RESEARCH ARTICLE

Clinicopathology Significance of p53 and p63 Expression in Indonesian Cervical Squamous Cell Carcinomas

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Abstract

Background: Human papilloma virus infection is associated with genesis and malignant potential of cervical cancer. E6 and E7 oncogens are known to bind to p53 and retinoblastoma gene products, abrogating their functions as tumor suppressors, leading to an abnormal cell cycle machinery. Roles of the p53 homolog p63 have also been postulated, E6 expression leading to TAp63b degradation allowing anchorage independent growth. Molecular studies correlated with clinicopathological factors are important to determine prognosis and treatment strategies, but results have been controversial and need to be clarified. Aim: To investigate expression of p53 and p63 in cervical squamous cell carcinomas in correlation with age, FIGO staging, morphology, and cancer cell proliferation. Materials and Methods: Expression of p53 and p63 immunohistochemical staining in a total of 56 paraffin-embedded tissues of cervical squamous cell carcinomas from Dr. Sardjito General Hospital Indonesia, was evaluated for correlation with clinicopathological parameters. The Mann-Whitney test was used to compare the percentage of p53 and p63 expression with patient age, FIGO staging and morphology and to compare mean p53 and p63 expression. The Spearman correlation test was applied to correlate p53 and p63 expression with that of Ki-67. A p-value of <0.05 was considered statistically significant. Results: There were significant associations between p53 expression with age (p=0.019) and FIGO staging (p=0.026), but not with with morphology or Ki-67 expression. There were no links between p63 expression and age, morphology, FIGO staging or Ki-67. Conclusions: This study indicated that p53 has a prognostic value in cervical squamous cell carcinomas given the relation with FIGO staging.

Keywords: p63 - p53 - Ki67 - Age - FIGO staging - morphology - cervical squamous cell carcinoma

Asian Pac J Cancer Prev, **14 (12)**, 7737-7741

Introduction

Cervical cancer is the third most common cancer in women worldwide, and the seventh overall, with an estimated 530,000 new cases in 2008. In Indonesia, an estimated incidence 13762 new cases and 7493 cause of death in 2008 (Ferlay et al., 2010). Although invasive cervical carcinoma has become rare in most European countries, in many countries in Africa, Southeast Asia, and Latin America reveal an incidence that is more than 10 times as frequent compared to the incidence in central Europe (Tavassoli et al., 2003). This fact may be caused by lack co-ordinated screening programmes in developed countries, therefore the burden of cervical cancer remains high (Nygard et al., 2002).

Histologically, the most frequent type of cervical carcinoma is squamous cell carcinoma followed by adenocarcinoma (Brinton et al.,1987 and Vizcaino et al., 2000). The treatment of cervical cancer depends on the stage of the disease according to International Federation of Obstetrics Gynaenocology (FIGO) classification. Most cervical cancer patients in early stage (Ib-IIa) can be successfully treated by radical hysterectomy and pelvic lymphadenectomy, with an overall 5-year survival rate 80-95%. However, approximately 10% of the patients without significantly adverse factors still develop tumor recurrence (Aoki et al., 2001). Until to date, there is no consensus about criteria for identifying patients with high risk of recurrence among those without the involvement of lymph nodes, parametrial tissue, or a surgical margin (Koh et al., 2000). So, prognostic markers that can identify aggressive cancer deserve careful study.

Human papilloma virus (HPV) infection has been associated with carcinogenesis and malignant potential of cervical cancer (Munger et al., 2002; Zur et al., 2002). It is well known that more than 95% of cervical carcinomas have high-risk HPV types. Two major viral proteins, E6 and E7, are known to bind p53 and retinoblastoma gene products (pRB), abrogating their functions as tumor suppressors, leading to an abnormal cell cycle machinery (Bosch et al., 1995). However, studies on p53 expression in cervical carcinoma have reported conflicting results

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with respect to prognostic significance (Lu Xin, 2004).

