Assessment of Possible HPV Association in Colorectal Cancer

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Article

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Abstract

Purpose of current study was to investigate HPV role in colorectal cancer development. The human papillomavirus (HPV) is one of the most prevalent sexually transmitted viruses, and its link to cancers of the cervix, penis, vulva, vagina, anus, and oropharynx has been well reported. Persistent infections can lead to premalignant and malignant tumours and HPV infection is responsible for about 4.8 percent of all malignancies worldwide. During this study a total of 135 histopathologically confirmed cancer paraffin embedded biopsy specimens were collected from different hospitals of Hazara division. These specimens were re-examined in histopathology lab for confirmation. Majority of samples were confirmed as Adenocarcinoma in various colon locations, rectal squamous carcinoma and colon adenoma.10 normal biopsy specimens without any abnormality was taken as controls. These specimens were processed in Abbot lab and after treating with liquid nitrogen specimens were subjected to GeneXpert PCR system for the detection of HPV DNA along with high risk genotypes (HPV-16, 18 and 45) of HPV DNA. There was no HPV DNA detection in all control specimens. Out of 135 specimens 85 (62%) were detected with HPV DNA 37 (43%) with HPV-16, 25(29%) with HPV-18 and 16(19%) with HPV-45 genotype. Seven specimens were detected with low risk genotypes

Introduction

The colon and rectum make up the large intestine (or big bowel), which is a part of the gastrointestinal (GI) system. The first portion is known as ascending colon. It all begins with cecum a pouch like structure, which receives undigested food from the small intestine [1]. It extends upward on the right side of the stomach (belly). The second part is called the transverse colon. It runs from the right side of the body to the left. The descending colon is named after the fact that it descends (travels down) on the left side. Because of its "S" form, the fourth portion is termed the sigmoid colon. The rectum connects with the sigmoid colon, which then joins the anus. The ascending and transverse colon sections make up the proximal colon. Distal colon is made up of the descending and sigmoid colons [1]. Human papillomavirus (HPV) infection of epidermal or mucosal epithelial cells results in benign and malignant neoplasms. HPV16, 18, 31, and 45, for example, are frequently discovered in anogenital malignancies, Specifically cervical and anal cancer As a result, they are classified as high-risk or carcinogenic. Infection by these HPVs is characterised by the integration of the viral genome into the cancer cell DNA. Other kinds of HPV, such as HPV6 and HPV11, which are low-risk or non-oncogenic, cause benign anogenital warts and are seldom seen in anogenital malignancies [2, 3]. Human papillomaviruses (HPV) infect stratified epithelium and are tiny double-stranded DNA viruses. There are at least 80 types, separated into those that infect cutaneous surfaces and those that infect mucosal surfaces. These kinds have traditionally been distinguished by sequence divergence however, antibodies to conformational epitopes on the major and minor capsid proteins are revealing that the virions are serologically different. While most viruses generate benign proliferative lesions or warts, a subset of viruses causes premalignant and malignant lesions [4]. Colorectal cancer is now regarded as a prevalent malignancy, and numerous studies have been conducted in this area. In 2011, Lorenzo and colleagues (Lorenzo *et al*.) performed a meta analysis of

various articles on the association between HPV and colorectal cancer that had been published in the last two decades. The researchers discovered a link between HPV and malignancies [5]. Liu et al. investigated the prevalence of HPV in a Chinese population with colorectal cancer. The frequency of HPV DNA identified in tumour tissues was higher than in non-tumor colorectal tissues and peripheral blood samples, according to their findings [6]. Damin *et al.* investigated the effect of HPV infection in colorectal cancer and prognostic variables in a 2007 study. HPV DNA was found to be positive in 60 (83.3%) of the 72 malignant colorectal samples. No HPV DNA was found in any of the noncancerous tissues (Fig. 1), however [7].

Figure 1

Methodology

Sample acquisition

The study was conducted at Abbott Laboratory, a private sector BSL-3 laboratory in Abbottabad city of the Khyber-Pakhtunkhwa province (Pakistan).135 histopathologically confirmed paraffin embedded tissue biopsy specimens were collected which include 65 colon, 25 ascending colon,20 descending,15 rectal polyps,10 rectal biopsies and 10 normal colorectal tissue were collected and included in the study (Fig. 2). The samples came from a histopathology department of Ayub Medical College (Abbottabad) and histopathology department of Abbott Laboratory and other Hospitals of Hazara Division.

