Cytological Features of Undifferentiated Nasopharyngeal Carcinomas (12 Cases Diagnosed Cytologically From Lymph Node Metastases)

Burcu ÖZCAN*, Merve CİN**

Abstract

Aim: Nasopharyngeal carcinomas (NPCs) are often diagnosed through fine-needle aspiration biopsy (FNAB) samples from metastatic lymph nodes. The differential diagnosis of cervical mass lesions includes a broad spectrum of lesions, which can complicate the diagnostic process. This study aims to analyze the clinical and cytological features of 12 cases of undifferentiated nonkeratinizing NPC diagnosed from metastases and identify key morphological findings that may aid diagnosis.

Method: This retrospective study included 12 cases of nasopharyngeal carcinoma diagnosed via fine-needle aspiration (FNA) in our department between 2021 and 2024. All cases were histologically classified as undifferentiated-type nasopharyngeal carcinoma. Clinical data of the cases were retrieved from the hospital information management system. Cytology smears were retrieved from the archives and re-evaluated in terms of cytomorphological characteristics.

Results: Eight of the cases were male, and four were female. Their ages ranged from 23 to 80 years. All smears were cellular. In half of the cases (6 cases), the tumor cells formed cohesive clusters, whereas in 5 cases, the cells displayed loose cohesion. In 7 cases, the tumor cells exhibited large nuclei, with pleomorphism in 10 cases. In half of the cases, the nuclei were oval-shaped, followed by oval-to-round nuclei in four cases. Naked nuclei were observed in most cases (9/12). Nucleoli were prominent in 8 cases. In 10 cases, the cytoplasm was scant with indistinct borders.

Conclusions: The cytologic diagnosis of NPC is complex and requires a multifaceted approach. FNA of cervical lymph nodes is a valuable diagnostic tool, but careful interpretation is crucial. The presence of cells exhibiting a mixed pattern—forming clusters as well as appearing isolated cells—characterized by large pleomorphic nuclei, prominent nucleoli, and scant cytoplasm, particularly in samples obtained from cervical lymph nodes, constitutes a significant clue in cytological diagnosis.

Keywords: Nasopharyngeal carcinomas, fine-needle aspiration (FNA), cytology.

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* Corresponding Author, Specialist Cytopathologist, University of Health Science, Istanbul Training and Research Hospital, Department of Pathology, Istanbul, Türkiye. E-mail: drburcuozcan@yahoo.com
ORCID https://orcid.org/0000-0002-7662-3306
** Specialist Pathologist, University of Health Science, Istanbul Training and Research Hospital, Department of Pathology, Istanbul, Türkiye. E-mail: merveates42@hotmail.com ORCID https://orcid.org/0000-0002-0656-497X

ETHICAL STATEMENT: This study was approved by the Istanbul Training and Research Hospital, Clinical Research Ethics Committee (Number: 25, Date: 07/02/2025) and was conducted under the principles of the Helsinki Declaration.

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Andiferansiye Nazofarenks Karsinomlarının Sitolojik Özellikleri (Lenf Nodu Metastazlarından Sitolojik Olarak Tanı Alan 12 Olgu)

Öz

Amaç: Nazofarenks karsinomları (NFK), sıklıkla metastatik lenf nodlarından alınan ince iğne aspirasyon biyopsisi (İİAB) örnekleri ile tanı almaktadır. Servikal kitle lezyonlarının ayırıcı tanısı, geniş bir spektrumu içermekte olup, bu durum tanı sürecini karmaşık hale getirebilmektedir. Bu çalışmanın amacı, metastazlardan tanı alan 12 undiferansiye nonkeratinize NFK olgusunun klinik ve sitolojik özelliklerini analiz etmek ve tanıya yardımcı olabilecek temel morfolojik bulguları belirlemektir.

