THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (September–October 2016) 66(S1):S383–S388
DOI 10.1007/s13224-015-0754-1

ORIGINAL ARTICLE

The Role of p53 Gene in Cervical Carcinogenesis

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Received: 12 May 2015 / Accepted: 27 July 2015 / Published online: 4 September 2015

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Abstract

Aim To investigate the role of p53 gene in cervical carcinogenesis.

Materials and Methods A total 50 cases and controls were taken after setting exclusion criteria. Venous blood (3 ml) samples were collected in sterile EDTA sterile vials. Both punch biopsy of cervical growth in cases and biopsy from cervix after hysterectomy in controls were performed. Genomic DNA was extracted from tissue and blood using standard protocol of Miller et al. 1994 using chloroform–phenol method. Gene was amplified using specific forward and reverse primers and p53 gene expressions were studied. The present study of p53 gene regulation analyzed the expression of 279-bp bands on 1.5 agarose gel.

Observations Out of the total 50 samples of cases and controls, we were able to isolate DNA from 38 cases and 28 controls in blood and in 22 cases and 22 controls in tissue. In cases of carcinoma cervix, p53 expression is either downregulated or absent in 71.06 % of cases compared to 50 % of controls in blood and 72.73 % of cases compared to 59.09 % of controls in tissue, but these figures were not statistically significant ($p = 0.67$ and $p = 0.167$, respectively). p53 positivity rate was only in 27.78 % of squamous cell cancer and 50 % of adenocarcinoma. Three out of nine patients (33.3 %) with L.N. positive status have p53 gene positivity, whereas 23 % (3 out of 13) with L.N. negative status have p53 gene positivity, which is not significantly associated. In our study, p53 overexpression increases with the various stages of cervical cancer.

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Conclusion In our study, we found that there is the increased frequency of upregulation or overexpression of p53 gene in control in both blood (50 %) and tissue (40.9 %), but this association is statistically nonsignificant. In the present study, there is a lack of relationship between p53 overexpression and prognosis in the cervical cancer patients. However, our study lacked larger sample size which otherwise would have been able to lend support to truly significant findings through much larger combined and comparative datasets.

Keywords p53 gene expression · Cervical cancer · PCR

Introduction

Cervical cancer is the fourth most common cancer in women. 528,000 new cases were diagnosed worldwide in 2012. Cervical cancer also contributed nearly 8 % of all cancers [1]. This tumor is associated to some specific types of human papillomaviruses (HPV), particularly of types 16, 18, 33, and 42. The peak incidence of the disease occurs in women over 40 years of age; however, the peak incidence for HPV infection is found in women in their twenties; therefore, there is a long latency period between the time of HPV infection and cancer appearance. In the pathogenesis of cervical carcinoma, there are three major components: two of them related to the role of human papilloma viruses (HPV). First, the effects of viral E6 and E7 proteins, and the second, the integration of viral DNA in chromosomal regions associated with the well-known tumor phenotypes. The third factor is the accumulation of cellular genetic damage, not related to HPV, needed for tumor development. HPVs have circular, double-stranded DNA genomes that encode eight genes, of which E6 and E7 have transforming properties. E6/E7 expression promotes chromosomal instability, foreign DNA integration, and other mutagenic events in the cell [2]. The identification of the specific genes involved, and their correlation with specific tumor properties and stages, could improve the understanding and perhaps the management of cervical carcinoma.

p53 protein degradation is induced by virus oncoprotein E6, a determinant step in the cervical HPV-induced oncogenesis. Interestingly, p53 is rarely mutated in early cervical tumors, an exception in cancer genetics. E6 acts as an inactivating mutation. The fact that some p53 mutant proteins are not susceptible to E6-mediated degradation suggests that p53 polymorphisms might be responsible for the variation of the epithelial cell response to the HPV infection.

This study aims

1. To evaluate the association of p53 gene expression with cervical carcinoma.
2. To study the effect of p53 gene expression in relation to disease prognosis.

