Association of Epstein Barr Virus Infection to Prognosis Factors of Invasive Breast Cancer in Semarang Kariadi General Hospital Population

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Keywords: Breast Cancer, Prognosis Factors of Breast Cancer, EBV Infection, Molecular Detection of EBV

Abstract: Breast cancer is the most common malignant disease in female. In addition to internal factors, external factors such as viral infections are thought to have a role in the carcinogenesis process of breast cancer. In the case of nasopharyngeal cancer, Epstein-Barr Virus (EBV) has been shown to be able to activate several signaling pathways so that cancer cells become more aggressive and have a worse prognosis. This study aims to determine the relationship of the detection status of EBV DNA with the prognosis factor of breast cancer. The research design used in this study was analytic observational with a cross-sectional approach, with 71 paraffin block samples from the Anatomical Pathology Laboratory of Kariadi General Hospital who obtained from mastectomy or Breast Conserving Surgery. Samples was examined using PCR to detect EBV DNA in tumor cells. age of breast cancer cases ranged from 23 to 78 years with median age of 52 years. From 71 samples, there were 28 positive samples (39.4%) of EBV DNA and 43 samples (60.6%) were negative. Significant results were obtained ($p < 0.05$) in the relationship between EBV infection and tumor size ($p = 0.002$), axillary lymph node metastasis ($p = 0.001$), and lymphovascular invasion ($p = 0.001$). Our research could find a significant statistical association in the status of axillary lymph nodes, lymphovascular invasion status and tumor size in breast cancer samples infected by EBV and those not infected by EBV..

1 INTRODUCTION

Breast cancer is the most common malignant disease in female (Bray et al.,2018). The incidence of breast cancer in Indonesia in female is the highest (Manuaba , Burmansyah, and Tjindarbumi, 2010; WHO, 2014) . In addition to internal risk factors such as genetic and sex hormones, external factors such as viral infections also play a role in the process of carcinogenesis (Alibek et al., 2013; Ahmed et al.,2019). The relationship of the epstein-barr virus (EBV) with breast cancer is based on several reasons, such as the high incidence of male breast cancer, which is reported in Mediterranean countries, endemic areas for EBV, the occurrence of several EBV-related lymphomas in the breast, and morphological similarities between breast medullary cancers and nasopharyngeal cancer (KNF) (Zekri et al., 2012). EBV is a family of herpesviruses and has two variations (type 1 and type 2) (Joshi and Buehring, 2012;. Hjalgrim, Friborg, and Melbye, 2007). In the human body, EBV will settle in B lymphocytes, and from B lymphocytes EBV will produce several viral proteins (LMP, EBNA, and EBER) that can affect cell growth signals from its host (Chang, Moore, and Weiss, 2017; Kim, Kim, Park, 2013; Kanda, 2018;He et al., 2003). Some authors believe that EBV might play a role in the oncogenesis of breast cancer not as a major etiology but as a cofactor in the development of breast cancer and can influence the aggressive nature of breast

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Yudisaputro, W., Handojo, D., Prajoko, Y. and Puspasari, D.
Association of Epstein Barr Virus Infection to Prognosis Factors of Invasive Breast Cancer in Semarang Kariadi General Hospital Population.
DOI: 10.5220/0010488701160120
In *Proceedings of the 1st Jenderal Soedirman International Medical Conference in conjunction with the 5th Annual Scientific Meeting (Temilnas) Consortium of Biomedical Science Indonesia (JIMC 2020)*, pages 116-120
ISBN: 978-989-758-499-2

cancer (Louise et al.,1 995;Murray et al.,2003; Arbach et al.,2006; Mazouni et al., 2011)

Treatment modalities for breast cancer include surgery, chemotherapy, hormonal therapy, immunotherapy, and external radiation (Panigoro and Purwanto, 2012). The success of the therapy given will be related to predictive factors and prognostic factors (Ramli, 2015; Cianfrocca and Goldstein, 2004) Several established prognostic factors such as hormone receptors, Her2 expression, tumor size, axillary lymph node metastases and lymphovascular invasion can affect the aggressive nature of breast cancer so that it will affect the survival rate and disease-free interval (Carter et al., 1989; Fisher et al., 1988)

With the development of molecular biology, EBV infection in breast cancer can be determined by using PCR examination by detecting the presence of EBV DNA in cancer cells22,23. The purpose of this study is to find out whether there is a relationship between the detection status of EBV DNA and the prognosis factor for breast cancer. The results of this study not only can find out the prevalence of EBV infection in breast cancer cases in Kariadi Hospital, but also can be the basis for further research on the role of EBV in the carcinogenesis of breast cancer.