Yöntem: Bu retrospektif çalışmaya, 2021-2024 yılları arasında bölümümüzde ince iğne aspirasyonu (İİA) ile tanı alan 12 nazofarenks karsinomu olgusu dahil edilmiştir. Tüm olgular histolojik olarak undiferansiye tip nazofarenks karsinomu olarak sınıflandırılmıştır. Olgulara ait klinik veriler, hastane bilgi yönetim sisteminden elde edilmiştir. Arşivlerden temin edilen sitoloji yaymaları, sitomorfolojik özellikler açısından yeniden değerlendirilmiştir.

Bulgular: Çalışmaya dahil edilen 12 olgunun 8'i erkek, 4'ü kadın olup yaşları 23 ile 80 arasında değişmektedir. Tüm sitoloji yaymaları hücre içeriği açısından yeterliydi. Olguların yarısında (6 olgu) tümör hücreleri koherent kümeler oluştururken, 5 olguda hücreler düşük kohezyon göstermiştir. Tümör hücreleri 7 olguda büyük çekirdekler sergilemiş olup, 10 olguda çekirdek pleomorfizmi saptanmıştır. Çekirdekler, olguların yarısında oval şekilli, dört olguda ise oval-yuvarlak olarak gözlenmiştir. Olguların büyük bir kısmında (9/12) çıplak çekirdekler izlenmiştir. Nükleoller 8 olguda belirgin olarak değerlendirilmiştir. Sitoplazma 10 olguda belirgin sınırları olmayan ve yetersiz miktarda olarak saptanmıştır.

Sonuç: NFK'nin sitolojik tanısı karmaşık olup çok yönlü bir değerlendirme gerektirmektedir. Servikal lenf nodlarından yapılan İİAB, değerli bir tanı yöntemi olmakla birlikte dikkatli yorumlanması gereklidir. Servikal lenf nodlarından elde edilen örneklerde, kümeler oluşturan ve aynı zamanda izole hücreler şeklinde dağılım gösteren, büyük pleomorfik çekirdekler, belirgin nükleoller ve az sitoplazma içeren hücrelerin varlığı, sitolojik tanıda önemli bir ipucu sunmaktadır.

Anahtar Sözcükler: Nazofarenks karsinomu, ince iğne aspirasyonu (İİA), sitoloji.

Introduction

Nasopharyngeal carcinomas (NPCs) are rare malignancies, accounting for 0.8% of cancer-related deaths[1] and representing 98% of all nasopharyngeal malignancies[2,3]. These malignancies originate where the Eustachian tube opens into the Rosenmüller fossa[4].

On physical examination, findings may be unremarkable, although subtle fullness, surface granularity, or a distinct mass can occasionally be detected. NPCs are often diagnosed through fine-needle aspiration biopsy (FNAB) of metastatic cervical lymph nodes[5-9], as cervical lymphadenopathy is the most common presentation[10,11]. Definitive cytological diagnosis is essential because nasopharyngeal tumors are frequently submucosal and not directly visible, and in young patients NPC may mimic lymphoma both histologically and cytologically[10-12].

NPCs are squamous cell carcinomas (SCCs) that exhibit squamous differentiation. These tumors are classified into three morphological subtypes: keratinizing SCC, nonkeratinizing SCC (differentiated and undifferentiated), and basaloid SCC. Etiological factors include viral, environmental, and genetic elements, with Epstein-Barr Virus

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(EBV) being the factor most consistently associated with NPC development, particularly in nonkeratinizing subtypes[1]. Nonkeratinizing and basaloid types are more common in younger patients, showing better responses to radiotherapy[13]. Notably, nonkeratinizing NPCs are more radiosensitive and have a higher survival rate than keratinizing carcinomas[10].

The defining characteristic of undifferentiated NPC is the lack of cellular differentiation, leading to anaplastic or poorly differentiated cells. This is reflected by significant cellular pleomorphism with marked variations in cell size and shape[14]. The undifferentiated subtype is typified by large cells with a syncytial growth pattern, prominent nucleoli, and round-to-oval nuclei. Additionally, a lymphocyte-rich stroma and intermingling lymphoid cells within epithelial clusters distinguish this subtype from the differentiated type[15].