Materials and Methods

This was a case–control study carried out in the Department of Obstetrics and Gynecology, Sir Sunderlal Hospital and Centre of Experimental Medicine and Surgery, The Institute of Medical Sciences, Banaras Hindu University, Varanasi from 2012 to 2013. Institutional Ethical Committee and departmental review board approval was obtained for this study.

Total number of cases and control: 50 each.

Inclusion criteria: Cases: Patients with histopathologically diagnosed cervical carcinoma. Control: Patients undergoing hysterectomy for some benign conditions without any evidence of HPV infection, PID, active infection, and histopathologically undetermined cervical abnormalities.

Exclusion criteria: Patients with active infections, HPV infections, chronic inflammatory disease, and histopathologically undetermined cervical abnormalities.

Collection and processing of sample: Venous blood (3 ml) was collected in sterile vials containing sterile EDTA and centrifuged within 4 h at 1500 rpm for 15 min at 40 °C. Isolated pellet was transferred into another polypropylene vial and stored at −80 °C until further study.

Punch biopsy of cervical growth in cases and punch biopsy from cervix after hysterectomy in controls were performed after obtaining written consent, and tissue is homogenized using mortar and pestle and stored at −80 °C until further study.

DNA extraction and real-time PCR: genomic DNA was extracted from tissue and blood using standard protocol of Miller et al. 1994 using chloroform–phenol method, and p53 expression were studied using both the forward and reverse primers as respective positive controls. Gene was amplified using specific forward and reverse primers with optimum conditions of the PCR.

Specific forward and reverse primers selected for PCR reaction were as follows:

p53

F = 5′-TGA AGT CTC ATG GAA GCC AGC-3′
R = 5′-GCT CTTT TTC ACC CAT CTA CAG-3′

Total reaction volume of 25 µl containing 50-100 ng of genomic DNA, 20 pmol of each primer, 200 µM of each dNTPs (Fermentas, USA) mixed with Taq buffer (10 mM

TrisHCl pH 8.3, 50 mM KCL), 3.0 mM MgCl2, and 3 units of Taq polymerase (New England, Biolabs) was prepared for Taq amplification. Amplification was carried out in a thermal cycler (BioRed, USA). Cycling conditions consisted of 3 min at 94 °C for initial denaturation, followed by 1 min at 60 °C for annealing, followed by 35 cycles, and 7 min at 72 °C for final extension to ensure a complete extension of all PCR products.

Anticontamination Measures

Strict precautions to prevent PCR products' contamination were enforced.

Statistical Analysis

Statistical analysis was performed using SPSS version-16. The various parameters studied during the observation period were compared using Chi-square test for noncontinuous variables. The critical value of 'p' indicating the probability of significant difference was taken as <0.05 for comparison.

Observations

Out of the 50 samples of cases and controls, we were able to isolate DNA from 38 cases and 28 controls in blood and in 22 cases and 22 controls in tissue. In cases of carcinoma cervix, p53 expression is either downregulated or absent in 71.06 % of cases compared to 50 % of controls in blood (Table 1); and 72.73 % of cases compared to 59.09 % of controls in tissue (Table 2), but these are not statistically significant ($p = 0.67$ and $p = 0.167$, respectively).

p53 positivity was found only in 27.78 % of squamous cell cancer and in 50 % of adenocarcinoma (Table 3). Three out of nine patients (33.3 %) with L.N. positive status have p53 gene positivity, whereas 23 % (3 out of 13) with L.N. negative status have p53 gene positivity, which is not significantly associated. L.N. positivity indicates poor prognosis (Table 4). In our study, p53 overexpression showed variable increases in different stages of cervical cancer (Table 5).

