International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.12 No.02, pp 197-202, 2019

Claudin-7 Expression In Triple Negative Breast Cancer Based On Histopathological Type And Grade

Rini Flora Doloksaribu[1]*, T Ibnu Alferraly[2], Betty[2]

[1]Resident in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.
[2]Supervisor in Department of Anatomic Pathology, Faculty of Medicine, Universitas Sumatera Utara, Indonesia.

Abstract : Triple negative breast cancer (TNBC) is an aggressive type of breast carcinoma that often invade to stroma or metastasis, that may be affected by claudin-7 protein in tight junction. Low expression of Claudin-7 considered to have worse outcome and prognose of TNBC patient. To know the immunohistochemical expressions of claudin-7 based on various clinicopathological parameters among TNBC patients, we collect formalin-fixed paraffin-embedded tissue blocks of 50 TNBC patients were immunohistochemically studied for claudin-7 expressions. Allclinicopathological characteristics were obtained through medical records and pathology archives. In our study found that 43 case (86%) of the TNBC patient showed claudin-7 high expression and 7 case (14%) showed low expression. The mean age for TNBC patients was 47,42 (±8,42) years old and the mean of claudin-low expression was 43 ± 8,28 years old with all of them was pra-menopausal. Claudin-7low expressions showed in the only one case with lung metastasis, in moderate clinical stage, tumor size, grade andvarious histopathology type in TNBC. Mostof claudin-7 low expression showed with no lymph node involment. Many of Claudin-7 low expression showed on grade 2 TNBC patient. Hence, application and work validation by using larger samples and equally distribution needed for a appropriate prognose determination andmetastatic tendency of TNBC patients with claudin-7 low expression.
Keywords : TNBC, Clinicopathological, Immunohistochemistry, Claudin-7.

Introduction

According to GLOBOCAN International Agency for research on Cancer (IARC)database, breast cancer is the second most common cancer in the world and the most common cancer in women with an estimated 1.67 million new cases diagnosed in 2012 (25% of all cancers).In Indonesia, breast cancer incidence 134 per 100.000 population. Cancer population in Indonesia increases along age. In North Sumatera, estimation of breast cancer case is about 2.682 based on Data Riset Kesehatan Dasar on 2013.[1,2,3]

DOI= http://dx.doi.org/10.20902/IJCTR.2019.120226

According to gene profiling from esterogen (ER+/ER-)/ progesterone receptor (PR+/PR-) and human epidermal growth factor receptor 2 (HER2+/HER2-), invasive breast carcinoma divided in to several subtype. Triple negative breast cancer (TNBC) aretype of breast cancer which lack of ER, PR, HER-2. It is accounting 12% from all breast cancer, usually seen in young age, advanced stage at presentation, unfavourable histopathology, higher grade, higher proliferative index, lack of tubule formation and higher rate of metastases.[4,5]

Metastatic breast cancer maybe affected by loss of cell adhesion one to another. Furthermore, tumor cell invaded to stroma.[4,5] Cell adhesion be regulated by 2 different junction, which is adherens juntion and tight junction. Many protein have role in tight junction one of them is claudin, integral membrane proteins found in tight junctions of all epithelial and endothelial.[6] Many research found overexpression, knockdown or knockout of claudins, and both naturally occurring and experimentally introduced mutations of claudins, consistently cause changes in paracellular permeability.[6.] There is may claudin subfamily, such as claudin-1 to claudin-27. This study, we will discuss about claudin-7, that expressed in various epithelial included breast epithelial. Claudin-7 can be a prognosis indicator and metastatic suspicion. Not many claudin-7 membrane protein studies in breast cancer.[6] Kominsky et al, reported claudin-7 low expression in invasive ductal carcinoma dan ductal carcinoma insitu compared with normal breast epithelium.[4] Therefore, we aim to evaluate claudin-7 expression in triple negative breast cancer (TNBC) based on histopathology feature and grading.[4]

Materials and method

We observed 50 cases of TNBC at the Department of Anatomical Pathology, University of Sumatera Utara/ H. Adam Malik General Hospital, Medan with cross sectional methode.We obtained clinical data consisting of age, menstrual status, stage, tumor size, lymph node involvement, and distant metastasis from pathology archives. Histological type and grade were independently assessed by three observers through microscopic examination of hematoxylin and eosin stained slides.

