The Relationship between Clinicopathological Features of Invasive Breast Cancer Patients Based on IGF-1R Expression

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***Abstract*-Background:** Breast cancer is the most common cancer found in women with approximately 23% cases of all cancer. It has been known that signaling through IGF-1R is involved in growth of many cancer especially breast cancer, for example by affecting cell proliferation, survival, invasion, and metastasis. One of the receptors in IGF pathway is IGF-1R. The research of IGF-1R expression and clinicopathological features in breast cancer patients is still controversial.
Objective: To analyze clinicopathological features of invasive breast cancer patients with IGF-1R expression.
Material and Method: This analytic study with cross-sectional study from 37 invasive breast cancer patients. Data about clinicopathological characterization were obtained from medical record. Then, re-cutting specimen paraffin blocks and immunohistochemical staining IGF-1R (Medasis company, rabbit monoclonal antibody, klon MD30R, dilution 1:50-100) were done. Results of analysis data were processed with statistical software and were presented in tables. Statistical test used in this research is chi-square or Fisher exact test.
Result: This study showed that there were significant relationship between menopausal status, body mass index, and histological grading with IGF-1R expression in invasive breast cancer patients (p value 0.044; 0.028; 0.002, respectively).
Conclusion: Immunohistochemistry of IGF-1R is needed in patients who is resistant to tamoxifen therapy.

Index Terms-: *Invasive breast cancer, Clinicopathological features, Body mass index, Menopausal status, IGF-1R*

I. INTRODUCTION

Breast cancer is the fourth most common cancer found in women with approximately 23% cases of all cancer.[1,2] American Cancer Society concludes that the incidence of breast cancer is increasingly every year.[3] Based on World Health Organization, it is estimated that the incidence of cancer in worldwide is projected to increase about 300 percents in 2030, and most of them will happen in developing countries including Indonesia.[4]

Insulin like growth factor (IGF) 1 is one of the IGF families that plays an important role in growth and development of many tissues, especially mammary gland. There are two receptors in IGF pathway such as IGF receptor type 1 (IGF-1R) and IGF-2R. Both IGF-1 and IGF-2 show biological activity through IGF-1R signaling. It has been known that signaling through IGF-1R plays an important role in some cancer, especially breast cancer.[5] IGF-1R expression has been associated with metatasis, drug resistance, radiotherapy resistance, and tumor recurrence.[6]

Obesity is a worldwide health issue and is well known risk factors for the development of certain cancer, including postmenopausal breast cancer.[7] Molecular mechanism relating cancer and obesity is still unknown.[8]

Eventhough researches about IGF-1 in breast cancer patients indeed have long been established, the studies of IGF-1R and clinicopathological features of breast cancer patients are still controversial. Considering the conditions mentioned above, the authors were interested to study about the relationship between clinicopathological features of invasive breast cancer patients and IGF-1R expression. This study was aimed to assess the association of age, menopausal status, body mass index, histopathology subtype and grade histology with breast cancer.

II. MATERIAL AND PRODUCT

This analytic study with cross sectional approach was conducted at the Department of Anatomical Pathology Medical Faculty, Universitas Sumatera Utara, Medan. The research was held from August 2018 until November 2019, after approved by Health Research Ethics Committee of Universitas Sumatera Utara.

The samples were paraffin blocks from breast specimen histopathologically diagnosed with invasive breast carcinoma fulfilling inclusion and exclusion criteria. Samples were recruited using consecutive sampling technique. Inclusion criteria was full detailed medical record such as age, menopausal status, and body mass index. Exclusion criteria for this study were paraffin blocks that couldn't be processed and immunostaining, and at least 100 tumour cells were found in Hematoxylin Eosin (HE) staining. Each samples were stained with HE and IGF-1R immunohistochemistry (Medasis company, rabbit monoclonal antibody, clone MD30R, dilution 1:50).

IGF-1R expression was interpreted based on allred scoring system. The interpretation was done by adding percentage score of positive tumour cells and staining intensity. Percentage score of positive tumour cells were assessed as follows. Absent or present in ≤ 1% positive in membrane/ cytoplasmic tumour cells was taken as score 1, 1-10% positive tumour cells was taken as 2, 11-33% was 3, 33-66% was 4, and 67-100% was 5. Meanwhile, staining intensity were scored as weak (score 1), moderate (score 2), or strong staining (score 3). After that, total addition were interpreted as negative (total score 0-2), low 1+ (total score 3-4), moderate 2+ (total score 5-6), and high 3+ (total score 7-8). Furthermore, IGF-1R expression was categorized as negative (negative and 1+) and positive (2+ and 3+).[9] The data obtained in the research was processed with help of statistical software and presented in tables. Statistical test used in this study were chi-square or Fisher exact test.