Figure 2

Histopathological Examination

Cutting

After labelling each tissue was cut in sections by using microtome (Leica RM 2135) first at 30 microns then at 20, 10 and 5 microns for rough cutting. Thin section will be cut on 2 to 3 microns (Fig. 3). Best sections were put in distilled water and picked with the help of slide (Fig. 5) and after treating with 70% alcohol put in tissue bath at 37 ^{0}C for 1 to 2 minutes (Fig. 4). After the addition of albumin on slide it was warmed at 60 to 65 ^{0}C on slide warmer for 30 minutes. The slide was put in xylene for 10 minutes. Then put in 70% alcohol for 30 minutes, 80% alcohol, 90% and finally in 100% alcohol for 30 minutes.

Figure 3

Figure 4

Figure 5

Hematoxylin and Eosin Staining

After washing with tap water and drying slide was put in Hematoxylin stain for 15 minutes again washed with tape water and dip 2 to 3 times in 1% acid alcohol. After washing again dip 2 to 3 times in 5% Eosin then put in 80% alcohol for 30 minutes, then in 90% and finally in 100% alcohol for 30 minutes. After treating with alcohol slide was put in xylene for 30 minutes (Fig. 6).

Figure 6

Mounting

DPX (Dibutylphthalate Polystyrene Xylene) oil was spread on slide and coverslip was placed on slide and gently pressed to expel trapped air. Now slide was studied under microscope.

Sample Processing for GeneXpert

Each paraffin-embedded specimen was treated with xylol (MERCK, Germany) to remove wax followed by washing with 100% ethanol after labelling (Fig. 7). Each specimen was treated with liquid nitrogen and by using mortar and pestle technique converted in to fine powder (Fig. 8). Each crushed specimen was then added to preserved Cyst solution provided (Fig. 9) after vortexing 1 ml solution was loaded in HPV cartridge and it was placed in GeneXpert system after completion of assay results were obtained.

Figure 7

Figure 8

Figure 9

Results

Histopathological Findings

Out of 65 colon biopsy specimens 35 showed adenocarcinoma of colon (Figure 10 & 11) 25 specimens were with carcinoma and 10 with squamous cell carcinoma. In ascending colon samples 15 were detected with adenocarcinoma, 7 with carcinoma (Figure 13) 3 with adenoma while in descending colon specimens 13 were with adenocarcinoma (Figure 12) 4 with metaplasia (Figure 14) and 3 with carcinoma. Rectal polyp specimens showed 8 specimens with adenoma, 4 with tubulovillous adenoma with severe dysplasia and 3 with carcinoma. In rectal specimens 4 were detected with adenocarcinoma, 3 with squamous cell carcinoma (Figure15) and 3 with metaplasia as shown in table I.

Figure 10

Figure 11

Figure 12

Figure 13

Figure 14

Figure 15

GeneXpert System

The GeneXpert Dx system uses real-time polymerase chain reaction to automate sample preparation, nucleic acid amplification, and target sequence detection in simple and complicated samples (PCR). The system is designed for in vitro diagnostic applications that need hand-off processing of patient samples (specimens), and it provides data in tabular and graphic representations for both summarised and comprehensive test findings. The samples are prepared and processed in GeneXpert cartridges that are designed for single-use assays. The sample and reagents are placed in a cartridge, which is subsequently put into the instrument module.

GeneXpert Cartridge

Each cartridge consists of 1) Processing chamber 2) Optical window 3) Valve and Reaction tube Total of 135 histopathologically confirmed colorectal paraffin embedded biopsy specimens were collected from histopathology department of Ayub Medical College (Abbottabad) and histopathology department of Abbott Laboratory and other hospitals of Hazara Division. The experimental work was carried out in Abbot Lab Histopathology and PCR departments. After melting the specimens to remove the wax, they were treated with xylol (MERCK, Germany), then washed with 100 percent ethanol. After removing wax tissues were cut in to pieces and treated with liquid nitrogen and by using mortar and pestle/tissue grinder tissues were crushed in to fine powder. Now each sample powder form was dissolved in preserved cyst solution provided and vortex for 10 to 15 seconds. After vortexing 1ml solution was loaded into HPV cartridge (Figure16) and after scanning bar code of cartridge it was lodged into PCR system and after 1 hour 50 minutes results were obtained (Figure17).

Figure 16

Figure 17

Out of 135 specimens 85 (62%) were detected with HPV DNA, 37 (43%) with HPV-16, 25(29%) with HPV-18 and 16(19%) with HPV-45 genotype (Table I). Seven specimens were detected with low risk genotypes and 43 samples were negative for HPV DNA there was no detection in normal control specimens as shown in Table II. Our results showed that HPV plays a definite role in carcinogenesis of colon and rectum so it should be considered in colorectal cancer cases like cervical cancer.