Given the rarity of NPCs, cytology-focused case series are scarce and typically include all subtypes. This study evaluates 12 metastatic cases of undifferentiated nonkeratinizing NPC to highlight key clinical and cytological features that may aid diagnosis.

Material and Methods

This retrospective study included 12 cases of nasopharyngeal carcinoma diagnosed by fine-needle aspiration (FNA) between 2021 and 2024. Corresponding biopsies, also reviewed in our department, confirmed undifferentiated-type nasopharyngeal carcinoma in all cases.

Clinical information was retrieved from the hospital database. Smears were re-evaluated for cytomorphological features; air-dried preparations were stained with May-Grünwald-Giemsa and alcohol-fixed with Papanicolaou. Available cell blocks underwent immunohistochemical analysis.

Cytological evaluation focused on cellularity, architecture, lymphoepithelial groups, background lymphocytes, nuclear and cytoplasmic details, and necrosis.

Results

The cohort included 8 males and 4 females (age range, 23–80 years; mean, 56.5). All aspirates were obtained from cervical lymph nodes with a 22-gauge needle. Two patients underwent simultaneous lymph node FNA and nasopharyngeal biopsy, resulting in concurrent diagnosis. Four cases were primarily diagnosed by FNA, while 6 had prior histologic confirmation. Systemic evaluation revealed no distant metastases in 10 patients; 2 had liver metastases, one of whom also had bone involvement. Clinical details are summarized in Table 1 and cytological features are summarized in Table 2.

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Table 1. Clinical characteristics of the cases

Case	Age	Gender	Clinical Findings	Localization	Biopsy Result
1	66	Male	Nasal congestion, neck swelling	Left submandibular lymph node	Undifferentiated type nasopharyngeal carcinoma (simultaneous cytological and histopathological diagnosis)
2	78	Male	Ear pain, neck swelling	Left cervical lymph node, level 2	Undifferentiated type nasopharyngeal carcinoma (previous tissue diagnosis)
3	78	Male	Ear pain, neck swelling	Left cervical lymph node, level 2A	Undifferentiated type nasopharyngeal carcinoma (previous tissue diagnosis)
4	47	Male	Neck swelling	Left parotid inferior lymph node	Undifferentiated type nasopharyngeal carcinoma (diagnosed after FNA biopsy)
5	51	Female	Neck swelling	Left submandibular + left parotid lymph node	Undifferentiated type nasopharyngeal carcinoma (previous tissue diagnosis)
6	23	Female	Neck swelling	Right cervical lymph node, level 2	Undifferentiated type nasopharyngeal carcinoma (diagnosed after FNA biopsy)
7	47	Female	Nasal congestion, snoring	Left cervical lymph node	Undifferentiated type nasopharyngeal carcinoma (previous tissue diagnosis)
8	49	Female	Neck swelling	Left cervical lymph node	Undifferentiated type nasopharyngeal carcinoma (simultaneous cytological and histopathological diagnosis)
9	60	Male	Nasal congestion	Left cervical lymph node	Undifferentiated type nasopharyngeal carcinoma (previous tissue diagnosis)
10	57	Male	Neck swelling	Right cervical lymph node	Undifferentiated type nasopharyngeal carcinoma (diagnosed after FNA biopsy)
11	80	Male	Neck swelling	Left cervical lymph node	Undifferentiated type nasopharyngeal carcinoma (previous tissue diagnosis)
12	42	Male	Neck swelling	Right submandibular lymph node	Undifferentiated type nasopharyngeal carcinoma (diagnosed after FNA biopsy)

Figure 1. 1A- Malignant basaloid pleomorphic cells are in clusters as well as dissociated. Note presence of lymphoid cells in background. (MGG x400) 1B,1C- Loosely clustered primitive pleomorphic cells with an oval nucleus and prominent nucleoli. (MGG x1000) 1D- Malignant cells with oval shaped nuclei arranged in a syncytium. (PAP x400)