Recently, Khalifa et al., 2011 observed in cervical carcinoma cells, repression of E6/E7 stabilizes the p53 transcription factor leading to activation of a large group of cellular p53 target genes. This evidence show that repression of E6/E7 also induces transcriptional activation of an additional large set of genes involved in cell adhesion including previously described p63 target genes. Indeed, further demonstrated that these p63 target genes are activated by TAp63b and not by p53 or by the DNp63a or b isoforms, even though these transcription factors are also expressed in these cells. In cervical carcinoma cells, E6 expression therefore leads to TAp63b degradation and thereby allowing anchorage independent growth. This work describes a new E6-dependent transformation pathway in HPV-associated carcinogenesis. TAp63b inhibition may also represent a common pathway to activate anchorage independent growth in cancers.

Both p53 and p63 expression were suggested to be prognostic markers associated with their molecular role in cervical carcinoma pathway. In this recent study, the expression of p63 and p53 in cervical squamous cell carcinoma will be investigated in correlate those with age, cancer cells proliferation (Ki-67), morphology, and FIGO staging.

Materials and Methods

The design of this research was a quantitative non-experimental, performed by cross sectional method. There was no follow up or reverse back intervention done in this research. Subjects used in this study were 56 paraffin-embedded tissues of cervical squamous cell carcinomas from Dr. Sardjito General Hospital (Pathology Laboratory archive) Jogjakarta, Indonesia, year 2010-2011. Informations recorded were medical record number, age, pathology laboratory number, methods of operation, clinical diagnosis, and pathological diagnosis. Identity of patients will only be witten as initial in raw data and unpublished in research paper. Samples taken from biopsy containing small focus of tumor were excluded from this study. Hematoxyllin Eosin slides were examined to classify cancer morphology into keratinized and nonkeratinized squamous cell carcinoma based on the WHO criteria (Tavassoli et al., 2003). The Stage of the disease based on clinical examination and confirmed by histopathology which coded according to International Federation of Gynaecology and Obstetric (FIGO) staging schemes. Patients with stage I-IIA had undergone a radical hysterectomy and thus the entire cervix was available for histological examination and were classified as early stage. While patients with stage IIB-IV had been treated with radiation and cervical biopsy obtained prior to therapy was available for assessing the histological examination and were classified as advance stage (FIGO Committee on Gynecologic Oncology, 2000).

Immunohistochemical staining

Paraffin-embedded tissue were stained immunohistochemically using monoclonal antibody anti p53 (CM 042 C, Biocare Med, dilution 1:100), p63 (CM 163 A, Biocare Med, dilution1:100), ki-67 (CRM 325 B, Biocare Med, dilution 1:100) using DAB chromogen and counter stain Hematoxyllin Mayer. Positive control was taken from squamous cell carcinoma of the skin tissue with positive IHC result for those markers. Stromal and normal columnar cells were used as negative internal controls.

Interpretation of results

All slides were examined under light microscopy by two independent observers. Strong nuclear staining was regarded to denote p53, p63 and Ki-67 positivity. For quantitative analysis, all cells were counted in 5 random fields at x 400magnification, and the p53, p63, ki-67 were expressed as a percentage of positive cells per total number of counted cells. The data were expressed as mean value (Shirendeb et al, 2009).

Statistical analysis

The statistical software SPSS 16.0 was used to analyze the data. Mann-whitney test was used to compare percentage of p53 and p63 expression with patient age, FIGO staging and morphology and to compare mean value of p53 and p63 expression. While, Spearman correlation test was used to correlate percentage of p53 and p63 expression with percentage of Ki-67 expression. A p-value of <0.05 was considered statistically significant.

Results

In this study, 56 cervical squamous cell carcinoma specimens were collected. Majority of tissue samples were collected from radical histerectomy (n=43). The rest of samples were collected from cervical biopsy (n=13). Mean of patients age at diagnosis was 54,23 years with minimum age 32 years old and maximum 81 years old. The number of patients ≤ 54 year old was 51,8% (n=29) and patients >54 year old was 48,2% (n=27). According to FIGO staging, patients could be grouped into 48,2% (n=27) in early stage and 51,8% in advance stage (n=29). Histopathology examination showed 76,8% (n=43) with morphology non keratinized squamous cell carcinoma and 23,2% (n=13) with morphology keratinized squamous cell carcinoma.