Table I

Table II

Discussion

Results of current study strongly showed that HPV can be consider as cause of colorectal cancer if not solely but its contribution in proliferation must be considered as there are multiple factors that influence the disease. As there were numerous studies showing causal relationship between colorectal cancer and HPV and different PCR systems and techniques were used but in our study we used GeneXpert system without human error as extraction and amplification is fully automatic in cartridge along with this it also type involved genotype in single run. As we have shown that HPV-16 is the most prevalent strain detected in our specimens same results were shown by other researchers despite of PCR equipment difference. In 2015 Mahmoudvand *et al*,[8] showed the same results in their study carried out in Iran with HPV-16 genotype most prevalent according to them, the findings show that some colorectal malignant tissues are infected with a high-risk HPV genotype. More research is needed into the findings. In another review conducted in 2010 Laura Lorenzon et al.[5] reviewed the literature on the possible function of human papillomavirus (HPV) infection in colorectal cancer to determine whether HPV infection plays an active role in colorectal carcinogenesis and to highlight evidence and problems of published studies. They discovered that, while HPV has been detected in the majority of reported series, definite data on standard techniques of examination and stratification of groups and populations is lacking in the available literature. These findings encourage more research into the virus's presence in larger groups, its possible role in oncogenesis viral oncoprotein expression, mutations in HPV positive tumours, and colon infection pathways (hematologic/lymphatic spreading or perineal diffusion). Marina K. *et al*. [9], in another study published in 2018, used data from 19 studies to investigate the link between HPV infection and colorectal cancer. Researchers calculated the statistically significant level of HPV infection in CRC tumour tissue and the resulting relative risk of developing CRC with HPV infection to be RR (95 percent CI) = 2.97 (1.42–6.22) with p = 0.0039, based on the provided data. In 2015 [10], Li *et al*. advised that because HPV is a risk factor for colorectal cancer, preventive measures such as vaccination and the use of antiviral medications suited for the afflicted communities' health plan should be undertaken According to recent studies, 15 percent of all cancers are caused by oncogenic viruses. The presence of papillomavirus is regularly identified in human tumours, and it can be an etiologic factor in cancers [11].

Declarations

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support

Ethical Approval: This study has been conducted **based on the guidelines in accordance** with the Declaration of AUST. Thus, the provided data by the participants remained confidential whilst maintaining the ethical standards during performing the study by the corresponding authors and co-authors. Furthermore, **all methods were carried out in accordance with relevant guidelines and regulations.** This study has been a**pproved by the ethical committee of Abbottabad University of Science & Technology** in accordance with all experimental protocols being approved by ethical committee.

Informed Consent: In current study the informed consent was waived by the competent authorities of Ayub Medical Complex and ethical committee of Abbottabad University of Science & Technology.

Data Availability: The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Tables

Table I: Histopathological investigation of Colorectal Biopsies

Sr. No	No. Samples	Type	Condition	
1	65	Colon biopsy	Adenocarcinoma	35 (53.8%)
			Carcinoma	25 (38.4%)
			Squamous Cell Carcinoma	10 (15.3%)
2	25	Ascending Colon	Adenocarcinoma	15 (60%)
			Carcinoma	7 (28%)
			Adenoma	3 (12%)
3	20	Descending Colon	Adenocarcinoma	13 (65%)
			Metaplasia	4 (20%)
			Carcinoma	3 (15%)
4	15	Rectal Polyps	Adenoma	8 (53%)
			Tubulovillous Adenoma	4 (26%)
			Carcinoma	3 (20%)
5	10	Rectal Biopsy	Adenocarcinoma	4 (40%)
			Metaplasia	3(30%)
			Squamous Cell Carcinoma	3 (30%)

Table II: HPV DNA detection (GeneXpert)

HPV	No	%	Genotype	No	%
Positive	85	62	HPV -16	37	43
			HPV-18	25	29
			HPV-45	16	19
Positive	7	5.1	Low Risk HPV		
Negative	43	31.8	No Detection of HPV DNA		
Control	10				
Total	135				

