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Original Research Article

Role of immunohistochemistry as a real mentor in diagnostic histopathology

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ABSTRACT

Objectives: The goals and objectives of this study are to find out the number of cases where IHC was used to solve discrepant cases of histopathology. This gives an idea as to what percentage of cases required IHC for diagnostic evaluation.

Materials and Methods: This research is a combined retrospective and prospective analysis of a three-year time frame, beginning in January 2017 and ending in December 2019. Only those cases of benign and malignant neoplasms which have undergone IHC in our department are part of this study. Total of 67 cases were studied using routine H&E and IHC stain. Slides were evaluated by light microscopy. Using specific monoclonal or polyclonal antibodies, paraffin sections were stained immunohistochemically (IHC) using a Peroxidase antiperoxidase (PAP) technique.

Result: There were a total of 23,558 biopsy reports made in the field of surgical pathology. Out of a total of 67 cases requested for IHC, 36 cases (53.7%) had histopathological diagnosis concordant with IHC diagnosis. While in 25 cases (37.31%) histopathological diagnosis was discordant with IHC diagnosis. In 6 cases (8.95%) conclusive diagnosis could not be derived.

Conclusion: From this study, we concluded that IHC plays a significant role in the definitive typing and grading of tumours. When trying to characterise a tumour, it's best to use an antigenic profile of both positive and negative markers, which may be achieved by a panel method consisting of well-chosen antibodies. It also brings about the conclusion that internal and external quality control plays an important part in routine Histopathology, which should not be undermined. Both QC and IHC must be made a routine part of Histopathology. Since we began comparing histology and IHC diagnosis three years ago, we've seen a significant uptick in the quality of our performance evaluations, patient care, and general laboratory practises.

1. Introduction

One of the longest lasting medical practises, Hematoxylin-eosin (H&E)-stained slides have not altered much over the years since they were first used. It is still considered the gold standard but has its own limitation. It helps in the accurate diagnosis of most of the tissue samples but in many cases, it poses a dilemma at the initial diagnostic level. Ancillary test is the answer to this and immunohistochemistry is one of them. To further separate the morphology that seems to be comparable in H & E section, IHC may be performed as a sanctioned supplementary investigation of the tissue in addition to basic H/E-staining. It's crucial in pinpointing the source of a tumour and determining how far along the spectrum of malignancy it falls. Based on the recognition between an antigen and an antibody, it may pinpoint where in a tissue or cell a certain antigen is located.

In the case of soft tissue neoplasms, overlapping morphology is common. It is helpful to use a holistic, applied approach to research the four most prevalent

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diagnostic scenarios, which include round cell tumours, monomorphic spindle cell tumours, epithelioid soft tissue tumours, and pleomorphic spindle cell tumours. IHC has shown that soft tissue cancer cells are very heterogeneous in their development, and it has also shown that no one stain is pathognomonic for any specific disease; as a result, conventional H&E stains should still be considered the gold standard.

The goals of this study are to find out the number of cases where IHC was used to solve discrepant cases of histopathology and to look for and identify areas of possible errors if any during root-cause analysis of discordant cases.

2. Materials and Methods

Pathologists at the Government Medical College in Kota, where the research was conducted, looked at both past and future data. There were a total of 23,558 biopsy reports made in the field of surgical pathology. The H&E slides were taken out and re-evaluated again by the two histopathologists. The IHC stained slides were studied.

Out of a total of 67 cases requested for IHC, 36 cases (53.7%) had histopathological diagnoses concordant with IHC diagnosis. While in 25 cases (37.31%) histopathological diagnosis was discordant with IHC diagnosis. In 6 cases (8.95%) conclusive diagnosis could not be derived.