Table 1. The Difference between p53 Expression with P63 Expression in Cervical Squamous Cell Carcinoma

Expression	n	mean (min-max)	p value
p53 expression	56	43.23 (0-87)	0.00
p63 expression	56	78.64 (3-98)	

Mann Whitney test

Table 2. Correlation between p53 Expression with Ki-67 Expression

		Ki-67 xpression
p53 expression	r	-0.099
	p	0.469
	n	56

Spearman correlation test

Table 3. Correlation between p63 Expression with Ki-67 Expression

		Ki-67 xpression
p63 expression	r	0.139
	p	0.308
	n	56

*Spearman correlation test

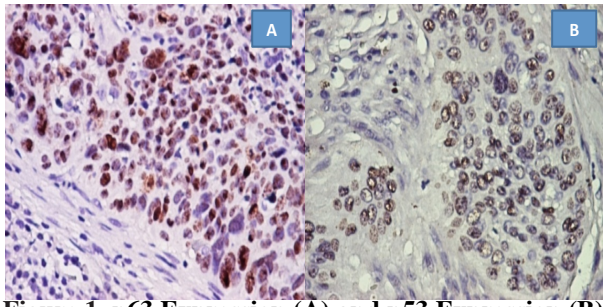

Figure 1. p63 Expression (A) and p53 Expression (B) in the Nuclei of Carcinoma Cells (x400)

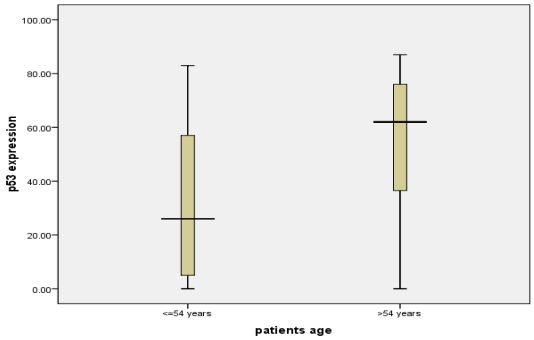

Figure 2. Association between p53 Expression with Patient Age (Mann Whitney test: p value=0.019)

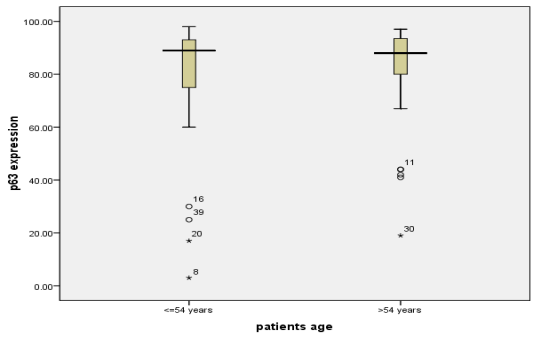

Figure 3. Association between p63 Expression with Patient Age (Mann Whitney Test: p value=0.818)

Discussion

Mean age of indonesian cervical cancer patients, exspecially squamous cell carcinoma was 54 years (range 32-81 years). While in other Asian country estimated to be 54 years in Korea (Cho et al., 2003), 48 years (range 32-73 years) in Japan, 46 years (range 29-53 years) in Mongolia, and 51 years (range 32-65 years) in Myanmar (Shirendeb et al., 2009), in contrast with study in Bucharest, showed the mean age of 35 years (Vasilescu et al., 2009). The age at diagnosis is important associated with early age at the start of the first sexual intercourse which regarded as one of high risk factor of cervical cancer. However, in development country such Asia, the mean age at diagnosed

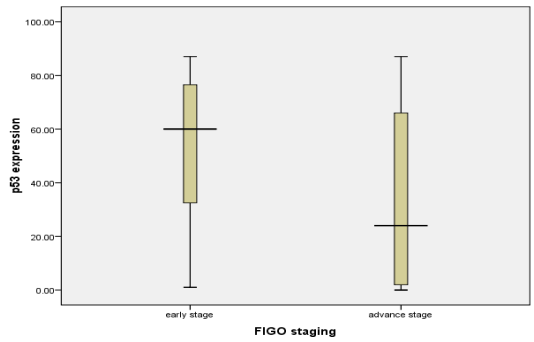

Figure 4. Association between p53 Expression with FIGO Staging (Mann Whitney test: p value=0.026)