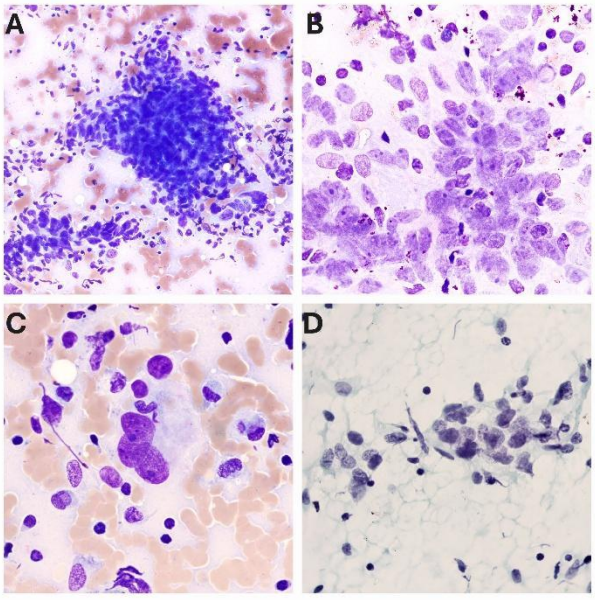

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Table 2. The cytological characteristics of the cases

Case	Architecture	Lymphoid Background	Nuclear Size	Pleomorphism	Bare Nuclei	Nucleolus	Cytoplasm	Necrosis
1	Cohesive	Present	Medium - Large	Present	Present	Multiple Large	Scant	Absent
2	Cohesive	Absent	Medium - Large	Absent	Present	Absent	Medium	Absent
3	Discohesive	Present	Large	Present	Present	Multiple Large	Scant	Absent
4	Loosely Cohesive	Present	Large	Present	Present	Single small	Scant	Present
5	Cohesive	Present	Large	Present	Present	Single Large	Medium-wide	Present
6	Cohesive	Present	Large	Present	Present	Multiple Large	Scant-Medium	Absent
7	Loosely Cohesive	Present	Medium-Large	Present	Present	Single Large	Scant	Absent
8	Loosely Cohesive	Absent	Large	Present	Present	Absent	Scant	Absent
9	Cohesive	Present	Large	Present	Absent	Single Large	Scant	Absent
10	Cohesive	Present	Medium-Large	Absent	Absent	Absent	Scant	Absent
11	Loosely Cohesive	Absent	Medium-Large	Present	Absent	Absent	Scant-Medium	Present
12	Loosely Cohesive	Present	Large	Present	Present	Single Large	Scant-Medium	Present

Immunohistochemistry was not feasible in 2 cases due to scant cellularity. At least one squamous marker (p63, p40, or CK5/6) was positive in 9 cases (Figure 2A–C). One case lacked squamous marker expression but was classified as metastatic nasopharyngeal carcinoma based on positive pan-cytokeratin and EBER-ISH, consistent with histopathology. EBER-ISH was positive in 8 cell blocks (Figure 2D), and in 4 additional cases positivity was confirmed on histologic samples.

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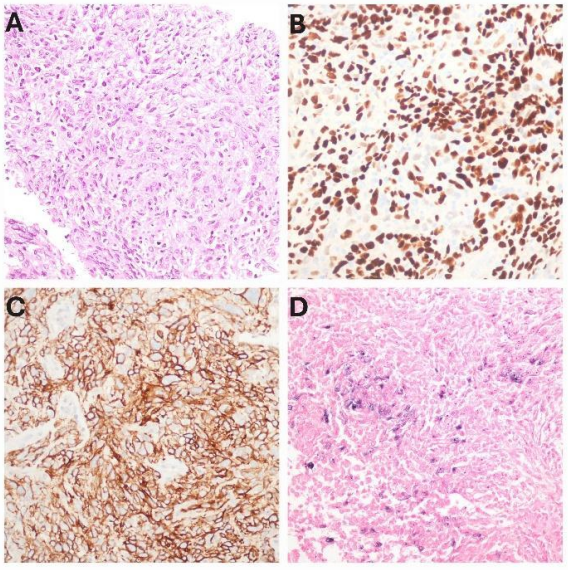
Figure 2. 2A- Large-sized, malignant cells with vesicular chromatin in cell block sections (x400) 2B- Strong nuclear staining with p63 immunstain (x400) 2C- Cytoplasmic staining with CK5/6 immunstain (x400) 2D- Positive staining with EBER-ISH (x400)



