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Original Article

Detection of Human Papilloma Virus in Colorectal Cancer by Polymerase Chain Reaction Technique in A Tertiary Care Hospital of Bangladesh

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Abstract

Background: *Human Papilloma Virus (HPV) infection is associated with the development of several cancers, including oral, esophageal, skin, lung, and uterine cervix. Association of HPV and colorectal cancers remains controversial. The aim of this study was to evaluate the association between HPV infection and colorectal cancer in Chattogram of Bangladesh.*

Materials and methods: *The study was carried out in the Department of Pathology, Chattogram Medical College, Bangladesh over a period of one year from July, 2013 to June, 2014. A total of 60 cases were included in the study during the time period. Histopathological type and grading was done. HPV type 16 and 18 DNA was examined from all samples by means of Real Time PCR using type specific primer named E7 protein. Finally association between HPV types 16/18 with colorectal carcinoma was assessed by Chi-square (c2) test.*

Results: *The mean age of 60 patients was 45.35 years (Range 17-75 years). Rectum was the common site of tumour 26 (43.3%). HPV type 16 and 18 was detected in 25 (41.7%) cases, out of whom 24 (96%) were associated with HPV16, and only one case was both HPV type 16 and 18. As regarding histological type, out of 25 cases 21 (84%) cases of adenocarcinoma, 3 (12%) case of signet ring carcinoma and only 1 (1.6%) case of mucinous carcinoma were positive for HPV.*

Conclusion: *HPV specially subtype 16, may play a role in the oncogenesis of Colorectal Carcinoma.*

Key words : Colorectal cancer; PCR technique; HPV.

INTRODUCTION

Colorectal Cancer (CRC) also called colon cancer or large bowel cancer includes cancerous growths in the colon, rectum and appendix[1]. It usually develops slowly over a period of 10 to 15 years. Patients with ulcerative colitis are at a 4.3 to 30-fold increased relative risk compared with the general population[2]. CRC is the third most common cancer in men (663,000 cases, 10.0% of the total cancers) and the second in women (570,000 cases, 9.4% of the total cases) worldwide in 2008[3]. It is more common in developed countries exhibiting Westernized lifestyle practices. High risk areas are considered North America, Europe and Australia. The developed world accounts for over 63 % of all cases. The incidence is determined largely by environmental exposure. The incidence is consistently higher among urban residents[4].

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There are more than 100 types of HPV, and about 40 of these types are known to infect genital epithelial cells[5]. At least 15 HPV types associated with malignancy of both genital tract and non-genital tract have been categorized as High Risk types (HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 59, 66, 68, 69, 73 and 82). In India, HPV type 16 alone in cervical cancer is 70-90 per cent while occurrence of HPV type 18 varies from 3 to 20 per cent[6].

The HPV viral oncogenes E6 and E7 have shown to be the main contributors to the development of HPV induced cancers. The HPV oncoprotein E7 is known to bind and inactivate hypophosphorylated retinoblastoma protein (pRB) which eventually leads to up regulation of p16INK4A. P16INK4A is a tumour suppressor protein that inhibits cyclindependant kinases (CDK)-4 or -6 binding to cyclin D, which regulates the G1 cell cycle checkpoints. E6 protein is known to functionally inactivate p53. By binding, it causes rapid degradation of p53. Thus p53 acts as a checkpoint control factor at the G1/S-phase of the cell cycle[7]. Inverse correlation between p53 mutations and HPV infection suggested that p53 inactivation caused by HPV infection play a role in the pathogenesis of colon cancer[8].

MATERIALS AND METHODS

This cross-sectional descriptive type of study was carried out in the Department of Pathology, Chattogram Medical College (CMC) Bangladesh over a period of one year from 1st July, 2013 to 30th June, 2014. By purposive sampling technique 60 specimens (Resected/ biopsy) of colon or rectum comprised the study population. Protocol was reviewed and approved by the Ethical review Committee of CMC. All the patients included in the study were informed of and explained about the nature of study, its purpose and risk benefits. Informed written consent was taken from each patient before data collection. Histomorphological diagnosis done from Hematoxylin and Eosin (H & E) stained section of paraffin embedded block. Histopathological types were done and tumour grading (Well, moderately, poorly differentiated or undifferentiated) was done according to Sobin et al[9]. HPV type 16 and 18 DNA was examined from all samples by means of Real Time PCR using type specific primer in Chevron Clinical Laboratory, Chattogram. It divided in two procedures: extraction and amplification.

