Detection of human papillomavirus infection by molecular tests and its relation to colonic polyps and colorectal cancer

Faten Gazzaz, MD, PhD, Mahmoud H. Mosli, MD, MSc, Hani Jawa, MD, Abdulrahman Sibiany, MD.

ABSTRACT

The objectives: To prospectively examine the association between human papilloma virus (HPV) colonization of the colonic mucosa and the development of colorectal polyps (CRPs) and colorectal cancer (CRC) in Saudi Arabia.

Methods: A case control study was performed between January 2013 and December 2014. All eligible patients underwent standard diagnostic colonoscopy. Patients with polyps or colorectal cancer were considered cases, while those with any other endoscopic findings were controls. Biopsy samples from polyps and tumors, and/or from normal colonic mucosa were acquired. Human papilloma virus colonization was detected using a hybrid capture technique of samples taken from both normal tissue, and CRPs and CRC. The association between HPV and CRPs/CRC was evaluated.

Results: A total of 132 patients were recruited. The mean age was 53 (±15.9) years. Sixty patients had endoscopically detectable CRPs/CRC, and 72 had either inflammation or normal endoscopic evaluations. Only 4 (0.8%) of the 132 samples that were collected and analyzed were positive for the HPV gene. Statistical analysis did not identify any significant association between HPV colonization and the presence of CRPs/CRC. The only significant predictor of detecting CRPs/CRC on colonoscopy was symptomatic presentation (odds ratio=11.072, 95% confidence interval 4.7-26.2, p<0.001).

Conclusion: Human papilloma virus colonic colonization is rare in Saudi Arabia. An association between HPV colonization and CRP/CRC development could not be identified in this cohort of patients.

Colorectal cancer (CRC) is one of the most common malignancies worldwide, and one of the leading cancers in the Saudi population. Human papillomavirus (HPV) infection has been associated...
with benign (warts)² and malignant (pre-cancer and cancer) genitals,⁴ perianal,⁹ and oral lesions¹⁰ as well as with rectal cancer through large cohort and case-control studies.¹¹⁻¹⁵ Preventive measures such as mass vaccination campaigns have reduced the incidence of HPV-related genital lesions.¹⁶,¹⁷ However, the role of HPV in the pathogenesis of colorectal polyps (CRPs) and adenomas is still undetermined despite some evidence that suggest an association between HPV and CRC exists.¹⁸,¹⁹ Based on the well-recognized etiologic role of HPV in cervical, ano-genital, and oro-pharyngeal carcinogenesis, a possible role of HPV 16/18 in the pathogenesis of colon cancers and polyps has been proposed, and western publications have estimated that 132 samples would be needed to detect HPV colonization in normal colonic mucosa may contribute to the development of CRC.²⁰,²¹ Other studies have contradicted this hypothesis.²² Such an association if proven may largely influence the development of preventive strategies against CRC, such as mass vaccinations and patient education campaigns. The aim of this study is to prospectively examine the association between HPV colonization of the colonic mucosa and the development of CRP and cancer in the Kingdom of Saudi Arabia.

**Methods.** We performed a prospective case control study involving 132 adult patients, who were recruited between January 2013 and December 2014. Included patients were those referred to the Endoscopy Unit, King Abdulaziz University Hospital (KAUH), Jeddah, Saudi Arabia for standard diagnostic flexible sigmoidoscopy or ileo-colonoscopy to investigate lower gastrointestinal symptoms or to undergo screening colonoscopy for primary or secondary prevention of CRC. Diagnosis of CRP or CRC was based on typical histological criteria. The only inclusion criteria was age from 18-85 years and the only exclusion criteria was patient refusal to consent to enrollment in the study. All patients were investigated with either colonoscopy or flexible sigmoidoscopy and patients unable to perform the diagnostic procedure were excluded.

Biopsy samples were collected from each patient recruited at the time of the endoscopic evaluation. Samples were taken from any polyp or mass found in any colonic segment and/or a single sample was retrieved from normal colonic mucosa if no abnormalities were detected. The board certified gastroenterologists and colorectal surgeons who performed the endoscopic evaluations for study participants were blinded to the results of HPV detection. Similarly, the laboratory personnel who received the colonic samples were blinded to the endoscopic reports. Clinical and demographic data were collected prior to the procedure.

**Outcomes.** The primary outcome of this study was the detection of HPV colonization in normal colonic mucosa and CRP/CRC lesions.

