ORIGINAL ARTICLE

Immunohistochemical Study of p53 Expression in Premalignant and Malignant Cervical Neoplasms

Tan GC[1], Sharifah NA[1], Salwati S[1], Shiran MS[3], Hatta AZ[2], Ng HO[2]

Department of Pathology[1] and Obstetric & Gynaecology[2], Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur
Department of Pathology[3], Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Kuala Lumpur

ABSTRAK

Salah satu faktor risiko penting dalam kanser serviks adalah jangkitan virus papiloma manusia (*human papillomavirus* - HPV). Gen p53 merupakan salah satu sasaran utama gen E6 HPV. Protein E6 mempunyai keupayaan untuk merangsang degradasi p53, menghentikan beberapa fungsi p53 jenis-liar dan bersaing dengan fungsi lain p53 seperti mencegah pertumbuhan kanser. Tujuan kajian ini adalah untuk mengenalpasti perbezaan pengekspresan p53 dalam neoplasia serviks pra-malignan dan malignan. Kajian retrospektif ini melibatkan 100 kes neoplasia serviks [21 neoplasia serviks intraepitelial 1 (*cervical intraepithelial neoplasia* 1- CIN1), 8 CIN2, 25 CIN3, 36 karsinoma sel skuamus, 7 adenokarsinoma dan 3 adenoskuamus karsinoma). Semua kes dikaji dengan kaedah immunohistokimia menggunakan antibodi monoklonal p53. Tiga puluh enam daripada 54 kes pra-malignan (66.7%) adalah positif terhadap protein p53 berbanding dengan 40 daripada 46 kes malignan (87.0%) yang menunjukkan kepositifan. Dua puluh sembilan daripada 54 (53.7%) kes CIN menunjukkan sama ada pengekspresan fokal atau tiada pengekspresan. Sebaliknya, 84.8% (39/46) kes karsinoma invasif menunjukkan pengekspresan regional atau pengekspresan keseluruhan. Pengekspresan p53 didapati lebih tinggi dalam neoplasia serviks malignan berbanding lesi serviks pra-malignan. Ini mencadangkan bahawa pengekspresan p53 bukan satu fenomena awal dalam patogenesis kanser serviks. Kajian ini juga menunjukkan terdapat peningkatan peratusan pengekspresan p53 dalam CIN2 dan CIN3 berbanding dengan CIN1. Akan tetapi, terdapat beberapa kes yang tiada pengekspresan p53 mencadangkan kemungkinan penglibatan faktor-faktor lain dan kajian HPV lanjutan adalah diperlukan.

Kata kunci: Serviks, kanser serviks, CIN, HPV, Immunohistokimia, p53

ABSTRACT

One of the most important cervical cancer risk factors is human papillomavirus (HPV) infection. The p53 gene is one of the most important targets of the HPV E6 gene. E6 protein has the ability to stimulate p53 degradation, inhibits several functions of wild-type p53 and it competes with its function including suppression of malignant growth. The aim of this study is to determine the differences in p53 expressions in pre-malignant and malignant cervical neoplasms. This is a retrospective study on 100 cases of cervical

Address for correspondence and reprint requests Dr Tan Geok Chin, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia. Jalan Yaacob Latiff, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Email: tan_geok_chin@yahoo.com

neoplasms. There were 21 cases of CIN 1, 8 cases of CIN 2, 25 cases of CIN 3, 36 cases of squamous cell carcinoma, 7 cases of adenocarcinoma and 3 cases of adenosquamous carcinoma. All cases were evaluated by immunohistochemistry using p53 monoclonal antibody. Thirty six of the 54 pre-malignant cases (66.7%) were positive for p53 protein, in contrast to the malignant cases in which, 40 of the 46 cases (87.0%) were positive. The majority of CIN showed absent to focal staining (29/54, 53.7%). In contrast, 84.8% (39/46) of the invasive carcinoma showed regional to diffuse staining. The expression of p53 is greater in the malignant cervical neoplasms than the pre-malignant cervical lesions, suggesting that p53 overexpression is not an early phenomenon in the pathogenesis of cervical cancer. It is also shown to be slightly higher in percentage in CIN 2 and 3 when compared with CIN 1. However, a number of cases were p53 negative, suggesting that other factors may be involved and further HPV studies are indicated.

