Research Article

Expression of Fas Ligand is Higher in Early Stage Cervical Cancer with Lymph Nodes Metastasis

Ekspresi Fas Ligand Lebih Kuat pada Kanker Serviks Stadium Awal dengan Metastasis Kelenjar Getah Bening

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Abstract

Objective: To know whether the expression of Fas Ligand has correlation with incidene of metastasis of pelvic lymph node and lymph-vascular stromal invasion (LVSI).

Methods: All patients diagnosed of cervical cancer stage IB or IIA who underwent radical hysterectomy in Dr. Cipto Mangunkusumo Central General Hospital from January 2008 until December 2009 were included in analytic cross sectional study. We tested expression of Fas Ligand in cervical cancer specimen by immunohystochemistry with monoclonal antibody. The expression of Fas Ligand was compared between the group of patients with a positive and negative pelvic lymph node and between LVSI positive and negative. The difference of expression in both group were statistically analized with Chi-square test and the correlation Spearman test.

Result: Ninety one patients underwent radical hysterectomy for two years and 43 patients were included in these study. The expression of Fas Ligand in 7 patients (16.3%) were negative and the others were positive, with weak, moderate and strong expression were 4 (9.3%), 27 (62.8%), and 5 (11.6%) respectively. The expression of Fas Ligand was significantly higher in the group of patients with positive pelvic lymph node compared to the group of the patients with negative pelvic lymph node (p=0.007) but there was no significant difference between group of the patients with LVSI positive and negative. With Spearman test, we found that the expression of Fas Ligand had a strong correlation with pelvic lymph nodes metastasis (coeff. correlation=0.519 and p=0.00) and have no correlation with LVSI (coef. corellation=0.112 and p=0.474).

Conclusion: These finding suggested that expression of Fas Ligand in cervical cancer patients has a strong correlation with the incidence of pelvic lymph node metastasis and none with LVSI.

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Keywords: cervical cancer, fas ligand, immunohystochemistry, LVSI, pelvic lymph node metastasis

Abstrak

***Tujuan**: Ekspresi Fas Ligand memungkinkan sel-sel karsinoma menginduksi apoptosis sel-sel imun sehingga dapat menghindarinya dan melanjutkan pertumbuhan dan bermetastasis. Dengan demikian, ekspresi Fas Ligand memiliki korelasi dengan insiden metastase kelenjar limfe pelvis dan limfovascular stromal invasion (LVSI).*

***Metode**: Semua pasien yang terdiagnosa kanker servik stadium IB atau IIA, yang menjalani histerektomi radikal di Rumah Sakit Umum Pusat Dr. Cipto Mangunkusumo dari bulan Januari 2008 sampai Desember 2009 diikutsertakan dalam penelitian analitis cross sectional. Kami menguji ekspresi Fas Ligand pada spesimen kanker serviks secara imunohistokimia dengan antibodi monoklonal. Ekspresi Fas Ligand kemudian diperbandingkan antara pada kelompok pasien dengan limfonodi pelvis positive dan negatif serta antara LVSI positif dan negatif. Perbedaan ekspresi kedua kelompok dianalisa secara statistik dengan Chi-square test dan analisa korelasi dengan Spearman test.*

***Hasil**: Sembilan puluh satu pasien yang telah menjalani radikal histerektomi selama dua tahun dan 43 pasien diikutsertakan dalam penelitian ini. Ekspresi Fas Ligand ditemukan negatif pada 7 pasien (16,3%) dan positif lemah, moderat dan kuat pada 4 (9,3%), 27 (62,8%), dan 5 pasien (11,6%). Ekspresi Fas Ligand lebih tinggi secara bermakna pada kelompok pasien dengan kelenjar limfe positif, namun tidak ditemukan perbedaan pada kelompok pasien dengan LVSI positif dan negatif, dengan Spearman test, ekspresi Fas Ligand memiliki korelasi kuat dengan metastasis limfonodi pelvis (koefisien korelasi=0,519 dan p=0,00) dan tidak memiliki korelasi dengan LVSI (koefisien korelasi=0,112 dan p=0,474).*

***Kesimpulan**: Penemuan ini menunjukkan bahwa ekspresi Fas Ligand pada kanker serviks memiliki korelasi kuat dengan metastase kelenjar limfe pelvis dan tidak memiliki korelasi dengan LVSI.*

