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CYCLIN D1 EXPRESSION RELATIONSHIP WITH CLINICAL STAGE, GRADING GLAND METASTASIS OF TUMOR CELLS AND SQUAMOUS CELL IN CERVICAL CARCINOMA

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ABSTRACT

To investigate the correlation between Cyclin D1 expression and the clinical staging, tumor cell grading and the lymph node metastasis of non kereatinizing squamous cell cervical cancer. The research design s observational study with cross sectional approach in Gynecologi Oncology Clinic and Pathology Anatomy Department of Dr Soetomo General Hospital, Surabaya. The samples are tissues (cervix and lymph node) covered with paraffin block collected from radical hysterectomies in Dr. Soetomo General Hospital, Surabaya Janurai 1st, 2003 to November 31st, 2006. 10 samples were collected consecutively for each clinical staging (IA, IB, IIA), thus 30 samples totally. All samples were processed by immunohistochemestry using monoklonal mouse anti-human cyclin D1, Glostrup Denmark and cyclin D1 expression were evaluated blindly, regardless of clinical staging, tumor cell grading and lymph node metastasis. Cyclin D1 expression were counted semiquantitavely with negative and positive controls. The correlation of cyclin D1, linical staging and tumor cell grading was analysed by Spearman's Rho. The correlation fo cyclin D1 expression and lymph node metastasis was analysed by logistic regreesion. There were significans difference in cyclin D1 expression with grading cell tumor (p : 0,000), clinical staging (p : 0,05) and lymph node metastasis (p : 0.03). In non keratinizing squamous cell cervical carcinoma, the higher cyclin D1 expression, the higher the tumor cell grading (r : 0,939) , the higher the clinical staging (r : 0.005). The higher cyclin D1 expression was assosiated with positive lymph node metastasis (r : 0,754).

Keywords: *Cyclin D1 expression, clinical staging, tumor cell grading, lymph node metastasis, non keratinizing squamous cell cervical carcinoma.*

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INTRODUCTION

According to the Federation of International Gynecology and Obstetrics (FIGO) in 2000, showed decrease in the number of cervical carcinoma and increase survival rates five years, but in developing countries and developing countries is still high prevalence of cervical carcinoma, while the number of living remains low resilience (Farid Aziz 2006, FIGO 2006).

There are several factors associated with prognosis of the disease stage cervical carcinoma, large tumor, grading of tumor cells, the type of histopathology, lymph nodes distribution and age, while the use of immunohistochemistry variable which describes the molecular changes in cervical cancer to know the prognosis is still rarely used (Berek JS et al. 2000, Sighn & Arif 2004).

In the last decade the main cause of cervical carcinoma is focused on infection of Human Papilloma Virus (HPV). The role of high-risk HPV in cervical carcinogenesis has become clear with the study of the epidemiology and natural history of cervical cancer gene that is a otential oncogenic high-risk HPV types such as E6 and E7. Laboratory evidence of oncogenic potential is based on the understanding of biomolecular cell cycle (Mark 1996, Arends et al. 1998, Sjamsuddin 2000, Sherr 2000, Sandal T 2002).

Definitive diagnosis of cervical carcinoma ditegakan with histopathologic examination. These checks have shown a weakness because there are processes at the cellular level while the ideal if the malignancy had been in the know at the molecular level. Because the occurrence of carcinoma cell proliferation result in uncontrolled cell cycle, then logically the ideal examination conducted is aimed at cell cycle regulatory proteins. Cyclin D1 as one of the cell cycle regulator is ideal turns out to represent this cell cycle regulatory proteins (Sherr 2000, Mendelsohn et al. 2001, Sandal 2002). High-risk HPV infection (HPV 16, 18) who settled on cervical epithelium and in the long term will

cause the body's defense reaction in the form of expression of p53, PRB, Cdk inhibitors with the intent to temporarily halt the cell cycle machinery. High risk HPV gene products issued by both E6 and E7 that will inhibit tumor suppressor genes and Cdk inhibitors. Cyclin D1 as a regulator unit acting on the G1-S phase cell cycle will be disrupted its function, because the catalytic pair CDK4/CDK inhibited by Cdk inhibitors work INK family of four, resulting in increased expression of cyclin D1. Cyclin D1 expression increased this in turn will inhibit tumor suprresor gene DRR work. This condition causes the failure of G1 arrest with the result that cells will continue to proliferate despite DNA lesions was found. (Sherr 2000, Mendelsohn et al. 2001, Sandal 2002, Berek et al. 2000, Chakrabarti et al. 2003, Cotran et al. 2005).

