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Correlation of Histopathology and Immunohistochemistry in Diagnosis of Malignancies

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Abstract
Introduction: *Immunohistochemistry has emerged as the most valuable adjunct to Hematoxylin and Eosin (H&E) staining in diagnostic histopathology. IHC has an essential role in the diagnosis of metastatic tumors. It is done on paraffin-embedded tissue and has a low cost compared to advanced imaging studies and molecular genetic analysis. The aim of the present study is to evaluate the diagnostic discrepancy between histopathology and IHC and to identify the root cause of discordance wherever possible.*
Materials and methods: *A retrospective analysis was done in Histopathology section of Government Medical College, Jammu. In this study, during the study period of one year (2022), cases for which IHC was requested were included. Total number of concordances and discordances are assessed to improve histopathological diagnostic accuracy. For each discordant case root cause analysis was done to identify areas of weakness.*
Results: *During the study period total 40 cases were sent for IHC. Out of 40 cases, for 33 (82.5%) cases histopathological diagnosis was in concordance with the IHC diagnosis. Whereas in 5 (12.50%) cases histopathological diagnosis was discordant with the IHC diagnosis. For 2 (5.0%) cases conclusive diagnosis could not be derived. The cases for which IHC was done were categorized as lymphomas, breast tumors, lung tumors etc.*
Conclusion: *IHC is a useful adjunct which helps in confirming the histopathological diagnosis and further subtyping the tumors as well as assessing certain prognostic factors. If we club the histopathology with IHC in our laboratories, we will definitely improve the diagnostic accuracy for the better patient care.*
Keywords: *Immunohistochemistry, histopathology, diagnosis*

I. INTRODUCTION

The histomorphological diagnosis of cancer and the exact categorization of the proper tumor type are very important for the adequate treatment of malignant tumors.[1] Immunohistochemistry has emerged as the most valuable adjunct to Hematoxylin and Eosin (H&E) staining in diagnostic histopathology.[2] The diagnosis for malignancy starts with biopsy of the tumor tissue after a medical history, physical examination, and several investigations including liver, kidney function test, blood test, stool for occult blood, chest radiograph, abdominal, chest CT scan and mammography for female, prostate-specific antigen test for male. Hematoxylin and Eosin (H/E) stain by identifying the general histomorphological features can categorize the tumors into adenocarcinoma, well/moderately or poorly differentiated carcinomas, squamous cell carcinomas, undifferentiated neoplasms, and carcinomas with neuroendocrine differentiation [3,4].

Histopathological examination and clinical correlation remain the cornerstone in morphologic diagnosis. IHC supports or rules out possible differential diagnosis. Immunohistochemistry combines anatomic and immunologic techniques to identify tissue components using a specific antigen-antibody reaction that can be detected through enzyme reactions with the antibodies being used. The enzyme reaction appears as a color at the site of antibody-antigen binding, so it permits the visualization and localization of specific cellular components within a cell or tissue [5]. After the biopsy, morphology is examined (first, the pathologist must prove cancers existence); a stepwise approach using IHC markers panels is followed to identify the tumor type and subtypes. Broadly site of origin is recognized using organ-specific markers.[6]

IHC has an essential role in the diagnosis of metastatic tumors. It is done on paraffin-embedded tissue and has a low cost compared to advanced imaging studies and molecular genetic analysis.[6] IHC is mainly indicated for following three purposes: a) Confirmation of diagnosis, b) Determination of site of origin of metastatic tumors and c) Detection of molecules having prognostic or therapeutic significance.[7]
The aims of the present study is:

- To evaluate the diagnostic discrepancy between histopathology and IHC.
- To identify the root cause of discordance wherever possible.

