



## African Journal of Biological Sciences



### SCULPTING PRECISION: IMMUNO HISTOCHEMISTRY'S ARTISTRY IN HISTOPATHOLOGICAL CANVASES

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**ABSTRACT****Introduction:**

Immunohistochemistry [IHC] is an integration of biochemical, immunological and histopathological techniques, which is used for the identification of specific tissue components of proteins and lipids (antigens) by specific antigen/antibody reactions. IHC is used to identify antigens present in cells or tissues, which can be anything from proteins and amino acids to infectious diseases and particular cell populations. The histopathological diagnosis of many tumors and diseases heavily relies on immunohistochemical staining. So the present study aimed to explain the significance of IHC in histopathology

**Materials and Methods**

It was a retrospective and cross sectional observational study carried out in the Department of Pathology, Saveetha Medical College and Hospital, Chennai, Tamil Nadu, from June 2022 to October 2023. Specimens of various large and small biopsies were received from departments of specialty (surgery, ENT, ortho, OBG, etc.) and superspecialties (surgical oncology, urology). The clinical, radiological, and other investigation data was obtained from the request form. As usual, routine histopathological processing and staining of tissue were done, and slides were seen under light microscopy to diagnose, classify, and grade the tumors. In problematic cases where the H&E sections did not provide a final diagnosis, IHC was carried out.

**Results**

The total number of IHC cases was 61, out of which GIST was 13, soft tissue tumors were 2, breast was 43, and the markers used were 270.

**Conclusion:**

In the present era, immunohistochemistry plays an important role in histopathology and also in hematopathology for the diagnosis of leukemia and lymphomas. Nowadays, it has been incorporated as routine investigation in histopathology.

**Key Words:** Immunohistochemistry, Histopathology, hematopathology Diagnostic and therapy.

Article History

Volume 6, Issue 5, 2024

Received: 22 May 2024

Accepted: 03 Jun 2024

doi: 10.48047/AfrJBS.6.5.2024.9323-9330

**Introduction:**

Immunohistochemistry (IHC) is an integration of biochemical, immunological and histopathological techniques, which is used for the identification of specific tissue components of proteins and lipids (antigens) by specific antigen/antibody reactions. FITC-labeled antibodies were employed by Coons et al. in 1942 to identify pneumococcal antigen in infected tissue, despite the fact that the IHC principle had been known since the 1930s. This was the first IHC study. The fundamental concept behind IHC is the demonstration of antigen within tissue sections by means of specific antibodies. Once Ag-Ab binding occurs, it is visible by light microscopy or fluorochromes by ultraviolet light. [1]

IHC is used to detect cell or tissue antigens that range from amino acids and proteins to infectious agents and specific cellular populations. [2] The histopathological diagnosis of many tumors [3, 4] and illnesses [5–9] benefits greatly from the use of immunohistochemical staining. Presently, many medical colleges and laboratories, for research or thesis work, have started immunohistochemistry, which is widely used to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of tissues for prognosis, classification of tumors, and therapy.

Immunohistochemistry [IHC] plays an important role in the use of polyclonal as well as monoclonal antibodies to determine the tissue distribution of proteins or lipids (antigens) by specific antigen/antibody reactions. Immunohistochemistry has an expanding role in diagnostics as well as in research/thesis studies. This article highlights the applications of IHC in various tissue biopsies for a definitive diagnosis or subclassification of cancer, and also gives more information or ideas to the clinician about the best type of therapy to the patient. So the present study aimed to explain the significance of IHC in histopathology

**Materials and Methods**

It was a retrospective and cross sectional observational study, carried out in the Department of Pathology, Saveetha Medical College and Hospital, Chennai, Tamilnadu from June 2022 to October 2023 after obtaining approval from the institutional ethics committee. Specimens of various large and small biopsies were received from departments of specialty (surgery, ENT, ortho, OBG etc) and superspecialties (surgical oncology, urology). The clinical, radiological, and other investigation data was obtained from the request form. The usual routine histopathological processing, staining of tissue was done and slides were seen under light microscopy to diagnose, classify, and grade the tumors. In troublesome cases where an H&E section did not provide a final diagnosis, IHC was carried out. Using 3µm thick sections on Poly-l-lysine coated slides, antigen retrieval was done using the pressure cooker TRIS-EDTA at PH 9. The required primary antibody and HRP-polymer detection system were used for

antigen detection. Specimen with inadequate material, inadequate fixation, overfixation, and autolysed specimens was excluded.

