International Journal of Research in Medical Sciences
Hasanah H et al. Int J Res Med Sci. 2019 Oct;7(10):3627-3631
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20194159

Original Research Article

c-Met overexpression in breast cancer with positive axillary lymph node

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Received: 21 August 2019
Revised: 27 August 2019
Accepted: 30 August 2019

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ABSTRACT

Background: Breast cancer is the second most common cancer in the world and is the most epidemic cancer in women, with approximately 1.67 million cases. Metastasis of tumor cells to other organs is a major cause of the increasing trend of mortality in breast cancer. This study aims to analyze the expression of c-Met associated with metastasis to axillary lymph nodes in invasive breast cancer.
Method: The research was conducted at the Laboratory of Anatomical Pathology of Hasanuddin University Hospital. Stratified sampling was performed from January 2014 - January 2019. Immunohistochemical staining technique was applied upon 66 collected samples, followed by evaluating the c-Met expression score in invasive breast cancer group with positive and negative lymph node status.
Result: c-Met overexpression was found among the invasive breast cancer incidence with lymph node metastasis. Among 50 cases with c-Met overexpression (c-Met positive), 40 cases (80%) of invasive breast cancer with lymph node metastasis were identified, while 10 cases (20%) were found in invasive breast cancer without metastasis to lymph nodes. On 16 cases with negative c-Met, 3 cases (18.8%) were found in invasive breast cancer with lymph node metastasis, and 13 cases (81.3%) in invasive breast cancer without metastasis to the lymph nodes. The statistical test results indicated a significant correlation between c-Met expression scores and metastasis to axillary lymph nodes in invasive breast cancer ($p < 0.001$).
Conclusion: As one of biomarkers, c-Met overexpression plays a vital role in the treatment of patients with invasive breast cancer to predict patient outcomes and to determine modalities. It is possible to apply c-Met overexpression to investigate aggressiveness of metastatic tumor cells in the future.

Keywords: Breast cancer, c-Met overexpression, Positive axillary lymph node

INTRODUCTION

Breast cancer remains the second most cancer in the world, the largest number in women with approximately 1.67 million new cases diagnosed in 2012, and the fifth leading cause of death with a mortality rate at 522.00 cases of all cases. The incidence of breast cancer has climbed by 20% along with the growth of death rate by 14% since 2008.[1]

According to the Data by Indonesian Ministry of Health and Information Center, breast cancer made up the highest rate in Darmais Cancer Hospital during 2010-2013. There was an upward trend in new cases of breast cancer: 711 cases in 2010, 769 cases in 2011, 809 cases in 2012, and 819 cases in 2013. These numbers also came up with an upward surge of mortalities caused by breast cancer.[2]

Invasive breast cancer is a heterogeneous disease with varied features and a group of histological characteristics and clinical outcomes. Currently, the management strategy focuses on the use of chemotherapy to induce apoptosis in some cases that are resistant to hormone and target therapies. However, the prognosis of invasive breast cancer is, by far, not satisfactory. Biomarker plays a vital role in the treatment of patients with invasive breast cancer to predict patient outcomes and to determine proper therapy.[3]

In breast cancer patients with clinical stages I and II, regional lymph node status is an essential prognostic factor in determining patient survival, regardless of tumor size, histopathological grading, and other clinicopathological parameters. Until today, thorough axillary lymph node resection has been routinely carried out, and about 30% positively contain metastatic tumor nests.[4]

c-Met receptor or HGF receptor (HGFR) can be used as a prognostic marker in the cases of invasive breast cancer. As previous studies has suggested that SF/HGF can be a prognostic marker, they have revealed that there was immunoreactive HGF (expressed) of the extracts of primary breast cancer tissue. Furthermore, the breast cancer cell tissue also responds to mitogen SF/HGF, which means breast cancer cells also express c-Met receptors.[5]

The involvement of Met/HGF such as breast, ovarian, lung, gastric, and prostate cancers in several types of cancers has frequently been investigated [3]. MET/HGF, however, plays a vital role in the development of cancer through several pathways by activating the oncogenic pathway such as RAS, PI3K, and STAT3: a role in angiogenesis, and a scatter factor that causes cell dissociation due to the production of metalloprotease, which results in the invasion and metastasis of tumor cells.[6]