Key Words: Cervix, Cervical cancer, CIN, HPV, Immunohistochemistry, p53

INTRODUCTION

Cervical cancer is one of the most common cancers in Malaysia. It is the second commonest cancer for women. According to the Malaysian National Cancer Registry, the percentage when compared with the rest of the cancer was 12.9% (Lim et al 2003). One of the most important cervical cancer risk factors is human papillomavirus (HPV) infection (Munoz et al. 2003). Up to now, more than 200 HPV types have been identified (Bernard 2005). The International Agency for Research on Cancer (IARC) studies has provided generic and type-specific risk estimates for HPV. They conclude that there is a strong association between HPV and cervical cancer development and the risk of developing cancer depends on the HPV type (Munoz et al. 2003). However, HPV infection alone is not sufficient for cervical carcinogenesis, and attention has been focused on other factors important to this process. The development and progression of cervical cancer are likely to be associated with loss of growth suppression (uncontrolled proliferation), increased cell growth rates, and angiogenesis (Araujo Souza et al. 2003, Tjalma et al. 2001, Stanley 2001).

The p53 gene is located on chromosome 17p13.1 and it functions as cell-cycle arrest and apoptosis in response to DNA damage. It is the most common target for genetic alteration in human tumours. Homozygous loss of p53 gene activity can occur in almost every type of cancer, such as carcinomas of the lung, colon and breast. Normal 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 (Finlay et al. 1988) and can be easily detected by immunohistochemical methods. Overexpression of p53 protein has been identified immunohistochemically in a variety of tumours.

The p53 gene is one of the most important targets of the HPV E6 gene. It was found that E6 protein of high-risk HPV, but not of low-risk HPV, could interact with p53 in vitro systems. In addition to the ability to stimulate p53 degradation, E6 protein inhibits several functions of wild-type p53 and it competes with it for the most important p53 protein functions including a major role in the suppression of malignant growth. It was also shown that E6 increased the level of mutagenesis and genomic instability (Storey et al. 1998, Kisseljov et al. 2000). The resultant loss of wild-type p53 increases cellular genomic instability after DNA damage.

The aim of the present study was to determine and compare the expressions of p53 protein in CIN and cervical cancers, which includes squamous cell carcinoma, adenocarcinoma and adenosquamous

carcinoma.

MATERIALS AND METHODS

Study Design

This is a retrospective study on cases diagnosed as CIN, squamous cell carcinoma (SCC), adenocarcinoma (AC) and adenosquamous carcinoma (ASC) obtained from the histopathology records of the Department of Pathology, Hospital Universiti Kebangsaan Malaysia (HUKM), for the past seven years from January 1, 1994 to December 31, 2000. The total number of cases was 100. There were 21 cases of CIN1, 8 cases of CIN2, 25 cases of CIN3, 36 cases of SCC, 7 cases of AC and 3 cases of ASC.

Antibody and immunohistochemistry

Three-micron thick sections were cut from the paraffin blocks and mounted on sialinized slides. Sections were then deparaffinized with 2 changes of xylene and rehydrated with four changes of alcohol in decreasing concentrations at 3 minutes each and then washed in water. The sections were then treated with target retrieval solution (Dako Corporation Denmark) in 1:10 dilution for 20 minutes in a water bath at 97^{o}C, followed by cooling for 20 minutes in room temperature. This is followed by staining using DAKO LSAB+ Kit peroxidase (Dako Corporation Denmark). The slides were treated with 3% hydrogen peroxidase for 10 minutes to block endogenous peroxidase activity. Then, the slides were washed in Tris-buffered saline (TBS) for 2 changes at 5 minutes each. They were then incubated in monoclonal antibody p53 protein (D07, Dako Corporation Denmark) at a dilution of 1:500, for 30 minutes. Streptavidin and diaminobenzidine (DAB) were added. Finally, the slides were counterstained with haemotoxylin and eosin. For all cases, the same technical personnel performed the immunohistochemical stainings. Sections of breast cancer were used as positive control.

Interpretation of result

All slides were examined under light microscopy. Distinct nuclear staining was regarded as p53 positivity. 100 cells were evaluated in representative high-power fields, to obtain the percentage of cell positivity. The pathologists were blinded to the clinical diagnoses and origin of the samples. The degree of nuclear staining was graded depending on the percentage of cells stained; grade 0 samples with no positivity (negative); grade 1 when less than 10% of cells showing positivity (mild expression), grade 2 with 11% to 50% expression (moderate expression), and grade 3 with greater than 50% expression (intense expression).

Statistical analysis

The difference in the degree of p53 protein staining between CIN (pre-malignant) and carcinoma (malignant) was assessed using the Pearson chi-square test. The percentage of reactivity in the different individual types of cervical neoplasms, i.e., CIN1, CIN2, CIN3, SCC, AC and ASC were categorical variables. P value was calculated by SPSS program version 12.0 (SPSS Inc., Chicago, IL, USA). Any p value < 0.05 was considered to be statistically signifycant.