### Results:

The total number of IHC cases was 61, among which male patients were 5 and 56 were female. Among the 5 male patients, 4 had soft tissue tumors, and one had a trucut biopsy of the left breast.

In our study population, the mean age was 54.8 years. Patients were more in the age group 61-70 years (n = 20). 17 patients were in 51-60 years age group. 21 patients were less than 20 years old. Out of which GIST was 13, soft tissue tumors 5, and breast cancer was in 43 patients among which invasive ductal carcinoma was in 39 patients.

Table 1: Demographic features

AGE IN YEARS	NO OF PATIENTS	PERCENTAGE
< 40	11	18%
41-50	10	16%
51-60	17	28%
61-70	20	33%
> 70	3	5%
SEX	NO OF PATIENTS	PERCENTAGE
MALE	5	8%
FEMALE	56	92%
ORGAN INVOLVED	NO OF PATIENTS	PERCENTAGE
BREAST	43	71%
GIST	13	21%
SOFT TISSUE TUMOUR	5	8%

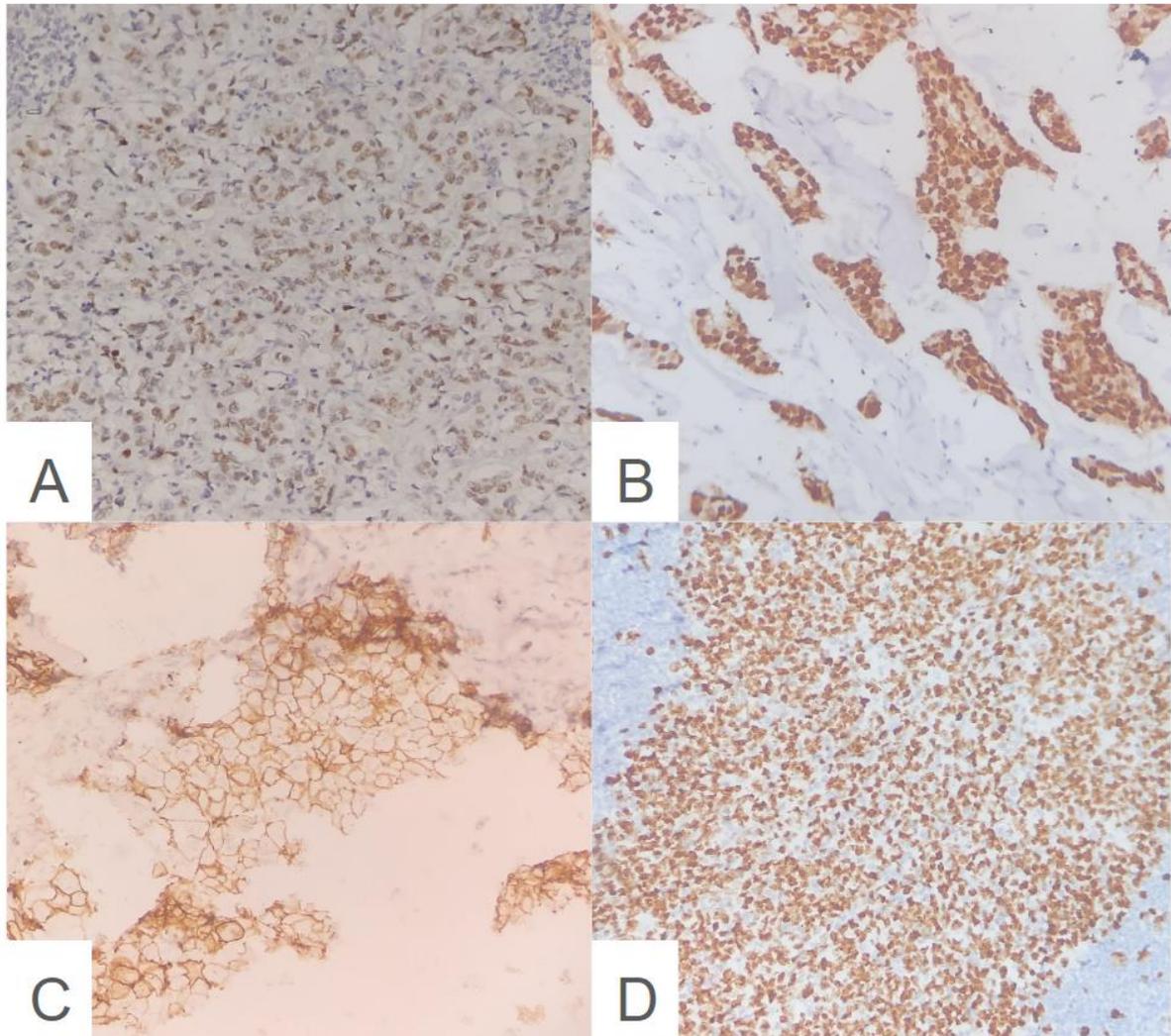
We analyzed using 270 markers. The markers were used predominantly for breast cancer followed by Soft tissue tumors and GIST. Positivity for marker in immunohistochemistry was analyzed and most commonly ER, PR was positive in 16 patients,