2 MATERIAL AND METHODS

2.1 Patients and Tissue

The research design used in this study was analytic observational with a cross-sectional approach. This study consisted of a Formalin-Fixed Paraffin-Embedded (FFPE) of cancer biopsy from 71 cases of invasive breast cancer stage I to III that had been performed mastectomy or Breast Conserving Surgery. The samples were taken from the pathology anatomy laboratory of Kariadi Hospital from January 2019 to September 2019. Each paraffin tumor block will be extracted DNA and then DNA amplification by PCR was done in the pathology anatomy laboratory of FKKMK UGM. The primers used were (forward primers 5‘GGCCTCCAAGGAGTAAGAC-C3‘ and reverse primers: 5‘CCCCTCTTCAAGGGGTC-TAC3') that were circulating on the market24. The DNA positive control for EBV comes from KNF where the virus was detected and the negative control uses nuclease-free distilled water instead of DNA. Histopathological data provided axillary lymph node metastasis, tumor size, receptor hormone status, Her2 overexpression status, degree of malignancy and lymphovascular invasion and were obtained from medical record data and anatomical pathology report of Kariadi Hospital.

2.2 Statistical Analysis

Data is processed using the SPSS 20.0 for Windows. The Chi square test was used for parametric test, and Mann Whitney test for non-parametric test to determine the relationship between the detection status of EBV DNA with the degree of malignancy, estrogen receptor status, progesterone receptor status, Ki 67 status, lymphovascular invasion status, Her 2 status, and axilla lymph node metastasis. The value of the significance is <0.05.

3 RESULTS

Study found that some relationship between EBV DNA status and prognosis factors for breast cancer among the patients (Table 1).

Table 1. Relationship between EBV DNA status and prognosis factors for breast cancer

Characteristics	EBV (+)	EBV (-)	Mean ± SD	Median (min – max)	P Value
Age			49,97 ± 11,99	52 (23 – 78)	
Tumor size					
T1	0	0			
T2	3	22			P = 0.002
T3	14	9			
T4	11	12			
Axillary lymph node metastasis					
Negative	1	17			
1 – 3	0	15			P = 0.001
4 – 10	18	8			
>10	9	3			
Estrogen Receptor					
Negative	10	18			P = 0.605
Positive	18	25			
Progesterone Receptor					
Negative	15	22			P = 0.843
Positive	13	21			
Her 2					
Negative	21	26			P = 0,673
Positive	11	7			
Ki 67					
< 20%	5	9			P = 0.750
> 20%	23	24			
Malignancy grade					
1	0	4			P = 0.808
2	21	24			
3	7	15			
Lymphovascular invasion					
Negative	4	24			P = 0.001
Positive	25	20			

4 DISCUSSION

In this study the prevalence of breast cancer samples which EBV Epstein Barr Virus (EBV) were detected was 28 samples (39.4%). According to epidemiological studies carried out by Huo Q et al. on the basis of PCR examination, the prevalence of EBV infection worldwide is 29.32% and prevalence at the Asian level is 35.25% (Huo, Zhang, and Yang, 2012)

The prevalence of EBV DNA detection status in this study has a higher rate than the prevalence worldwide, or in Asia. The higher incidence can be caused by demographic differences and the characteristics of the research sample (Hjalgrim,

Friborg, and Melbye, 2007)

This study proves the link between the EBV infection and the incidence of breast cancer, but only a small proportion of positive EBV cases develop into breast cancer. This shows that only EBV alone is not enough as an etiological factor in carcinogenesis, a number of biological and environmental cofactors are also needed for the occurrence of breast cancer (Alshammari, 2017).

The results of our study have significant statistical association of EBV infection with tumor size. This result is in accordance with some studies conductedpreviously. Arbach in his research found that EBV infection in breast cancer cells can increase the ability of these tumor cell mutations such as invasion, angiogenesis, and metastasis. As in the case of nasopharyngeal cancer, the LMP-1 oncoprotein produced by EBV induces cyclooxygenase 2 (COX-2) which then induces vascular endhotelial growth factor that plays a role in the process of angiogenesis through NF-κB signaling (Arbach et al., 2015).

LMP-1 is directly related to oncogenesis, because it is able to activate several cellular signaling pathways such as Nuclear Factor-κB, c-Jun NH2-terminal kinase (JNK), p38 kinase, phosphatidylinositol 3-kinase (PI3K), and several other possible pathways. thereby resulting in continuous proliferation and inhibits apoptosis (Sun et al.,2015)

One of the oncoproteins found in Epstein-Barr virus is EBNA-1. The oncogenic role of EBNA-1 protein has been reported by some previous researchers. EBNA-1 can reduce p53 levels by triggering ubiquitin specific protease USP7 so that p53 becomes unstable. In the end the breast gland epithelium is infected with EBV so that the cells become proliferated continuously and anti-apoptotic (Frappier, 2012)

The results of our study have significant statistical association of EBV infection with The metastatic status of axillary lymph nodes and lymphovascular invasion status. These findings come in agreement with studies conducted by Fessahaye et al., 2017. Our study shows that tumor cells contained in the EBV gene have the potential for metastases to occur, which suggest aggressive tumor behaviour, and making them a poor prognostic factor. Arbach et al. who showed that EBV infection of breast tumor enhances its mutagenic properties, such as invasion, angiogenesis, and metastasis.

5 CONCLUSION

The conclusion of this study is, there are significant statistical association in the status of axillary lymph nodes, lymphovascular invasion status and tumor size in breast cancer samples infected by EBV and those not infected by EBV.

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