Role of Immunohistochemistry in Metastatic Bone Diseases

Farukh Mir Muhammad, Farhan Akhtar, Nighat Jamal, Rabia Ahmed, Muhammad Asif, Fatima Wagan
Department of Pathology, Pakistan Naval Ship, Shifa Hospital, Karachi Pakistan,

ABSTRACT

Objectives: To identify the role of immunohistochemistry in correctly identifying metastasis of bone disorders.

Study Design: Cross-sectional study

Place and Duration of Study: Department of Histopathology Pakistan Naval Ship, Shifa Hospital, Karachi Pakistan, from Jun 2019 to Jun 2020.

Methodology: Patients aged 18-75 years, of either gender, already diagnosed case of the primary tumour, and now diagnosed with bone metastasis on biopsy were included. Various imaging techniques were used to locate the site of metastases. The histopathological reports of all the intra-operative specimens of bone metastases were reviewed.

Results: The mean age of 60 patients was 59.5±28.8 years and mean weight was 67.4±13.5 kg. The period of presentation from diagnosis of the primary tumour was 34±15.4 months, and the period from onset of symptoms was 8.2±5.8 months. The most common site of the primary tumour was the breast in 18(30%) of patients, followed by the kidney in 14(23.3%) of patients. The most common site of metastasis was the femur in 30(50 %) of patients, followed by the spine in 15 patients.

Conclusion: The most common site of primary tumour identified by immunohistochemistry was the breast, followed by the lungs. The femur was the most common site of metastasis, followed by the spine.

Keywords: Immunohistochemistry, Metastatic bone disease, Primary tumour, Secondary tumour.

INTRODUCTION

Malignancies most commonly observed in the bones are metastasis, with approximately 33% of all the sites of metastases following malignant metastasis to the lungs and liver. In around 25-30% of patients, bone metastasis might be the most initial manifestation of malignancy. In recent years, the frequency of cancer survivors has risen. A prime example is the 5-year survival rates of 60% and 85%, respectively, among patients of breast cancer and colorectal cancers. Per the reports, the 5-year survival rate in patients with prostate cancer is observed to be greater than 95%; therefore, in such settings where survivors from cancers are rising, the overall frequencies of such survivors having bony metastasis may also be on the rise.

Generally, a past medical and surgical history along with general physical examination laboratory test with imaging modality are mostly enough for establishing the diagnosis of bony metastasis. Determining the type and exact site of malignant metastasis is difficult in pathology. Evaluation through microscopy might reveal the morphological features specific to a certain disease or help in determining the origin and lineage of a tumour.

Moreover, when such an examination fails to report any distinctive features, an immunohistochemical (IHC) evaluation might aid in diagnosing the disease or tumour. For IHC, panels of tissues or markers specific to organs are mostly utilized. Therefore, many researchers regard the vital part played by cytokeratins and other organ-specific markers that help discriminate primary tumours’ lineage and organ and help determine the metastatic disorder a patient is suffering from to ascertain a diagnosis and plan treatment protocols accordingly for better outcomes.

In this regard, to aid in providing further insight into the role of the expression of specific markers using IHC in cases of metastatic bone disease, this study was carried out to evaluate and determine the effectiveness of such specific markers using IHC to help identify the site of the primary tumour.

METHODOLOGY

The cross-sectional study was carried out at the Histopathology Department PNS SHIFA Karachi from June 2019 to June 2020, after receiving approval from the Institutional Review Board of the Hospital. The sample size was calculated using the prevalence formula and keeping a prevalence rate of 7%, as reported in a study.

Inclusion Criteria: Patients aged 18-75 years, of either gender, as an already diagnosed case having a primary
tumour and now diagnosed with bone metastasis on biopsy were included.

**Exclusion Criteria:** Patients having a primary tumour other than carcinoma were excluded. In addition, patients with incomplete pathological or clinical information or with missing records were also excluded.

The data source was the medical records of patients admitted and managed under the diagnosis of bone metastases in the hospital. All patients in the study using a non-probability convenient sampling technique had undergone palliative orthopaedic surgery. The histopathological reports of all the intra-operative specimens of bone metastases were reviewed.

A total of 60 patients were included in the study. Various imaging techniques were used to locate the site of metastases, such as plain radiographs, CT scans, MRI and bone scans. In addition, the nature of lesions and their relationship to surrounding tissues was also observed and recorded. The pathological report of biopsy done from bone metastases was then analysed through immunohistochemistry (IHC) analysis. During surgery, the sample of tumour tissues collected was fixed for 2-5 days in buffered formalin and then processed according to decalcification protocols and paraffin embedding. Decalcification of fixed specimens was done in 20 % Ethylene Diamine Tetra Acetate (EDTA) mixed in NaOH at a pH of 7.4 for days to weeks, intermittently shaking to ensure the solution flowed around the bone and depending upon the specimen’s size and degree of demineralization, the time required for decalcification varied. A glass slide was then prepared using 5 micrometre thick sections covered with 2% silane solution in acetone. For standard histology, dewaxing was done in xylene and rehydration using ethanol. After which, the slides were stained for Hematoxylin and Eosin staining (H&E). For IHC, antigen retrieval was done on slides through heating in a microwave oven in 0.02 M citrate buffer at a pH of 6.0. The prepared glass slides were then incubated in 3 % Perhydrol solution after cooling to block endogenous peroxidase reaction. Patients having an already diagnosed primary tumour were examined for the association between the origin of metastasis and the one indicated by initial diagnosis at histological examination.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages.

