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Detection of *Helicobacter pylori* and Human Papilloma Virus in Laryngeal Papillomatosis of Patients in Rasul Akram in Tehran in 2011

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Laryngeal papillomatosis **is the most common of benign laryngeal neoplasms, different factor may play role in incidence of laryngeal papiloma but** ***Human papillomavirus*** **and may be** ***Helicocabacter Pylori*** **one of the most important of them. Aim in this study is the presence of** ***Helicobacter pylori*** **and** ***Human papillomavirus*** **in laryngeal papilloma. Polymerase chain reaction was employed for detection of** ***Helicobacter pylori*** **and** ***Human papillomavirus*** **DNA from 41 biopsy samples of laryngeal papilloma. Three samples of 41 biopsy samples for the presence of** ***HP*** **DNA and 19 samples from 41 biopsy of patient for** ***HPV*** **DNA were positive. At this study we evaluate the role of** ***Helicobacter pylori*** **and** ***human papillomavirus*** **on laryngeal papilloma. The author found that although,** ***Helicobacter pylori*** **is no dominant agent in incidence of laryngeal papilloma but** ***Human papillomavirus*** **can be a dominant risk factor for development of it.**

Key word: *Helicobacter pylori* (HP), *Human Papilloma Virus* (HPV), Polymerase Chain Reaction (PCR).

Laryngeal papillomatosis (LP) is one of the most common of laryngeal tumor in the world. It is able to recurrence in patient especially in children so that it is also known as recurrent respiratory papillomatosis (RRP). It has been estimated that about 84% of laryngeal tumors that related to *LP* are benign, that some patients developed to some signs like asthma, croup and chronic bronchitis. Primary symptoms of *LP* is noisy breathing, chronic cough, difficult swallowing, snorting, snoring and breathing problems during sleep but occasionally convert to respiratory distress and air way obstruction[1]. Different rick factor are recognized for development of *LP*, Such as alcohol, smoking, *Human Papilloma Virus* (HPV) and *Helicobacter pylori*. In several studies, it has been revealed that *HPV* infection increased the risk of developing *LP*. *HPV* is a DNA virus that causes a variety of skin and mucous lesions including skin and vaginal wart and some cancers in oral cavity. Until now about 100 types of *HPV* were recognized, and of them, *HPV* 6, 11 is the most important cause of development of *LP*[2-5]. This is a hypothesis that *Helicobacter pylori* (HP) can be a primary factor to *HPV* for developing *LP*[6-9]. *HP* is a motile, gram negative, catalase and oxidase positive bacteria that produce strong urease[10]. *HP* can increased

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proliferation and induce apoptosis cells, and through destruction of mucosal immunity can facilitate to exposure of laryngeal tissue to some carcinogen agents such as *HPV*[6]. The CagA protein is a pathogenesis factor that can activate tyrosine kinase and can restructure host cell's skeleton[11,12,13]. Various methods have been used for detection of *HP* and *HPV*[18,19], because of the sensitivity and specificity of their methods is not the same, their results are different. The aim of this study was to investigate the effect of *HP* and *HPV* in development of *LP* individually or synergistically.

MATERIALS AND METHODS

41 biopsy samples of patient with *Laryngeal papillomatosis* were collected in normal saline. All samples were stored at -80°C until they were used. phenol-chloroform method was employed for DNA extraction of both of *HP* and *HPV* from 41biopsy samples[14]. Extractracted DNA was diluted in final volume of 50 ml elution buffer.

PCR

HP DNA was detected by PCR assay using pair primers associated with the cagA gene: P1 5"'AAAATGGAAGCGAAAGCTCA"3' and P2 5"' CGGCTAGCACTTGCTCTACC at -3' (P1, sense nucleotides 1921-1941, P2 antisense nucleotides 2406-2426). PCR reaction were done in 25µl reaction contained the following: 0.5µl of the dNTP, 1µl of $MgCL_2$ (50 mM), 2.5µl PCR buffer, 0.2µl Taq DNA Polymerase (5U), 13.8µl of deionized water, 1µl of both of the primers (10pmol/µl), and 5µl template DNA. Temperature profile of PCR was comprised 5min for preancubation at 94°C, followed by 40 cycles of 30 sec at 94°C, 30 sec at 50°C and 40 sec at 72°C, final extension was performed for 5 min at 72°C. *HPV* DNA was detected by PCR assay using pair primers associated with the My 09:
5'-CGTCC(A/C)A(A/G)(A/G)GGA(A/T)ACTGATC - 3'
My11: 5' – GC(A/C)CAGGG(A/T)CTATAA(C/T)AATGG - 3'. PCR reaction were done in 25µl reaction contained the following: 0.2µl of the dNTP, 1.5µl of $MgCl_2$ (50 mM) 2.5µl PCR buffer, 0.5µl Taq DNA Polymerase (5U), 18.3 µl of deionized water, 0.5µl of both primer, and 1ml template DNA. temperature profile of PCR for *HPV* was comprising 5min for preancubation at 95°C, followed by 35 cycle of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, final extension was performed for 1 min at 72°C. the PCR product were isolated by 1.5% agarose gel electrophoresis, Then stained with ethidium bromide and therefore visualized by UV transluminator.