Discussion

p53 is a tumor suppressor protein and transcription factor present in a latent state in all cells and activated by various stresses such as hypoxia, free radicals, DNA damage, and UV light. Some of the first molecular explanations for the malignant potential of the high-risk HPV types, 16 and 18, came with the observations that their E6 and E7 oncoproteins could interact with the key cellular tumor suppressors, p53 and pRB. p53 is a multifunctional transcriptional modulator and inducer of apoptosis. In response to DNA damage, nucleotide depletion, or hypoxia, it becomes activated by acetylation and phosphorylation, functioning as a nuclear transcription factor to induce genes involved in cell-cycle inhibition or apoptosis, or it can induce apoptosis more directly by interacting with proteins in the cytoplasm at mitochondrial sites. Similarly, pRB is also a key regulator, interaction of which with the members of the E2F family of transcription factors regulates both cell-cycle progression and apoptosis. Both p53 and pRB mutations are common in many types of cancer, but occur very rarely in early-stage cervical cancers, inviting the speculation that their functional abrogations by the E6 and E7 oncoproteins from high-risk mucosal HPVs are functionally equivalent to mutations in other cancer types to some extent. The E7 proteins encoded by the high-risk type HPVs, such as HPV 16 and HPV 18, bind Rb with a much higher affinity compared to those encoded by the low-risk type HPVs, such as HPV 6 and HPV 11. E7 disrupts the interaction between Rb and E2F, resulting in the release of E2F factors in their transcriptionally active forms. The E7-mediated conversion of E2Fs to their activator forms stimulates replication and cell division [3]. Subsequently, it was shown that high-risk, but not low-risk, E7 could induce the proteasome-mediated degradation of pRB.

The present study of p53 gene regulation analyzed the expression of 279-bp bands on 1.5 agarose gel as shown in Fig. 1. The p53 activity was analyzed in terms of expressions in three different forms: 1. complete disappearance (null) of 279 bands, 2. overexpression, or 3. regression (underexpression) compared with controls. Interestingly, in our study, we found that there is the increased frequency of

Table 1 p53 gene expressions in blood in cervical cancer cases and controls

Expression	Case ($n = 38$)		Control ($n = 28$)	
	No.	%	No.	%
Up regulation	11	28.94	14	50.0
Down regulation	14	36.84	9	32.15
Null (absent)	13	34.22	5	17.85
Total	38	100	28	100

$\chi^2 = 3.569$, $p = 0.67$

Table 2 p53 gene expressions in tissue in cervical cancer cases and controls

Expression	Case ($n = 22$)		Control ($n = 22$)	
	No.	%	No.	%
Up regulation	6	27.27	9	40.91
Down regulation	9	40.91	11	50.00
Null (absent)	7	31.82	2	9.09
Total	22	100	22	100

$\chi^{22} = 3.577$, $p = 0.167$

Table 3 p53 gene expressions in tissue in cervical cancer cases and their correlation to histopathology

Histopathology	Case ($n = 22$)		p53 positive		p53 negative	
	No.	%	No.	%	No.	%
Squamous cell cancer	18	81.8	5	27.78	13	72.22
Adenocarcinoma	2	9.1	1	50	1	50
Adenosquamous	2	9.1	0	0	2	100
Total	22	100	6	27.27	16	72.7

$\chi^{2} = 1.273$, $p = 0.529$

Table 4 p53 gene expressions in tissue in cervical cancer cases and their correlation to lymph node status

Histopathology	Lymph node mets			
	Positive ($n = 9$)		Negative ($n = 13$)	
	p53 positivity	p53 negative	p53 positive	p53 negative
Squamous cell cancer ($n = 18$)	2	6	3	7
Adenocarcinoma ($n = 2$)	1	0	0	1
Adenosquamous ($n = 2$)	0	0	0	2

Table 5 p53 gene overexpressions with respect to stages of carcinoma cervix

Stages	Cases (n)	p53 overexpression	Percentage
Stage I	5	1	20
Stage II	10	2	20
Stage III	4	1	25
Stage IV	3	2	66
Total	22	6	27.27

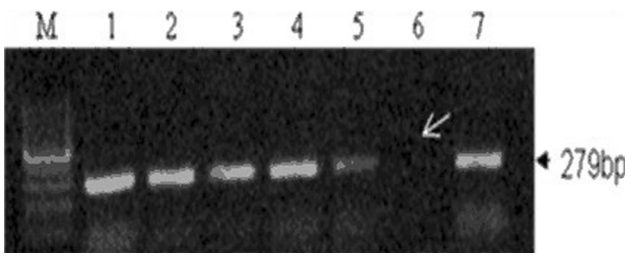

Fig. 1 PCR based DNA analysis of p53 gene showing overexpression in lane 1, underexpression in lane 5 and complete disappearance of 279bp amplicon in lane 6 in carcinoma cervix

upregulation or overexpression of p53 gene in control both in blood (50 %) and tissue samples (40.9 %), but this association is statistically nonsignificant. These observations had the possibility of a predisposive effect due to the small sample size of this study.