RESULTS

Clinical Presentation

The age of clinical presentation of patients with cervical neoplasm (CIN and invasive carcinoma) ranged from 20 to 72 (43.8) years. Patients with cervical carcinoma (32 to 72, mean 50.3 years) were older than those with CIN (20 to 58, mean 37.8 years); four of our patients who had CIN were younger than aged 30 years. The distribution of cervical neoplasm in different

ethnic groups in Malaysia is 53% Chinese, 37% Malays and 10% Indian.

p53 Expression

Of the 100 samples obtained, 54 cases were pre-malignant lesions (21-CIN1, 8-CIN2, 25-CIN3), 46 cases were malignant (36-SCC, 7-AC and 3-ASC. Positive staining for p53 overexpression was localized in the nuclei of carcinoma cells (Figure 1). The relationship between the percentage of cells with p53 expression and histological diagnosis is summarized in Table 1.

Thirty six of the 54 pre-malignant cases (66.7%) were positive for p53 protein, in contrast to the malignant cases in which, 40 of the 46 cases (87.0%) were positive. When the intensity of staining is evaluated, grade 3 or intense expression of p53 protein was observed in 30/46 (65.2%) of the malignant cases, while only 8/54 (14.8%) of the pre-malignant cases were immunoreactive. The majority of CIN showed negative to grade 1 (mild expression) (29/54, 53.7%) immunostaining. In contrast, 84.8% (39/46) of the invasive carcinoma showed grade 2 to 3 (moderate to intense) immunostaining. The difference in the intensity of p53 protein immunoexpression was statistically significant (p value <0.05). (Table 1)

In the pre-malignant lesions, grade 3 or intense expression of p53 was noted to be higher in CIN3 (6/25, 24%) when compared to CIN1 (1/21, 5%) and CIN2 (1/8, 12.5%) (Figure 2). However, 7 of the 25 CIN3 cases were found to be negative for p53 expression. In malignant cervical lesions, SCC (26/36, 72.2%) was observed to have the highest percentage of cases with intense p53 expression, this is followed by AC (3/7, 42.9%) and ASC (1/3, 33.3%) (Figure 3).

DISCUSSION

p53 overexpression is linked with the control of cell growth, cell cycle, and apoptosis (Levine 1992, Levine et al. 1991, Greenblatt et al. 1994). Wild-type p53 protein usually resides in normal cell nuclei. This protein is unstable and has a half-life of only 20–30 min, while the mutant p53 protein is more stable and has a prolonged half-life, resulting in detectable immunohistochemical staining (Levine 1992, Greenblatt et al. 1994). In uterine cervical carcinoma, the detection rate of p53 overexpression by immunohistochemistry has been reported to range from 8 to 74%, (Huang et al. 2001, Avall-Lundqvist et al. 1997, Chen et al. 2000, Oka et al. 1993, Brenna et al. 2002, Helland et al. 1993, Cavuslu et al. 1997, Dejonge et al. 1999, ter Harmsel et al. 1998, Holm et al. 1993) and some reports have suggested that p53 overexpression is important as a prognostic marker (Huang et al. 2001, Avall-Lundqvist et al. 1997, Chen et al. 2000, Oka et al. 1993, Holm et al. 1993).

In the present study, p53 overexpression was detected in 87% of all invasive cervical carcinoma cases (40/46). Other investigators have reported similar findings (Ikuta et al. 2005). Our study did not show a significant difference in p53 expression

Table 1: The percentage of p53 expression in pre-malignant and malignant cervical neoplasms

Histological Diagnosis	Percentage Of p53 Expression (%)				TOTAL
	0 (Negative)	1-10 (Grade 1)	11-50 (Grade 2)	>50 (Grade 3)	
Pre-Malignant (CIN 1 -3)	18	11	17	8	54
Malignant (SCC, AC, ASC)	6	1	9	30	46
Total					100

(p value <0.05)

CIN- cervical intraepithelial neoplasia, SCC- squamous cell carcinoma, AC- adenocarcinoma, ASC- adenosquamous carcinoma