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***Kata kunci**: fas ligand, imunohistokimia kanker servik, LVSI, metastase limfonodi pelvis*

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INTRODUCTION

Cervical cancer is still a major health problem in the developing countries like Indonesia. It was reported that there is more than 2500 new cases every year and the mortality ratio was 55%. Despite the success of cytologic techniques in diag-

nosing precursor lesions and the early diagnosis of invasive disease, the coverage of screening program remains low.[1,2]

Among the prognostic factors, pelvic lymph node metastasis and lymphovascular invasion (LVSI) are considered the important factors that influence on management after radical hysterectomy and affecting overall survival, recurrence rate and disease free survival in early stage cervical cancer after surgical treatment.[3]

Fas Ligand, one of the tumor necrosis factor family proteins, is a type II membrane protein. It is known that Fas L expressed on activated T lymphocytes or natural killer cells, which triggers apoptosis on the cells that express Fas (CD95), one of the tumor necrosis factor receptor families. FasL-expressing tumors have been reported to have a significantly worse prognosis. It is hypothesized that these tumor cells can escape from immune surveillance via a counter-attack on activated T cells, natural killer cells and TILs (Tumor Infiltrating Leucocytes) that express Fas and cause cancer cell able to continuing the growth and have ability to invasion and metastasis.[4,5]

Many investigation both in vitro and in vivo have reported that Fas Ligand could induced apoptosis in lymphocyte cell at the microenvironment of tumor cells and some studies found any relations between increasing expression of Fas Ligand and cancer metastasis and progression of tumor cells.[5-7]

There have also been reported at many type of cancer like breast, melanoma and colorectal cancer, expression of Fas Ligand (CD95L) have significant correlation with lymph-node metastasis and lymph-vascular stromal invasion.[8,9]

However, no research is available on the clinical significance Fas Ligand in local lymph nodes and Lymph-vascular stromal invasion (LVSI). To explore the relation between cervical cancer and regional lymph node metastasis and LVSI, FasL protein expressions were detected using immunohistochemistry method in this study.

METHODS

Specimens were obtained from 43 patients with early stage cervical cancer (Stage IB_1,IB_2 and IIA) who was admitted to the Department of Pathology Dr. Cipto Mangunkusumo Central General Hospital Jakarta, from January 2008 to December 2009. The specimens were collected from patients that underwent radical hysterectomy surgery. The specimens were reviewed hystopatologically to determine the type of cancer and evaluation was done to detect lymp-node metastasis and Lymp-vascular stromal invasion (LVSI).

Immunohistochemistry

Specimens, fixed with 10% neutral formaldehyde solution and embedded in paraffin, were cut into 4 μm thick sections. Briefly, endogenous peroxidase was blocked by 3% hydrogen peroxide following deparaffinization. Novocastra Lyophililized Mouse Monoclonal Antibody (production code NCL-FAS-L) produce by Leica Biosystem Newcastle was used as primary antibodies with 1:40 dilution and incubated for 6 hours. Positive control was taken from prostate specimens following its manufacturer's instructions.

Evaluation of score

Intensity of staining was scored as 0: negative, 1: light yellow, 2: brown-yellow and 3: brown. Eight random highpower fields were observed under optical microscope and 4 high-power fields were recorded as the percentage of positive cells. Extent of staining was scored as 0: < 5%, 1: 5%-25%, 2: 26%-50%, 3: 51%-75% and 4: > 75%. The final score was determined by multiplying the scores of intensity and extent of staining, ranging 0-12. Scores 9-12 were defined as strong staining (+++), scores 4-8 as moderate staining (++), scores 1-3 as weak staining (+), and 0 as negative staining (–).

Statistical analysis

Statistical analysis was performed using SPSS 15.0. The data were analyzed using Spearman correlation test and Chi-square test. $P < 0.05$ was considered statistically significant.