Research also focuses on the study of cyclin D1 with the following considerations (Cotran et al. 1999, Stoler 1996, Weinberg 1996, Chakrabarti et al. 2003) cyclin D1 is a key regulator of the cell cycle progression machinery, cyclin D1 is the first koezim which acts on the G1-S phase cell cycle and maintained constant levels from early G1 phase to late S phase, cyclin D1 is the only cyclin that is directly regulated by extracellular signals, cyclin D1 is an upstream regulator retinoblastoma protein that serves as a goalkeeper for the G1 to S phase, expression of cyclin D1 excessive known to correlate closely with an early onset of malignancy and the risk of tumor progression and metastasis of a malignancy, HPV oncoprotein catching point in the cell cycle based on the epidemiology and natural history of cervical cancer has been unclear research on the expression of cyclin D1 as a prognostic factor of cervical carcinoma is still small and the results are still controversial.

The purpose of this study was to determine the increase in cyclin D1 ekspersi of clinical stage, tumor grading and metastatic lymph cells in cervical squamous cell carcinoma by radical hysterectomy at Dr Soetomo Hospital. .Assessment of expression of cyclin D1 was done semiquantitatively, and analyzes's rho Correlation to see the correlation between cyclin D1 expression with clinical stage, tumor cell grading and logistic regression to examine the relationship between the expression of cyclin D1 with lymph node metastases.

MATERIALS AND METHODS

Research carried out by the analytic observational study design in the form of latitude pontong observed (cross sectional). Cervical carcinoma patients who had radical hysterectomy operations performed clinical history, and traced the paraffin tissue blocks of the operating result is then performed immunohistochemical staining to determine expression of cyclin D1. The degree of cyclin D1 expression then compared between stage, grading and the presence or absence of tumor cell metastasis tisue.

Research held during January 1, 2003 until 31 November 2006 found 114 cases of radical hysterectomy performed for indications of stage IA, IB and IIA. From this amount was included in the category of cervical squamous cells and as many as 83 cases were classified as many as 77 cases of non keratin. Of the 77 cases found as many as 51 blocks of paraffin block and a proper examination of 47 blocks.

Distribution of 47 blocks as follows: Stage IA were: 11 samples, stage IB: 14 samples and IIA: 22 samples. Then, from the total sample was taken 30 blocks (based on calculation of total sample) and divided equally in each category. Staining performed on 30 samples, in which each paint sample done twice, ie on the tissue containing the tumor and lymph nodes in patients.

Because the purpose of this study is to see the expression of cyclin D1 based on clinical staging, grading and the presence or absence of tumor cell metastasis, the way of sampling based on unfounded withdrawal opportunity (non-probability sampling), ie, consecutive sampling means when the number of samples for each category will be considered sufficient The sampling has been fulfilled.

Equitable division of the number of samples into each category is selected in accordance with the least number of investigators for each of the 10 samples katogori. Distribution of sample mean in order to get the same proportion as well as statistical calculations will be clearly visible differences in the expression cyclin D1.

RESULTS

Cyclin D1 expression differences of clinical staging, grading and the presence or absence of tumor cell metastasis glands as follows:

In table 1 shown, that the amount of cyclin D1 expression with stage IA low grade of 60%, the degree of being with a 50% stage IB and stage IIA with a high degree of 57.1%. Statistical analysis showed that the degree of expression of cyclin D1 was significantly different for each stage of the clinic. (P: 0005). There is a significant correlation between the degree of cyclin D1 expression with clinical stage operative with strong relationships (r: 0.5), namely the higher the degree of expression of cyclin D1 higher operative clinical stage.