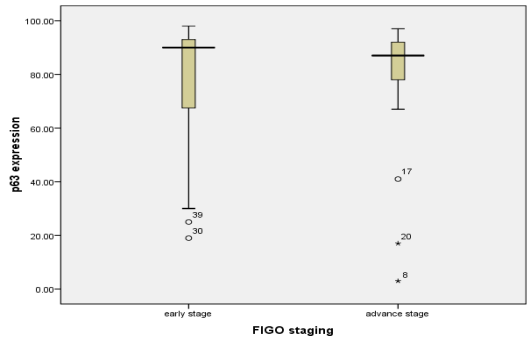

Figure 5. Association between p63 Expression with FIGO Staging (Mann Whitney Test: p value=0.736)

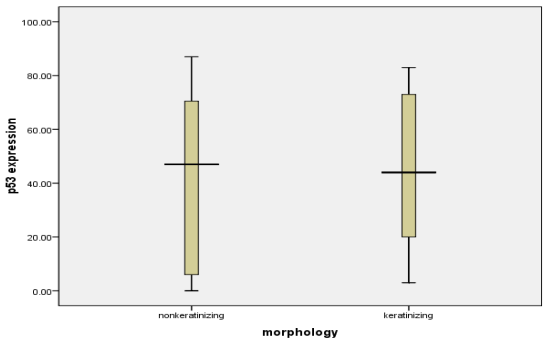

Figure 6. Association between p53 Expression with Morphology (Mann Whitney test: p value=0.778)

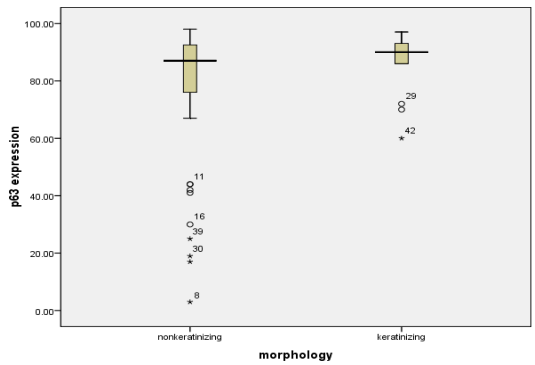

Figure 7. Association between p63 Expression with Morphology (Mann Whitney test: p value=0.307)

could be older due to less awareness of cervical cancer.

Histopathology examination showed 76,8% of cervical cancer patients has morphology of non keratinized squamous cell carcinoma and 23,2% keratinized squamous cell carcinoma. This result was similar with some previous

study by Cho et al, 2003 and with WHO (Tavassoli et a.l, 2003). According to morphology, after radiation therapy, non-keratinized carcinomas had 5-year survival of 61% compared to 40% for keratinized tumours. While, patients treated by radical surgery show no significant difference in outcome between keratinizing and non-keratinizing tumours (Tiltman et al., 2005).

In invasive cervical cancer, the clinical stage is the single most important parameter determining the outcome. However, within a given stage, other variables can affect prognosis, such as the tumor volume and lymphovascular space invasion (Jain et al., 2003). In this study, percentage of patient with FIGO staging between early stage (Ia-IIa) and advance stage (IIb-IV) are nearly similar number 48.2% and 51.8% respectively.

The p53 gene functions include cell-cycle arrest and apoptosis in response to DNA damage. The p53 gene is one of the most important targets of the HPV E6 gene. It was found that E6 protein has the ability to stimulate p53 degradation, and inhibits several functions of the wild-type p53 including the suppression of malignant growth (Storey et al., 1998; Kisseljov et al., 2000). Wild-type p53 protein has a very short half-life, and thus the protein level is too low to be identified immunohistochemically. In contrast, mutant p53 proteins have a longer half-life and can be easily detected by immunohistochemical methods (Finlay et al., 1988).