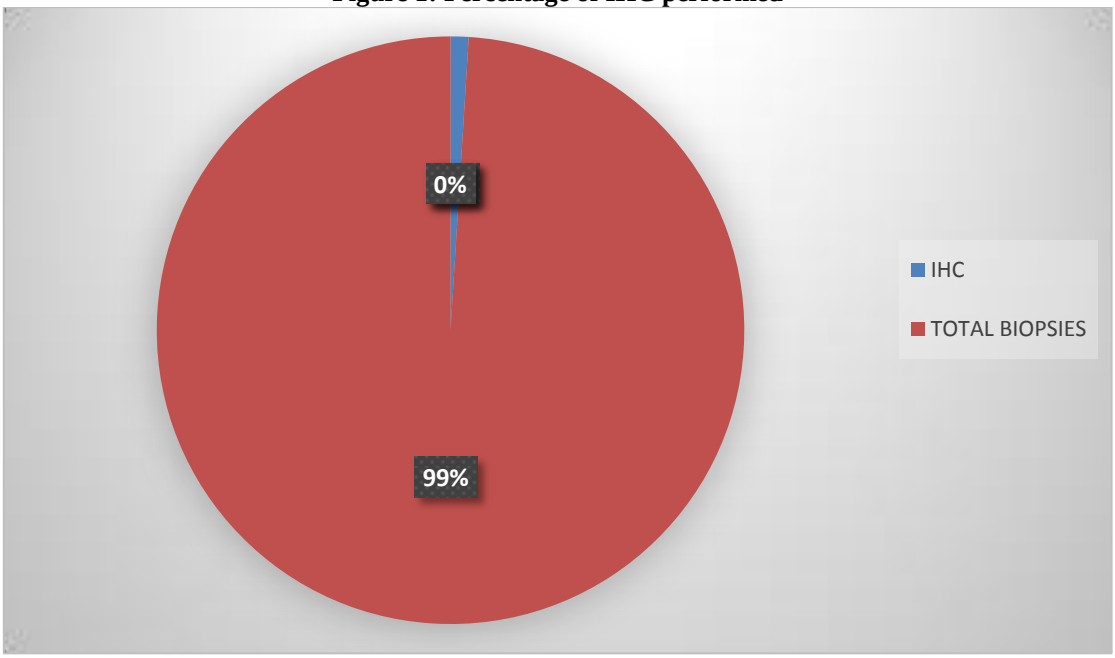
II. MATERIALS AND METHODS

A retrospective analysis was done in Histopathology section of Government Medical College, Jammu for a period of one year (2022).
• Inclusion criteria: Histopathology cases for which IHC is requested by oncologist for diagnostic confirmation were included.
• Exclusion criteria: Cases for which IHC is requested for prognostic and therapeutic markers were excluded.
Data of all the malignancies reported on biopsies and review blocks for which IHC were requested during the year 2022 were retrieved retrospectively and a detailed history was reviewed from the archival material in the pathology department. Total number of concordances and discordances are assessed to improve histopathological diagnostic accuracy. For each discordant case root cause analysis was done to identify areas of weakness.

III. RESULTS

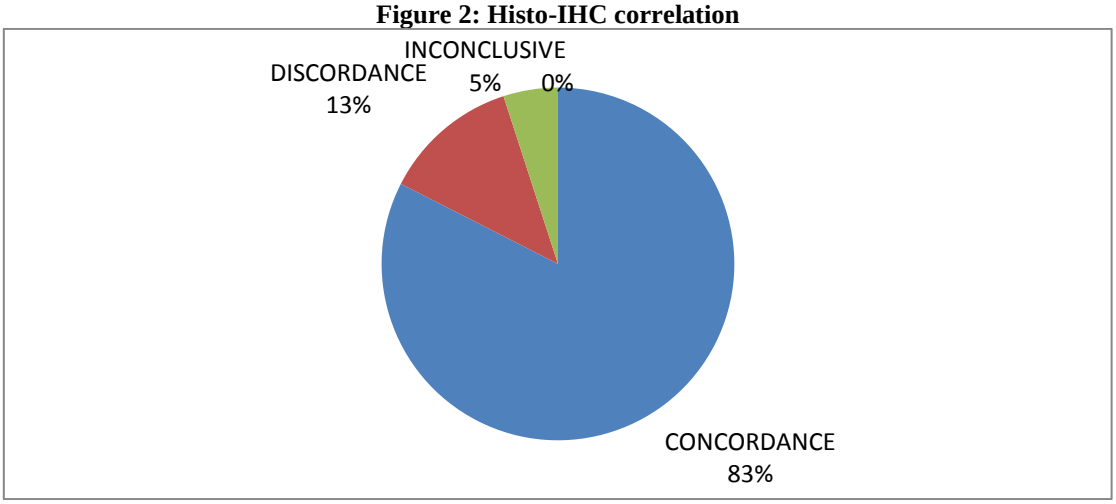
During this study period, a total of 4000 biopsies were reported in surgical pathology section of Government Medical College, Jammu. Out of which IHC for diagnostic confirmation, exact categorization or sub-typing were requested for only 40 biopsies (1%).

Figure 1: Percentage of IHC performed



Out of 40 cases, for 33 (82.5%) cases histopathological diagnosis was in concordance with the IHC diagnosis. Whereas in 5 (12.50%) cases histopathological diagnosis was discordant with the IHC diagnosis. For 2 (5.0%) cases conclusive diagnosis could not be derived.

Figure 2: Histo-IHC correlation



The cases for which IHC was done were categorized as lymphomas, breast tumors, lung tumors etc. The findings are given as follows:

Table 1: Diagnosis wise concordance and discordance

CATEGORY	IHC DONE	CONCORDANCE	DISCORDANCE
Lymphomas	13	10	3
Breast	12	12	0
Lung	5	4	1
Bone	1	1	0
Female genital tract	5	5	0
Small round cell tumor	2	1	1

Root cause analysis was done for all discordant cases. Maximum discordant cases were noted with diagnosis of lymphoma due to inadvertent attempt at typing of the lymphoma on histopathology examination. The discordant cases were reviewed by senior consultants and interpretational error in histopathology reporting was concluded as the reason for discordance with IHC.