A) DNA Extraction

Three to five sections in 6 µm thickness were cut from the paraffin blocks containing representative tissue in microtome. Then the material was scraped with a sterile needle and transferred to eppendorf. The microtome blade was changed between cases to avoid contamination. Then following six steps were done

i) **Remove Paraffin:** Paraffin was dissolved in xylene and removed

ii) **Lyses:** Sample was lysed under denaturing conditions with proteinase K

iii) **Heat:** Incubation was done at 90^0 C reverses formalin cross-linking

iv) **Bind:** DNA binds to the membrane and contaminants flow through

v) **Wash:** Residual contaminants were washed away

vi) **Elute:** Pure, concentrated DNA was eluted from the membrane.

B) Real Time Amplification

HPV type 16 and 18 were amplified and detected by mean of Real Time PCR machine named Rotor Gene 3000/6000/Q (Corbett research Qiagen) using type specific primer (Amplification kit of Sacace biotechnologies, Italy). The E7 region of the HPV genome was chosen to prepare the primers. Sample was transferred into the Real Time Thermal Cycler. It denatured for 15 minutes at 95^0 c temperature and subjected to 45 cycles of amplification. A cycle represents primer annealing and extension for 30 sec at 60^0 c temperature and denaturation for 15 sec at 95^0 c temperature. Amplified DNA was visible by ultraviolet light after staining with ethidium bromide within Real Time Thermal Cycler. The results were analyzed with the software of instrument through the presence of crossing of fluorescence curve with the threshold line. Internal Control (Human DNA) was detected on the Yellow channel; HPV 16 was detected on the Green channel and HPV 18 on Orange channel. Data were analyzed by Computer using SPSS version 15 software. The association between HPV types 16/18 with colorectal carcinoma was analyzed using Pearson's Chi square test (χ^2) test. Association between tumour site and histopathological grading with HPV type 16/18 were also tested by Chi square test.

RESULTS

The age range of 60 patients was 17 to 75 years with mean age was 45.35 years (SD ± 14.15). It was revealed from the study that majority of the patients 16 (26.7%) were in age group 41-50 years. Another important age group was 31-40 and 51-60 years that constituted 13 (21.7%) of total patients. 33 (55%) patients were male and 27 (45%) patients were female and male to female ratio was 1.2:1 (Table I).

Out of 60 patients HPV were detected in 25 cases. HPV types 16 positive were in 24 (96%) cases and only one case was both 16 and 18 positive. Out of 33 male patients, 14(23.3%) cases were positive for HPV. Rectum was involved in majority of the (48%) patients. 32% patients had sigmoid colon involvement, 12% patients showed caecum and 4% patients had ascending and transverse colon involvement each. As regarding histopathological type, out of 25 cases, 21(84%) cases of adenocarcinoma, 3 (12%) cases of signet ring carcinoma and only 1(1.6%) cases of mucinous carcinoma were positive for HPV. All SCC were negative for HPV type 16/18.

Table I : Relationship between HPV infection and parameter of colorectal carcinoma patients

		HPV type 16 & 18				Total	%
		Positive		Negative			
	Years	n	%	n	%		
Age	<20	1	4.0	1	2.7	2	3.3
	21-30	3	12.0	7	20.0	10	16.6
	31-40	4	16.0	9	25.9	13	21.7
	41-50	9	36.0	7	20.0	16	26.7
	51-60	6	24.0	7	20.0	13	21.7
	61-70	1	4.0	2	5.7	3	5.0
	71 & above	1	4.0	2	5.7	3	5.0
Sex	Male	14	23.3	19	31.7	33	55.0
	Female	11	18.3	16	26.7	27	45.0
Site	Caecum	3	12.0	3	8.6	6	10.0
	Ascending colon	1	4.0	3	8.6	4	6.7
	Transverse colon	1	4.0	2	5.7	3	5.0
	Descending colon	0	0.0	2	5.7	2	3.3
	Sigmoid colon	8	32.0	11	31.4	19	31.7
	Rectum	12	48.0	14	40.0	26	43.3
Type	Adenocarcinoma	21	84	27	77	48	80.0
	Signet ring cell carcinoma	3	12	4	12	7	11.6
	Mucinous carcinoma	1	4	2	5.5	3	5.0
	SCC	0	0	2	5.5	2	3.4
Grade	Grade-1	17	47	19	53	36	60.0
	Grade-2	2	25	6	75	8	14.0
	Grade-3	6	38	10	62	16	26.0