Discussion

FNAB of cervical lymph nodes is a widely used method for diagnosing metastatic NPC[16-19]. NPC typically exhibits a bimodal age distribution, peaking in the second and sixth decades[20]. In our series, one patient was in the second decade, whereas the others were older, with a mean age of 56.5 years. Male predominance (3:1) was consistent with previous reports[5,16,21,22]. Cervical swelling was the most frequent clinical presentation, followed by nasal and otologic symptoms[16].

Cellularity is a characteristic feature of NPC smears. All cases in our series were cellular, consistent with Mohanty et al.[5], who reported cellularity in all 15 cases, and Viguer et al.[10], who observed this feature in 74% of their 43 predominantly undifferentiated cases.

Our cases exhibited diverse architectural patterns. In half of the cases, cohesive cell clusters were observed, whereas the remaining cases demonstrated predominantly singly scattered cells with loose cohesion. Bare nuclei were present in 9 cases, a finding highlighted in previous studies[5,10]. NPC cells can be arranged in clusters[5,16], and syncytial groups with nuclear overlapping are frequently observed[16]. Viguer et al.[10] identified mixed patterns of clusters and single cells as the most common, followed by discohesive and carcinoma-like patterns. Chan et al.[6] in their study of 40 metastatic NPC cases predominantly of the undifferentiated subtype, reported cohesive groups in all cases.

In 9 cases, lymphoid cells were interspersed within the epithelial component. Lymphocytes intermingled with tumor cells is a well-known hallmark of NPC cytology[5,6,16,23]. This dense lymphocytic infiltrate reflects the immune response against NPC but may also cause diagnostic confusion, mainly mimicking reactive lymphoid

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hyperplasia or lymphomas[23,24]. While lymphoid elements are expected in metastatic tumors to lymph nodes, extensive residual lymphoid tissue is uncommon in large metastatic deposits, as most of the nodal architecture is replaced by tumor. In contrast, lymphoid-rich backgrounds are rarely observed in SCC, thyroid, and salivary gland carcinomas[10].

Large nuclei were noted in 7 cases, and pleomorphism in 10 of 12, consistent with prior reports. Viguer et al.[10] reported a lower incidence (16.7%), likely due to inclusion of differentiated subtypes. Nuclear shapes were predominantly oval, with oval-round nuclei in 4 cases and spindle-shaped nuclei in 1, aligning with descriptions of oval–spindle nuclei[16].

Nucleoli were prominent in 7 cases, with single large nucleoli in 4 and multiple nucleoli in 3 cases. Previous studies have emphasized nucleolar prominence as a characteristic finding[5,6,10,16]. Alongside a single central nucleolus, small multiple nucleoli were frequently observed[10,16].

Most of our cases (10/12) comprised cells with scant cytoplasm and indistinct cell borders, consistent with prior studies[5,16]. Necrotic cell debris was present in 3 cases. Grenko et al.[16] reported necrosis and inflammatory cells in 2 of 18 cases.

Additional findings included crush artifacts in 1 case and prominent capillary networks in 3 cases. Microvascular density (MVD), a parameter that quantifies tumor angiogenesis, plays a crucial role in tumor growth and metastasis. Studies have explored the relationship between MVD and the proliferation index (Ki-67) in undifferentiated NPC[25].

