

Original Article

HISTOPATHOLOGICAL ASSESSMENT OF ARCHIVED FORMALIN-FIXED PARAFFIN-EMBEDDED SAMPLE QUALITY FROM NASOPHARYNGEAL BIOPSY CASES

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ABSTRACT

Formalin-fixed paraffin-embedded (FFPE) tissue samples are widely used for diagnostic and research purposes. However, many factors can affect the quality of these samples, potentially compromising subsequent tissue analyses. This study aimed to assess the quality of archived FFPE samples from nasopharyngeal biopsy cases using systematic histopathological evaluation to determine sample suitability for tumour microenvironment (TME) studies. We retrospectively analysed 124 archival FFPE nasopharyngeal biopsy specimens. FFPE tissue sections were stained with routine staining (haematoxylin and eosin) and reviewed by a histopathologist. Histopathological assessment systematically evaluated tissue morphology, tumour presence, stromal component, and lymphoid tissue. From the 124 FFPE samples evaluated, only 97 samples, comprising 55 nasopharyngeal carcinoma and 42 non-tumour samples, fulfilled the inclusion criteria. A systematic histological assessment was crucial for determining the suitability of a sample for cell and tissue structure analysis. This study established a histological quality control framework for assessing archival FFPE samples in the TME study, ensuring reliable and reproducible data for future investigations.

KEYWORDS: FFPE, nasopharyngeal biopsy, nasopharyngeal carcinoma, sample quality control, tumour microenvironment

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a malignant tumour arising in the nasopharynx, a region located behind the nasal cavity and above the oropharynx. It is strongly associated with the Epstein-Barr virus (EBV) and is classified as a type of head and neck cancer (1). NPC shows a distinct geographic and ethnic distribution, with higher prevalence in southern China, Southeast Asia, and parts of the Middle East and North Africa (2, 3). According to the Globocan 2022 report, the Maldives recorded the highest incidence rate of NPC, followed by Brunei, Indonesia, Malaysia, and Singapore (4). In Malaysia, NPC was identified as the fifth most common cancer, particularly affecting males, as reported by the Malaysia Cancer Registry (2012–2016) (5).

Formalin-fixed paraffin-embedded (FFPE) tissues are extensively used in clinical and research settings due to their preserved tissue morphology and biomolecules, such as protein markers. FFPE samples can also be stored long-term in room temperature conditions, making archiving cost-effective and convenient in most laboratory settings. They serve as an invaluable resource for prospective and retrospective studies and have become a widely used tool in biomedical research, particularly in oncology (6). Consequently, FFPE samples have facilitated the discovery of novel biomarkers and therapeutic targets, which contributed to the advancement of cancer therapies (7, 8).

Understanding the tumour microenvironment (TME) holds immense potential for developing more effective cancer therapies. Researchers have used cutting-edge techniques to improve their understanding of the TME, including immunohistochemistry-based, cytometry-based (imaging, mass, and flow cytometry), and spatial-omics methods (9, 10). It is possible to obtain comprehensive data on the abundance, phenotypes, and spatial distribution of the components of the TME using FFPE tissues (11, 12). Hence, prior evaluation of the quality of FFPE tissues is crucial as it could affect the accuracy and reliability of the subsequent analyses.

Archival FFPE tissues are typically leftover samples that were originally collected for clinical and diagnostic purposes (13). Various factors can affect the quality of archival FFPE tissues for subsequent studies, such as the method used for tissue sampling (surgery or biopsy), tissue fixation and processing. Therefore, assessing their quality depends on the intended downstream analysis (14). The current gold standard for cancer diagnosis is histopathological examination of a tissue biopsy. This procedure involves sampling a limited piece of the suspicious tissue to identify and further analyse the malignant cells (15). As a result, biopsy tissue may lack tumour heterogeneity and other critical cellular components for TME study (16).

In this report, we present a systematic histopathological evaluation method to assess the quality of archived FFPE tissues from nasopharyngeal biopsy cases. This assessment could provide information on the sample's suitability for morphological TME study.

