Survivin Expression in Uterine Cervical Exfoliative Cells as Diagnostic Test of Cervical Malignancy Process
(Pernyataan Survivin dalam Sel-sel Eksfoliatif Pangkal Rahim sebagai Ujian Diagnostik daripada Proses-proses Malignan Pangkal Rahim)

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ABSTRACT

Papanicolaou test is a diagnostic test for uterine cervical cancer screening and routinely examined. It has limitations. A better technique is needed to identify true cervical malignancy process. Molecular cancer marker detection may have high the sensitivity and specificity in detecting cancer. Survivin, a marker candidate, is upregulated in many malignancy processes. Ninety women have joined in this cross sectional study by consecutive sampling. Survivin expression was examined by indirect immunoperoxidase method. It was predominantly found in metaplastic cells. Correlation between survivin expression and Papanicolaou test results was calculated by Fischer's-exact test. Using Papanicolaou test result as an indicator for the presence of uterine cervical abnormalities, the performance indicators were calculated. Fischer's-exact test showed that survivin expression was not significantly useful as cervical cancer molecular marker. Survivin expression of the uterine cervical exfoliative cells cannot be used as a diagnostic test for uterine cervical malignancy process.

Keywords: Diagnostic test; indirect immunoperoxidase; Papanicolaou test; survivin; uterine cervical cancer

ABSTRAK

Ujian Papanicolau adalah sebuah ujian diagnostik bagi pemeriksaan barah pangkal rahim (kanser serviks) dan dilakukan secara rutin. Ujian ini mempunyai beberapa had. Suatu teknik yang lebih baik diperlukan bagi mengenal pasti proses malignansi serviks yang sebenarnya. Survivin, satu calon penanda kanser, meningkat kadarnya dalam banyak proses malignansi. Sembilan puluh wanita telah menyertai kajian keratan lintang ini melalui pensampelan berturutan. Pernyataan survivin diperiksa dengan menggunakan kaedah imunoperoksidas tidak langsung. Korelasi antara ekspresi survivin dengan keputusan ujian Pap dikira dengan menggunakan ujian Fischer's-exact. Seterusnya, dengan menggunakan keputusan yang diperoleh daripada ujian Papanicolau sebagai penunjuk adanya keganjilan serviks, penunjuk prestasi daripada immunositokimia daripada survivin boleh dikira. Ujian Fischer's-exact menunjukkan bahawa ekspresi survivin tidak berguna secara penuh makna sebagai penanda kanser serviks secara molekular. Pernyataan survivin daripada sel-sel eksfoliatif serviks tidak boleh digunakan sebagai ujian diagnostik bagi proses malignan serviks.

Kata kunci: Imunoperoksidas tidak langsung; kanser pangkal rahim; survivin; ujian diagnostik; ujian Papanicolaou

INTRODUCTION

Morbidity and mortality rates in women worldwide are predominantly caused by cervical cancer. In many developed countries, cervical cancer incidence has been successfully decreased by the routine Papanicolaou test examination. However, this achievement has not appeared yet in Indonesia, one of developing countries, owing to many reasons (Tjindarbumi et al. 2002). Papanicolaou test has some limitations. These limitations due to inadequacy of specimen (Cannistra et al. 1996) low sensitivity, and high false-negative rate (Williams et al. 1998). However, this test is still the most cost-effective test in screening of cervical malignancy process (Chen et al. 2003). A better technique is needed to identify the true cervical malignancy process. Early detection of true cervical malignancy process is needed. This cancer can be treated easily in the early stage of the disease (Williams et al. 1998).

Cancer molecular markers attracted researchers attention. For cervical cancer, a good marker is a marker that overexpressed in all cervical cancer cases, majority of high-grade squamous intraepithelial lesion (HSIL) and small percentage of low-grade squamous intraepithelial lesion (LSIL) that will progress to cervical cancer (Chen et al. 2003). A candidate of cancer molecular marker is survivin. Survivin is a member of inhibitory of apoptosis protein (IAP) family. It has some activities such as regulator of cell cycle, cell proliferation and inhibition of apoptosis. It is overexpressed in embryonic, development, and many cancer tissues, but undetectable in normally differentiated adult tissues (Li, 2005). Overexpression of survivin has been linked to radiotherapy resistence (Lu et al. 2004), biomarker for aggressive tumor behavior before the appearance of tissue abnormalities (Salz et al. 2005), and higher risk for local tumor recurrence (Rodel et al. 2005).

Two previous studies evaluated the usefulness of survivin overexpression as a marker of cervical cancer (; Branca et al. 2005; Frost et al. 2002). In the present study, we reexamined the correlation between the Papanicolaou test and survivin immunoreactivity of the uterine cervical exfoliative cells to confirm the usefulness of survivin as a molecular marker of the early stage of the cervical carcinoma.