RESULT

Subject characteristic

In the recent study the average of age of the sample was 48.35 year old, with the range of 33-63 year old. The most frequent cases were at the group of 35-50 year old (55.8%), followed by the group of over 50 year old (39.5%) and less frequent in the

group of under 35 year old (4.7%). The mean of parity was 4.09 with the range between 1-10 children. Most patients admitted at Dr. Cipto Mangunkusumo Hospital were from low social economy group. Almost all patients had never had the screening program (Pap Smear) (95.3%). The first complain happened more or less 3.91 month before admission to the hospital. More than 70% patient first married before 20 years old. (Table 1)

Tabel 1. Demographic profile

Demographic profile	n=43				
	n	%	Range (min-max)	mean	SD (std deviaton)
age					
<35	2	4.7			
35-50	4	55.8	3 – 63	48.35	7.486
>50	17	39.5			
Parity					
0	0	0			
1-3	18	41.8	1 – 10	4.09	1.986
4-5	15	34.9			
≥ 6	10	23.3			
Education					
6 years	35	81.4			
9 years	6	13.9			
12 years	2	4.7			
Pap smear history					
Ever	2	4.7			
Never	41	95.3			
Length of complain					
1–3 months	21	48.8			
> 3–6 months	18	42.9	1 – 8	3.91	1.887
> 6 months	4	8.3			
Age of marriage					
≤ 20	31	72			
21-30	11	25.6	14 – 33	19.23	3.804
> 30	1	2.4			

Clinicopathological characteristic

Clinicopathological feature showed at Table 2. The histologic type of cancer found in this study were squamous 24 cases (55.8%), adenocarcinoma and adenosquamosa are 12 cases (27.9%) dan 7 cases (16.3%) respectively. 25 cases (58.1%) are well differentiated, poorly differentiation are 12 cases (27.9%) and 6 cases are mild differentiation (14%).

There were 23 cases of stage 1B (53.5%), followed by 17 cases (39.5%) of stage IIA and 3 cases of stage IB2 (7%). The average amount of pelvic lymph node was 17.02 with range of 4 to 38. The most common found was 11-19 lymph nodes in 18 cases (41.9%), more than 20 lymph nodes in 16 cases (37.2%) and less than 10 lymph nodes in 9 cases (20.9%).

The size of tumor more than 4 cm only found at 6 cases (14%) and less than 4 cm were 37 cases (86%). Long metastasis was found at follow up in 3 cases (6.97%).

The cutting margin of vagina and parametrium that not free from tumor was found in 5 cases (11.6%) dan 9 cases (20.9%) respectively while the rest of cases have free margin of vagina in 38 cases (88.4%) and parametrium 34 cases (79.1%).

Positive pelvic lymphnode metastasis was found in 18 cases (41.9%) and 25 cases were negative (58.1%). 27(62.8%) cases have positive LVSI and 16 cases (37.2%) have no LVSI.

Table 2. Clinicopathological Features

Clinicopathological Characteristic	n = 43	
	n	%
Stage		
IB_1	23	53.5
IB_2	3	7
IIA	17	39.5
Cell type		
Squamous	24	55.8
Adenocarcinoma	12	27.9
Adenosquamous	7	16.3
Differensiation		
well	6	14
moderate	25	58.1
poor	12	27.9
Amount of lymphnodes		
≤ 10	9	20.9
11–19	18	41.9
≥ 20	16	37.2
mean	17.02	
range	4-38	
Size		
≤ 4	37	86
More than 4 cm	6	14
Distance metastasis		
positive	3	6.97
negative	40	93.03
Vaginal margin		
Negative	38	88.4
Posititive	5	11.6
Parametrium		
negative	34	79.1
positive	9	20.9
Lymphnodes metastasis		
Positive	18	41.9
Negative	322	51.2

Fas Ligand Expression

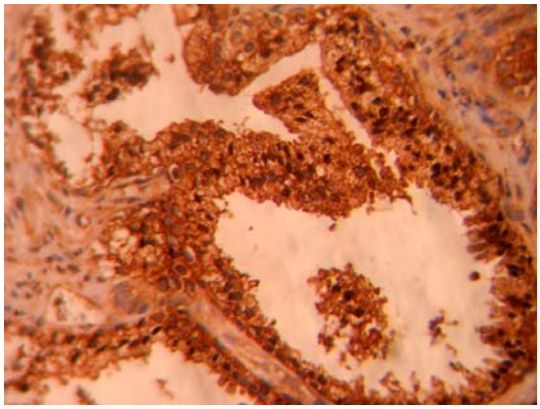
FasL expression was observed in cervical cancer cells with membranous and cytoplasmic staining. This study showed the expression of Fas Ligand regarded as positive on 36 cases which 4 cases (9.3%) weak expression, 27 cases (62.8%) moderately expression and 5 cases (11.6%) have strong expression. Negative Fas Ligand expression just found at 7 cases (16.3%).