Figures

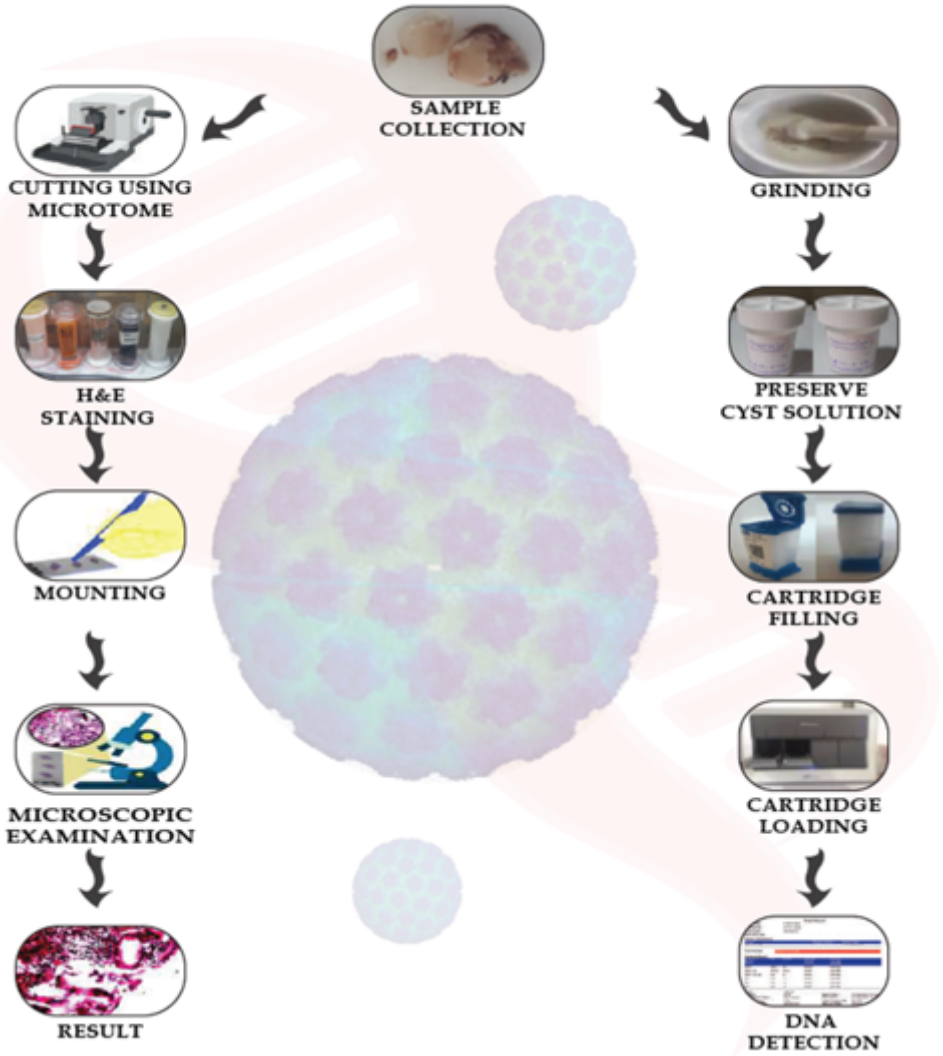

Figure 1

Methodology overview

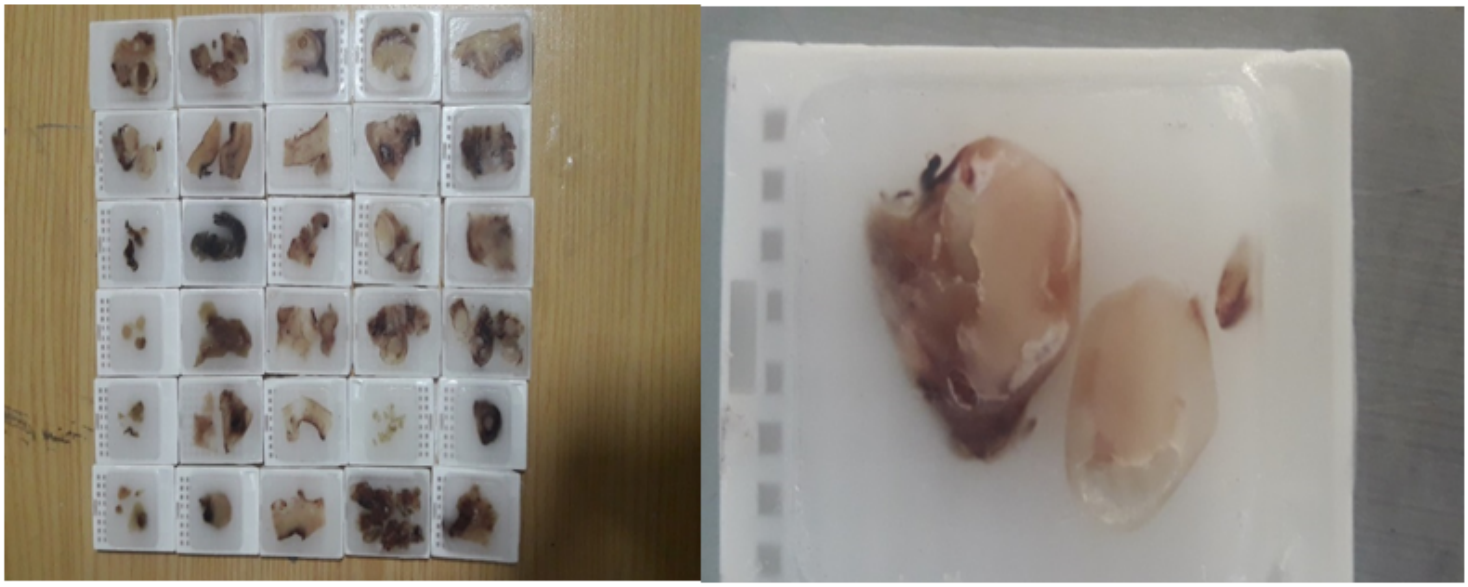

Figure 2

Paraffin embedded tissue biopsy specimens

Figure 3

Microtome

Figure 4