METHODS

PATIENTS AND SAMPLES

Cervical smears were collected from outpatients visiting cytology and oncology clinics of Obstetrics and Gynecologic Department, Cipto Mangunkusumo Hospital in Jakarta, Indonesia between November 19, 2007 and February 28, 2008. Every patient was scrapped twice in her cervix in order to obtain two samples: one for conventional Papanicolaou test and the other for survivin expression test. Ethical clearance for the collection of cervical smears was provided by The Committee of the Medical Research Ethics of the Faculty of Medicine, University of Indonesia.

IMMUNOCYTOCHEMISTRY

Cervical smears were fixed in absolute ethanol and acetic acid (95:5) for 15 min on silane-coated slides. Smears were incubated with a solution of 3.0% H_2O_2 in absolute methanol for 30 min. Non-specific staining was blocked using normal goat serum (G9023, Sigma) for 10 min. The slides were incubated overnight at 4ºC with a specific polyclonal primary antibody (antisurvivin antibody produced in rabbit, S8191, Sigma), at 1:100 dilution. This antibody detects human survivin by immunoblotting (16 kDa). The samples were then rinsed in phosphate-buffered saline (PBS) and incubated for 30 min with secondary antibody (antirabbit antibody-peroxidase conjugated produced in rabbit, A9169, Sigma) at 1:100 dilution. Finally, the samples were developed with diaminobenzidine tetrahydrochloride substrate (Sigma*Fast*™ DAB, D4293, Sigma) for 5 min, slightly counterstained with hematoxylin, dehydrated and mounted with Entellan™.

The validation of the use of the antibody against survivin for immunocytochemistry was carried out in 24 h with absolute ethanol and acetic acid (95:5)-fixed testes of Sprague Dawley rat. Testes was dehydrated by graded alcohol, cleared in xylol and embedded in paraffin. The paraffinized testes tissue block was sectioned 5 μm thin and the sections were mounted on silane-coated slides. After deparaffinization process, the sections were rehydrated and incubated with a solution of 3.0% H_2O_2 in absolute methanol for 30 min. Non-specific staining was blocked using normal goat serum (G9023, Sigma) for 10 min. A section was incubated overnight at 4ºC with a specific polyclonal primary antibody (antisurvivin antibody produced in rabbit, S8191, Sigma), at 1:100

dilution. The omission of the primary antibody on another incubated section was used as negative control. Sections were then rinsed in phosphate-buffered saline (PBS) and incubated for 30 min with secondary antibody (antirabbit antibody-peroxidase conjugated produced in rabbit, A9169, Sigma) at 1:100 dilution. Sections were developed with diaminobenzidine tetrahydrochloride substrate (Sigma*Fast*™ DAB, D4293, Sigma) for 5 min, slightly counterstained with hematoxylin, dehydrated and mounted with Entellan™.

STATISTICAL ANALYSIS

Due to ethical issue, we decided to use Papanicolaou test result as an indicator for the presence of cervical abnormality. Interpretation of the Papanicolaou test was based on The 2001 Bethesda System. The cancer, high-grade squamous intraepithelial lesion (HSIL), low-grade squamous intraepithelial lesion (LSIL), and atypical lesion were then classified as positive for the presence of disease. Normal Papanicolaou test result was classified as negative for the presence of disease.

The percentage of immunoreactive cells was assessed on all samples. Positive samples were determined and analyzed as proposed previously. Positive samples were analyzed semi-quantitatively in at least twenty five areas at x450 magnification. Cases were scored positive when >5% of the cells reacted with the anti-survivin antibody. Assessment of all staining was blinded to knowledge of the Papanicolaou test result.

Correlation of survivin expression of positive cases with the presence of disease based on Papanicolaou test results was calculated using Fischer's-exact test. P value ≤0.05 was considered to indicate statistical significance.

RESULTS

VALIDATION IN SPRAGUE DAWLEY RAT TESTES

Survivin was overexpressed in the testes of Sprague Dawley rat. Immunolocalization of survivin in rat testes is in cytoplasm. No immunoreactivity was identified in the control (no primary antibody) section (Figure 1).

SURVIVIN EXPRESSION IN CERVICAL EXFOLIATIVE CELLS

Survivin is expressed in parabasal cells, intermediate cells and metaplastic cells (Figure 2). Survivin expression can be found both in cytoplasm and nucleus or only in cytoplasm. Survivin expression is found in many, but not all, samples with normal and intraepithelial lesions in Papanicolaou test results (Table 1). The same results were found in samples from post-therapeutic cancer samples. We did not find any samples with cancer lesion for survivin expression test.

