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The Effect of human papilomavirus on esophageal cancer: A double blind study in north of Iran

Maryam Taghi Zoghi[1]*, Anna Boyajyan[2], Misagh Bastani[3], Kimia Sharifi[4]

1. Medical Doctor, PhD candidate in molecular and cellular biology, Science Academy of Armenia, Yerevan Branch, Co responding author， Adress: Department of genetic, school of science academy, Tonekabon, Iran
Email: glad_tidingss@yahoo.com

2. Professor in molecular and cellular biology, Institute of Molecular Biology of the National Academy of Sciences of Armenia

3. Medical Doctor, Resident of anesthesiology, Shiraz University of Medical Sciences, Shiraz, Iran

4. Medical Doctor student, Tehran University of Medical Sciences

Abstract

The virus HPV is involved in some kinds of cancers and the DNA of this virus has been observed in skin , genitalia and oral tumors. Among them nearly 30-40 kinds are transfered sexually which have the ability to make sexual wart and cervical cancer. HPV is one of the affective factors in esophageal cancer as well. the aim of this study was to examine the Effect of human papilomavirus on esophageal cancer in north of Iran. This was a double blinded case control study conducted in north of Iran. The total of 40 tumor biopsies plus 40 samples of the control groups have been analysed applying primer $Gp5^{+}$ &$Gp6^{+}$ which are capable of detection high risk types. Results of this study indicates that there were no results confirming the affection of HPV in 40 examined patients as the case group comparing with their couples as the control arm. Non existing HPV virus can be referred to different life style for example different sexual habits ,and the other reason is the aging of affected people. According this fact hat affection to virus HPV, as an environmental factor, is extremely influenced by people life style, and capability of this virus in induction esophageal cancer.

Key word: Human papilomavirus, esophageal cancer, north of Iran.

Introduction

Cancer of the esophagus is the eighth most common cancer world wide with more than 400.000 case per year incidence [1,2].The two main types of esophageal cancer are squamous cell carcinoma and adenocarcinoma [3]. The cause of esophageal cancer is unknown however, epidemiologic studies in several areas of the world suggest a relationship with alcohol, tobacco, nitrosamine, vitamin deficiencies, aflatoxin, candidal and viral infectious [4-6].

Esophageal cancer is often diagnosed at an advanced stage and has a poor prognosis. Most patients with advanced esophageal cancer have significant dysphagia that contributes to weight loss and malnutrition. Esophageal stenting

is a widespread palliation approach, but unsuitable for cancers near the upper esophageal sphincter, were stents are poorly tolerated [7].

High- risk human papilomavirus (HPV) infection is important for carcinoma in the esophagus [8]. The incidence of esophageal cancer shows certain geographic variation for instance south African countries,Iran ,china, India, Ceylon and Puerto- Rico are high incidence areas [9-11].Human papillomaviruse (HPV) has been implicate as one of the risk factors of esophageal cancer. HPV is kind of papiloma virus which pollutes human. Nearly 130 subset of this virus has been known. Some kinds of them cause wart and cancer while most of its kinds do not show any clinical signs. In polluted cells, virus provides the basis of its mass assimilation with interfering in cell cycle. This action is done by expressing primary virus genes which we can mention the most important of them as E6 and E7 [12, 13].

HPV virus has a clear role in cervical cancer and has been known in many patients, in a way that the virus is observed in %90 of patients [14]. Some studies have reported a high infection rate of HPV in esophageal cancers [15-17].While others have detected only a low rate or absence of HPV infection [18-20] .A Variable infection rate of HPV has also been reported by different research groups within the same geographic region [15,18].

Covsiderig all the above stated the aim of this study was to examine the Effect of human papilomavirus on esophageal cancer in north of Iran.

Methodology

This was a double blinded case control study conducted in north of Iran. The total of 40 tumor biopsies plus 40 samples of the control groups have been analysed. None of the patients or the researchers knows that which patient belongs to case or the control groups. Biopsy specimens were collected from operation theatre of gastro endoscopies in the internal department of Shahid Rajai Tonekabon hospital. These samples were collected from 2008 to 2011. Tissue samples were stored in -20 -70.