Tissue Bath

Figure 5

Section in distilled water

Figure 6

Hematoxylin & Eosin Stains

Figure 7

Removal of wax

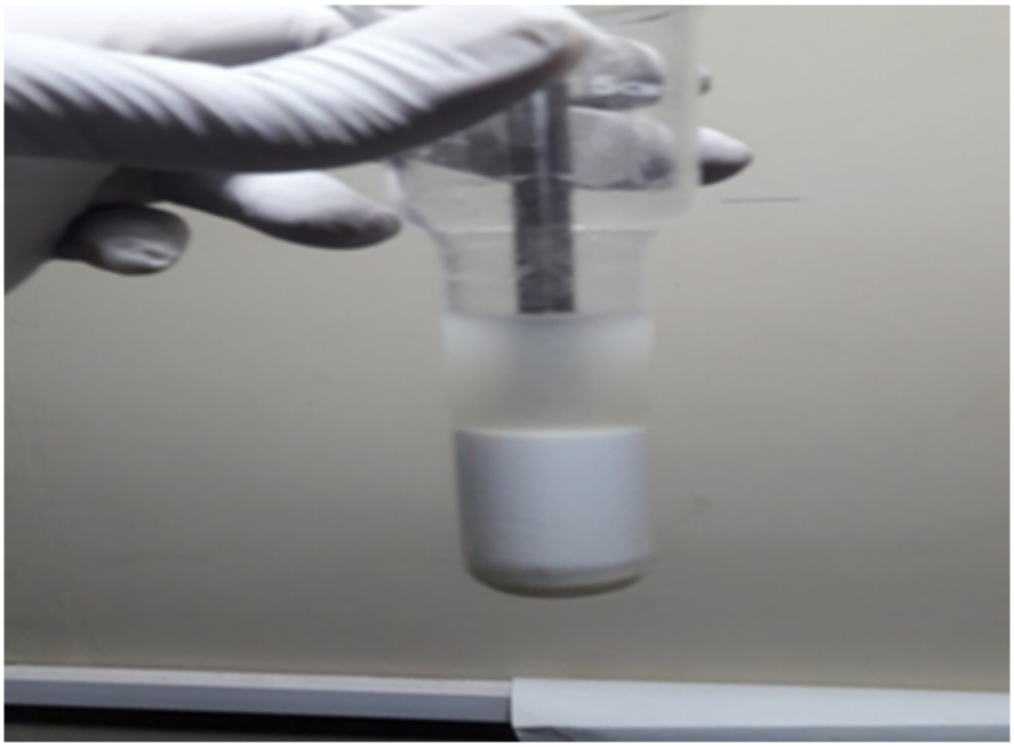

Figure 8

Specimen crushing

Figure 9

Preserve cyst solution