The mechanism of c-Met overexpression can occur due to: gene mutation, gene amplification, protein overexpression, increased ligand-dependent paracrine stimulation, and the presence of autocrine signals. According to some previous studies, c-MET overexpression in breast cancer is not promoted by gene amplification but an increase in transcription.[7]

In breast cancer, clinical and preclinical studies prove that c-MET dysregulation occurs in the carcinogenesis process and the development of malignant phenotypes of breast tumors. There are a variety of c-MET overexpressions among the incidence of breast cancer with around 36-100% of cases analyzed. Some investigations suggest that c-Met tends to be more expressed in the basal subtype group (triple negative breast cancer).[8]

Recently, several targeted therapy strategies for the HGF-MET pathway have also been developed. This approach includes using small molecules MET tyrosine kinase inhibitors (TKI), antibodies that can neutralize anti-HGF, and antibodies that neutralize anti-MET.[9]

METHODS

This study was conducted at the Laboratory of Anatomical Pathology of Hasanuddin University Hospital in Makassar during July 2018 - January 2019. The subject of this study was mastectomy spicemens dispatched to the Anatomical Pathology Laboratory of Hasanuddin University Hospital, Anatomical Pathology Laboratory of Dr. Wahidin Sudirohosodo Hospital, and the Pathology Diagnostic Center Laboratory in Makassar, which were diagnosed as grade 1, 2 and 3 of invasive breast cancer, including metastatic and non-metastatic cancer throughout regional lymph nodes with Eosin Hematoxylin staining.

Subjects undergoing mastectomy resection without lymph node removal and post-cemoptheraphy were excluded. The total of 66 samples were collected during January 2014 - January 2019, which included 5 well differentiated cases of invasive breast carcinoma, 43 moderately differentiated cases, and 18 poorly differentiated cases.

Immunohistochemical staining of breast specimens

The tissue in paraffin blocks was cut to a size of 4 um and glued to the object glass of a poly-L-lysine before deparaffinized. A simple avidin-biotin peroxidase complex (ABC) method was applied in immunohistochemical staining. On the other hand, the unstained slide was incubated with peroxidazed-1 in 5 minutes at room temperature before referred to the standard ABC procedure. Immunohistochemical staining used c-Met receptor concentrated polyclonal antibodies with 1:50 dilution. Afterwards, the results of immunohistochemical staining were evaluated using a light microscope by a pathologist and a researcher.

c-Met expression is the accumulation of protein in the cell cytoplasm detected by immunohistochemical methods. When the expression is indicated positive, the membrane and cytoplasm was stained brown on the light microscope. C-Met immunoexpress ion is expressed in semi-quantitative estimation with a scoring system based on staining intensity and the proportion of stained cells.

The intensity and proportion of the stained area were combined and scaled into a score level of 0 for unstained or <50% of tumor cells stained with weak intensity; a score of +1 was set for >50% of tumor cells stained with mild intensity, or <50% of tumor cells stained with moderate intensity; score +2 was valued for >50% of tumor cells stained with moderate intensity or <50% of tumor cells stained with strong intensity; and a score of +3 was marked for >50% of tumor cells stained with strong intensity. c-Met expression was positive if the total score of expression was +2 and +3. In contrast, it was negative if the total score of c-Met expression indicated +1 and 0.[10]

Statistical analysis

The Chi-square statistical test was performed to identify the association between c-Met expression in invasive breast cancer with positive lymph nodes and invasive breast cancer with negative lymph nodes. The value of p <0.001 indicates that there was a significant correlation.

RESULTS

Among 66 cases obtained, 43 cases (65.2%) were invasive breast cancer with positive lymph nodes, whereas 23 cases (34.8%) were diagnosed with negative lymph nodes. Positive c-Met expression (c-Met overexpression) constituted 50 cases (75.7%), while negative c-Met expression made up (24.3%) respectively.

Table 1: Sample Characteristics.