Immunostaining was performed by using Claudin-7 (clone Ab27487, Abcam, Singapore) rabbit polyclonal antibodies. Detection with Diagnostic BioSystems (Diagnostic BioSystems, Pleasanton, CA, USA) polymer kit was done according to manufacturer protocol. The positive control was colon tissue whereas negative control was obtained by omission of primary antibodies.[7,8]

Claudin-7 expressions was determined independently by three observers, used Allerd's score methode, which assess how many cell presentage (score 0-5)expressed and intensity of the imunohistochemical staining (scala 0-3), with total score is 8.the cut off-point is 4, when 4 or less described as claudin-low and above 4 described as claudin-7 high. Claudin-7 expression was considered positive in membrane cells.[7]

Results and Discussion

In this study,43 cases TNBC patients with claudin-7 high expression and 7 cases (14%) with low expression(Table1).

Table 1.Sample distribution of Claudin-7 expression

Claudin-7 expression	**Jumlah (n)**	**Persentase (%)**
High	43	86
Low	7	14
Total	50	100

Mean age of our TNBC patients is 47,4 years old (range 39-56 years),with younger was 26 and the oldest was 63 years old. The majority about 29 cases (58%) were 35-49years old, followed by 19 cases (38%) were above 50 years old and 2 cases (4%) were under 35 years old. Meanwhile, mean age TNBC patients with claudin-7low expression were 43 ± 8,28 years old, younger than mean age TNBC patient with claudin-7 high expression. All of TNBC patient with claudin-low expression were premenopausal woman.

Most of the patient have stage III accounting 33 cases (66%), followed by stage II accounting 17 cases (34%). Most of Stage III group (8%) showed claudin-7 low expression, than stage II (4%). The most tumor size group was T3 (33 cases/66%), followed by T4 (16 cases/32%), and T2 (3 cases/6%). Most of claudin-7 high expression showed in T3 (52%), and most of claudin-7 low expression showed in T3(10%). Lymph node involment (N1-3) found 28 cases (56%) and the remains 22 kasus (44%) without lymph node involment. Cases with lymph node involment (50%) showed claudin-7 high expression compared with without lymph node involment group (36%). However, from 7 case claudin-7 low expression found 4 case without lymph node involment, followed by 3 cases with lymph node involment. Most cases with distant metastatic showed claudin-7 high expression (86%), followed by claudin-7 low expression (12%). Meanwhile, the only 1 case (2%) lung metastatic that showed claudin-7 low expression (Table 2).

Table 2. Claudin-7 expression with clinicopathological features of TNBC.

Variable	Claudin-7		Total (n)	Persentage (%)
	High	Low		
Age				
<35	1	1	2	27,5
35-49	24	5	29	43,38
50-64	18	1	19	55,68
≥65	0	0	0	0
Menstrual status				
Pra-menopause	30	7	37	74
Post-menopause	13	0	13	26
Clinical Stage				
Stage I	0	0	0	0
Stage II				
IIA	1	0	1	2
IIB	13	2	16	32
Stage III				
IIIA	14	1	15	30
IIIB	15	3	18	36
Stage IV	0	0	0	0
Tumor size				
T1	0	0	0	0
T2	3	0	3	6
T3	26	5	31	62
T4	14	2	16	32
Lymph node involment	18	4	22	44
negative (N0)				
positive (N1-3)	25	2	27	54
N1	0	1	1	2
N2	0	0	0	0
N3				
Ditant Metastasis				
M0	43	6	49	98
M1	0	1	1	2
Total	43	7	50	100

Microscopical evaluation of Haematoxilin and Eosine preparations showed most of them were invasive carcinoma NST, 37 cases (74%), while other 13 cases (26%) were special type invasive carcinoma, consist of 5cases (10%) carcinoma with medullary feature, 4 cases (8%) invasive lobular carcinoma, 2 cases(8%) metaplastic carcinoma, 1 case (2%) mucinous carcinoma, 1 case (2%) mixed invasive NSTandlobular carcinoma. Among of all, claudin-7 low expression were showed in 5 cases (10%) invasive carcinoma NST, 1 case (2%) invasive lobular carcinoma, and 1 case (2%) carcinoma with medullary feature. Claudin-7 high

expression were showed in 32 cases (64%) invasive carcinoma NST, 4 cases (8%) carcinoma with medullary feature, 3 cases (6%) invasive lobular carcinoma, 2 cases (4%)metaplastic carcinoma, 1 case (2%) mucinous carcinoma, and 1 case (2%) mixed invasive NST and lobular carcinoma.