III. RESULT

In this study, there were initially 50 breast tissue paraffin blocks histopathologically diagnosed as invasive breast carcinoma in Department of Anatomical Pathology, Medical Faculty, Universitas Sumatera Utara, Medan. From these 50 paraffin blocks, there were only 40 samples had full detailed medical record. From these 40 samples, only 37 paraffin blocks could be assessed.

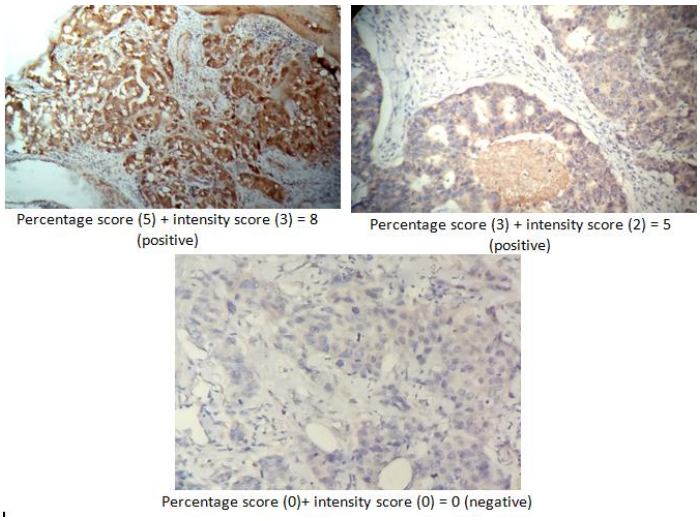

Figure 1. Interpretation of IGF-1R immunohistochemical staining.

In this study, analysis of association of age, menopausal status, body mass index, histopathological subtypes, and grading hsitology with breast carcinoma were done. Statistical test showed that there were significant relationship between menopausal status, body mass index, and grading histology with breast cancer (p value 0.044, 0.028, 0.002, respectively). Meanwhile, there were no significant association between age and histopathological subtypes with breast cancer (p value 0.823 and 1.000, respectively) (Table 1).

Furthermore, postmenopause invasive breast carcinoma patients had 3.048 higher risk to have positive IGF-1R expression than premenopause (95%CI 1.030-9.021). Overweight patients had 0,294 risk than normal weight to have positive IGF-1R expression (95% CI 0.099-0.873). Last, patients with grade histology 3 had 4.706 risk than grade 2 to have positive IGF-1R (95% CI 1.586-13.961).

Variables	*IGF-1R* expression				Total	OR (95% CI)	p
	Negative	%	Positive	%			
Age (years old)							
– <40	2	40	3	60	5	-	0.823
– 40 - 49	4	33.3	8	66.7	12		
– 50 - 59	7	50	7	50	14		
– ≥60	2	33.3	4	66.7	6		
Menopausal status						3.048	
– Premenopause	12	57.1	9	42.9	21	(95%CI:1.030-	
– Postmenopause	3	18.8	13	81.3	16	9.021)	0.044
Body mass index							
• 3groups							
– Normal	12	60	8	40	20	-	0.028
– overweight	0	0	2	100	2		
– obesity	3	20	12	80	15		
• 2 groups						0.294	
– Normal	12	60	8	40	20	(95%CI: 0.099-	0.023
– Over	3	17.6	14	82.4	17	0.873)	
Histopathology subtypes						0.884	
– IC NST	11	39.3	17	60.7	28	(95%CI:0.373 -	1.000
– Special type	4	44.4	5	55.6	9	2.096)	
Grading histology						4.706	
– Grade 2	12	70.6	5	29.4	17	(95%CI:1.586-	
– Grade 3	3	15	17	85	20	13.961)	0.002

Table 1. The relationship between clinicopathological features of invasive breast cancer patients with IGF-1R expression

IV. DISCUSSION

As mentioned before, IGF family plays an important role in development and dissemination of several types cancer, such as by influencing cell proliferation, cell survival, invasion, and metastasis.[10,11] Bruchim, et al. (2013) reported that IGF-1R upregulation, in vitro and in vivo, had been correlated with certain types cancer, including breast.[12] IGF-1 coud affect breast cells through 3 mechanisms as follows. First, endocrine mechanism which is from IGF-1 serum (especially produced by hepar and other tissues) can stimulate breast cancer cells. Second, paracrine mechanism leads to normal breast cells and adjacent stroma including fibroblasts, that can produce IGF-1 to e adjacent breast cancer cells. Third, autocrine mechanism which is by the tumour cells.[13] This study showed that 22 samples out of 37 had positive IGF-1R expression (59.5%).