MATERIALS AND METHODS

Sample collection

Convenience sampling was carried out at Hospital Universiti Teknologi MARA (UiTM), Sungai Buloh, Malaysia. A total of 124 archived FFPE samples of nasopharyngeal biopsy tissues from cases diagnosed between 2013 and 2022 were collected for this study.

Sample preparation, staining and slide scanning

All FFPE samples were sectioned at 4 µm thick and stained with haematoxylin and eosin (H&E) using an autostainer Leica XL (Leica Biosystems, Melbourne, Australia). Slides were scanned at 20x objective magnification using a tissue scanning microscope Vectra Polaris (now known as Phenomager™ HT, Akoya Biosciences, Massachusetts, USA) for image archiving. Images were viewed and processed using the Phenochart software (version 1.0.8).

Histological assessment

A histological review of the H&E-stained samples was performed by a single experienced diagnostic histopathologist following a flowchart of predetermined criteria. Reproducibility of assessments by other histopathologists was not evaluated. Figure 1 shows the flowchart for the histological assessment. Firstly, the FFPE samples' quality was assessed based on the tissue morphology, and only tissues with good morphology and adequate tissue for analysis were included. Criteria for samples with good morphology include the presence of intact, well-preserved cellular and tissue structures for tumour or lymphoid tissue. Adequate lymphoid tissue includes the presence of distinct lymphoid follicles and diffuse infiltration of lymphocytes. Secondly, the samples were further screened based on the percentage of tumour viability for NPC cases or the presence of lymphoid tissue for non-tumour cases. Only samples showing more than 50% viable tumours were included for the NPC cases, and for the non-tumour cases, only samples with adequate lymphoid tissue were included. The cut-off value (50% viable tumour content in NPC cases) was to ensure adequate tissue regions (tumour and stroma) for further analysis. Samples that did not follow these criteria were graded as poor or histologically inadequate for further evaluation and were excluded from the study.

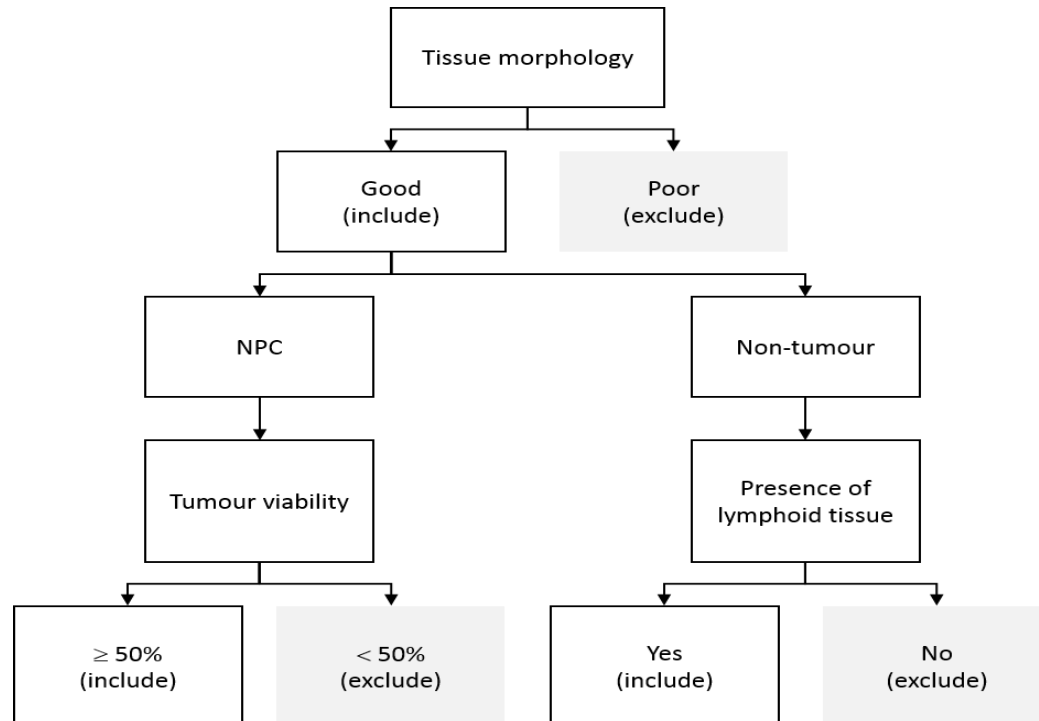


Figure 1. Flowchart for nasopharyngeal biopsy FFPE samples quality assessment

Data analysis

Data analysis was done using Microsoft Excel.