Filtering DNA from samples (by fermentase kit) :First prior to DNA extraction , the sample should be digested for a night with digestion buffer 100ml and 2.5ml proteinase k.Mixing binding solution with tissue samples has been carried out with the ratio 1 to 3 (100ml to 300ml),and then 5ml of silica was added.Ancubation was carried out for 5miniutes in the temperature of 55c.washing buffer was added to the settle and Then vortex was performed . The quick centrifusion 3 times, for 5-10′ was performed and then DNA was extracted during this process and the result was analysed in Agarose gel 0.8 %.

HPV detection and identification : This step also is done the same as previous treatment with this difference that GP5+/GP6+Primers were used .The following primers have been used :
Gp5+:5'TTGGATCCTTTGTTTACTGTGGTAGATACTAC_3',GP6+:5'_TTGGATCCGAAAAATAAACTGTAAATCATATTC_3'

Result and disscusion

In this study 40 patient suffering from esophageal cancer and 40 normal biopsys for control group have been sampled. The samples were kept in -20 °C – 70 °C.All the samples as mentioned in the last part, have been DNA extracted and then the result has been examined in gel Agarose 0.8 % .

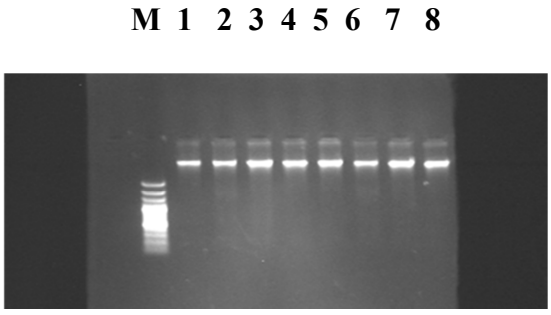

Figure 1-3: Extracted DNA in gel Agarose %0.8 column M, in related to molecular weight marker, and the other columns with numbers 1-8 are related to samples.

The use of molecule weight marker, was due to making sure of the suitable quality of extracted DNA for PCR. As observed in Figure 1-3, the amount of extracted DNA fragility is little .

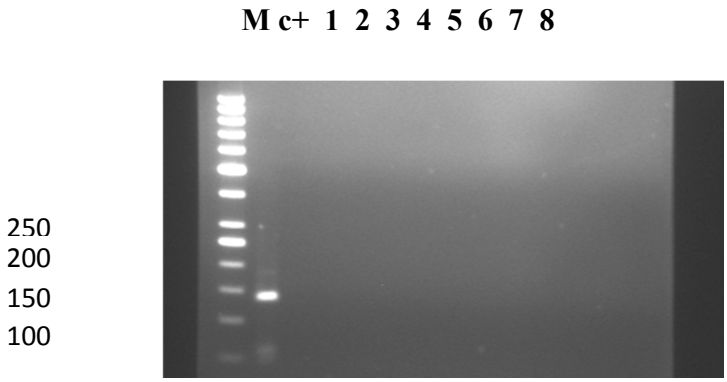

Figure 2-3: PCR result of the sample with primer GP5+/GP6+. M(molecular weight) C+ is positive control, and the other columns are for samples 1 to 8.

Analysis of HPV virus in esophageal cancer samples, revealing of the existence of HPV virus in esophagus cancer samples was done by PCR method. In this regard a pair of primer called Gp5+ Gp6+ has been used, and joined to a segment in the area ORF, gene L1 HPV virus and produced a segment 140 long. Protein L1 among the capsid virus proteins, and is observed in all types HPV virus. Also primers Gp5+/Gp6+ pair, has the ability of linking to all type of HPV and is considered as a universal primer.There is no HPV affected area in esophagus cancer samples.

Conclusion

In this research primers $Gp5^{+}$ &$Gp6^{+}$ have been used ,which are capable of detection high risk types. In 40 examined patients , there are no results confirming the affection of HPV.Non existing HPV virus can be referred to different life style for example different sexual habits ,and the other reason is the aging of affected people. At the same time this diffrences can be justified by the variation of settings and the restricted sample size that it is recommended to design complementry studies in other patrs of the country to increase the robustness of the study.

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