3. Observation and Results

It consisted of 45 cases (67.16%) of malignant neoplasm, while benign lesions included 13 cases (19.4%). Cases with Inflammatory lesions / inconclusive results were 9 (13.4%). Among all the diagnosed cases, the most common concordant tumour belonged to the Nerve sheath tumour about 100% and the Neuroendocrine tumour, about 75%. While maximum discordant cases were noted in diagnosis of CNS tumors about 70% followed by Germ cell tumors of the ovary, 50% and soft tissue tumors about 43.7%. (Table 1)

Table 1: Cases broken divided by diagnosis

S.No.	Diagnosis	Cases	Percentage
1.	CNS tumors	10	14.92%
2.	Lymphomas	12	17.91%
3.	Soft tissue tumor	16	23.89%
4.	Germ cell tumor(ovary)	2	2.98%
5.	Nerve sheath tumor	3	4.47%
6.	Neuroendocrine tumors	4	5.97%
7.	Carcinomas	12	17.91%
8.	Inflammatory Lesion	2	2.98%
9.	Inconclusive	6	8.95%
	Total	67	100%

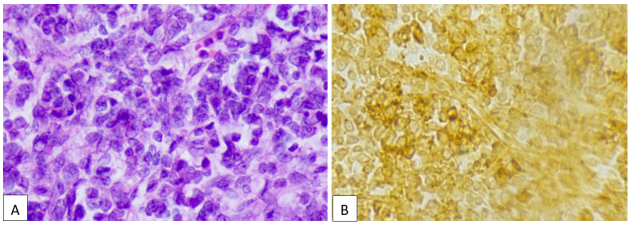

Fig. 1: A): Granulosa cell tumour. Small to medium sized cells arranged in sheets, cords and nest with fine sparse chromatin, inconspicuous nucleoli. H&E(400X); **B**): Granulosa cell tumor CD99+. IHC (400X);

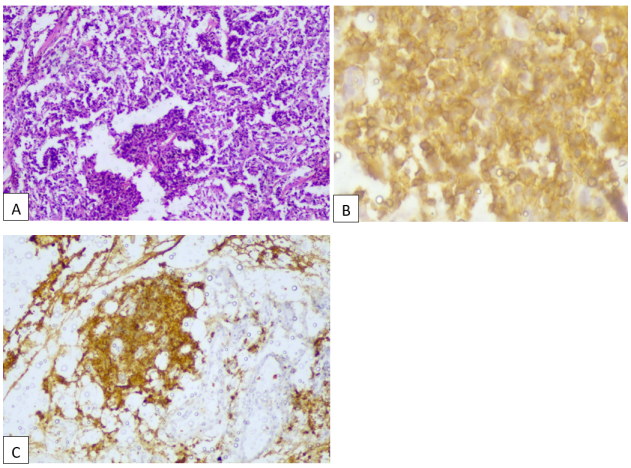

Fig. 2: A): Lymphoma originating in B cells, or blasts. Round, tiny cells that form sheets and divide often. These cells have little cytoplasm, mild nuclear pleomorphism, and fine chromatin. H&E(100X); **B)**: Blastic B-cell lymphoma, LCA+. IHC(400X); **C**): Blastic B-cell lymphoma, CD20+. IHC (100X)

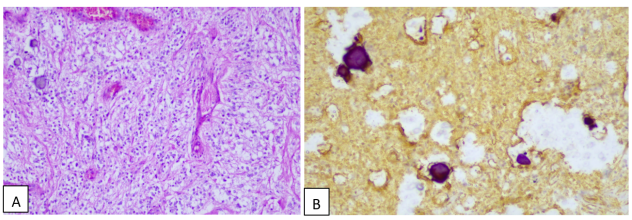

Fig. 3: A): Mixed Oligoastrocytoma. Mixed pattern of oligodendrocytes and areas showing spindled astrocytic component, large dilated congested vessels and psammoma bodies are also seen. H&E(100X); **B**): Mixed Oligoastrocytoma, GFAP+. IHC (100X)

Table 2: Histopathology-IHC correlations case distribution

S.No.	Histopathology – IHC correlation	No of cases	Percentage
1.	Concordance	36	53.73%
2.	Discordance	25	37.31%
3.	Inconclusive	6	8.95%
	Total	67	100%

Table 3: Diagnosis and systemic evaluation the instances' concordance or discordance

