Open Access

Original Article

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.

How to Cite This Article: *Sarwar M, Mirza IA, Imtiaz A, Hussain W, Khurshid U, Chaudhry AH. Changing Trends of Antimicrobial Resistance in Clinical Isolates Yielded from Lower Respiratory Tract Specimens of ICU Patients-A Two-Year Study. Pak Armed Forces Med J 2023; 73(4): 1223-1226. DOI: https://doi.org/10.51253/pafmj.v73i4.6462*

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 .[1] 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.[2] A prime example is the 5-year survival rates of 60% and 85%, respectively, among patients of breast cancer and colorectal cancers.[3] 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.[4]

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.[5] 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.[6]

Correspondence: Dr Ayesha Arooj, Department of Pathology, Armed Forces Institute of Pathology, Rawalpindi Pakistan
Received: 07 Mar 2021; revision received: 03 Sep 2021; accepted: 03 Sep 2021

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.[7] 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.[8,9]

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.[8]

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 *p*H 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-I: Baseline Demographics of Study Patients (n=60)

Variables	Mean±SD
Age (Mean±SD)	59.5±28.8 years
Weight	67.4±13.5kg
Time Lapse from Diagnosis of Primary Tumor	34±15.4 months
Time Lapse from onset of Symptoms	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, 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. 0(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-II: Breakdown of Primary Tumors of Patients having Bone Metastases found on Immunohistochemistry (IHC) Analysis (n=60)

Primary Tumor	n(%)
Breast	18(30)
Kidney	14(23.3)
Lungs	04(6.7)
Liver	04(6.7)
Bladder	03(5)
Small Intestine	02(3.3)
Stomach	02(3.3)
Colon	01(1.7)
Prostate	01(1.7)
Unidentified	11(18.3)

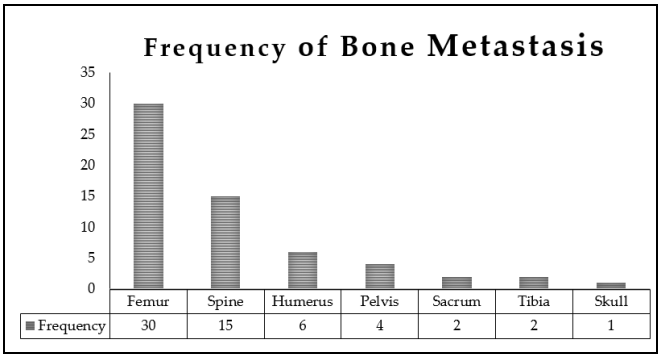

Figure: Graphical representation of Frequency of Bone Metastasis

DISCUSSION

Several studies have observed and reported that, more often than not, IHC helps determine the site of origin of the carcinomas.[9] 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.[10] 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.[11] 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.[12] In such patients, the usual management mode is through chemotherapy, while other treatment protocols have not reported conclusive results.[13] 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.[14] 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 .[15]

Metastasis of bone is the most frequently reported neoplasm of bone tissue, mostly occurring in the axial skeleton and proximal limbs.[16] 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.[17] 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.[18]

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

1. Duraiyan J, Govindarajan R, Kaliyappan K, Palanisamy M. Applications of immunohistochemistry. J Pharm Bioallied Sci 2012; 4(2): 307-309. https://doi.org/10.4103%2F0975-7406.100281
2. Kim LD, Bueno FT, Yonamine ES, de Próspero JD, Pozzan G. Bone metastasis as the first symptom of tumors: Role of an immunohistochemistry study in establishing primary tumor. Rev Bras Ortop (English Edition) 2018; 53(4): 467-371. https://doi.org/10.1016%2Fj.rboe.2018.05.015
3. Piccioli A, Maccauro G, Spinelli MS, Biagini R, Rossi B. Bone metastases of unknown origin: epidemiology and principles of management. J Orthop Trauma 2015; 16(2): 81-86. https://doi.org/ 10.1007%2Fs10195-015-0344-0
4. Allemani C, Weir HK, Carreira H, Harewood R, Spika D, Wang XS, et al. CONCORD Working Group. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). Lancet 2015 ; 385(9972): 977-1010. https://doi: 10.1016/S0140-6736(14)62038-9.
5. Shibata H, Kato S, Sekine I, Abe K, Araki N, Iguchi H, et al. Diagnosis and treatment of bone metastasis: comprehensive guideline of the Japanese Society of Medical Oncology, Japanese orthopedic association, Japanese Urological Association, and Japanese Society for Radiation Oncology. ESMO Open 2016 ; 1(2): e37-47. https://doi.org/10.1136/esmoopen-2016-000037
6. Varadhachary GR, Abbruzzese JL, Lenzi R. Diagnostic strategies for unknown primary cancer. CA Cancer J Clin 2004 ; 100(9): 1776-1785. https://doi.org/10.1002/cncr.20202
7. Krishna M. Diagnosis of metastatic neoplasms: an immunohistochemical approach. Arch Pathol Lab Med 2010; 134(2): 207-215. https://doi.org/10.5858/134.2.20

8. D'Oronzo S, Coleman R, Brown J, Silvestris F. Metastatic bone disease: Pathogenesis and therapeutic options: Up-date on bone metastasis management. J Bone Oncol 2018 ; 15: 004. https://doi: 10.1016/j.jbo.2018.10.004.
9. Schrijver WA, Van Der Groep P, Hoefnagel LD, Ter Hoeve ND. Influence of decalcification pro-cedures on immunohisto-chemistry and molecular pathology in breast cancer. Mod Pathol 2016; 29(12): 1460-1470. https:// doi.org/10.1038/modl.2016.116
10. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. Diagn Pathol 2014 ; 9: 221. https://doi: 10.1186/s13000-014-0221-9.
11. Antonescu CR, Erlandson RA, Huvos AG. Primary leiomy-osarcoma of bone: a clinicopathologic, immunohistochemical, and ultrastructural study of 33 patients and a literature review. Am J Surg Pathol 1997; 21(11): 1281-1294. https://doi.org/ 10.1097/ 00000478-199711000-00003
12. Pavlidis N, Khaled H. A mini review on cancer of unknown primary site: a clinical puzzle for the oncologists. J Adv Res 2015; 6(3): 375-382. https://doi.org/10.1016/j.jare.2014.11.007
13. Sporn JR, Greenberg BR. Empirical chemotherapy for adenocarcinoma of unknown primary tumor site. Semin Oncol 1993; 20(1): 261–267.
14. Schaefer IM, Hornick JL. Diagnostic immunohistochemistry for soft tissue and bone tumors: an update. Adv Anat Pathol 2018; 25(6): 400-412. https://doi.org/10.1097%2FPAP.0000000000204
15. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat. Rev. 2001; 27(3): 165-176. https://doi.org/10.1053/ctrv.2000.0210
16. Anract P, Biau D, Boudou-Rouquette P. Metastatic fractures of long limb bones. Orthop Traumatol Surg Res 2017; 103(1): S41-S51. https://doi.org/10.1016/j.otsr.2016.11.001
17. Kandalaft PL, Gown AM. Practical applications in immuno-histochemistry: carcinomas of unknown primary site. Arch Pathol Lab Med 2016; 140(6): 508-523. https://doi.org/10.5858/ arpa.2015-0173-cp
18. Roskams T. The role of immunohistochemistry in diagnosis. Clin Liver Dis 2002; 6(2): 571-589. https://doi.org/10.1016/s1089-3261(02)00012-0