Bivariate analysis

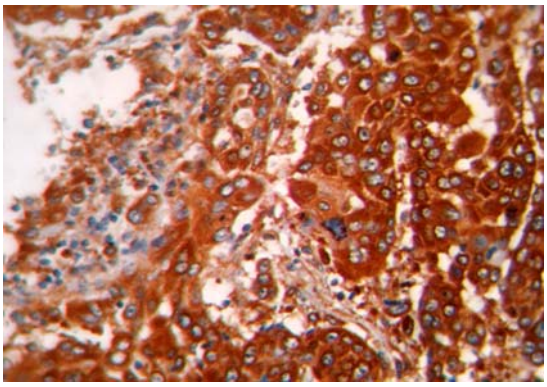
This study showed that among the clinicopathological factors, lymphnode metastasis have significantly different of Fas Ligand expression between negative lymphnodes matastasis cases and positive lymphnode metastasis cases (p=0.007). The other factors, LVSI, differensiation, and cell type were not showed not significant different of Fas Ligand expression with p =0.108 (LVSI), grade p=0.945, and cell type p=0.669. (Table 3)

Table 3. Chi-Square test between Fas Ligand expression and clinicopathologic factors

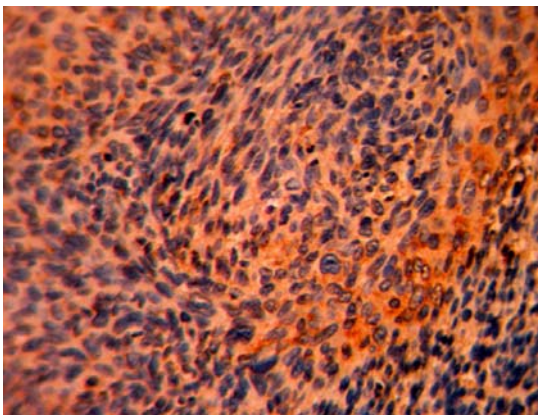
	Fas Ligand Expression (n=43)				p=0.05
	negative	weak	moderate	strong	
Lymph nodes					0.007 (S)
Negative	7	4	13	1	
Positive	0	0	14	4	
LVSI					0.108 (NS)
Negative	5	0	9	2	
Positive	2	4	18	3	
Different					0.945 (NS)
mild	1	1	4	0	
moderate	4	2	5	4	
poor	2	1	8	1	
Cell type					0.669 (NS)
Squamous	4	3	15	2	
Adeno	3	1	6	2	
Adeno skuamous	0	0	6	1	
Stage					0.817 (NS)
IB_1	5	2	14	2	
IB_2	0	0	2	1	
IIA	2	2	11	2	
Parametrium					0.701 (NS)
Free	6	3	22	3	
Not free	1	1	5	2	
Vagina					0.670 (NS)
Free	6	3	25	4	
Not free	1	1	2	1	



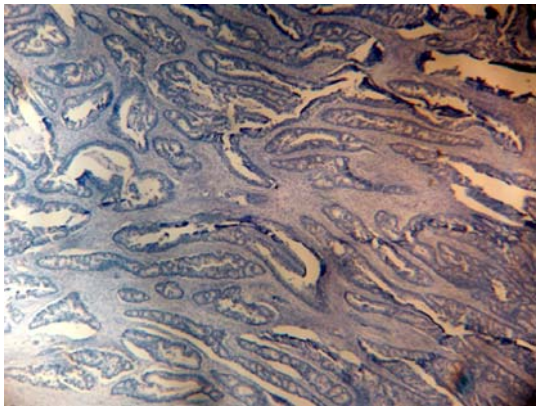
A. Positive control showed expression Fas Ligand expression on cytoplasma was taken from prostat tissue



B. Strong expression of Fas Ligand in cervical cancer cell



C. Negative/weak expression of Fas Ligand at cervical cancer tissue



D. Negative control taken from normal cervical tissue

DISCUSSION

There was 91 patients with early stage cervical cancer (Stage IB_1, IB_2 and IIA) who underwent radical hysterectomy at Dr. Cipto Mangunkusumo Hospital, Jakarta for two years periode (2008-2009), or 12 patients every month. Among these cases just 43 cases met the criteria of this study.