Category	Total no. of biopsy requested for IHC	Concordance	Discordance
CNS tumors	10	3(30%)	7(70%)
Lymphomas	12	8(66.7%)	4(33.3%)
Soft tissue tumor	16	9(56.2%)	7(43.7%)
Germ cell tumor(ovary)	2	1(50%)	1(50%)
Nerve sheath tumor	3	3(100%)	0
Neuroendocrine tumor	4	3(75%)	1(25%)
Carcinomas	12	7(58.3%)	5(41.7%)
Total	59		

(Inflammatory lesions/Inconclusive cases are not included)

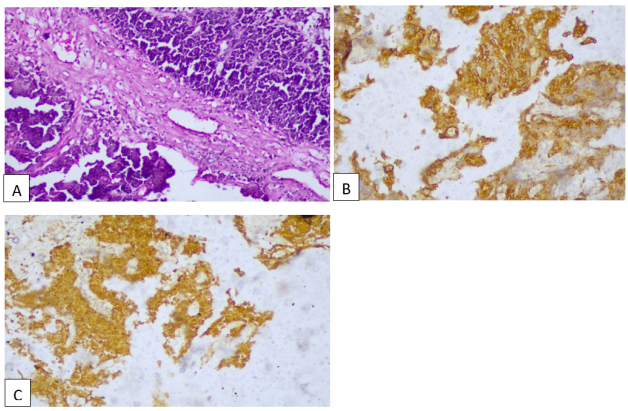

Fig. 4: A): High grade Transitional cell carcinoma. Irregular cords of small to medium sized closely packed cells showing stratification in certain areas. H&E(100X); **B**): Cancer of the transitional cell (CK+) of high grade (IHC, 100X); **C**): A high-grade transitional cell carcinoma, positive for elastin and keratin (EMA) by immunohistochemistry (100X)

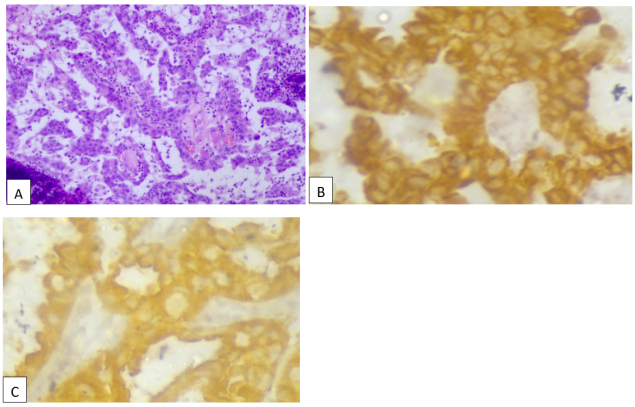

Fig. 5: A): Metastatic papillary adenocarcinoma. Haphazardly arranged irregular branching and complex networking papillary structure lined by cuboidal to columnar cells, high N:C ratio and intracytoplasmic vacuoles present. H&E(400X); **B**): Metastatic papillary adenocarcinoma, CK+.IHC (400X); **C**): Metastatic papillary adenocarcinoma, EMA+. IHC(400X)

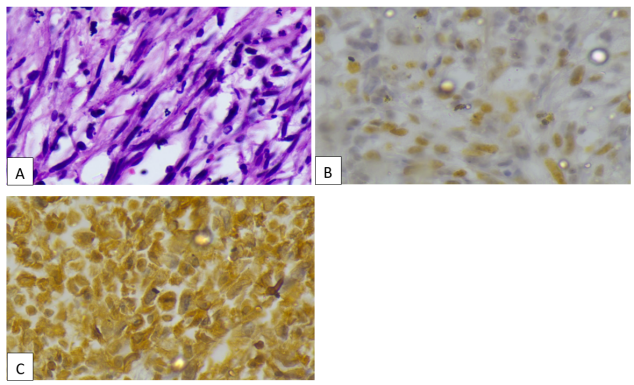

Fig. 6: A): Spindle cell sarcoma, high grade. Haphazardly arranged spindle cells having cigar shaped nuclei, small indistinct nucleoli with frequent mitosis. H&E(400X); **B**): High grade spindle cell sarcoma, Ki67(50-55%). IHC (400X); **C**): High grade spindle cell sarcoma, Ki67(50-55%). IHC (400X)

4. Discussion

Appropriate therapy can only be administered once a correct histologic diagnosis of cancer and classification of the tumour type has been made. Modern diagnostic tools allow for precise subtyping of malignant tumours. IHC is a very important tool in exact typing of tumours and assessing their true malignant potentials. In the current research, the histology unit of Government medical college Kota reported 23,558 biopsies during a three-year period. Only 231 (0.98%) of the samples examined were sent for IHC testing (Tab.5) in order to confirm or subtype the diagnosis. Among 231 samples, there were 164 in which immunohistochemistry (IHC) was requested to look for prognostic markers.