Table 1 shows the positive cases of survivin expression related to the presence or absence of the cervical abnormality detected by the Papanicolaou test. Positive cases were found in 13 of 90 women.
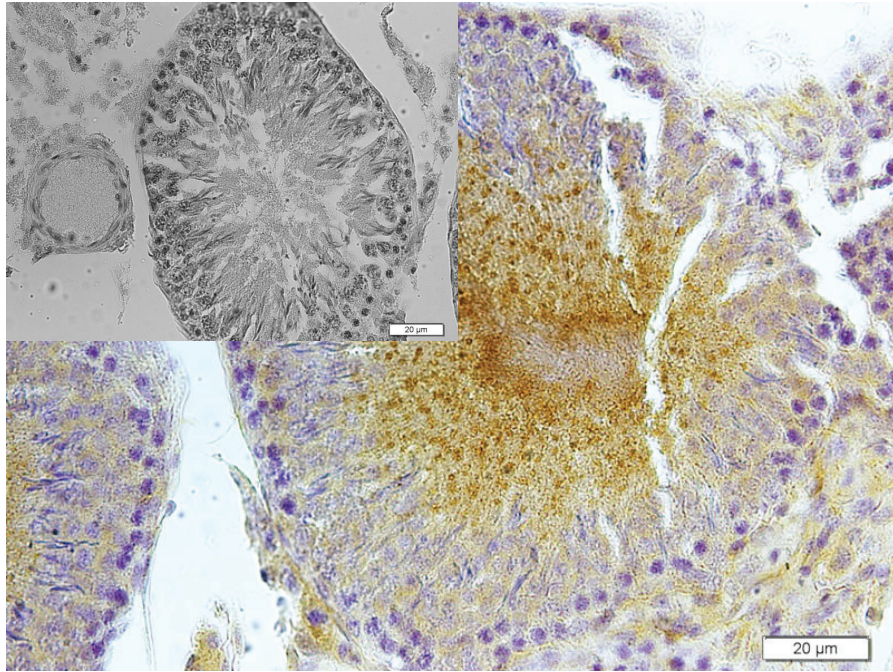
FIGURE 1. Positive and negative control of survivin expression in rat testes.
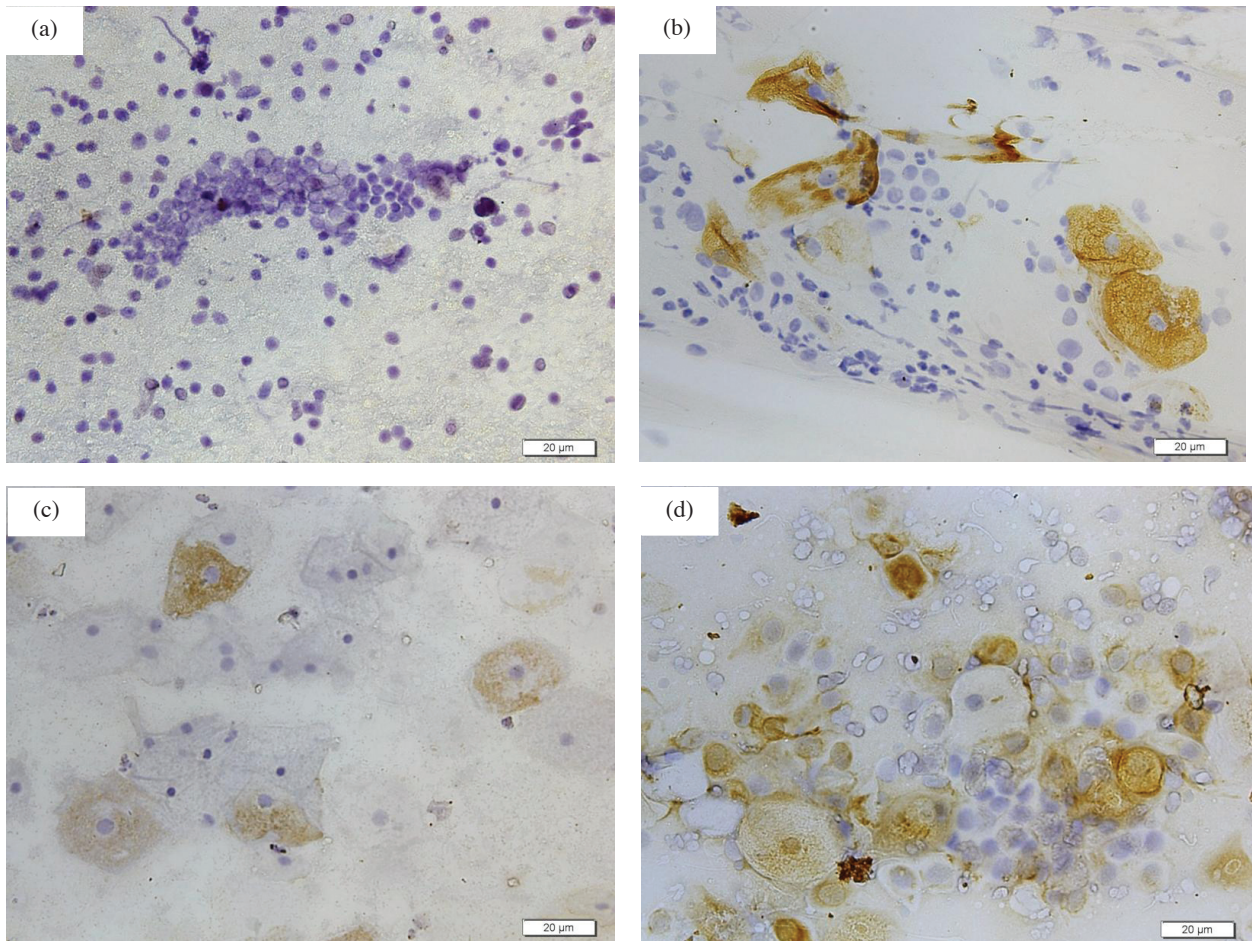Inset: negative control