RESULTS

Figure 2 shows different types of nasopharyngeal biopsy cases. The non-keratinising differentiated NPC subtype (Figure 2a) shows tumour cells with well-defined borders, variable intercellular bridges, and focal or no keratinisation. The intensity of immune-stromal infiltration and the presence of desmoplastic stroma varies. The histology of the non-keratinising undifferentiated NPC subtype shows either a nest of cohesive tumour cells with indistinct cell borders (Regaud pattern, Figure 2b) or sheets of diffuse, non-cohesive tumour cells (Schmincke pattern, Figure 2c). The non-keratinising undifferentiated NPC usually shows high lymphoplasmacytic infiltration and absence of keratinisation, while the presence of desmoplastic stroma varies. In lymphoid hyperplasia (Figure 2d), the histology shows the presence of lymphoid follicles with prominent, reactive germinal centres. Normal lymphoid tissue, typically composed of mononuclear cells is usually presented with a focal or absence of prominent lymphoid follicles (Figure 2e).

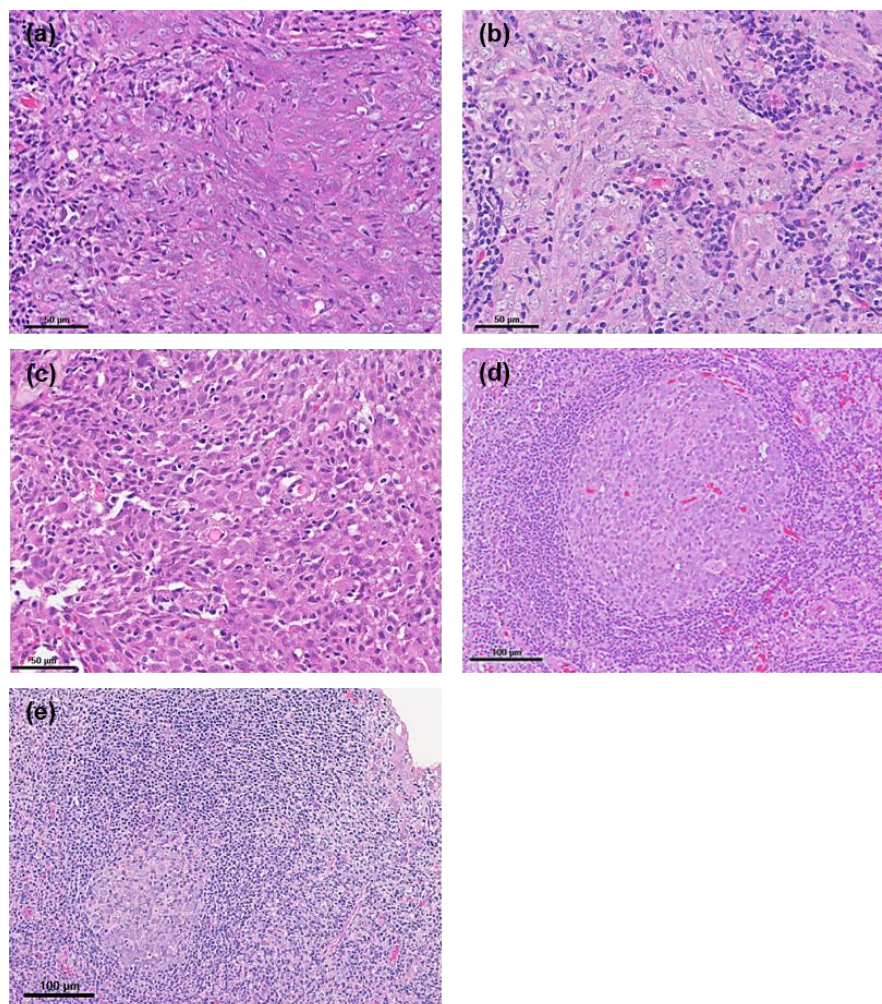


Figure 2. H&E images of nasopharyngeal biopsy cases: (a) non-keratinising differentiated NPC; (b) non-keratinising undifferentiated NPC (Regaud pattern); (c) non-keratinising undifferentiated NPC (Schmincke pattern); (d) lymphoid hyperplasia, and (e) normal lymphoid tissue