The results of the present study and their comparison with the results obtained by different previous studies are discussed here.

4.1. CNS tumors

In our study, the most common errors were seen with typing (interpretational error) and grading of tumours.

4.1.1. Rhabdoid meningioma (Grade III)

Sheets of highly pleomorphic round to polygonal cells with rich eosinophilic cytoplasm, vesicular nuclei with conspicuous big nucleoli, 0-2 mitosis/hpf, and regions of geographical necrosis are seen under the microscope in histopathology. Recent research by Mondal et al.*Large, round to oval cells with rich eosinophilic cytoplasm, eccentrically positioned nuclei, and prominent nucleoli characterise rhabdoid shape in[1] rhabdoid meningioma.[2] Eosinophilic inclusions in the cytoplasm around the nucleus are a common finding.[2] Histological examination of most instances reveals rhabdoid shape with meningothelial differentiation.[2] Differentiation of the meninges (whorls, nuclear characteristics, and eosinophilic inclusion bodies) and immunohistochemistry results are used to provide a histological diagnosis.

In our study the meningothelial component was absent and on the basis of IHC findings, the diagnosis of Glial tumour (WHO Grade IV) was concluded. Typing and grade of tumour was changed.

4.1.2. Clear cell ependymoma (WHO Grade III)

Section shows sheets of clear cells surrounded by vascular channels showing endothelial proliferation mitosis and mild pleomorphism seen. Many perivascular pseudo rosette and areas of necrosis also seen.

Morphological picture closely resembled Ependymoma. But on IHC it was diagnosed as oligodendroglioma. According to research by Hikaru Sasaki et al. 3, oligodendrogliomas exhibiting classical traits were more likely to indicate deletion of 1p. Therefore, calcifications and ”chicken wire” vasculature were looked for in addition to the usual oligodendroglioma hallmarks of homogenous and spherical nuclei, sometimes with tiny nucleoli.

4.1.3. Mixed glioma (WHO Grade II)

Section reveals predominant areas of sheets of polygonal cells with clear ill marginated cytoplasm. The nuclei show moderate pleomorphism and a few mitotic activity and spindle cell proliferation also seen.

Initially a low-grade tumour (Mixed glioma) was reported which came out to be of higher grade (Anaplastic Oligodendroglioma) due to high Ki67 index. Important histologic findings for glioma grading include nuclear atypia, mitotic activity, microvascular proliferation (MVP), and necrosis, as shown in investigations by Sarah Zhang et al.[3] Nuclear atypia refers to nuclei that exhibit both size and shape variation (nuclear pleomorphism) as well as unusually thick chromatin (hyperchromasia). Grade II gliomas, the lowest subtype of gliomas, are characterised by little nuclear atypia and infrequent, if any, mitotic activity. Increased nuclear pleomorphism, cellularity, and mitotic activity characterise anaplastic gliomas of grade III. The Ki-67 proliferation index is an immunohistochemistry stain used in conjunction with histologic grading. Increases in the proliferation index are often seen in tumours with a more aggressive development pattern, as well as a higher histologic grade.

4.1.4. Pleomorphic xanthoastrocytoma (Grade I)

Histomorphology reveals scanty fragmented biopsy material showing areas of haemorrhage and necrosis. A small viable area showing moderate nuclear pleomorphism, mitosis with giant cell formation were seen. Features favour Pleomorphic xanthoastrocytoma (Grade I). The diagnosis was changed to Anaplastic Astrocytoma (WHO Grade III) on the basis of IHC findings. Grade of tumour was higher due to increased ki67 index.

