

## Relationship between Human Papilloma Virus and Colorectal Cancer in Northern Iran

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### Abstract

**Background:** Colorectal cancer is one of the most common malignancies worldwide with more than one million new cases. According to the Ministry of Health and Medical Education of Iran, colorectal cancer is the third most common cancer in Iran. Many risk factors are known causes of this disease. However, the molecular mechanisms associated with colorectal cancer are still under investigation. Recent studies have shown that some viruses, particularly human papilloma virus, may be associated with the pathology of colorectal cancer.

**Methods:** This case-control study examined 95 colorectal cancer and 95 normal colon tissue paraffin blocks (control) to identify the relationship between human papilloma virus and colorectal cancer by polymerase chain reaction.

**Results:** Clinicopathological data that included sex, age, tumor grade, stage and location were recorded. All tumor and control groups (totally: 190 samples) were negative in terms of the human papilloma virus genome. No relationship between clinicopathological data and human papilloma virus genome was identified.

**Conclusions:** Regardless of other risk factors for colorectal cancer, a number of studies in different parts of the world have shown that human papilloma virus may be an important factor in the increasing incidence of colorectal cancer. However, we have found no association between human papilloma virus and colorectal cancer in this study.

**Keywords:** Colorectal cancer, Human papilloma virus, PCR

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### Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide. According to the Ministry of Health and Medical Education of

Iran, CRC is the third most common cancer in men and second in women.<sup>1-3</sup> Risk factors such as sedentary lifestyle, obesity, high body and abdominal fat, hormone

replacement therapy, smoking, alcohol, and inflammatory bowel diseases are known causes. In addition, genetic predisposition has recently been introduced. However, the molecular mechanisms associated with CRC are still under investigation.<sup>4,5</sup> Researchers have shown that a few viruses, particularly human papilloma virus (HPV), may be associated with the pathology of CRC.<sup>6-10</sup>

HPV is a DNA virus whose role in the etiology of cervical and anogenital cancers is well known.<sup>11-13</sup> More than 100 different types of this virus have been isolated from various human samples thus far which can cause benign (wart) and malignant tumors (cervical cancer).<sup>14</sup> Among these, types 16 and 18 have been classified as high risk viruses for cervical cancer. Today, these two are considered as human carcinogens and separated from 7.99% of cervical cancers.<sup>15</sup> Virus types 6 and 11 are associated with benign or subclinical diseases and considered low-risk viruses.<sup>16,17</sup>

The most common types, HPV 16 and 18, are associated with anogenital malignancy. They are the most common types that can be detected in other types of cancer.<sup>18-22</sup> In this regard, researchers have detected HPV DNA in colorectal adenocarcinomas by in situ hybridization or PCR,<sup>7,11</sup> whereas others have been unable to demonstrate the presence of HPV in CRC.<sup>23-26</sup> As far as viral infection in cancer etiology is concerned, the relation of HPV with CRC carcinogenesis can have a significant impact on patient care. However conflicting results have been reported. This study attempted to identify an association of HPV with CRC by polymerase chain reaction (PCR).

## Materials and Methods

This case-control study examined the relationship between HPV and CRC, and HPV genotype by PCR. Inclusion and exclusion criteria for the case group were CRC patients without age restrictions and those with incomplete file information. Inclusion and exclusion criteria for the control group were patients with normal

colorectal tissue with no age restrictions and those with normal colorectal tissue with no previous history of CRC. Paraffin blocks of CRC specimens were obtained from the Pathology Department at Imam Khomeini Hospital, Sari, Iran. We prepared 5-7  $\mu\text{m}$  sections which were subsequently maintained in  $-80^{\circ}\text{C}$  refrigerators. After paraffin removal by the xylene-ethanol method, the following procedure was performed.