Hunt et al. study [4] showed p53 gene product was overexpressed in 17.1 % (14/82) of all carcinomas and also in areas of cervical intraepithelial neoplasia grade III adjacent to invasive squamous carcinoma. Inactivation of wild-type p53 gene product represents the most common genetic alteration in human carcinogenesis, and its overexpression, as detected by immunohistochemistry, has been proposed to indicate a worsened prognosis in some malignancies [5, 6]. Loss of p53 function is believed to play an important role in the pathogenesis of carcinomas of the uterine cervix, where it has been shown that the p53 gene product may be inactivated in three principal ways: (1) after somatic point mutation; (2) after loss of heterozygosity; and (3) following human papillomavirus (HPV) infection, as the viral oncoprotein E6 is known to bind to, stabilize, and ultimately degrade wild-type p53.

In the present study, p53 upregulation was found in 28.94 % of cases in blood and 27.27 % of tissue. p53 overexpression was present in 25.5 % of cases in Oka et al.'s study [7], 33.6 % in Hanprasertpong J et al.'s study [8], and 14.0 % in Busby-Earle et al.'s study [9].

p53 Expression and Histopathology of Cervical Cancer

In our study, p53 positivity was present in 27.78 % of cases with squamous cell cancer ($n = 18$), in 50 % of cases with adenocarcinoma ($n = 2$), and 0 % of cases with adenosquamous carcinoma ($n = 2$). The results are different from Hunt et al.'s [4] study in which p53 positivity was present in 22.7 % of cases with squamous cell cancer ($n = 22$), in 14.3 % of cases with adenocarcinoma ($n = 35$), and 16 % of cases with adenosquamous carcinoma ($n = 25$). Helland et al.'s study [10] showed p53 positivity in 44 % of cases with adenocarcinoma ($n = 9$) and 57 % of cases with adenosquamous carcinoma ($n = 7$). In Holm et al.'s study [11] and Bosari et al.'s [12] study, rates of positivity were 11 % ($n = 36$) and 100 % ($n = 1$), respectively, in adenocarcinoma.

Lymph node status helps in predicting the prognosis of carcinoma of cervix. Our study tried to correlate the positivity of p53 gene with lymph node status. 33.3 % with L.N. positive status , whereas 23 % with L.N. negative status have p53 gene positivity, which is not significant. Hunt et al.'s study [4] showed the corresponding figures as 33 and 36 %, respectively. There was no relation between p53 overexpression and prognosis in cervical cancer [4, 8]. The present study shows lack of relationship between p53 overexpression and prognosis in the cervical cancer patients.

At various stages, p53 overexpression increased from 20.1 % in stage IB to 60 % in stage IV, according to Bremer et al. [13]. In our study also, p53 overexpressions showed variable increases with different stages, i.e., 20 % in both stage I and stage II, 25 % in stage III, and 66 % in stage IV, but were not statistically significant.

Conclusion

Interestingly, In our study, we found that frequency of upregulation or overexpression of p53 gene increased in control in both blood (50 %) and tissue (40.9 %). Although this association is statistically nonsignificant, this study's findings will, however, help in meta-analysis studies done on p53 gene expression in carcinoma cervix. The study had the possibility of a predisposive effect due to its small sample size or unknown environmental factor. In most of the earlier studies on cervical cancers, the function of p53 is downregulated and perhaps failed to regulate the process of carcinogenesis. The present study shows lack of relationship between p53 overexpression and prognosis in the cervical cancer patients. Traditional parameters such as tumor size and lymph node status still seem to be the most important determinants of prognosis in the carcinoma of the uterine cervix. However, our study lacked larger sample size, which otherwise would have been able to lend support to truly significant findings through much larger combined and comparative datasets.

Compliance with Ethical Standards

Conflict of interest No conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval Institutional ethical clearance was taken. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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