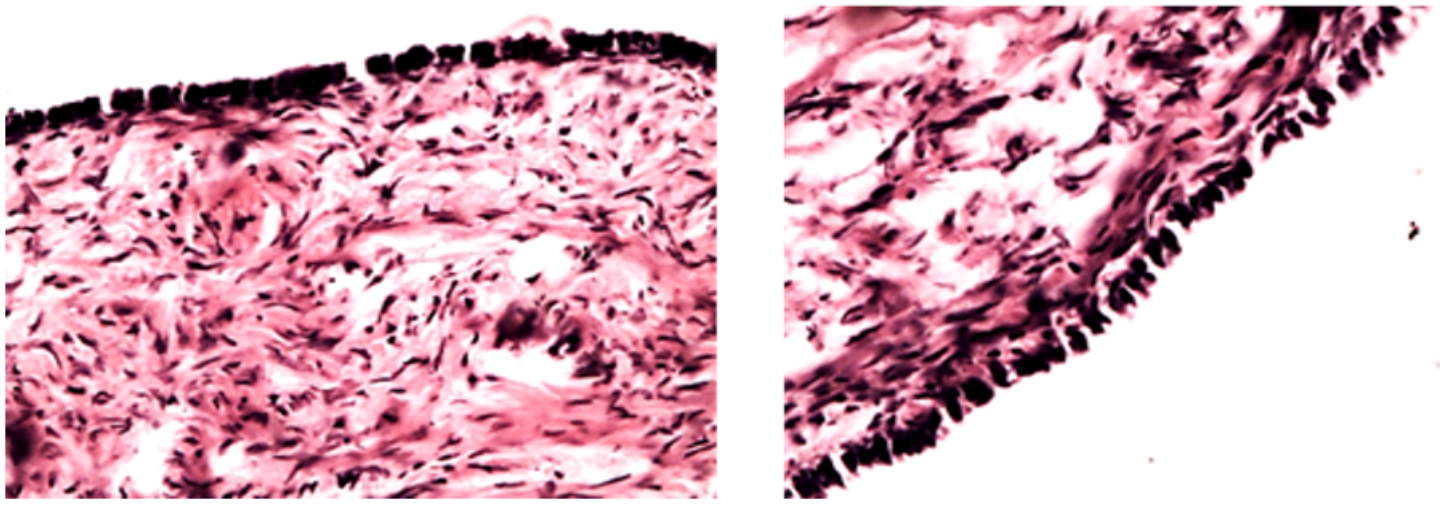

Figure 10

Adenocarcinoma of colon

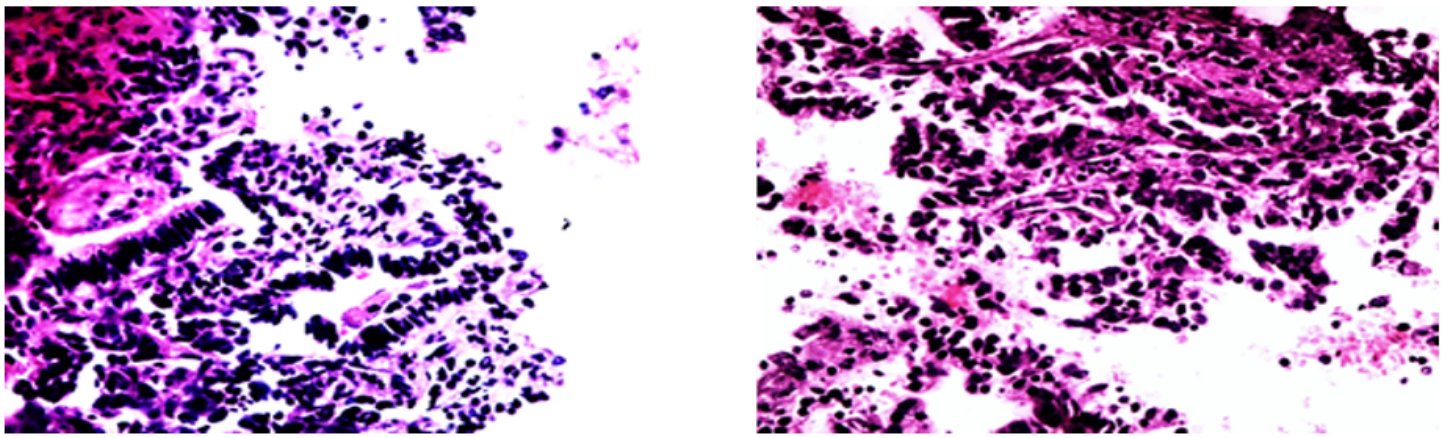

Figure 11

Adenocarcinoma of colon

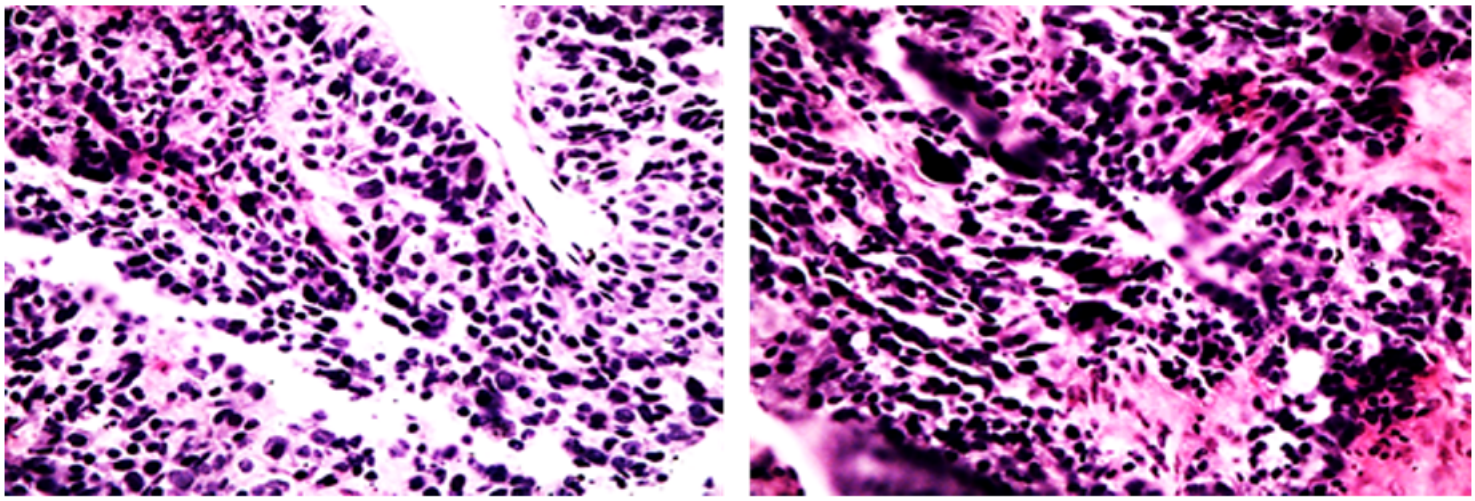

Figure 12