Figure 3 shows some detailed histological features observed in NPC samples. Figure 3a shows the morphology of viable tumour versus tumour necrosis (Figure 3b). We also observed different types of epithelial lining, namely the respiratory-type epithelium (Figure 3c) and squamous epithelium (Figure 3d). The tumour cells were intermixed with immune cells (Figure 3e). Sometimes desmoplastic reaction in the stroma was observed (Figure 3f). There is also the presence of inflammatory cells (Figure 3g) and adjacent lymphoid tissue (Figure 3h).

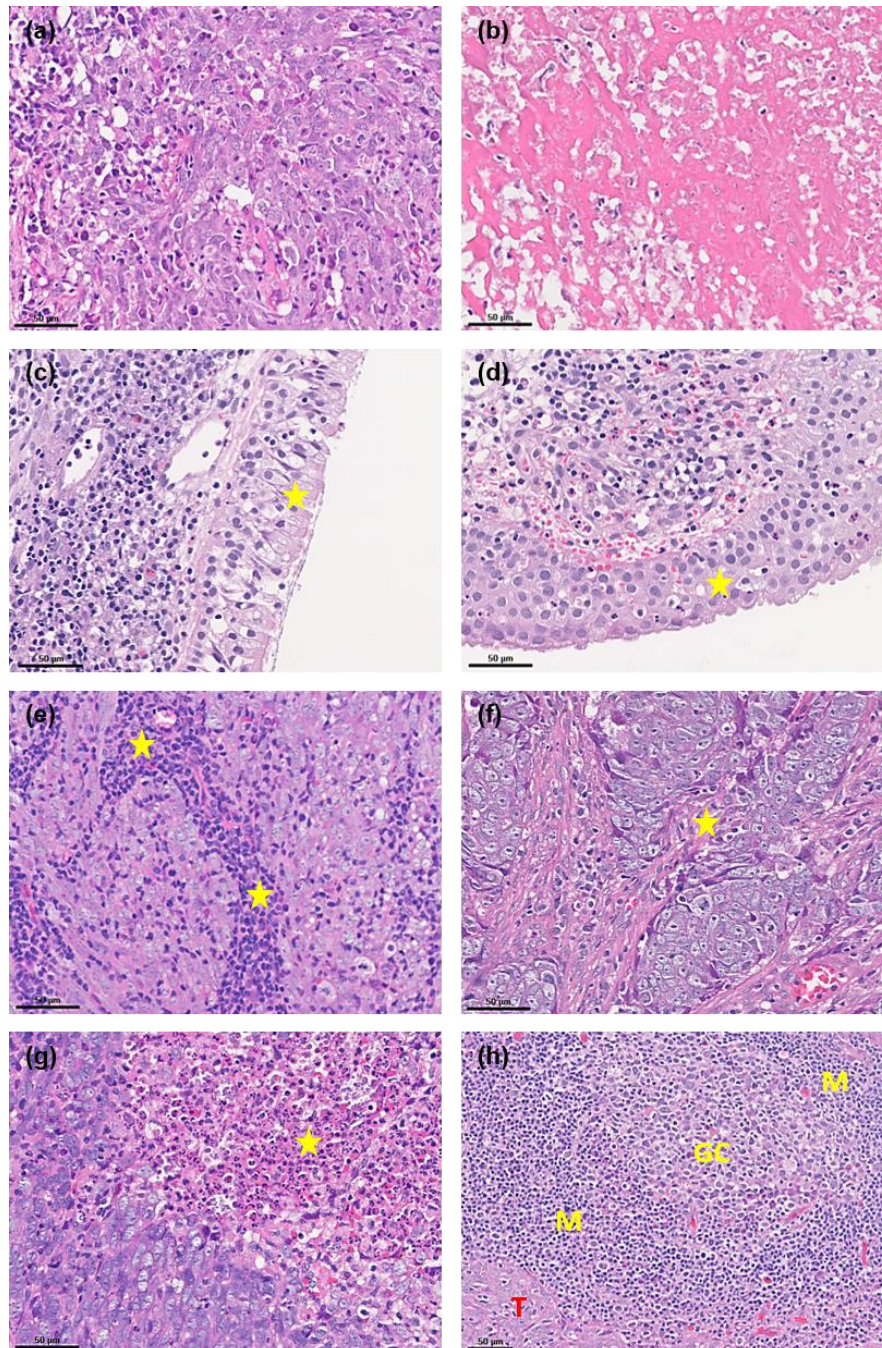


Figure 3. Histological features observed in NPC samples. (a) viable versus (b) necrotic tumour. The star symbol highlighted specific structures: (c) respiratory epithelium, (d) squamous epithelium, (e) inflammatory cells/lymphocytes, (f) reactive fibroblasts, and (g) inflammatory cells. (h) lymphoid structure is adjacent to the tumour. GC = germinal centre, M = mantle zone, T = tumour

Table 1 summarises the histological assessment of the FFPE samples. A total of 124 samples were examined, out of which only 97 passed the inclusion criteria. These criteria included good tissue morphology, the presence of more than 50% tumour tissue (NPC cases), and the presence of lymphoid tissue (non-tumour cases). Based on the first criterion, which is good tissue morphology, a total of 101 samples (56/59 NPC cases, 45/45 non-tumour cases) were included for further filtering. Further analysis of the NPC cases showed that 55 out of 56 samples had more than 50% viable tumour tissue, whereas 42 out of 45 non-tumour cases (lymphoid hyperplasia and lymphoid tissue) had adequate presence of lymphoid tissue for analysis. Overall, this result shows that approximately 22% (27/124) of the cases were excluded based on the histological assessment.

Table 1. Summary of samples before and after the histological assessment

Number of samples before assessment	Number of samples after assessment			
	a. Inclusion by morphology	b. Inclusion by % viable tumour (NPC)	c. Inclusion by the presence of lymphoid tissue (non-tumour)	Total number of samples after assessment (b+c)
124	101	55	42	97

DISCUSSION