Table 1. Expression of Cyclin D1-Degree Distribution Based on the Clinical Stage.

Stage	Expression of Cyclin D1			Total	Statistic
	Low	Average	High		
IA	6 (60 %)	1 (17 %)	3 (21.4 %)	10 (33.3 %)	Spearman rho
IB	4 (40 %)	3 (50 %)	3 (21.4 %)	10 (33.3 %)	Correlation Test:
IIA		2 (33 %)	8 (57.1 %)	10 (33.3 %)	(r : 0.500; p :
Total	10 (100 %)	6 (100 %)	14 (100 %)	30 (100%)	0.005)

Table 2. Expression of Cyclin D1-Degree Distribution Based on the Grading of Tumor Cells.

Grade	Expression of Cyclin *D1*			Total	Statistic
	Low	Average	High		
Grade 1	8 (80%)			8 (26.7%)	Spearman rho
Grade 2	2 (20 %)	6 (100%)	1 (7.1%)	9 (30 %)	Correlation Test
Grade 3			13(92.9%)	13 (43.3 %)	(r : 0.939; p :
Total	10 (100%)	6 (100%)	14 (100%)	30 (100%)	0.000)

In table 2 shows that the amount of cyclin D1 expression with grading low grade tumor cells of 80% first degree, second degree is a degree of 100% and higher degrees by 3 degrees at 92.9%. Statistical analysis showed that the degree of expression of cyclin D1 was significantly different for each tumor cell grading. (P: 0000). There is a significant correlation between the degree of expression of cyclin D1 to the grading of tumor cells with strong relationships (r: 0.939), ie the higher the degree of expression of cyclin D1 higher the grading the degree of tumor cells.

Table 3. Distribution of Cyclin D1 Expression Based on the Degree of Lymph Node Metastases.

Metastasis	Expression of Cyclin D1			Total	Statistik
	Low	Average	High		
Positif	1 (9,1%)	6 (75 %)	11 (100 %)	18 (60%)	Logistic
Negatif	10 (90,9%)	2 (25 %)		12 (40 %)	Regression :
Total	11 (100 %)	8 (100 %)	11 (100 %)	30 (100 %)	(r : 0.754 ; p : 0.03)

In Table 3 shown, that the amount of cyclin D1 expression with low degrees without metastases at 90, 9%, the degree of being with positive metastases and high grade of 75% with positive metastases at 100%. Statistical analysis showed that the degree of expression of cyclin D1 was significantly different with the absence of metastasis (p: 0.03). There is a significant correlation between the degree of expression of cyclin D1 lymph node metastases with a strong correlation (r: 0.754), namely cyclin D1 expression was higher in cervical cancer with metastasis than without metastasis of lymph glands.

DISCUSSION

Cyclin D1 is not the direct cause of cervical cancer, but due to continuous exposure of HPV will cause the failure of G1 arrest with cell proliferation due to take place even obtained DNA lesions. This condition will cause mutations in the genes of somatic cells is the cyclin D1 satuhnya. Several types of cyclin D1 mutations that might occur on the first cervical cancer is, amplification (multiplication) on chromosome 11 Q13 band (11q13) that resulted in excessive expression. Causing excessive expression of other oncogenes through the cell cycle, and the second is a somatic mutation in the form of inversion (reversal) that involves tape Q13 and Q15 on chromosome 11: inv (11) (Q15: Q13). How cyclin D1 expression correlation and prognosis of cervical cancer? Research conducted by Cheung TH by Kaplan-Mayer method to get that poor prognosis of cervical cancer associated with tumor grade, stage, and exaggerated expression of cyclin D1. While using Cox regression analysis: tumor grading, staging and excessive expression of cyclin D1 is an independent prognostic factor for cervical cancer (Cheung Tet al. 2001).