Bhardwaj et al. (2019) stated that in premenopause women, estrogen is mainly produced in ovarium. Hipothalamus releases gonadotropin releasing hormone (GnRH) that will stimulate the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH stimulates biosynthesis of estrogen by developing ovarium follicles, that will induce LH production in hypothalamus. Acute LH surge will cause ovulation. After menopause, ovarium produce negligible amount of estrogen. The importance of steroidogenesis in gonad gland in the development of normal breast and origin of breast cancer is confirmed by facts stating that early menstruation and late menopause has been associated with higher risk of breast cancer. In postmenopause women, the one that plays a role as source of androgen to produce estrogen in the peripheral area is dehydroepiandrosterone sulfate (DHEA-S) from adrenal gland. Local biosynthesis of estrogen in breast and circulating serum level of estrogen, is believed as the representative of steroid

producation derived from adipose tissue, that is directly associated with proliferation of breast tumour cells. This illustrates there is increased risk of breast cancer after rmenopause. In particular, breast expresses all enzymes needed to convert DHEA-S into estradiol (E2), including steroid sulfatase, 3β-HSD, 17βHSD1 and aromatase. Of all these enzymes, the one that the most important role in obesity is aromatase. Aromatase could be found in many tissues, such as breast, adipose, gonad, and etc. Its expression in breast adipose tissue is hypothesized as main driver causing breast cancer due to estrogen after menopause. After menopause, adipose tissue is the primary source of estrogen production in human body. The higher fat mass and higher body weight, the higher aromatase and estrogen level in postmenopausal women.[14]

Song et al. (2007) has revealed that 17β-estradiol can activated IGF-1R activation pathway, that ultimately promote MAPK pathway.[15] Overexpression and amplification of IGF-1R will activate PI3K/Akt/mTOR and Raf/Mek/Erk pathway and has proven to associated with continuous tumour proliferation apart from estrogen. This pathway gives an alternative stimulus to tumour, and can occur as tumour development driver without any connection with ER. This will cause resistance to therapy.[14] Upregulation of IGF-1 pathway causes resistnace breast cancer cell line to tamoxifen and fulvestrant through upregulation of MAPK and PI3K signaling.[16]

This study revealed that there were significant relationship between menopausal status, body mass index, and grading histology with IGF-1R expression in invasive breast cancer patients (p value= 0.044, 0.028, and 0.002). However, there were no significant association between age and histopathological subtypes with IGF-1R in invasive breast cancer patients (p value= 0.823 and 1.000). Studies about the relationship between IGF-1R expression and clinicopathological features of invasive breast cancer patients to date is still controversial. Some literature showed there were significa nt relationship, but some weren't. Engels et al (2015), and Sun et al. (2015) found that IGF-1R expression didn't significant associated with age, body mass index and grading histology.[10,17] On the contrary, Shin et al (2014) and Reinholz et al (2017) showed that there were significant relationship between IGF-1R with grading histology and menopausal status.[18,19]

The difference in study results might be occured due to different research location that can be influenced by race living in the areas. IGF-1 level may be affected by environment and lifestyle factors. Discrepancy in results obtained from different measurement method of IGF-1R expression often cause inconsistent results about IGF-1R expression. IGF-1R molecules might be controlled between cytosol and membrane cell and IGF-1R cytoplasma representing boun, internalized receptor. Depending on grade and pathology features of invasive tumour, there might be a failure in transporting IGF-1R to the surface of cells.

There were few limitation in this study. First, to date, there was no uniform cut-off levels for IGF-1R expression. Therefore, researchers categorized IGF-1R immunohistochemical staining results using allred score. Second, body weight of patients obtained in this study was measured at initial diagnosis. Those patients maybe previously obesity, but due to suffer from cancer, they had lost weight. This could induce bias in this study.

V. CONCLUSION

After conducted this study, we highlighted several points in the following:

1. There were significant associations between menopausal status, body mass index, and grading histology with IGF-1R expression in invasive breast cancer.
2. There were no significant association between age and histopathological subtypes with IGF-1R expression.
3. Clinicians should do IGF-1R immunohistochemical staining in invasive breast cancer cases that are resistant to tamoxifen therapy.

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