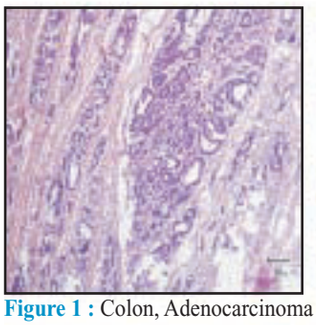

Figure 1 : Colon, Adenocarcinoma Grade-1 x 100 (H& E stain)

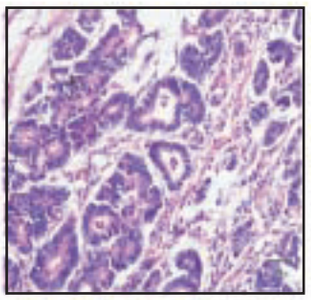

Figure 2 : Colon, Adenocarcinoma Grade-1 x400 (H& E stain)

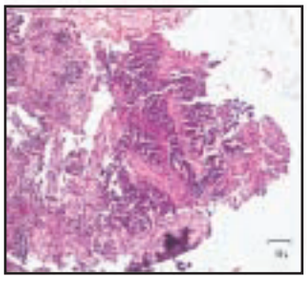

Figure 3 : Rectum Adenocarcinoma Grade-2 x 100 (H& E stain)

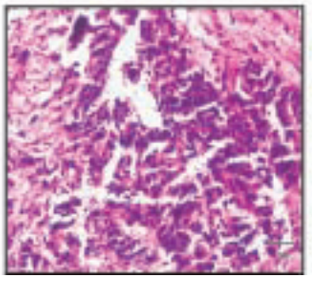

Figure 4 : Rectum Adenocarcinoma Grade-2 x 400 (H& E stain)

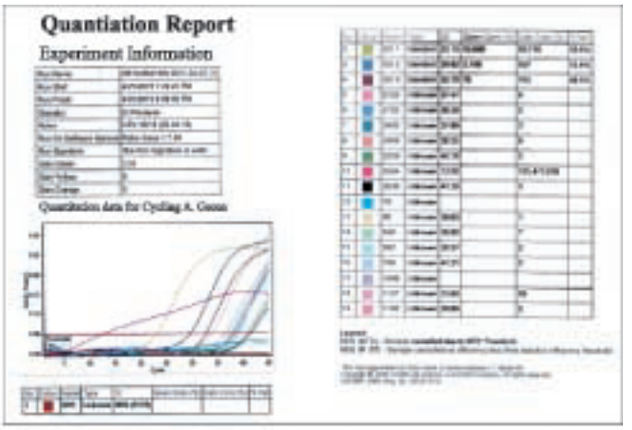

Figure 5 : PCR amplification of HPV type 16 by real time

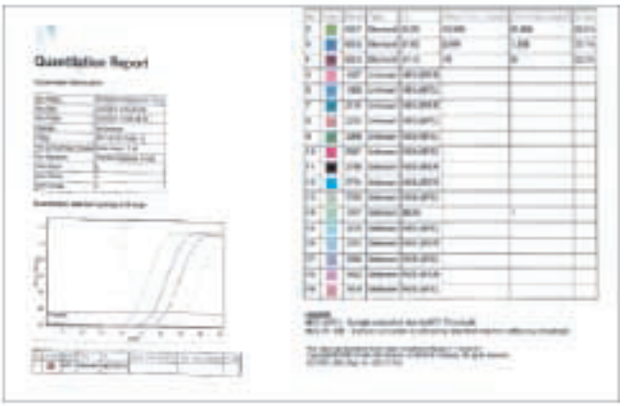

Figure 6 : PCR amplification of HPV type 18 by real time

DISCUSSION

The age range of 60 patients was 17 to 75 yrs. Mean age was 45.35 yrs (SD ±10.57) similar to study conducted by Doosti et al, where mean age of the patients were 48.56 yrs. Among HPV positive cases mean age was 46.04 yrs[1]. Deschoolmeester et al showed mean ages was 56 years in HPV positive cases[7]. This may be due to more age range (30-88 years) in comparison to my study.

Regarding sex, out of 60 patients, 33 (55%) cases were male and 27 (45%) cases were females. Male to female ratio was 1.2:1. Doosti et al showed male-female ratio was 1.1:1 which consistent with this study[1]. Overall, CRC incidence and mortality rates are about 35% to 40% higher in men than in women. The reasons for this are not completely understood, but likely reflect complex interactions between gender-related differences in exposure to hormones and risk factors. In our study, out of 25 HPV 16 and 18 infected patients, 14(56%) were male and 11 (44%) were female with male to female ratio was 1.3:1.