For inconclusive cases repeat biopsy and IHC were advised. However, due to high cost of IHC and patients refusal to undergo repeat biopsy procedure were major hurdles in proceedings for deriving conclusive diagnosis.

Fig 3: A case of Infiltrating ductal carcinoma, Breast with its IHC markers (ER,PR&Her-2/neu)

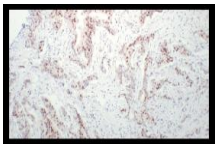
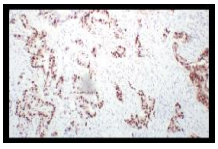
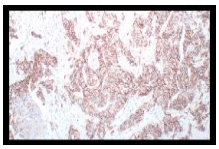
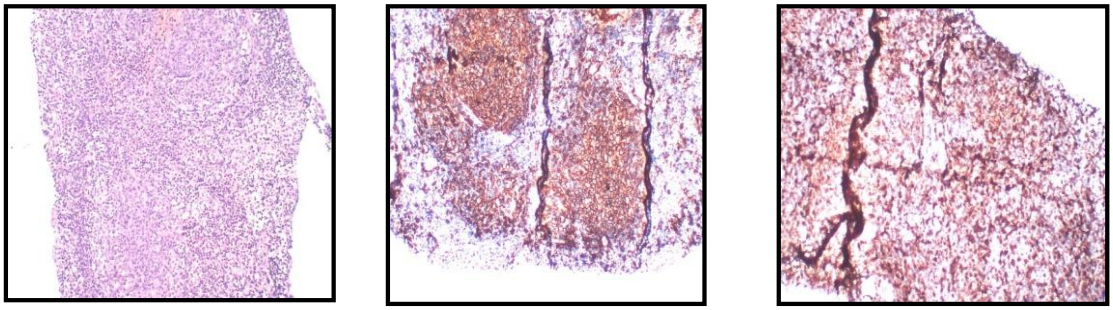
MARKERS(CLONES)	RESULTS		INTERPRETATION	IMAGE
ER (EP-1)	% of cells with nuclear staining in the invasive component of the tumor	**90%**	**Positive**	
	Intensity of staining	**Strong**		
	Allred score	**8**		
PgR (EP-2)	% of cells with nuclear staining in the invasive component of the tumor	**90%**	**Positive**	
	Intensity of staining	**Strong**		
	Allred score	**8**		
Her-2/neu (EP-3)	Complete membrane staining that is intense and >10% of tumor cells	**Score 3+**	**Positive**	

Fig 4: A case of Follicular lymphoma(H&E) along with its IHC markers (CD20 & BCL2)



H&E CD20 BCL2

IV. DISCUSSION

In present study, it was observed that IHC for diagnostic confirmation and sub-typing was done for only a small fraction of biopsies reported in the histopathology section. However, for the cases of lymphoma, where IHC is of utmost importance for confirmation and sub-typing; it was done for considerable number of cases. For breast lesions, IHC were mainly done for receptor study.

In the present study, only a small fraction of biopsies were requested for IHC which is similar to the study done by Thanky et al. [7]

A study done by Thanky et al showed concordance in Histo-IHC diagnosis in 80.98% cases and discordance in 15.49% cases. These results are comparable to our findings which showed concordance in 82.5% cases and discordance in 12.5% cases. Only two cases were reported as inconclusive.

Majority of the biopsies sampled were from lymphomas and breast tissue. This is also comparable to the above study. Other biopsies sampled were from lung and female genital tract; these cases rarely require IHC confirmation for diagnosis.

V. CONCLUSION

From our study we conclude that IHC is an important adjunct to the histopathological diagnosis. It not only confirms the morphological diagnosis but at times helps in sub typing the tumors. At the same time it is impressed that histopathological examination is the first step in the diagnostic algorithm of the malignant tumors and IHC always follows the histopathology. The morphological examination helps in getting the primary impression of the tumor type, tumor origin, it's grade and extent of involvement of the organ involved. The architectural patterns are also better appreciated on morphology. IHC is a useful adjunct which helps in confirming the histopathological diagnosis and further subtyping the tumors as well as assessing certain prognostic factors.

Subject to standardization of IHC, proper optimisation, accurate interpretation: IHC can be a very important auxiliary method and can become an indispensable tool for validation in cancer biomarker study. This has proved to be a very important milestone in targeted therapy of certain cancers and improving the quality of life of cancer patients on and after the therapy.

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