Characteristics:	n (%)
Histopatology Diagnosis:	
Well differentiated	5 (7,6)
Moderately differentiated	43 (65,2)
Poorly differentiated	18 (27,3)
Lymph Node Metastasis:	
Positive	43 (65,2)
Negative	23 (34,8)
c-Met Expression:	
Positive	50 (75,7)
Negative	16 (24,3)

According to the histopatology diagnosis (Table 1), the majority of patients with invasive breast carcinoma indicated a moderately differentiated grade with 65.2 %, while the well differentiated and poorly differentiated grades made up just 7.6% and 27.3% respectively. On the other hand, more than half of the subjects were identified Lymph Node Metastasis positive, and 75.7% of the total samples reveals positive c-Met expression.

Table 2 shows the Chi-square statistical test result which indicates the correlation of c-Met expression and axillary lymph node status (p <0.001). The statistical anaylsis indicated that there was a significant difference between c-Met expression in invasive breast cancer with positive lymph nodes and invasive breast cancer with negative lymph nodes. In particular, c-Met overexpression tended to show higher rate in lymph node metastasis (positive lymph nodes) with 40 cases (80%).

Table 2: c-Met expression in invasive breast cancer associated with axillary lymph node status.

c-Met Expression	Axillary lymph node		Total	
	Positive	Negative		p
Positive	40(80)	10(20)	50(100)	<0,001
Negative	3(18,8)	13(81,3)	16(100)	
Total	43(65,2)	23(34,8)	66(100)	
Chi-Square Test				

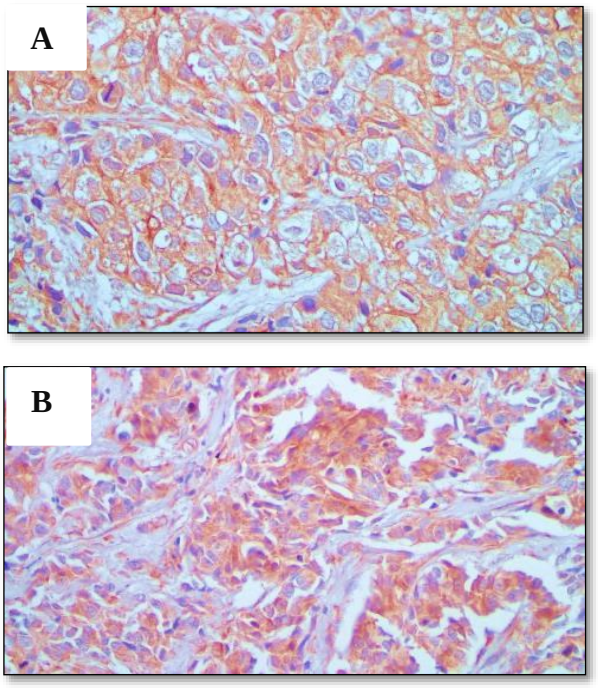

Figure 1: (A): C-Met overexpression: Stained with strong intensity on the membrane and cytoplasm in invasive breast cancer with negative lymph nodes (Obj. 40X) (B): C-Met overexpression: Stained with strong intensity on the membrane and cytoplasm in invasive breast cancer with positive lymph nodes (Obj. 40X).

DISCUSSION

This research reported that higher level of c-Met overexpression was detected among invasive breast cancer with lymph node metastasis (positive lymph nodes). In 50 cases with c-Met overexpression (c-Met positive), 40 cases (80%) of invasive breast cancer with lymph node metastasis (positive lymph nodes) were obtained, while only 10 cases (20%) were found in invasive breast cancer without metastasis to lymph nodes (negative lymph nodes). On the other hand, among 16

cases with negative c-Met, 3 cases (18.8%) were found in invasive breast cancer with lymph node metastasis (positive lymph nodes), and 13 cases (81.3%) in invasive breast cancer without metastasis to the lymph nodes (negative lymph nodes).