### *DNA extraction*

We used commercial kits according to instructions provided by the manufacturer. Samples were purified with an Invitex<sup>®</sup> Spin DNA Mini Kit (Invitex Company, Germany). The extracted DNA was maintained at  $-20^{\circ}\text{C}$ . By using tissue lysis buffer, the extraction was prepared according to the manufacturer's instructions.<sup>10</sup>

Extracted DNA could become severely crushed, hence amplification of human  $\beta$ -globin gene fragments with PC03, PC04 and PC04, GP20 primers were used to qualify and evaluate the physical condition of the extracted DNA, as quality control analysis.

### *Polymerase chain reaction (PCR) for HPV screening (PGMY)*

We performed a search operation according to the global network of papilloma virus - World Health Organization. We used the FastStart PCR Master kit (Roche Company, Germany). The sense and antisense primer sets were used to generate a mixture of 5  $\mu\text{mol}$  in a volume of 50  $\mu\text{l}$  and maintained at  $-20^{\circ}\text{C}$ . The PCR products of the PGMY primers were approximately 450 base pairs in size. Distilled water and HPV-16 were the negative and positive controls, respectively. The PCR process was carried out in 45 cycles that consisted of three programs. Electrophoresis of PCR products was performed in a 1.5% agarose gel and examined with UV Pyrotechnic devices.

### *Statistical analysis*

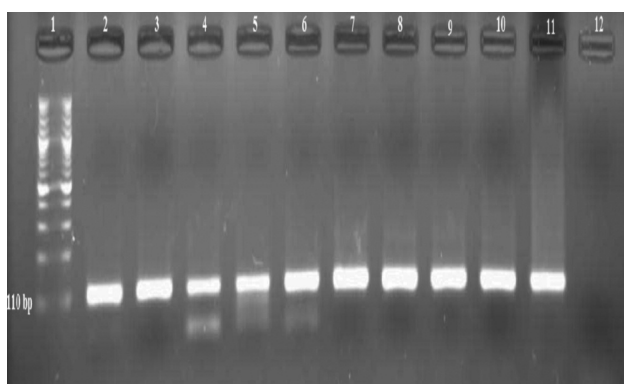
Descriptive and analytical data were analyzed by SPSS 16.0. For quantitative variables between the two groups (case and control), we used the t-

test; for qualitative variables, the chi-square test was used. For the odds ratios, we used the logistic regression model. For the central tendency and dispersion indicators, descriptive statistics were used.

## Results

All 190 CRC paraffin blocks in the case and control groups equally underwent DNA extraction and were investigated in terms of HPV genome by PCR. Amplification of the human  $\beta$ -globin gene fragments in both groups was used to qualify the extracted DNA assay (Figure 1). In the case group, 92 samples were positive for  $\beta$ -globin and 3 samples were negative for  $\beta$ -globin. In the control group, 94 samples were positive for  $\beta$ -globin and 1 sample was negative for  $\beta$ -globin. All case and control samples were negative for the HPV genome (Figure 2).

There were 55 (57.9%) men and 40 (42.1 %) women (in case group). A total of 53 patients were over 60 years of age and 42 were less than 60 years of age. The mean age was  $60.82 \pm 1.52$  years. Most tumors were grade I (51.6%), stage II (43.2%) and located in the rectosigmoid (48.4%). There was no significant association between clinicopathological data in terms of sex, age, tumor grade, stage and location (the colorectal region was considered as the same location) to HPV DNA. There was no HPV DNA discovered, hence we were not able to make a comparison of clinicopathological data.