4.1.5. Pilocytic astrocytoma and diffuse astrocytoma (Grade III)

In both the cases, the diagnosis was changed to Mixed oligoastrocytoma. Tumours of low to moderate cellularity, as described by V. Peter Collins,[4] have both compact, densely fibrillated areas rich in Rosenthal fibres, composed of cells with long bipolar (hair-like) processes and elongated cytologically bland nuclei, and loosely textured areas, composed of multipolar cells (protoplasmic astrocyte-like), with bland, round to oval nuclei and multiple, relatively short cytoplasmic extensions. In some cases, areas morphologically similar to oligodendrogliomas may be found.

Sarah Zhang’s research shows that Histopathological differences exist between diffuse astrocytomas and oligodendrogliomas, as stated in.[3] Increased nuclear pleomorphism (variability in size and form) and elongated, hyperchromatic (excessively dark nuclear staining) nuclei characterise diffuse astrocytomas. Dispersed tiny cystic spaces, known as microcystic alterations, may be seen as a distinct architectural pattern in.[5] In our studies, earlier, the Oligodendrocyte component was overlooked.

4.1.6. Cellular ependymoma

On the basis of higher Ki67 index (15-20%) and other IHC markers, the diagnosis of Anaplastic Ependymoma was made, Grade higher as compared to Morphological diagnosis. Aparna Singh et al.[6] found that subependymoma (WHO grade I) had a Ki-67 positive labelling index of 2% and a p53 labelling index of 8% across all ependymoma grades. In cases of ependymoma (WHO stage II), the

percentage of cells labelled with Ki-67 was 2% and the percentage of cells labelled with p53 was 2%. The Ki-67 index was 12% in one instance of Anaplastic Ependymoma (WHO grade III), whereas the p53 index was 40% in the same case.

Comparable research was conducted by Ken Aldape et al.,[7] who found that of 34 cases of neuroepithelial neoplasm initially diagnosed as Astrocytoma, 2 were diagnosed as glioblastoma, 3 were diagnosed as juvenile pilocytic astrocytoma (JPA), 1 was diagnosed as anaplastic ependymoma, 1 was diagnosed as astrogliosis, 1 was diagnosed as a congenital. 1 case of Ependymoma as Oligoastrocytoma. In our study 1 case of Glial tumour was misinterpreted as Rhabdoid Meningioma, this is similar to the studies done by Janet M. Brunner[8] where 1 case of Pleomorphic xanthoastrocytoma and 1 Anaplstic oligodendroglioma was misinterpreted for Meningioma.

4.2. Soft tissue tumors

4.2.1. DFSP 2 cases

Microscopically, the cells have spindled nucleus with moderate anisokaryosis. Mitosis is 8 to 10 per hpf, no giant cells or histiocytes are seen.

Differential diagnosis of Dermatofibrosarcoma protuberans and Diffuse neurofibroma were considered. On the basis of immune-staining, the diagnosis of Dermatofibrosarcoma protuberans was concluded. This can be simillar to the studies done by Sameer Rastogi et al[9] where Benign Mesenchymal tumour? DFSP was confirmed to DFSP after doing IHC.

4.2.2. Low grade fibromyxoid sarcoma

Tumour was infiltrating around the skeletal muscles and mitotic activity was scant. Area of thick collagenous bundles are also seen. Review of the slide shows no typical myxoid areas, only hypocellular areas were present. These areas showed fibrillary background in contrast to ground glass seen in myxoid area.

On the basis of IHC panel used, the diagnosis of Fibromatosis was given. Two cases of myofibroblastic lesions were found in the major discrepancy group; one was found in the abdominal cavity and was initially diagnosed as an inflammatory myofibroblastic tumour; the other was found in the calf and was initially diagnosed as nodular fasciitis; after review, both were classified as fibromatosis. Error was likely caused by a lack of acquaintance with the characteristic fascicular morphology of fibromatosis, which was present in both instances.

4.2.3. Stromal sarcoma with chronic mastitis and xanthogranulomatous inflammation

Section reveals areas of mild chronic mastitis with areas of xanthogranulomatous inflammation. A small area of spindle cell proliferation showing irregular infiltration into surrounding tissue, nuclei were oval to elongated along with 3-4 mitosis per high power field. No ductal cells seen.