Table 3. Claudin-7 expression according to histopathological type and grade of TNBC

Tipehistologi	**Claudin-7**				**Total (n)**	**Persentase (%)**
	High		**Low**			
	n	**%**	**n**	**%**		
Non-spesifik Invasive carcinoma NST Mixed invasive NST and lobular carcinoma	 32 1	 64 2	 5 0	 10 0	 37 1	 74 2
Spesifik					4	
Invasive lobular carcinoma	3	6	1	2	0	6
Tubular carcinoma	0	0	0	0	0	0
Cribiform carcinoma	0	0	0	0	1	0
Mucinous carcinoma	1	2	0	0	0	2
Carcinoma with signet ring cell	0	0	0	0	5	0
Carcinoma with medullary feature	4	8	1	2	0	8
Invasive micropapillary carcinoma	0	0	0	0	2	0
Metaplastic carcinoma of no special Type	2	4	0	0	0	4
Carcinoma with apocrine differentiation	0	0	0	0	0	0
Grade histologi Grade 1	 0	 0	 0	 0	 0	 0
Grade 2	27	54	5	10	32	64
Grade 3	16	32	2	4	18	36
Total	43	86	7	14	50	100

The majority of all case showed grade 2 was 32 cases (64%), followed by grade 3 was 18 cases (36%). Claudin-7 low expression were showed in 5 cases in grade 2, followed by 2 cases in grade 3. Claudin-7 high expression were showed in 27 cases in grade 2, followed by 16 cases in grade 3.

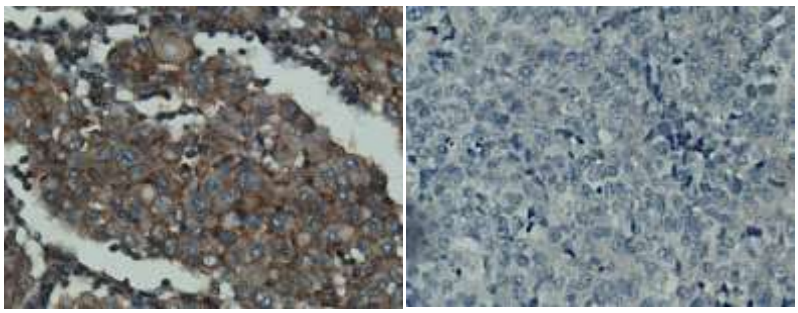

Figure 2.Immunohistochemical expression. (a) Claudin-7 high expression (b) Loss of membranous staining of Claudin-7

Claudin-low subtype breast cancer associated with worse survival rate. Dias et al., found only a few TNBC cases show claudin-low expression (21%). However most of claudin-low cases are TNBC (67-71%). Claudin-low associated early onset of age, higher grade, larger tumor size, abundant lymphocyte infiltrat, and higher metastastic rate.[4,9,10]Claudin-low patient have worse survival rate compared with luminal A. But almost

same with Luminal B, HER-2 enriched and basal-like.[9]Earlier studies can not ensure claudin-low subtype associated with worse prognose of TNBC.[11]

Our study found claudin-7 low expression breast cancer tend to younger age.the mean age TNBC patient with claudin-7 low expression was 43 years old (Tabel2), younger than mean age TNBC patient with claudin-7 high expression. Dias et al. foundthat claudin-low expression generrally in younger age, with mean age 58 years [9].In contrast, Ma et al. found that TNBC patient show claudin-7 low expression more above 50 years (79,1%).[11]

All of TNBC patient with claudin-7 low expression were pra-menopausalwoman (Table2). Cause of the mean age ofclaudin-7 low expression patient in this study were 43 years old, that is reproductive period groupwoman.

Claudin-low expression can't determine clinical stage, tumor size and histopathological type (Table2and3). It caused by the sample not evenly distributed and also so many subfamili claudin and histopathological type.