However, Zhao Xixi et al, obtained different results in which there was no significant association between c-Met expression with menopausal status, hormone status, Ki-67 expression label, the incidence of lymph node metastasis (lymph node status) and histopathological type of breast cancer. Thus, it suggests that target therapy with Anti c-Met can be applied for breast cancer in all subtypes of invasive breast cancer without taking into account of hormonal or lymph node status.[3,5]

In cases of metastasis, the rate of survival stood at just 2-3 years. One of the critical steps in detecting the presence of metastases and staging of breast cancer is by detecting the presence of tumor nests of metastases in regional lymph nodes. Regional lymph nodes are primary lymphatic drainage from all areas of the breast, and the involvement of lymph is a strong predictive factor to determine the rates of recurrence and survival among patients.[11]

Perhaps not surprisingly, a new marker, which has a strong and independent predictive score is needed to determine the prognosis value. Therefore, c-Met, one of the receptor tyrosine kinases (RTKs) that play an important role in cell survival, process of angiogenesis, growth, and metastasis, is one of potential markers.[5,12]

This study also revealed that positive c-Met expressions (c-Met overexpression) constituted 50 cases out of a total of 66 cases (75.7%), while that in negative expressions accounted for 16 cases (24.3%). In line with previous studies, this research presented that c-Met overexpression was found in 73% of the incidence of invasive breast cancer [13]. However, the study conducted by Edakuni et al, reported that 45 out of 64 cases (70.3%) showed c-Met overexpression. Likewise, a study conducted by Zagouri et al. revealed that c-Met overexpression was detected in 52% among the subjects with triple negative breast cancer (TNBC).[12]

The findings of this study are in line with the research by Lengyel et al., which reported that there was a significant association between c-Met overexpression and an increased risk of disease progression. This study also found that c-Met overexpression manifested itself in invasive breast cancer groups with positive lymph nodes in which c-Met expression occurred more frequently with higher rates in metastatic tumor cells in the lymph nodes compared with primary tumor cells. Most patients with c-Met overexpression revealed an increased relapse incidence within 14 months after treatment. This indicates a failure of conventional systemic therapy.[13]

In addition, c-Met tyrosine kinase plays a role in invasive tumor growth, through a series of complex physiological programs that regulate cell proliferation activity, survival, invasion, and angiogenesis. According to the most recent preclinical study of invasive breast cancer, tumor invasion and metastasis occurred after inhibition of the VEGF signal pathway. The mechanism for an increase in aggressiveness is not sufficiently understood, but the involvement of factors such as the increased condition of intratumor hypoxia forces the tumor to form new blood vessels where hypoxia can increase c-Met expression.[14]

The study conducted by Edakuni et al. also found a significant association between HGF/c-Met co-expression in invasive breast cancer with a histopathology grade, lymph node status, and Ki-67 index label where the expression pattern of HGF/c-Met was expressed in an autocrine, paracrine, or combination pattern.[15]

The activation of HGF/c-Met signal runs essential function in the stromal interaction of the tumor under hypoxic conditions. Hypoxic conditions will induce molecular responses in both healthy and neoplastic cells, which trigger the activation of transcription factors such as hypoxia-inducible factor-1 alpha (HIF-1α). An upward rate of HIF-1α activates target genes involved in tumor cell growth, angiogenesis, metabolism, apoptosis, and metastasis. HIF-1α upregulates the HGF/c-Met signal, which consecutively precipitates cell migration through blood vessel microcirculation and lymphatic vessels. The expression of HGF by stromal tissue is significantly related not only to stromal HIF-1α but also to c-Met expression as well as in PK8 pancreatic cancer cells.[16]

The HGF/c-Met complex also magnifies the potential of tumor malignancy by inducing VEGF-A production and suppressing Thrombospondin-1 that interacts synergistically with VEGF receptors through downstream molecular signals to enhance neovascularization activity. VEGF prompts angiogenesis and lymphangiogenesis in primary tumors, providing a route for tumor dissemination. VEGF also induces changes in vascular integrity and permeability, eliciting intravasation and extravasation, while VEGF also induces angiogenesis in secondary tissues needed for cell proliferation and stabilizes metastatic lesions.[16]