**Statistical analysis.** Data was collected and entered into a standard data entry sheet. Subsequently, data was cleaned and prepared for analysis. Descriptive statistics were expressed as mean±SD for continuous variables and as proportions for categorical variables. Student t-test or Mann-Whitney U test was used to compare means, and Chi-square or Fishers exact test was used for comparisons between frequencies, where appropriate. These tests were carried out with the assumption of normal distribution. Otherwise, Welch's t-test for 2 group means was used as an alternative. The presence of CRPs/CRC lesions was identified as the dependent study variable and defined as a binary outcome. Patients presenting with symptoms were considered cases, while asymptomatic patients undergoing screening were controls. A Binary Logistic Regression Model (BLRM), with Backward Conditional Elimination with

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company. This project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Saudi Arabia (Grant No 305/599/1433).
Enter Criteria=0.05 and Elimination=0.10 was used to determine the significant predictors of CRPs/CRC with 95% confidence intervals (95% CI). We used IBM SPSS statistical software, version 22.0 (IBM Corp, Armonk, NY, USA) for statistical analysis with a 2-sided 5% significance level that were used for all statistical inferences and precision of estimates measured using the 95% CI.

Ethical considerations. The King Abdulaziz University Research Ethics Board for research involving human subjects approved this clinical study, and all recruited patients provided written informed consent for participation. The study was conducted in accordance with the principles of the Helsinki Declaration.

Results. Patients characteristics. Baseline characteristics of the study population are summarized in Table 1. One hundred and thirty-two samples were collected. Clinical data was missing for 2 patients. Only 15 (11%) patients had inflammatory bowel disease (IBD).

Primary end points. A total of 132 colorectal biopsies specimens were received in the Virology Laboratory from the Endoscopy Unit. A total of 4 (3%) specimens tested positive for the HPV Gene. Twenty-six patients were found to have polyps (20%) and 34 (26%) had CRC, of which only 2 tested positive for HPV (Table 2).

Statistical analysis did not identify any significant association between HPV colonization of the colon and the presence of CRPs or CRC (Table 3). The only significant predictor of CRPs/CRC was symptomatology.

Discussion. Human papillomavirus infection of epidermal or mucosal epithelial cells causes benign and sometimes malignant neoplasms. Certain types of HPVs such as HPV 16, 18, 31, and 45 have been detected in ano-genital cancers, particularly cancers of the cervix, and anus and are considered high-risk (H-R) genotypes or oncogenic sub-types of HPV. Integration of the viral genome into the cancer cell genome is a characteristic of the infection by these HPVs. Other types of HPV, such as low-risk (L-R) or non-oncogenic HPV6 and HPV11, induce benign ano-genital warts and are rarely found in ano-genital malignancies. The HPV DNA has been detected in tumor tissues of head and neck cancers, oral cancers, esophageal cancers, and skin cancers, as well as lung cancers. Even though HPV DNA has been previously detected in CRC tissues by in situ hybridization and PCR, 18,32,33
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it was not detectable by regular PCR in another study by Snietura et al34 and a survey of HPV16 virus-like particle antibodies in patients with epithelial cancers also failed to provide an association between HPV and CRC.35 Similarly, our results show no association between HPV and CRPs/CRC detection.

Colorectal cancer is largely preventable through intensive, mass screening programs to remove premalignant colonic polyps as proposed by many cohort studies.36-38 Since CRC mostly arises from adenomas, recognized as CRPs, removing such lesions reduces the overall risk. While CRC in advanced and incurable stages often produce clinical findings, premalignant adenomatous polyps and early, highly curable, CRC are often asymptomatic. Cappell39 suggested also that the persistently high incidence and mortality is largely due to ineffective implementation of established screening protocols due to patient fears regarding screening tests, physician under-referral for screening, and test costs. Cappell,39,40 also reported a screening protocol where adenomas or early cancers are difficult to be detected by clinical presentation and provides the rationale for mass screening of asymptomatic adults over 50 years for early detection and prevention of CRC by colonoscopy, which is considered the primary screening test worldwide. All polyps identified during colonoscopy should be removed by endoscopic polypectomy. Endoscopic mucosal resection (EMR) is required for deeply penetrating non-cancerous polyps. According to the American Society guidelines,41 colonoscopy is repeated every 10 years if the index colonoscopy revealed no lesions, but is repeated more frequently if adenomatous polyps were identified at the first colonoscopy due to an increased risk of subsequent CRPs or CRC. Identifying an association between CRC and a potentially preventable infectious agent, such as HPV, may theoretically reduce the incidence of pre-malignant lesions and ultimately the incidence of CRC. However, our results do not support the presence of such an association, at least in patients of this specific cultural background.

Our study is limited by its small sample size and case control design, which has many inherent biases such as selection bias. Future large randomized control studies are needed to further elucidate this association.

In conclusion, HPV colonic colonization appears to be rare (<1%) in Saudi Arabia, which might be influenced by the cultural background. No association between HPV colonization and CRP/CRC could be depicted in this cohort of patients. Based on these results, HPV colonization is not considered a risk factor for CRP/CRC in Saudi Arabia and in countries of similar cultural background.

Acknowledgment. We would like to acknowledge and thank all the nurses and hospital staff who assisted in acquiring the study samples and the Virology Laboratory technical team for their much appreciated professional work that helped us accomplish this project.
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