Adenocarcinoma of descending colon

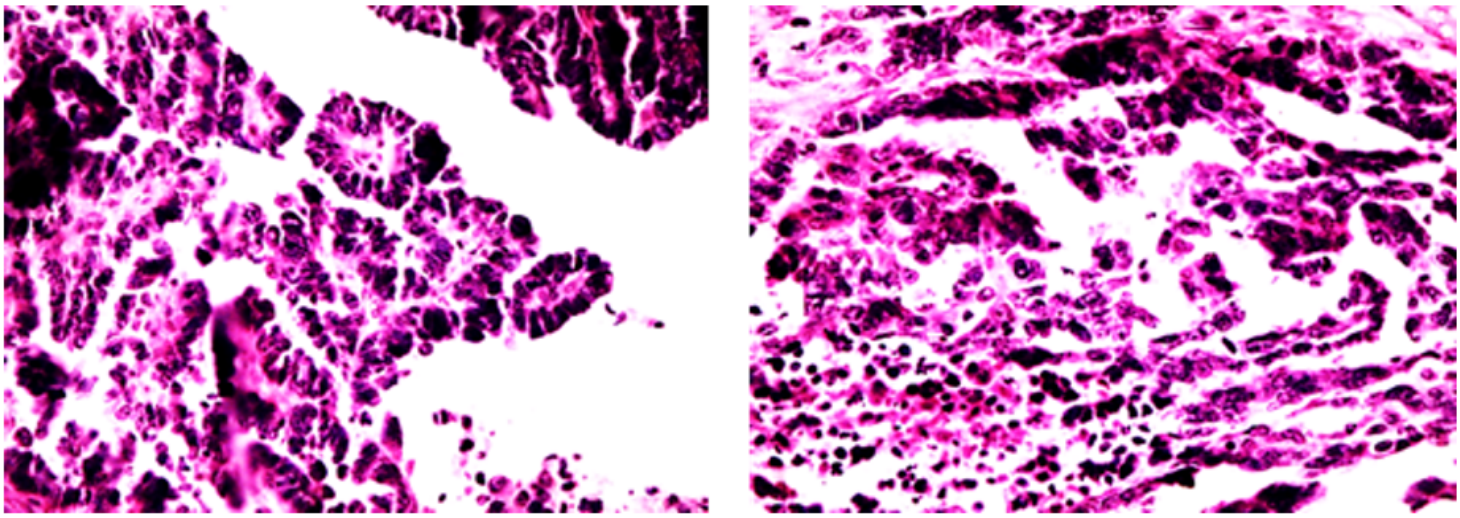

Figure 13

Carcinoma of ascending colon

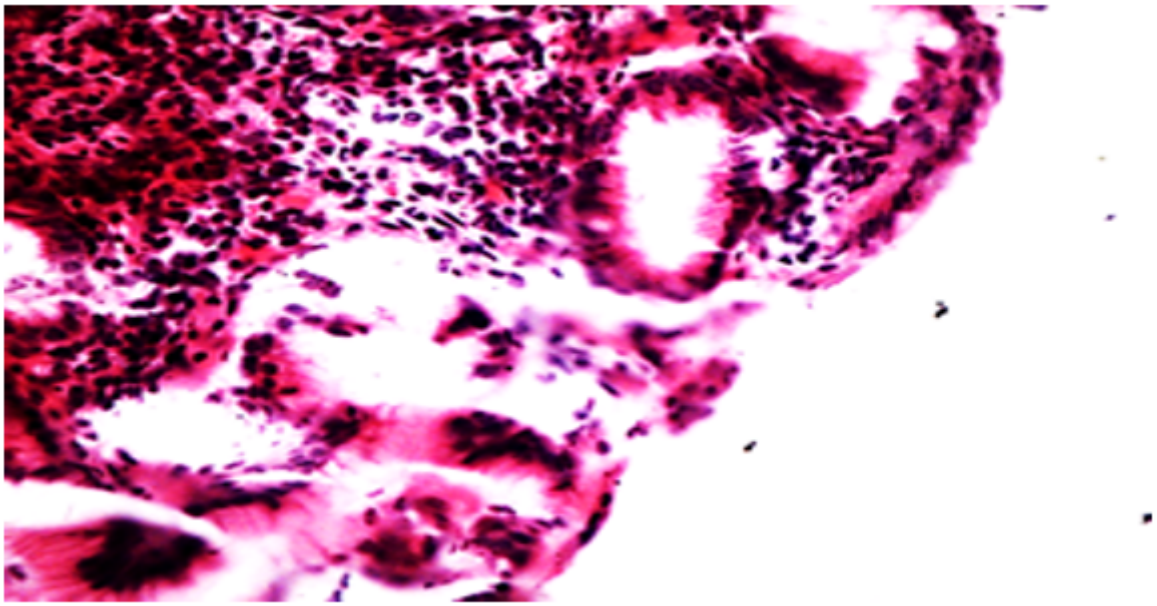

Figure 14

Metaplasia of Intestine