CONCLUSION

In conclusion, this study has revealed a relationship between c-Met and metastasis to axillary lymph nodes in invasive breast cancer. The results support the case for distinguishing positive and negative lymph nodes in breast cancer in particular.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. IARC 'Latest world cancer statistics Global cancer burden rises to 14 . 1 million new cases in 2012 : Marked increase in breast cancers must be addressed Latest world cancer statistics Global cancer burden rises to 14 . 1 million new cases in 2012 : Marked incr', Dec 2013:1-3.
2. Kementrian Kesehatan RI. Situasi kesehatan reproduksi remaja. Jakarta: Pusat Data dan Informasi Kemenkes RI. 2015.
3. Zhao X, Qu J, Hui Y, Zhang H, Sun Y, Liu X, et al. Clinicopathological and prognostic significance of c-Met overexpression in breast cancer. Oncotarget. 2017 Aug 22;8(34):56758.
4. Sophia Ran,1 Lisa Volk, Kelly Hall, and Michael J. Flister. Lymphangiogenesis and Lymphatic Metastasis in Breast Cancer, Pathophysiology. 2010 Sep; 17(4): 229-51.
5. Ghoussoub RA, Dillon DA, D'Aquila T, Rimm EB, Fearon ER, Rimm DL. Expression of c-met is a strong independent prognostic factor in breast carcinoma. Cancer: Interdisciplinary Int J Am Cancer Society. 1998 Apr 15;82(8):1513-20.
6. Elliott BE, Hung WL, Boag AH, Tuck AB. The role of hepatocyte growth factor (scatter factor) in epithelial mesenchymal transition and breast cancer. Canad J physiol pharmacol. 2002 Feb 1;80(2):91-102.
7. Ho-Yen CM, Jones JL, Kermorgant S. The clinical and functional significance of c-Met in breast cancer: a review. Breast Cancer Research. 2015 Dec;17(1):52.
8. Minuti G, Landi L. MET deregulation in breast cancer. Annals of translational medicine. 2015 Aug;3(13).
9. Parikh RA, Wang P, Beumer JH, Chu E, Appleman LJ. The potential roles of hepatocyte growth factor (HGF)-MET pathway inhibitors in cancer treatment. Onco Targets and therapy. 2014;7:969.
10. Watermann I, Schmitt B, Stellmacher F, Müller J, Gaber R, Kugler C, et al. Improved diagnostics targeting c-MET in non-small cell lung cancer: expression, amplification and activation?. Diagnostic pathol. 2015 Dec;10(1):130.
11. Rahman M, Mohammed S. Breast cancer metastasis and the lymphatic system. Oncology letters. 2015 Sep 1;10(3):1233-9.
12. Zagouri F, Brandstetter A, Moussiolis D, Chrysikos D, Dimitrakakis C, Tsigginou A. Low protein expression of MET in ER-positive and HER2-positive breast cancer. Anticancer research. 2014 Mar 1;34(3):1227-31.
13. Lengyel E, Prechtel D, Resau JH, Gauger K, Welk A, Lindemann K, et al. C-Met overexpression in node-positive breast cancer identifies patients with poor clinical outcome independent of Her2/neu. International J cancer. 2005 Feb 10;113(4):678-82.
14. Sennino B, Ishiguro-Oonuma T, Schriver BJ, Christensen JG, McDonald DM. Inhibition of c-Met reduces lymphatic metastasis in RIP-Tag2 transgenic mice. Cancer research. 2013 Jun 15;73(12):3692-703.
15. Edakuni G, Sasatomi E, Satoh T, Tokunaga O, Miyazaki K. Expression of the hepatocyte growth factor/c-Met pathway is increased at the cancer front in breast carcinoma. Pathol Int. 2001 Mar;51(3):172-8.
16. Spina A, De Pasquale V, Cerulo G, Cocchiaro P, Della Morte R, Avallone L, et al. HGF/c-MET axis in tumor microenvironment and metastasis formation. Biomedicines. 2015;3(1):71-88.

Cite this article as: Hasanah H, Masadah R, Nelwan BJ, Achmad D, Miskad UA, Rahawarin H, et al. c-Met overexpression in breast cancer with positive axillary lymph node. Int J Res Med Sci 2019;7:3627-31.