Table 1. The result of the PCR assay

Number of samples	Male	Female	HP DNA Positive	HPV DNA Positive	HP&HPV DNA Positive simultaneously
41	30	11	3	19	Zero(0)

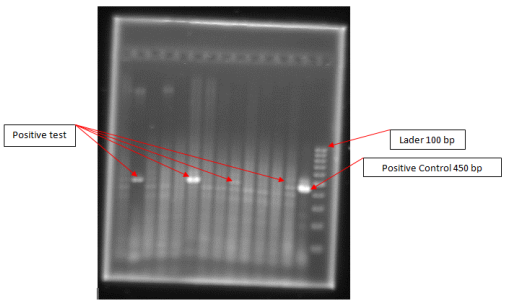

Fig. 1. The result of PCR for samples HPV DNA

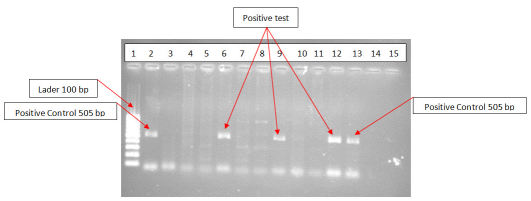

Fig. 2. The results of PCR for cagA of HP

RESULTS

Amplification of DNA with PCR disclosed that of 41 biopsy samples, *HP* DNA was detected in 3 (%7.3) samples (Table 1). And *HPV* DNA was detected in 19(%46.34) samples (Fig. 1, 2). Moreover none of samples were not positive for both of *HP* and *HPV* infection .

CONCLUSION

HP and *HPV* infection was documented as risk factor for *LP* development. In several studies for *HP* detection from *LP*, various methods are employed. Urease assay is unreliable because it is not specific for *HP* and some bacteria such as some species of corynebacterium, streptococcus, proteus can show positive reaction as the same as *HP*. In serological diagnoses are used various kits that their sensitivity and specificity of them are different, so that the results of them are not the same[16-19]. For both of the *HP* and *HPV* agent, new molecular assay have been developed which included the PCR, Real-time PCR, Immonohisto chemical methods and test[15, 18-20]. Detection of DNA by PCR is method of rapid, reliable and more ever at this method there is no need to radioactive material or complex apparatuses. In this study, PCR reaction was employed for detection of *HP* and *HPV* DNA from 41 biopsy samples of laryngeal papillomatosis. 3 samples of 41 were positive for *HP* DNA and 19 samples of them were positive for *HPV* DNA. Several studies evaluated only one of the *HP* or *HPV* DNA from LP[16-20]. In a study, Dr. Paul Diclas analysed 27 cases of Laryngeal Papilloma for the genome of *HPV*6, 11, 16, 18 by slot-blot hybridization, that finally 48% from samples was positive about *HPV*11 and 11% of samples was positive for *HP*V6, 11, 16 simultaneously[2], at the same project Nicollas showed a co-infection with *HPV*6, 11 in the 11 year old girl with *laryngeal papillomatosis* by real-time PCR[3]. But about *HP*, Alititiz research the presence of *HP* DNA in normal tissue and squamous cell carcinoma (SCC) of larynx by PCR that their results showed that *HP* can play a role in SCC of larynx[6],or at the other study Xian-Lu indicated that *HP* can be a risk factor for neoplasm of larynx[22]. In conclusion in our study we find that, *HP* has no potential role in incidence of LP but *HPV* can play a role in occurrence or accelerate incidence of *LP*. But *HP* with destruction of mucosal immunity can facilitate to exposure of laryngeal tissue to some carcinogen agents such as *HPV*[6] however it is need to more epidemiological and molecular study for better and more reliable result.

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