Most patients came from low social economy status, which was shown by the fact that more than 80% was not graduated from elementary school. It can be understood because this hospital was aimed mainly for low social economy class patients, since the cost of treatment was paid by government health assurance program. Almost all patients have no history of screening test like Pap smear or VIA test, and only 4.7% patients stated had ever done screening test. This is appropriate with the fact that coverage of the screening program is still low (below 5%) especially at the group of low social economy class.[1]

In this study the mean age of the patients 48.35 with the youngest patient was 33 years old and the oldest patient was 63 years old. The most frequent cases are at the group 35-50 years old. This finding is appropriate with former study by Azis et al, stated that cervical cancer occurs more frequent in the group 35-50 years old.

More than 70% patient had married when they were less than 20 years old year old (probably the first year intercourse). This finding is not surprising because the patients came from middle low social economy and have low education. In this group the marriage tend to happen at the very young age. The mean parity of patiens in this study was 4.09 children this fact supported the assumption that multiparity is one of risk factor in cervical cancer.

Cancer cells expressing high level of FasL are able to attack tumor-infiltrating immune cells expressing high Fas levels by promoting apoptosis of immune cells, thus allowing cancer cells to escape immune attack. In our study, the Fas expression level was significantly higher in positive lymph nodes patient than in negative lymph nodes, suggesting that cancer cells with a high Fas Ligand expression level may be more susceptible to self-apoptosis, thereby promoting lymph node metastasis.

Many malignant tumors have been reported to express FasL. Hahne et al reported that melanoma cells expressing FasL grew more rapidly in wild-type mice than in Fasmutant lpr mice. From their experiment, it was expected that FasL expression in tumor cells would affect tumor progression and the prognosis of patients. In fact, FasL expression had a significant association with lymph node metastasis in breast cancer, gastric cancer, and colorectal cancer.

Our study showed Fas Ligand expression regarded as moderate and strong were found in 32 patients (74.4%) and negative or weak expression were found in 11 patients (25.6%). Munakata et al found similar result, in which 74.4% cervical carcinoma express Fas Ligand.[9] Ibrahim et al studied Fas Ligand expression in squamous cervical carcinoma and intraepithelial neoplasia stated 64% cases had positive Fas Ligand expression, while Kase et al observed Fas Ligand expression in cervical adenocarcinoma found 62.5% primary tumour had positive Fas Ligand expression.[3,5,6] It seems that expression of Fas Ligand in this study was consistent with other studies.

This recent study found expression Fas Ligand was significantly higher in patients with positive lymph nodes metastasis compared to patients with negative lymph nodes ($p=0.007$) but not significantly different in LVSI positive or negative ($p=0.108$). Kase et al found Fas Ligand expression was significantly higher in both positive lymph nodes metastasis and positive LVSI.[3] These different finding was probably caused by different study sample that did not include the patients who received neoadjuvant chemotherapy before surgery.

For the other clinicopathological factors (stage, cell type, grade, parametrium and vaginal involvement), expression of Fas Ligand was not significantly different. Kase et al stated that all lymph nodes metastasis had strong expression of Fas Ligand, thus indicated that Fas Ligand have important role for evasion of tumor from immune surveillance because only cell expressed Fas Ligand can successfully achieve the lymph nodes.[3] Munakata et al found that high expression of Fas Ligand in cervical cancer cell ascociated with poor prognosis, while Ibrahim et al stated only cancer cell that have Fas Ligand expression can make apoptosis of jurkat and T cell.[8] These finding support the theory of Fas Ligand's important role via counter-attack mechanism on progressivity and behavior of cancer cell.

Metaanalysis study stated that limph nodes metastasis was a constant prognostic factor in 22 from

25 article (88%), while LVSI has significant prognosis factors just in 12% article, this fact supports the assumption that Fas Ligand expression has significant ascociation with prognosis of cervical cancer patients as had been investigated by Munakata et al.[8]

CONCLUSION

Expression of Fas Ligand was significantly higher in the group of patients with positive lymph nodes metastasis cervical cancer compare with negative patients and not significantly different between patients with positive LVSI and negative LVSI. Increasing of Fas Ligand have significant correlation with lymph nodes metastasis and have not significant association with LVSI.

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