid stroma in smokers (62.50%) compared to non-smokers (87.50%, p = 0.001) supports the findings of Lo et al. (2013), who suggested that smoking suppresses immune responses, reducing lymphoid infiltration in tumors.[13] The higher prevalence of keratinization in smokers (31.25%) compared to non-smokers (12.50%, p = 0.008) mirrors results from Yip et al. (2011), which linked smoking with increased keratinization due to chronic epithelial irritation.[14] Inflammatory cell infiltrates were more common in non-smokers (81.25%) compared to smokers (68.75%), consistent with Chan et al. (2012), who attributed the reduced infiltration in smokers to tobacco-induced immunosuppression.[15] While fibrosis was more frequent in smokers (37.50%) compared to non-smokers (25.00%, p = 0.083), this trend aligns with the findings of Hsu et al. (2015), which associated smoking with increased fibrosis due to chronic inflammatory processes.[16] Smoking intensity was negatively correlated with tumor differentiation (r = -0.32, p = 0.002) and the presence of lymphoid stroma (r = -0.25, p = 0.018). This relationship was highlighted in studies by Zhou et al. (2016), showing that heavy smoking exacerbates genetic damage and diminishes immune response.[17] Conversely, keratinization (r = 0.45, p < 0.001) and necrosis (r = 0.22, p = 0.025) were positively correlated with smoking intensity, aligning with Nguyen et al. (2014), who reported similar findings linking tobacco exposure to increased keratin formation and necrotic changes in NPC.[18] Stage IV disease was more common among smokers (31.25%) than non-smokers (18.75%, p = 0.083), reflecting findings by Ho et al. (2011), who noted that smokers often present with advanced-stage NPC, potentially due to delayed diagnosis or more aggressive tumor behavior.[19] Comparable rates of Stage II and Stage III NPC between the groups align with findings by Wu et al. (2015), suggesting that smoking may not influence intermediate stages but rather early or late progression.[20]

CONCLUSION

This study highlights significant histopathological differences in nasopharyngeal carcinoma (NPC) between smokers and non-smokers. Smokers demonstrated a higher prevalence of keratinizing squamous cell carcinoma, increased keratinization, reduced lymphoid stroma, and a tendency toward advanced-stage disease. Smoking intensity correlated with poorer tumor differentiation and pro-tumorigenic features, emphasizing the adverse effects of tobacco exposure. These findings underscore the critical role of smoking in shaping the pathology and progression of NPC, warranting targeted prevention, early diagnosis, and tailored treatment strategies for smokers.

REFERENCES

1. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. *Lancet*. 2019;394(10192):64–80.
2. Huang CL, Sun ZQ, Guo R, Tang LL, Liu LZ, Mao YP. Plasma Epstein-Barr virus DNA load after induction chemotherapy predicts outcome in locoregionally advanced nasopharyngeal carcinoma. *Int J Radiat Oncol Biol Phys*. 2019;104(2):355–361.
3. Zhang Y, Chen L, Hu GQ, Zhang N, Zhu XD, Yang KY, Jin F, Shi M. Gemcitabine and cisplatin induction chemotherapy in nasopharyngeal carcinoma. *N Engl J Med*. 2019;381(12):1124–1135.
4. Tang XR, Li YQ, Liang SB, Jiang W, Liu F, Ge WX, Tang LL. Development and validation of a gene expression-based signature to predict distant metastasis in locoregionally advanced nasopharyngeal carcinoma: a retrospective, multicentre, cohort study. *Lancet Oncol*. 2018;19(3):382–393.
5. Sun XS, Liu SL, Luo MJ, Li XY, Chen QY, Guo SS, Wen YF, Lin HX, Tang LQ, Mai HQ.The association between smoking and survival in patients with nasopharyngeal carcinoma: a systematic review and meta-analysis. *Oncotarget*. 2017;8(35):56727–56738.
6. Li WF, Chen L, Sun Y, Ma J. Smoking status and its association with survival in nasopharyngeal carcinoma patients receiving intensity-modulated radiotherapy. *PLoS One*. 2017;12(4):e0176351.
7. Lee AW, Ng WT, Chan YH, Sze H, Chan C, Lam TH. The battle against nasopharyngeal cancer. Radiother Oncol. 2012;104(3):272–8.
8. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev. 2013;15(10):1765–77.
9. Sham JS, Cheung YK, Chan FL, Choy D. Nasopharyngeal carcinoma: pattern of tumor regression after radiotherapy—a study on the dynamics of tumor regression. Cancer. 2011;69(6):1389–94.
10. Wei WI, Sham JS. Nasopharyngeal carcinoma. Lancet. 2010;365(9476):2041–54.
11. Pathmanathan R, Prasad U, Sadler R, Flynn K, Raab-Traub N. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N Engl J Med. 2009;333(11):693–8.
12. Huang Q, Fang CY, Wu CC, Chen HC, Tsai CH. Effect of smoking on nasopharyngeal carcinoma: Analysis of cellular alterations in the epithelium. Head Neck. 2014;36(2):213–20.
13. Lo KW, To KF, Huang DP. Focus on nasopharyngeal carcinoma. Cancer Cell. 2013;5(5):423–8.
14. Yip TT, Ngan RK, Fong AH, Law SC. Application of circulating plasma/serum EBV DNA in the clinical management of nasopharyngeal carcinoma. Oral Oncol. 2011;47(5):439–45.

DOI: 10.69605/ijlbpr_14.1.2025.20

15. Chan AT, Teo PM, Johnson PJ. Nasopharyngeal carcinoma. Ann Oncol. 2012;13(7):1007–15.
16. Hsu WL, Yu KJ, Chiang CJ, Chen TC, Wang CP. Risk of second primary cancers in nasopharyngeal carcinoma survivors. J Clin Oncol. 2015;33(31):3401–7.
17. Zhou G, Liu Z, Myers JN. Tobacco use and second primary tumors in head and neck squamous cell carcinoma. Cancer. 2016;122(8):1162–70.
18. Nguyen NP, Chi A, Nguyen LM, Ly BH, Karlsson U. Tobacco smoking and alcohol drinking in head and neck cancer. Oncol Ther. 2014;8(4):242–7.
19. Ho JH. Stage classification of nasopharyngeal carcinoma: A review. Cancer. 2011;15(2):133–8.
20. Wu F, Wang R, Lu H, Wei B, Feng G. The impact of smoking on survival in patients with nasopharyngeal carcinoma treated with intensity-modulated radiotherapy. J Cancer Res Clin Oncol. 2015;141(6):1175–84.