The diagnosis of stromal sarcoma with chronic mastitis and xanthogranulomatous inflammation was rendered. On the basis of IHC, diagnosis of inflammatory myofibroblastic tumor was given. Area of lesion was small, localised and morphologically revealed features of low-grade stromal sarcoma.

4.2.4. Malignant spindle cell tumor

H&E section reveals hypo and hypercellular areas with swirling bundles of thin delicate and elongated spindle cells. The nuclei are rounded, lengthy, and taper at both ends. A few mitosis are present.

Malignant spindle cell tumor was changed to Low grade spindle cell tumor due to low ki67 index.

4.2.5. Malignant phyllodes

H&E section reveals sheets of loosely arranged plump ovoid to spindled cells showing moderate pleomorphism and mitotic activity. Tumour cells are infiltrating into the surrounding fat spaces. No epithelial component seen.

Morphological Diagnosis: - Malignant phyllodes

On the basis of IHC markers done, the diagnosis of Malignant Myofibroblastic tumor was given. It was difficult to differentiate between these two conditions on morphological basis.

4.3. Spindle cell neoplasm

H&E section reveals haphazardly arranged bundles of moderately pleomorphic spindled cells, nuclei are cigar shaped, nucleoli are small to indistinct. Mitosis are frequent, few bizarre nuclei are also seen.

4.3.1. Morphological diagnosis: Spindle cell neoplasm

Since SMA was positive and high Ki67 index, it was diagnosed as Leiomyosarcoma. This can be compared to the studies done by Khin Thway and Cyril Fisher[10] where 2 cases of Atypical Spindle cell proliferation was reviewed and finalized to Leiomyosarcoma grade I and Spindle cell Sarcoma, NOS.

4.4. Carcinomas

Differential diagnoses of squamous cell carcinoma, rhabdomyosarcoma, and adenocarcinoma were made due to a lack of sufficient tumour tissue.

Rhabdomyosarcoma and adenocarcinoma were ruled out by immunohistochemical analysis since tumour cells were negative for myogenin and positive for p63. p63 Squamous cell carcinoma with moderate differentiation may also be identified by immunostaining. Tissue was scanty for morphological diagnosis. IHC was necessary for proper diagnosis.

4.4.1. Small cell carcinoma

H&E section revealed totally necrotic fragments, a single viable bit shows irregular cords of small to medium sized closely packed tumour cells showing stratification in certain areas and pinkish material. Morphological Diagnosis: Small cell carcinoma.

IHC was used to diagnose a case of high-grade transitional cell carcinoma of the urinary bladder. Studies done by Zhi Chen et al[11] analyzed retrospectively nine cases with small cell carcinoma bladder (SCCB). Approximately 50% of patients with small cell carcinoma of the bladder (SCCB) also have other types of bladder cancer, such as transitional cell carcinoma, adenocarcinoma, or squamous cell carcinoma. They claim that SCCB is linked to epithelial atypical hyperplasia and cancer in situ. It's probable that this is due to the fact that SCCB comes from a poorly differentiated stem cell population found in the submucosa of the brain[12]. Cytologically, SCCB is difficult to distinguish from poorly differentiated squamous cell carcinoma, adenocarcinoma, and transitional cell carcinoma,[13] thus electron microscopy or immunohistochemistry is required for diagnosis.

4.4.2. Poorly differentiated carcinoma

H&E section reveals numerous cords and nodular sheets of atypical spindled cells showing severe anisocytosis, atypical bizarre large nuclei some surrounding the nerve bundle. Mitosis is frequent. Morphological Diagnosis: Poorly differentiated carcinoma.

Squamous cell carcinoma was first diagnosed but was changed to Poorly differentiated carcinoma after immuno-histochemistry was performed. Typing of lesion changed.

4.4.3. Metastatic carcinoma

Report on H&E section reveals haphazardly arranged irregularly branching and complex networking papillary structures lined by cuboidal to columnar cells. Increased nucleocytoplasmic ratio with distinct nucleoli and showing secretory activity.