Most of claudin-7 low expression cases found without lymphnode involment (Table 2). In contrast to Constantinou et al. found that most TNBC patient with claudin-low expression was with lymph node involment, so associated with worse prognosis.[14]

Claudin-7 low expression ussually have higher metastatic rate.[10,11,12]The only one lung metastatic case (M1) in our study showed claudin-7 low expression (Tabel2).but, 1 case metastatic case can not represent all metastatic case.Szasz et al. found claudin-7 expression corelated with regional lymph node metastatic breast cancer.[15]Suported by Tabarieset al.found loss of claudinexpression in liver metastatic breast cancer.[16]

In our study, moderate histology grade showed claudin-7 low expression (Table 3).Constantinouet al.found higher expression of claudin-7 significant corelated with higher grade.However, previous study associated with worse outcome, according with Ma et al. found that TNBC patient with claudin-7 low expression tend to have higher histological grade.[11,14]

Conclusion

Claudin-7 low expression showed in early onset age, pramenopausal woman and metastatic case. But it is not in accordance with clinical stage, tumor size,and histopathological type. Most of TNBC patient were grade 2 with many of them showed claudin-7 high expression. For better assesment, we expected to make prospective study with longer interval time, numerous sample and evenly sample distribution. So we can see metastatic level and reccurency in TNBC patient based on claudin-7 expression.

References:

1. Kementrian Kesehatan RI Pusat Data dan Informasi 2015 Pusat Data dan Informasi Kementrian Kesehatan RI
2. American Cancer Society 2015 Breast cancer fact and figure 2015-2016 Atlanta: American Cancer Society, Inc
3. Kementrian Kesehatan RI2015 Hasil riskesda tahun 2013 Kementrian Kesehatan RI Hal 85-87.
4. Yadav R, Sen R and Chauhan P 2016 Role of cytokeratin biomarkers in breast carcinoma Asian *JPharm Clin Res*9(6) 293-6
5. World Cancer Research Fund International [Internet] Breast cancer statistic 2012Available from: https://www.wcrf.org/int/cancer-facts-figures/data-specific-cancers/breast-cancer-statistics
6. Gunzel D, Alan S and Yu L Claudins and the modulation of tight junction permeability. Physiol Rev. 2013 Apr; 93(2) 525–569.
7. Claudin-7 Antibody [product data sheet] 2011 Chesire, UK: Thermo Fisher Scientific
8. Staining Protocol [product data sheet] 2015 Pleasanton, CA: Diagnostic Biosystem
9. Dias K, Gheva AD, Hallet RM, Wu Y, Hassell J, Pond GR, Levine M, Whelan T, and Bane AL2017 Claudin-low breast cancer; clinical & pathological characteristics*Plos one* 1-17.
10. Thike AA, Cheok PY, Jara-Lazaro AR, Tan B, Tan P and Tan PH 2010 Triple negative breast cancer:

clinicopathological characteristics and relationship with basal-like breast cancer *Mod Pathol*23(1) 123-33

11. Ma F, Ding X, Fan Y, Ying J, Zheng S, Lu N, Xu B 2014 A CLDN1-Negative Phenotype Predicts Poor Prognosis in Triple-Negative Breast Cancer *Plos One*9(11): e112765
12. Prat A, Parker JS,Karginova O, Fan C,Livasy C,Herschkowitz JI, he X, and Perou CM 2010 Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer*Breast Cancer Research*12:R68
13. Lu S, Singh K, Mangray S, Tavares R, Noble L, Resnick MB, and Yakirevich 2013 Claudin expression in high-grade invasive ductal carcinoma of the breast: correlation with the molecular subtype *Modern Pathology*26 485–495
14. Constantinou C, Papadopoulos S, Karyda E, Alexopoulos A, Agnanti N, Batistatou A, and Harisis H 2018 Expression and Clinical Significance of Claudin-7, PDL-1, PTEN, c-kit, c-Met, c-Myc, ALK, CK5/6, CK17, p53, EGFR, Ki67, p63 in Tiple negative breast cancer- A single centre prospective obsevational Study*In vivo*32 303-311
15. Szasz AM, Tokes AM, Micsinai M, Krenacs T, Jakab C, Lukacs L, Nemeth Z, Baranyai Z, Dede K, Madaras, et al 2011 Prognostic significance of claudin expression changes in breast cancer with regional lymph node metastasis *Clin Exp Metastasis*28 55–63
16. Bernardi MA, Logullo AF, Pasini FS, Nonogaki S, Blumke C, Soares FA and Brentani MM 2012 Prognostic significance of cd24 and claudin-7 immunoexpression in ductal invasive breast cancer *Oncol Rep*27(1) 28-38.

*****