Her2neu was positive in 13 samples. Ki-67 was positive in 5 samples other markers like EMA, S100, SMA were positive in few cases. (Figure 1)

Table 2: IHC markers

IHC MARKERS	NO OF PATIENTS	PERCENTAGE
HER2NEU	13	21%
ER,PR	16	26%
Ki-67	5	7.50%
EMA	2	3%
S100	2	3%
SMA	1	1.50%
CD34	1	1.50%
CD138	1	1.50%

Figure 1: IHC markers



**Figure 1:** A - ER, B - PR, C - HER2NEU, D - Ki67

#### Discussion:

Neoplasms are classified as benign, intermediate (locally aggressive), intermediate (rarely metastasizing), and malignant. In our routine practice, before giving a definitive diagnosis of any neoplasm, we first evaluate the clinio-radiologic, histomorphologic, and cytomorphologic features of the neoplasm to give some pertinent differential diagnoses. It includes whether the tumor is benign or malignant, and whether it is low or high grade. Although molecular/genetic testing is increasingly finding its applications in characterizing many tumors, immunohistochemistry plays an important role in defining tumor origin.

IHC is routinely used in the diagnosis of lesions in stomach, duodenum, and colon, lesions of lung [7,10,11], differential diagnosis and classification of soft-tissue tumors [3,4] diagnosis of genitourinary (bladder and prostate) cancer [12], prognosis and therapy in breast cancer [13], diagnosis of dermatological lesions [9] and occasionally in oral pathology [14]. It can be used to differentiate hyperplasia from neoplasia [15]. With the use of particular

antibodies against microbial DNA or RNA, such as in the case of *H. pylori* in gastric biopsy, Cytomegalovirus, and Hepatitis B and C virus, IHC can be used as a prognostic marker in cancer, to predict therapy response, and identify infectious agents in tissues. [16]

**Conclusion:**

The present study concluded that histopathological examination of various biopsies is of prime importance for diagnosis and grading in most of the tumors but immunohistochemistry plays an important role in difficult cases where diagnosis, classification, and grading, is not possible on histological examination alone. IHC is also useful in providing vital information, which aids in the prognosis, diagnosis and treatment, which ultimately leads to better patient care.

**Funding:** This research received no external funding.

**Informed Consent Statement:** Not applicable.

**Conflicts of Interest:** The authors have no conflicts of interest to disclose.

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