Only 2(3.3%) patient had habit of anal sex. Sexual activity has been considered to be a major route of transmission for HPV. There is evidence of HPV infections in infants and female university students who are virgins, revealing that HPV transmission via other routes than sexual intercourse may exist. In addition, Peripheral Blood Lymphocytes (PBLs) from healthy donors have been shown to be infected with HPV.

Therefore, it has been suggested that HPV infection in internal organ tissues might occur through blood circulation[10]. Gillison and Shah indicated that HPV associated malignancies would occur at anatomic sub sites of exposure by direct contact, since there is no viremic phase in the pathogenesis of HPV, so the infection is not widely disseminated in the body[11]. Bodaghi et al reported that HPV infection in the tumour tissues which obtained from the caecum and ascending colon is as common as in the tissues obtained from rectosigmoid locations and this indicated that the HPV infection might not be a result of the direct spread from anogenital sites[12]. Chen et al also found that the transmission of the HPV to the colorectal tissues might occur through blood circulation[10]. Since higher frequency of HPV infection in caecal and ascending colonic tissues has been determined in compare to frequency of infection in rectosigmoid tissues, this study supports the hypothesis that investigated HPV infection in colon and rectum might not occurs through direct infection[13].

HPV16 was the most frequent type in CRC in this study (24 out of 25 positive samples, 96%). Damin et al found HPV- 16 as the most frequently detected HPV type in patients with CRC 68.3% (41 out of 60 cases) 73% (8 out of 11 cases) respectively[10,14]. But Lee et al showed high frequency HPV18 infection in patients with CRC in Taiwan (84% 16 out of 19 cases, 90% 19 out of 21 cases)[15,16]. However Cheng et al have shown that HPV16 infection is common in Taiwanese patients with CRC (70% 26 out of 37 cases, 81.5% 31 out of 38 cases, 100% 41 out of 41 cases respectively)[12,17,18]. We found HPV18 infection was much less prevalent in our patients (Present in only 1 of 25 samples, 4%) one of whom had dual infection with HPV16 and 18.

Rectum was involved in majority of the cases, i.e 12 (48%) patients. It was found 8 (32%) patients had sigmoid colon involvement, 3 (12%) patients showed caecum and 1(4%) patient had ascending and transverse colon involvement each. Similarly Miasko et al showed rectum was the most common than caecum, 15 (48%) cases and 7 (39%) cases respectively[7,14]. But study showed rates of viral detection were similar (30.3%) in tissues taken from the proximal colon, the distal colon or the rectum[3].

Out of 60 cases 48 cases showed adenocarcinoma, 7 cases signet ring carcinoma, 3 cases mucinous carcinoma and 2 casess Squamous Cell Carcinoma (SCC). Mi sko et al showed most common CRC is adenocarcinoma (95%) remaining 5% include SCC, mixed carcinoma (Adenocarcinoma +SCC) and undifferentiated carcinoma[19]. Out of 25 HPV positive cases 21 (84%) cases of adenocarcinoma, 3 (12%) cases of signet ring carcinoma and 1 (4%) cases of mucinous carcinoma were positive for HPV. All SCC were negative for HPV. But presence of HPV was detected also in SCC. Bognar et al detected HPV16 in sigmoid SCC in 94-year-old women[20]. Kong et al described 3 cases of SCC (2 invasive rectal tumours, 1 colonic tumour in situ) in which HPV 16 was also detected[21]. The tumours were collected from women aged 48, 53 and 49 years. Matsuda et al presented a case of 55-year-old, HIV-positive man, in whom presence of HPV was determined in rectal SCC[22]. Also, Sotlar et al confirmed presence of HPV in SCC in 87-year-old man[23]. In this study we didn't find any significant relationship between HPV infections with SCC. Because number of SCC patient was too small.

CONCLUSION

In conclusion we were able to document the presence of HPV DNA type 16 & 18 in CRC (41.6%). HPV DNA type 16 is more frequent type (96% cases). We also found that colorectal HPV18 infection was much less frequent and present in only 1 of 25 samples (4%) of which had dual infection with HPV16 and 18. This strengthens the theory that HPV subtype 16, which previously had been identified in squamous lesions of the female genital tract, and oral cavity may play a role in the oncogenesis of CRC.

DISCLOSURE

All the authors declared no competing interest.

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