Morphological diagnosis: 1. Metastatic carcinoma, 2. Papillary Meningioma.

On the basis of immunostaining, the diagnosis of Metastatic Adenocarcinoma was confirmed.

4.4.4. Renal cell carcinoma

Morphology: H&E section reveals a capsulated tumor, tumor cells are large polygonal to columnar. Cells are haphazardly forming papillae and acinar structure; nuclei are small and central; nucleoli are small visible.

Differential diagnosis: 1. ? Renal cell carcinoma ? 2. Adreno cortical carcinoma (ACC).

Adreno-cortical carcinoma was given as differential diagnosis and IHC was used to rule out Adreno cortical carcinoma: CKAE1/AE3 Focal+, EMA Focal+, Chromogranin-, Synaptophysin-, Vimentin-. Mark R. Wick et al.[14] found that clear-cell cytologic appearance was present in 10 instances of renal cell carcinomas. Tumour cells in some cases grew in clusters or in a medullary pattern, and there was localised necrosis and bleeding. One to two mitoses occurred on average every high-intensity field. All cases of RCC express cytokeratin positivity especially with selected monoclonal antibodies (AE1/AE3 and PKK1). In their studies, 10 cases of ACCs were vimentin-positive and EMA- and BGI-negative. Sun et al[15] and Nagle and colleagues[16] have also observed a lack of cytokeratin reactivity in adrenocortical neoplasms, using nontrypsinized tissue.

5. Lymphomas

Other discrepant cases were from Lymphoma category (4 cases) (16%) (Tab.13) including both Hodgkin's and Non-hodgkins Lymphoma. In HL error was in sub-typing, 1 case of NLPHL was misinterpreted for classical HL. while in NHL cases (3), 1 Follicular Lymphoma, the section showed only surface follicles, possibly a slice from the Cortical area of node, not covering the medulla or hilum (and was reported as follicular lymphoma which after IHC report was corrected as Reactive follicular hyperplasia. Other cases were to exclude NHL (1) from Undifferentiated malignant neoplasm on basis of IHC. One case of Atypical Ewing's sarcoma which on IHC came out to be Centroblastic Lymphoma (1). Similar to the research conducted by Neval Zkaya,[17] we found that in twenty-nine instances, the original diagnosis of lymphoma was revised to benign/reactive, and in twenty of those cases, the underlying pathology was reactive hyperplasia (RH). We found that Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) was seldom misdiagnosed as cHL (n=11), while they found the converse to be true. According to their findings, following further assessment, 28 of 33 patients initially diagnosed as undifferentiated malignant tumour (UMT) in the first and second time periods were reclassified as lymphoma, respectively. Moreover, DLBCL (n=17) was the most common histological subtype. In addition, they looked at 21 previously misdiagnosed instances of cancer that were ultimately determined to be lymphoma following further investigation. Cancers that look like lymphoma most often are poorly or not at all differentiated carcinomas.

Neval Zkaya et al.[17] found that Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) was commonly misdiagnosed as cHL (n=11), and this finding is consistent with their findings. Whereas in our case HL-Classical type was misdiagnosed for HL-NLPHL.

Discussion: Morphological findings of clearcut normal mantle zones and tingible body macrophages in follicular centres was missed, leading to mis-diagnosis. These observations also indicate that for a proper sampling of

Lymph nodes by LS along the hilum so that medullary areas are also included. A sectioning along the cortical areas will include only follicles and can prompt a wrong diagnosis if made in haste. According to studies done by Neval Özkaya et al[17] There were 29 samples that were first diagnosed as lymphoma but were later deemed to be benign/reactive, 20 of which were found to be Reactive follicular hyperplasia. This is similar to the research conducted by M. J. Matasar,[18] whereby in both 2001 and 2006, a single case with a reported diagnostic of low-grade FL was considered to reflect benign pathology (follicular hyperplasia or gradual transformation of germinal centres).

5.1.

5.1.1. Atypical ewing's sarcoma

Sheets of tiny, spherical cells with minimal cytoplasm, mild pleomorphism in the nuclei, and fine chromatin are seen in an H&E slice; no definitive rosette like structure seen. Mitosis is frequent.