**Figure 1.** PCR results for  $\beta$ -globin.; Lane 1: Marker Lanes 2-10: Samples Lane 11: Positive control Lane 12: Negative control

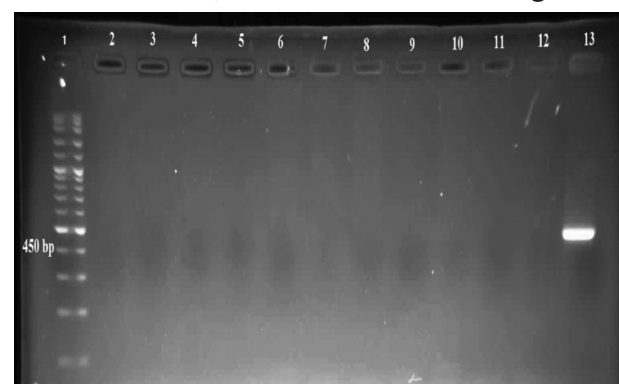
## Discussion

Cancer is the third most common cause of death in Iran after death by accident and cardiovascular disease.<sup>27,28</sup> Each year approximately 51000 new cases of cancer are diagnosed. In Iran, about 35000 deaths are cancer-related of which 38% originate from the digestive system.<sup>1-3</sup> Many risk factors are known causes of CRC and include sedentary lifestyle, obesity, high body and abdominal fat, hormone replacement therapy, tobacco smoking and viral infections.<sup>4</sup>

Some studies reported detection of oncogenic HPV DNA in a number of colorectal carcinomas.<sup>29,30,31</sup> A few studies showed low positivity of a high risk HPV (HPV-16 DNA).<sup>31</sup> However, others reported no HPV DNA in colorectal tumors.<sup>29</sup> HPV was reported in other tumors, such as lung and skin cancers.<sup>27,28</sup>

Recent studies have shown that some viruses, particularly HPV, may be associated with the pathology of CRC.<sup>7,11</sup> We examined 95 normal colorectal and 95 CRC tissues for the presence of HPV DNA by PCR. According to our results, HPV DNA was extracted.

In support of the current study results, Gornick et al. analyzed 279 CRC and 30 normal samples from three different countries for detection of 37 HPV types. All samples were negative for all HPV types. Thus, they concluded that infection with carcinogenic HPV types had no significant association with CRC.<sup>25</sup> The presence of HPV in CRC was negative in other studies both outside<sup>23,24</sup> and inside Iran,<sup>32,33</sup> however a low degree of



**Figure 2.** PCR electrophoresis for HPV (PGMY); Lane 1: Marker Lanes 2-11: Samples Lane 12: Negative control Lane 13: Positive control

HPV DNA in CRCs was reported by Meshkat et al. (1%) and Ranjbar et al. (6.25%) in Iran.<sup>34,35</sup>

However HPV positivity in CRC was reported in Brazil (83.3%), Turkey (81.2%), Argentina (74%) and America (51%).<sup>10,26,27,35</sup> Likewise, an analysis of five case-control studies showed an increase in CRC risk with HPV positivity.<sup>33</sup>

Medically speaking, the frequency of HPV-positive CRC is about 43% worldwide.<sup>36</sup> It seems that there are several reasons for discrepancies in reported viral prevalence of this virus that include geographical variation, cultural, religious and economic differences in different communities as well as insufficient numbers of samples examined in studies. Other reasons for contradictory results in different studies include previous contamination of patients by HPV and tropism of this virus to tumor cells. In our study, we have examined as many as qualified CRC specimens in our national zone (Northern Iran), followed the commercial kit instructions and observed accurate quality control in different phases in an attempt to decrease the possibility of false negative results.

## Conclusion

Colorectal cancer is one of the most common malignancies worldwide. Hence numerous risk factors exist which include viral infections such as HPV. We have studied the HPV genome in CRC tissues in Iran. We reported no association between HPV and CRC. Due to the contradictory results from different studies, research on HPV and its association with CRC is at the beginning and more complete studies are needed. Additional samples, simultaneous investigation of CRC (or rectal itself) and anogenital tumors, HPV and its tumor creator mechanisms in patients with CRC, and the use of other cytogenic methods can be recommended.

## Acknowledgment

We would like to express our appreciation to Dr. Kamyar Khodadadi who reviewed the manuscript and assisted us in reaching our goal. Mazandaran University provided us with financial support.

## Conflict of Interest

No conflict of interest is declared.

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