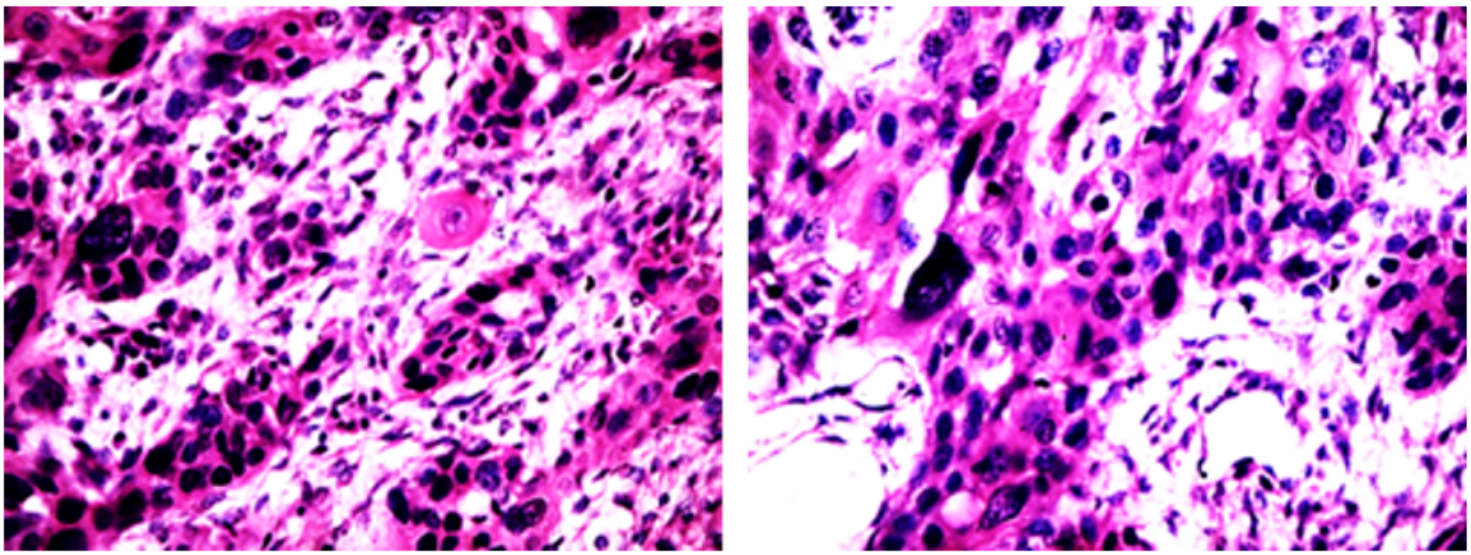

Figure 15

Squamous cell carcinoma

Figure 16

HPV Cartridge

Figure 17

GeneXpert System

Supplementary Files

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- GenexpertCartidge.png