Earlier a diagnosis of Atypical Ewing's sarcoma was given. However, on the basis of IHC, the diagnosis of Centroblastic lymphoma was finalised. Typing of lesion changed.

5.1.2. Suspicious for malignancy

H&E section shows three tissue fragments, two fragments show stratified squamous epithelium lining with evidence of chronic inflammation. One fragment shows dispersed population of atypical cells with lymphocytes.

Morphological diagnosis: Highly suspicious for malignant neoplasm.

IHC markers were used to confirm a diagnosis of Non-Hodgkin Lymphoma.

5.2. Juvenile granulosa cell tumor

Section shows a solid to cystic lesion showing small to medium-sized cells with fine sparse chromatin, inconspicuous nucleoli, cells arranged in nests, cords, perivascularly and around small and large cystic spaces. There is necrosis, haemorrhage, brown pigment, macrophages and atypical mitosis.

Morphological diagnosis: Juvenile granulosa cell tumor

IHC Panels used: inhibin+, alpha+, vimentin+, CD99+, HMB45 -, CK -, Calretinin -, EMA -.

IHC confirms the diagnosis of Granulosa cell tumor. According to research conducted by Belghith C,[19] inhibin, vimentin, CD 99, and smooth actin are the most prominent markers expressed by granulosa tumour cells. However, calretinin is not as specific as inhibin and might falsely read as positive. Tumour cells, in 90% of instances, also express E-cadherin. Shah et al. in 2009 discovered a somatic mutation in the FOXL2 gene, which codes for the transcription factor.[20]

5.3. Carcinoid tumor

Morphological report on H&E section: Histologically undifferentiated neoplasm, tumor consists of numerous delicate capillary channel with cells around the vessels. The cells range in size from tiny to medium, and their pale-staining nuclei indicate mitotic activity. Cells appear to be projecting into the perivascular spaces.

5.3.1. Diagnosis: Carcinoid tumor

IHC Panels used: Vimentin+, SMA-, CD117-, CD34-, Synaptophysin-, Chromogranin-, NSE-, CK-, LCA-, Ki67(35-40%)

The diagnosis of carcinoid tumor was made on the basis of morphological findings which on IHC was concluded as an Undifferentiated malignant tumor of the ileum. There was a typing error.

6. Conclusion

The present study has significantly highlighted and created awareness about the role of QC in Histopathology. It has made us understand that mistakes must have been committed in past and a pathologist has to be extra cautious while making morphological diagnosis. One can learn from mistakes committed earlier by maintaining a record of such cases. It is also important to remember that morphology alone cannot suffice for exact typing and grading of some lesions. Some cases must undergo IHC analysis for this.

It was also noted that a wider and complete panel of IHC's is required for arriving at a conclusive diagnosis. As in our case we are equipped with a limited panel due to financial restraints. Low economic status of some patients is also a restraining factor in conducting IHC studies.

In order to characterise a cancer as precisely as possible, it is best to use a panel method consisting of carefully chosen antibodies that together provide a complete antigenic profile of positive and negative markers. Training, knowledge, and sensitization of authorised signatories and physicians towards interpretational (analytical) abilities should be considered as preventative measures to reduce the number of discordant instances. Additional biopsies and IHC testing were recommended for patients where the first results were equivocal. While IHC's ability to provide a definitive diagnosis was crucial, the patient's unwillingness to undergo a second biopsy operation and the expensive expense of the test both slowed down the diagnostic process. Through a three-year comparison of histology and immunohistochemistry diagnosis, we were able to greatly enhance our laboratory services in terms of performance assessment, patient care, and overall quality.

In situ hybridization (IHC) is crucial for accurately classifying tumours. It also brings about the conclusion that internal and external quality control plays an important part in routine Histopathology, which should not be undermined.

Both QC and IHC must be made a routine part of Histopathology. Also, IHC can detect some of the diagnostic histopathology errors. It is a positive way of creating insight among pathologists that sometimes morphological diagnosis may differ from the actual pathology and making them believe that QC in Histopathology should be an integral part of any good pathology department.

7. Source of Funding

None.

8. Conflict of Interest

None.

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