**RESULTS**

The mean age of 60 patients was 59.5±28.8 years and mean weight was 67.4±13.5 kg, with 55% females and 45% males. The period of presentation from diagnosis of the primary tumour was 34±15.4 months, and the period from onset of symptoms was 8.2±5.8 months Table-I.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean±SD)</td>
<td>59.5±28.8 years</td>
</tr>
<tr>
<td>Weight</td>
<td>67.4±13.5 kg</td>
</tr>
<tr>
<td>Time Lapse from Diagnosis of Primary Tumor</td>
<td>34±15.4 months</td>
</tr>
<tr>
<td>Time Lapse from onset of Symptoms</td>
<td>8.2±5.8 months</td>
</tr>
</tbody>
</table>

The most common site of the primary tumour was the breast in 18(30%) of patients, followed by the kidney in 14(23.3%) of patients, after which were lungs and liver, observed in 04(6.7%) patients each. 03(5%) of patients had bladder carcinoma as their primary tumour site. 02(3.3%) patients were correctly identified to have a small intestine and stomach as their primary tumour site. 01(1.7%) each was observed to have prostate and colon carcinoma as their primary tumour site (Table-II). The primary site of the tumour in 11(18.3%) patients was unidentified by IHC. The most common site of metastasis was the femur in 30(50 %) of patients, followed by the spine in 15 patients, the humerus in 06 patients, the pelvis in 04 patients, the sacrum and tibia in 02 patients and the skull in 1 patient (Figure).

<table>
<thead>
<tr>
<th>Primary Tumor</th>
<th>n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>18(30)</td>
</tr>
<tr>
<td>Kidney</td>
<td>14(23.3)</td>
</tr>
<tr>
<td>Lungs</td>
<td>04(6.7)</td>
</tr>
<tr>
<td>Liver</td>
<td>04(6.7)</td>
</tr>
<tr>
<td>Bladder</td>
<td>03(5)</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>02(3.3)</td>
</tr>
<tr>
<td>Stomach</td>
<td>02(3.3)</td>
</tr>
<tr>
<td>Colon</td>
<td>01(1.7)</td>
</tr>
<tr>
<td>Prostate</td>
<td>01(1.7)</td>
</tr>
<tr>
<td>Unidentified</td>
<td>11(18.3)</td>
</tr>
</tbody>
</table>

**Figure:** Graphical representation of Frequency of Bone Metastasis
DISCUSSION

Several studies have observed and reported that, more often than not, IHC helps determine the site of origin of the carcinomas. After the determination of metastasis is confirmed to be from a carcinoma based on the screening of immune stains, a variety of tissues or specific markers may be tested, which can help suggest or even confirm the origin of the primary tumour. Nonetheless, various organ or tumour-specific markers might cause a reaction with different types of tumours. In cases where the origin of the metastatic tumour is not known, a panel of different IHC markers is strongly recommended. As in our study, IHC helped correctly identify primary tumour sites, with the most commonly observed primary tumour site being the breast in 18(30%) of patients, followed by 14(23.3%) of patients having primary tumour sites being lungs.

In diagnosing metastatic carcinoma, corresponding to an unknown site of origin has since passed many years but with a poor prognosis, with an average survival time ranging from 6 to 12 months. In such patients, the usual management mode is through chemotherapy, while other treatment protocols have not reported conclusive results. However, the method of identification using IHC has been upheld in most of the studies, as mentioned in a study by Schaefer et al. done in 2018. Similarly, in our study, most patients were correctly identified with their primary tumour site using IHC. Expression of IHC phenotype determination among high-grade tumours may correspond to better differentiation, with a better prognosis. With the facts considered, further research is needed to identify undifferentiated phenotypes correctly, which might have poorer prognoses.

Metastasis of bone is the most frequently reported neoplasm of bone tissue, mostly occurring in the axial skeleton and proximal limbs. In accordance with published literature reported by Kandalaft et al. in 2016, the most common locations reported are the skull, ribs, spine and pelvis, while the humerus and femur are most commonly observed proximal limbs bone metastasis. In our study, the findings were consistent with the above, where the most common bone metastasis was to the femur in 50% of patients, followed by the spine in 20%. IHC in metastatic bone disease was termed as a major advantage over other staining methods such as immunofluorescence, since in the latter; the darker background does not allow detailed morphological analysis.

LIMITATIONS OF STUDY

Even though our study correctly identified the role of IHC in metastatic bone diseases, the study was not immune from selection and observer bias. Furthermore, due to the limited sample size and single-centered study, the findings of the study also resulted in bias.

CONCLUSION

According to the findings of this study, the most common site of primary tumour identified by IHC was the breast, followed by the lungs. The femur was the most common site of metastasis, followed by the spine.

Conflict of Interest: None.

Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

FMM: & FA: Conception, study design, drafting the manuscript, approval of the final version to be published.

NJ: & RA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

MA: & FW: Critical review, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES


Immunohistochemistry in Metastatic


Pak Armed Forces Med J 2023; 73(5): 1226