FIGURE 2. Survivin expression in cervical exfoliative cells. No immunoreactivity is identified in the control smear (photograph (a)).
Photograph (b), (c) and (d) show survivin-immunopositive cells. Bars in (a)-(d) = 20 um

TABLE 1. Positive cases of survivin expression related to presence or absence cervical abnormality

		Presence of disease (Papanicolaou test result)		Total
		Positive	Negative	
Survivin expression	Positive	4	22	26
	Negative	9	55	64
Total		13	77	90

Fischer's-exact test showed that immunocytochemistry of survivin expression was not significant as cancer molecular marker (1-sided, p = 0.553; 2-sided, p = 1). The performance indicators are: sensitivity = 30.76%, specificity = 71.42%, PPV = 15.38%, and NPV = 85.93%.

DISCUSSION

World Health Organization recommended that a good diagnostic test is a test with specificity of 98-99% (WHO 2002). Diagnostic test in a country with low prevalence should be directed to increasing specificity. However, diagnostic test in a country with high prevalence should be directed to increase sensitivity (Pusponegoro et al. 2006).

Survivin has been studied in many cancers such as lung (Nakanishi et al. 2003), colon (Ashanuma et al. 2004) and cervix (Branca et al. 2005; Frost et al. 2002; Li 2003). Therefore, survivin is not a tissue specific of cancer molecular marker. However, The Prognostic Factor Committee of the European Society of Gynecological Oncology has concluded that there is an urgent need for specific prognostic biomarkers in cervical carcinoma (Frost et al. 2002).

Many molecules have been proposed as biomarker. Using microarray, Carlsson studied 140 genes that represent many biomarkers. Keratin 19 increases 21.2 fold (Carlson 2006). However Maddox et al. (1999) using immunohistochemistry, showed that keratin 19 expression was not significantly different in invasive cervical carcinoma, cervical intraepithelial neoplasia (CIN), metaplasia, and normal epithelium. Using northern blot analysis it was found that in many cases, survivin mRNA level has increased 2 fold compared to normal cervix tissue (Maddox et al. 1999). The result of this present study has similarity with Maddox et al. (1999)study. However, high false negative rate of Papanicolaou test is still needed to be considered as a factor that influenced the result. Another factor is, may be, the low sensitivity of immunostaining compared to microarray test.

Frost et al. (2002) showed that there is a staining pattern of survivin expression in normal benign squamous mucosa, cervical dysplasia, and invasive squamous carcinoma. However, Branca et al. (2005) reported that survivin expression is not found in normal or metaplastic squamous epithelium. This study is consistent with Frost et al. (2002) study. Metaplastic cells that show survivin expression are found in normal and abnormal Papanicolaou test samples. Survivin expression is regulated by many factors and survivin gene promoter domain. Human papilloma virus (HPV)-infected cervical cells will results in degradation of p53 and releasing of E2F-1. These results can interfere with survivin gene promoter domains resulting in overexpression of survivin. Therefore, we suggest that overexpression of survivin can be found in the HPV infected cervical cells.

Many cervical tumors are in infected forms and partially necrotic (Geisler et al. 2001). Chemotherapy and radiotherapy kill cancer cells by inducing apoptosis. In the lung squamous carcinoma, there is no significant correlation between chemotherapy response and survivin expression or localization. There is no correlation therapy modality and chemotherapy (Vischioni et al. 2004). These features may explain why survivin expression is not highly expressed in post-therapeutic cancer exfoliative cells.

CONCLUSION

Although it is difficult to decide whether survivin expression is an early sign of abnormal change toward cancer or related to normal cell cycle, the present study revealed that survivin is not suitable for the molecular marker of the cervical cancer. We need a prospective study to examine survivin expression role as a cancer molecular marker that appears early in malignancy process.

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