In this study, we have established a histological quality control framework for assessing archival FFPE samples for nasopharyngeal biopsy. Our findings show that histological assessment is critical in determining sample suitability for downstream work that involves cell and tissue structure analysis. Without prior knowledge of this aspect of tissue quality, further downstream work will be negatively impacted. This aspect is of utmost importance, especially when working with biopsies, since tissue may lack critical cellular components for the intended study (16). A previous study demonstrated the importance of histopathological assessment for selecting appropriate samples for downstream genomic studies (17).

This framework also aids in estimating sample size by indicating potential dropout rates. However, we acknowledge several limitations. First, the use of convenience sampling may introduce selection bias, and the study did not assess reproducibility across multiple histopathologists.

Second, while our study focused on histological evaluation, alternative methods such as molecular profiling could complement tissue assessment. For example, samples with a low tumour content may still be suitable for other downstream applications like RNA/DNA sequencing, depending on the methods used for tumour enrichment strategy (for example, laser capture microdissection), integrity and quantity of nucleic acids. Additionally, the adequacy of tissue remains a subjective measure. Future work could explore standardised criteria, such as the estimation of total surface area, to improve consistency.

Lastly, we did not include the assessment of protein or nucleic acid integrity (RNA/ DNA). For future consideration, immunohistochemistry can be considered for in situ protein assessment, while nucleic acid integrity can be assessed by in situ hybridisation staining of housekeeping genes or by fragment analysis of extracted RNA/DNA from the FFPE sample to determine the percentage or quality of RNA/DNA fragments (18). This will add useful information on the overall quality of the FFPE tissues.

CONCLUSION

By establishing quality criteria for archival FFPE NPC biopsy samples, this study ensures reliable and reproducible data for future TME investigations.

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Institutional Review Board Statement: This study was performed in accordance with the tenets of the Declaration of Helsinki and approved by the UiTM Research Ethics Committee (Approval num.: REC/03/2022 (PG/FB/5)) and Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia (Approval num.: NMRR ID-22-01930-6NH (IIR), 22-00178-2BC).

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