The results of p53 expression in our study are in the range of 0%-87% with mean 43,23%. This result was consistent with the previous report which the frequency of p53 expression has ranged from 9% to as high as 75% of cervical cancers (Graflund et al., 2002) and 0-90% of cervical cancer (Vosmik et al., 2013) and different with Shiohara et al 2005. These result suggest that p53 may be play an important role in this cancer, and possibly on the basis of abnormal accumulation of non-mutant p53 protein due to altered p53 homeostasis in tumor cells rather than p53 mutation (Ikuta et al., 2005). In addition, Akasofu et al. reported that if more than 50% of the tumor cells showed strong p53 staining is assumed take p53 mutation gene. In this study, 26 from 56 samples with p53-positive percentage more than 50%. Therefore, the expression of p53 in the present study may represent an accumulation of p53 mutation, therefore we have to perform the sequencing of p53 gene. More over, in this study found there is association between p53 expression with age (p=0,019) and FIGO staging (p=0,026). p53 expression was high in patients with early stage and age before 54 years, while, Khunamornpong et al, 2006 assumed that immunohistochemistry for p53 protein appears to provide no prognostic information in the patient with early stage cervical cancer treated by surgery.

Recently cloned transcription factor for p63 is a promising marker to regulate epithelial proliferation and differentiation of the epidermis (Tsujita et al, 2003). p63 expression has been found in SCC of the oral cavity, lung, head and neck, skin and esophagus. In the cervix, p63 was expressed in basal and parabasal cells of ectocervix, maturing transformation zone, and cervical intraepithelial neoplasia (Quade et al, 2001). p63 is a unique biomarker, and although its function remains unresolved, it is strongly that p63 has linked to epithelial differentiation and maturation. p63 expression by immunohistochemistry was lost in typical adenocarcinoma, consistent with divergence from basal or squamous differentiation. P63 is not induced during the normal cell cycle, like p53; moreover the absence of either does not prevent proliferation.

The results for p63 expression in our study were in the range of 3%-98% with mean 78,64%, 41 of 56 samples were highly positive (80-98%), 11 of 56 samples were moderately positive (30-<80%), 3 of 56 samples were low positive (15- <30%) and 1 was negative (0-<15%) in p63 staining. This result was consistent with the previous report which 64 of 70 samples were highly positive (80-100%), in 40 cases 100 %, two samples were moderately positive (both 60 %) and four samples were negative (0-10%) in p63 staining (Vosmik et al., 2013). p63 was a powerfull marker for squamous differentiation and when diffusely expressed, excludes a glandular or neuroendocrine differentiation (Wang et al., 2001). Therefore, for 1 sample with p63 expression in this study was negative, immuhistochemistry for cromoganin done which negative result. We assumed this sample may be undifferentiated adenocarcinoma.

In our study, we found that there were no association between p63 expression with age, morphology and staging. This result similar with previous study in Myanmar patient and different with Japan and Mongolia patient which p63 expression was high in advance stage (Shirendeb et al, 2009).But, positivity index of p63 higher than p53 (p value=0,000). Therefore this study assumed that p63 have important role in cell differentiation than prognostic factor.

Ki-67 protein is a cellular marker for proliferation which can be detected within the cell nucleus. Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Ki-67 is an excellent marker to determine the growth fraction of a given cell population (Gerdes et al., 1983). Ki-67 staining has been evaluated extensively as a prognostic parameter in squamous cell carcinoma.

In our study, we found that there were no correlation between p53 and p63 expression with Ki-67 expression. This result was different with previous study which p63 expression has positive correlation with Ki-67 expression (Shirendeb et al, 2009).

In summary, although limited sample numbers and methods were used, however this research may contribute a brief description regarding that p53 has a prognostic value in cervical squamous cell carcinoma and that this gene has associated with FIGO staging. While p63 has a diagnostic value in cervical squamous cell carcinoma because its role in squamous cell differentiation. Further study is demanded to reveal that the expression of p53 in squamous cell carcinoma is an accumulation of p53 mutation or non-mutation.

Acknowledgements

This work was supported by Faculty of Medicine, Gadjah Mada University (GMU); DANA MASYARAKAT, GMU 2012.

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