At least one squamous marker (p63/p40/CK5/6) was positive in 9 cases. Undifferentiated NPCs exhibit a squamous epithelial phenotype and can express P63, P40, CK5/6, and AE1/AE3[26]. EBER-ISH was applied in eight cases, all of which were positive. The strong association between Epstein-Barr virus (EBV) infection and NPC is well established[27], making EBER detection a useful diagnostic tool[26]. The cytological findings have been summarized in Table 3 in comparison with the literature.

Table 3. Comparison of the findings with the literature

Parameter	**Findings (Current Series, n=12)**	**Literature Comparison**
Age	Range: 2nd–6th decades; Mean: 56.5	Bimodal peak: 2nd & 6th decades[20]
Sex ratio	Male: Female = 3:1	Male predominance reported [5,16,21,22]
Clinical presentation	Cervical swelling (most common), nasal symptoms, otologic complaints (5 cases)	Similar patterns reported[16]
Cellularity	All cases cellular	Mohanty et al.: 100% (15/15)[5]; Viguer et al.: 74.1% (43 cases, mainly undifferentiated)[10]
Architecture	6 cases: cohesive clusters; 6 cases: singly scattered/loose cohesion; bare nuclei in 9	Mixed patterns (clusters + single cells) most common[10,16]

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Lymphoid background	9/12 with lymphocytes intermixed; 3 exclusively tumor cells	Lymphocyte admixture is a hallmark [5,6,21,23]; uncommon in SCC/thyroid/salivary[10]
Nuclear morphology	Large nuclei: 7 cases; Pleomorphism: 10/12; Shapes: 6 oval, 4 oval-round, 1 spindle	Viguer et al.: lower pleomorphism (16.7%)[10]; oval-spindle nuclei reported[16]
Nucleoli	Prominent in 8 cases (4 single large, 3 multiple); Inconspicuous in 33.3%	Prominence emphasized in prior studies[5,6,10,16]
Cytoplasm	Scant cytoplasm, indistinct borders in 10/12	Consistent with prior reports[5,16]
Necrosis	3 cases with necrotic debris	Grenko et al.: 2/18[16]
Other features	Crush artifact (1 case), prominent capillaries (3 cases)	MVD linked to tumor growth/metastasis[25]
Immunohistochemistry	Squamous markers positive in 9 cases (p63/p40/CK5/6)	Undifferentiated NPC expresses squamous phenotype[26]
EBER-ISH	Performed in 8 cases, all positive	EBV association well established[27]

Differential diagnoses include squamous cell carcinoma (SCC), metastatic carcinoma, and lymphomas[5]. NPC and Hodgkin lymphoma (HL) share a reactive lymphoid background and may contain eosinophils or granulomas. NPC cells can mimic Reed–Sternberg cells, but the latter show abundant pale cytoplasm, vesicular chromatin, and distinct nucleoli, unlike the irregular nucleoli and frequent bare nuclei typical of NPC[10,23].

In contrast to the monotonous lymphoid cells of non-Hodgkin lymphoma (NHL), NPC demonstrates neoplastic aggregates against lymphoid backgrounds[23]. Small cohesive clusters in discohesive smears are diagnostically helpful[10]. Bizarre cells, plasma cells, and eosinophils are rare in NHL[23]. Immunohistochemistry, including cytokeratin, EMA, and EBV-related assays, further aids distinction[5].

Distinguishing NPC from metastatic SCC can also be difficult. Both may show pleomorphism, but keratinization—typical of SCC—is absent in NPC. SCC often displays keratinized cells with eosinophilic cytoplasm and altered nuclei, whereas NPC more often shows plasma cell–rich backgrounds. EBV detection further supports NPC[23].

FNAC of cervical lymph nodes is valuable but requires careful interpretation. For undifferentiated nasopharyngeal carcinoma, a mixed pattern of clusters and isolated cells with pleomorphic nuclei, prominent nucleoli, scant cytoplasm, and a lymphoid background represents a key diagnostic clue. Further studies should refine cytological criteria and identify specific markers to improve diagnostic accuracy.

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