In this study, a significant difference in cyclin D1 expression with clinical stage of cervical cancer. Some authors offer rationale to explain this situation is (Stoler

1996, Weinberg R 1996, Berek et al. 2000, Chakrabarti et al. 2003, Cotran RS et al. 2005). Expression of cyclin D1 would lead to excessive hypofosforilasi PRB. This situation will further hypofosforilasi working menghabat a tumor suppressor gene PRB to E2F is not quit. Expression of cyclin D1 over-worked with high-risk HPV E7 inhibits PRB work. Expression of cyclin D1 induces excessive number of other oncogenes that play a role in carcinogenesis. Cyclin D1 over-expression causes abnormal cells able to "escape" check-point mechanism.

Employment restriction of PRB, induction of a number of other oncogenes as well as the ability to escape causing abnormal cells to uncontrolled cell proliferation despite a consequence of DNA lesions showed progression of cervical cancer cells will be a marked increase with increasing clinical stage. Significant correlation between cyclin D1 expression with clinical stage can then be used as a prognostic factor for cervical cancer (Arends MJ et al. 1998, Heilmann V et al. 2002, Chakrabarti et al. 2003).

Grading is defined as an estimate of tumor cell aggressiveness and malignancy of a tumor that is based on how much the tumor cells look under mikrsokop. In this study, based on mitotic index of cells. While the mitotic index is defined as a measure of proliferation status of the cell population that is the ratio between the number of cells in mitosis and the total number of cells. Increased mitotic index associated with the possibility of Distant metastases and low survival rates. Patients with lower tumor grading generally have a good prognosis compared with patients with high tumor grading. (Berek et al. 2000, Cotran et al. 2005).

In this study, the results, namely the degree of expression of cyclin D1 was significantly different for each tumor cell grading. Rational explanation to explain this circumstance is the activity of cervical ancer caused by uncontrolled cell division and the rate of malignant carcinoma correlated linearly with cell division activity so that more and more cells that divide the more proliferatiflah populations of these cells. Researchers argue that the activity of cell division is represented by the cyclin D1 as proliferative rate index is represented by mitosis. Thus it is understandable that there is a correlation between cyclin D1 expression with grading tumor cells, which in turn both these parameters can be used as a prognostic factor for cervical cancer. If you want to compare between the two variables which are most affected by cyclin D1 is seen the value of r (strong relationship) are high. It appears that the grading of tumor cells (r: 0.939) were affected more cyclin D1 than the clinical stage (r: 0.500). Why did this happen? We argue that the clinical stage of cervical cancer described changes in the levels of tumor tissue while the grading of cervical cancer represents a sea change at the cellular level. Significant correlation between cyclin D1 expression with prognostic factors of cervical cancer and grading the clinical stage of tumor cells supports the findings of scholars Cheung tumor grading, staging and excessive expression of cyclin D1 is an independent prognostic factor for cervical cancer. (Cheung et al. 2001).

Why in the table two-stage IA found three samples with high expression of cyclin D1? To explain this we refer to the model of cervical HPV infection. Integration of HPV in the host that occurs in the E1-E2 provides two implications of which inhibit the expression of E2 as well as causing local deletion. This will cause the local deletion E6 and E7 directly joined with enhancers or promoters. How heavy is what determines the focal deletion of E6 and E7 expression. This in turn will determine the severity of the barriers to tumor suppressor genes and Cdk. Researchers argued that high expression of these three samples because the expression of E6 and E7 are so high that strongly inhibit the work of p53, p21 and Cdk 4 / 6 consequently cyclin D1 expression was high (Weinberg 1996, Stoler 1996, Sherr 2000, Berek et al. 2000, Sandal 2002, Cotran et al. 2005).

Whether cyclin D1 can be used as the gold standard marker to determine prognosis of cervical cancer? The author can not dare say. This is due to the design and methodology of this study is cross sectional. But what's interesting is the analysis conducted graduate Cheung and Bae (Cheung et al. 2001, Bae et al. 2001). He discovered from 21 patients with positive expression of cyclin D1, 14 patients died (66.7%) while overall survival rate for cyclin D1 positive by 33%, whereas according to the degree Bae cyclin D1 in carcinoma is associated with cervical disease free survival and overall lower survival (P: 0.0175 and 0.0189). Of the 80 patients with grading I and II found in 24 patients who died (30%) but the overall survival rate of 30% ratenya, sementera for grading III of 41 patients 20 patients died (48.8%) with overall 51% survival rate. Of the 48 patients with stage I only found 9 patients who experienced the death (18.7%) with overall survival rate of 81%, while for stage II of 44 patients 15 patients died (34.1%) with overall 66% survival rate.