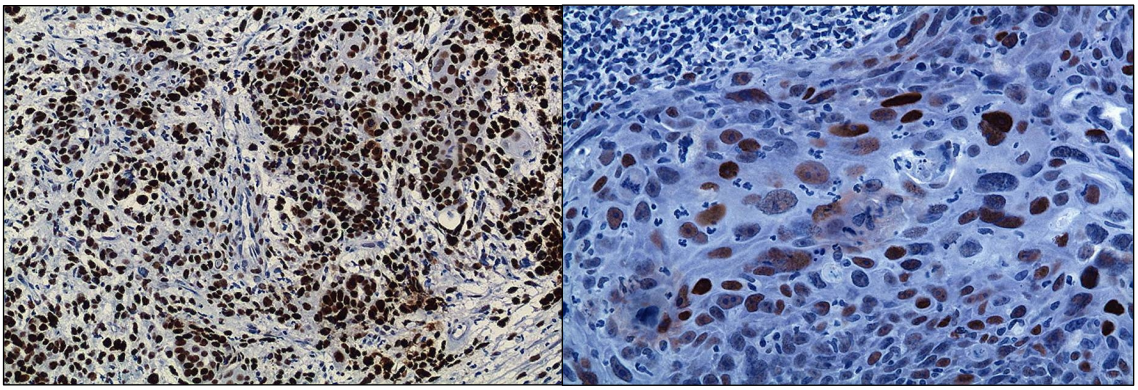
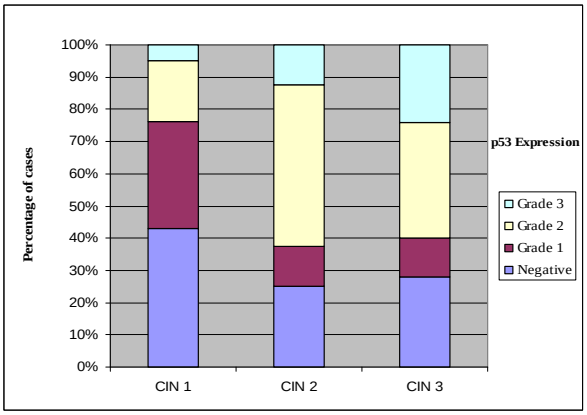
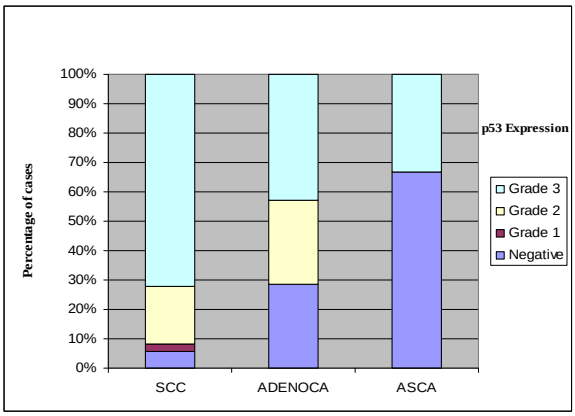


Figure 1: Positive staining for p53 expression in the nuclei of carcinoma cells (A- adenocarcinoma, B- squamous cell carcinoma)



CIN- cervical intraepithelial neoplasia

Figure 2:. The distribution of grades of p53 expression in pre-malignant cervical lesions



SCC- squamous cell carcinoma, ADENOCA- adenocarcinoma, ASCA- adenosquamous carcinoma

Figure 3: The distribution of grades of p53 expression in malignant cervical neoplasms

between the degrees of CIN, although a slight increased in the percentage of cases with intense p53 expression was observed in CIN3. On the contrary, when pre-malignant lesions were compared with malignant lesions, there is a significant difference. This suggests that p53 alteration is not an early event in the pathogennesis of cervical cancer.

AC (Suzuki et al. 2004) and ASC are thought to carry a less favourable prognosis compared to SCC, therefore p53 expression would be expected to be greater in the former. However, p53 expression was conversely noted to be greater in SCC. This finding does not reflect the true population because there were only 7 cases of AC available for evaluation. Many reports have demonstrated the correlation between p53 gene mutation and unfavorable outcome in various human cancers, including lung (Mitsudomi et al. 2000), breast (Rahko et al. 2003), bladder (Lorenzo-Romero et al. 2003), and cervical (Oka et al. 2000) cancer. Previous reports have shown that p53 overexpression has a statistically significant correlation with poor outcome of cervical SCC (Oka et al. 2000) and AC (Suzuki et al. 2004).

Epidemiological studies have shown that HPV infection, especially high-risk HPV such as HPV types 16 and 18, is involved in the carcinogenesis of uterine cervical carcinoma (Walboomers et al. 1999, Zur Hauzen 2002, Munoz et al. 2003). It is known that high-risk HPV produces E6 oncoprotein, which binds to wild-type p53 protein to inactivate and degrade tumor suppression (Graflund et al. 2002, Kedzia et al. 2002, Crook et al. 1992, Werness et al. 1990, Akasofu et al. 1995). It is therefore postulated that loss of p53 function due either to binding of HPV coding proteins or mutations of the p53 gene is an important event in the pathogenesis of cervical carcinomas (Akasofu et al. 1995). Further investigations to determine the HPV status in our population is crucial.

CONCLUSION

In conclusion, the expression of p53 is significantly higher in malignant cervical neoplasms than in pre-malignant cervical lesions, suggesting that p53 overexpression is not an early phenomenon in the pathogenesis of cervical cancer. It is also shown to be slightly higher in percentage in CIN2 and CIN3 (high-grade lesion) compared to CIN1 (low-grade lesion). This may be due to the fact that high grade lesions are more likely to have high risk oncogenic human papillomavirus infection as compared to low grade lesions.

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