Lack of Cheung's study is not explained specifically by statistical analysis which of these three variables most strongly linked with cervical cancer prognosis. However, if only to see the numbers there are researchers who believe that the expression of cyclin D1 "more representative" variable prognosis of cervical cancer. And to prove these researchers suggested that the

prognostic test conducted by using multiple parameters fixed. Why continue to use multiple parameters? because in the staging of cervical cancer clinical staging is used instead of surgical staging. Cut-off could be known whether cyclin D1 expression in cervical cancer? this seems difficult because of valuation expression of cyclin D1 is semikuantitatif. Whether the expression of cyclin D1 is ideal as a prognostic factor for cervical cancer? Researchers think if seen from the viewpoint of science, the expression of cyclin D1 is ideal as a prognostic factor because it depicts the changes that occur at the molecular level but if viewed from the side of everyday practice is still to be debated, because it must meet the requirements are easy, inexpensive, safe, effective and efficient.

Metastasis begins when the cells regardless of the tumor and invaded individually or in groups of normal stroma and penetrate the walls of blood vessels, lymphatics and can be life after risky journey that could turn it off in the circulation and then be able to grow at distant places. Cells that survived had to live through endothelial cells into the organ recipient, forming blood vessels and grow there. Furthermore, in order to be able to penetrate into the surrounding cancer cells must be able to separate themselves with their mother while the tumor to metastasize, the tumor cells must be able to penetrate into blood vessels or lymph vessels (Berek et al. 2000, Mendelsohn et al. 2001, Azis 2004, Cotran et al. 2005).

In this study, the occurrence of lymph node metastases greater expression of cyclin D1 high degree of cyclin D1 expression compared with medium and low degrees. Whereas between cyclin D1 expression and cyclin D1 low grade high grade there was no significant difference in the occurrence of lymphatic metastasis. The next question is how the translation of cyclin D1 expression of theoretical significance to the occurrence of lymph node metastases. As already known before the cancer cells apart from the primary tumor to form new colonies elsewhere, then preceded by a series of events that includes the stages of initiation, promotion and progression, uncontrolled proliferation, angiogenesis through local invasion, vascular or lymph nodes. The author argues that the sequence of events that cyclin D1lah role. What to look for is whether there is a relationship between cyclin D1 with a number of mediators that contribute to these events such as VEGF on angiogenesis or cell adhesion molecules misalanya kadherin, integrin and katenin. (Berek et al. 2000, Mendelsohn et al. 2001, Aziz 2004, Cotran et al. 2005).

The other hand, the author believes this study still has some deficiencies which are not carried out inspection of high risk HPV type 16.18. This is important because expression of cyclin D1 theoretical model in this study is based on the model of HPV infection in the cervix. The second, unknown type of mutation in the cyclin D1 whether it is an amplification that ended with excessive expression or somatic mutations in the form of inversion. (Cotran et al. 1999, Berek et al. 2000)

CONCLUSION

In patients with cervical squamous cell carcinoma by radical hysterectomy found cyclin D1 expression for each of the different grading of tumor cells was significantly and positively correlated with the degree of grading of tumor cell expression of cyclin D1 (p: 0.000), the higher the degree of expression of cyclin D1, the higher grading of tumor cells (r: 0.939), cyclin D1 expression for each clinical stage was significantly different (p: 0.005) and there was positive correlation between cyclin D1 expression with clinical stage operabel ie higher cyclin D1 expression, the higher the clinical stage operabel (r: 0.005), relations cyclin D1 expression with grading of tumor cells was stronger than relationship with clinical stage (r: 0939> r: 0500) the positive correlation between the degree of expression of cyclin D1 with kelenjer metastatic lymph nodes (p: 0.03). Cyclin D1 expression was higher in cervical cancer with metastasis than without metastasis of lymph glands (r: 0, 754).

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