

Investigation of *Helicobacter pylori* in Laryngeal Papillomatosis

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Background: *Helicobacter pylori* (HP) is a motile, gram negative, catalase and oxidase positive bacteria that produce strong urease. *H. pylori* has been recognized as an etiologic agent of gastritis, gastric ulcer, gastric adenocarcinoma and gastric MALT lymphoma. *H. pylori* infections have been correlated with many throat and larynx disease. More recently it is introduced as group 1 carcinogen. The existence of *H. pylori* within buccal cavity and dental plaque has been reported. The nasal cavity can be colonized easily with *H. pylori*.

Objectives: The aim of this Study is to report the result of an investigation of *H. pylori* genome within the larynx papillomatosis biopsy samples.

Materials and Methods: 41 biopsy samples were provided from papillomatosis cases during therapeutic surgery. The biopsy samples were preserved at -80°C until use. The DNA extractions were achieved by the phenole- chloroform method. The *cagA* of *H. pylori* were amplified by specific primer sets.

Results: *H. pylori* DNA were detected from 3 out of 41 (0.7%) papilloma samples.

Conclusions: This result is in agree with another reports about correlation between laryngeal papillomatosis and *H. pylori* infections.

Keywords: *Helicobacter pylori*; Papillomatosis; Caga Protein, *Helicobacter pylori*

1. Background

Helicobacter pylori is a gram-negative, microaerophilic and motile bacteria that have been identified in 6 serotypes and 25 species. *Helicobacter pylori* and *heilmannii* are two pathogenic species for human. This Bacterial infection exists around the world and more than half of the world's population are infected (1, 2). The infection rate is about 80% in Iran (3). The bacteria have been observed in 95% of patients with duodenal ulcers and 70-80% of patients with gastric ulcers. The risk of the gastric cancer that are associated with *H. pylori* infection in industrialized countries is estimated about 70% whereas in developing countries is estimated about 80% (4). Strains of *H. pylori* with CagA+ have been reported as a risk factor for gastric carcinoma in infected patients (5, 6). The risk factors for *H. pylori* infection such as poverty, using common sleeping place, living in very crowded places like boarding raise the possibility of infection also cluster infection occurs among families. Also there is transmission via endoscopy and aerosols. This bacterium was isolated from saliva, dental plaque and feces. Dental plaque is noted as secondary storage for *H. pylori* (7-10). In developing countries contaminated water with feces is the most important factor to infection (11, 12). The high prevalence of *H. pylori* infection through the world and

its role in malignancies of stomach and other diseases also the emergence of antibiotic resistances have led to offer the various methods for identification, treatment and prevention against this infection. Among them identity is recognized very momentous (13-15). CagA protein is present in 60-70% of all strains of *H. pylori* that is associated with increasing the risk of cancer, developing of atrophic gastritis, forming the ulcer and deferring in recovery, inducing of expression of oncogenes and mutation in p53 gene, atherosclerosis, ischemic heart disease, coronary heart disease, producing of PGE2 and increasing the production of reactive forms of oxygen (5, 16-21). In several studies, the presence of *H. pylori* has been confirmed in oral cavity, dental plaque, saliva and its colonization in the nose and along with a number of diseases of the throat and larynx (21-24). *H. pylori* causes increased proliferation and decreased apoptosis in the tissues of the larynx. Based on the findings, *H. pylori* destructs the mucosal immune barriers therefore it facilitate the exposure of the aero-digestive system to variety of carcinogens (25). The larynx is an organ that belongs to both the upper respiratory and digestive systems, so its damages are important for the body health. Laryngeal Papillomatosis which is known as Recurrent Respiratory

Papillomatosis is one of the most common neoplasms of the larynx that constitute more than 84% of cases of benign tumors in larynx. These warty lesions that grow out of mucus can cause symptoms such as noisy breathing, chronic cough, difficulty swallowing, snorting, snoring and breathing problems during sleep and sometimes in untreated cases may grow out of control and cause obstruction of the airway and can progress to the mucosal cell carcinoma of the upper respiratory tract (26).

2. Objectives

This study investigated the relationship between *Helicobacter pylori* and Laryngeal Papillomatosis.

3. Materials and Methods

In this study PCR was employed for detection of *H. pylori* in 41 biopsy samples of laryngeal papillomatosis from the patients that were undergone laryngeal surgery in Rasoulakram hospital in Tehran. Because the *cagA* gene product plays an important role in pathogenesis of *H. pylori*, pair primers for the synthesis of *cagA* gene were designed by Primer 3 software. Blast software was employed to determine the homology with the genomes of other microorganisms and also Oligo and Gene Runner software programs were used to assess the characteristics of the formed loop primers. The nucleotide sequences of the primers were ordered to research company synazhen to synthesis. All samples were stored at -80°C until they were used. phenole-chloroform method was employed for DNA extraction. HP DNA was detected by PCR assay with using pair primers associated with the *cagA* gene: P1 5' "AAAATGGAAGCGAAAGCTCA"3' and P2 5' "CGGC-TAGCACTTGCTCTACC at"3' (P1, sense nucleotides 1921-1941, P2 antisense nucleotides 2406-2426).polymerase chain reaction were done in 25µL reaction contained the following: 0.5 µL of the dNTP, 1 µL of MgCl₂ (50 mM), 2.5 µL PCR buffer, 0.2 µLTaq DNA Polymerase (5U), 13.8 µL of deionized water, 1 µL of both of the primers (10pmol/µL), and 5 µL template DNA. Temperature profile of PCR was comprised 5minutes for preincubation 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 minutes at 72°C. The PCR products were isolated by 1.5% agarose gel with 80 voltage electrophoresis and a 505 bp band was observed expectantly.

4. Results

Amplification of DNA with PCR disclosed that of 41 biopsy samples, HP DNA was detected in 3 (%7.3) samples.

5. Discussion

In this study PCR was employed for detection of *H. pylori* in 41 biopsy samples of laryngeal papillomatosis that had been maintained at -80 °c in normal saline until to use. Undeniable role of *H. pylori* in creation and regression

of some gastric disease especially gastritis, gastric and duodenal ulcer has been proven. Moreover that is associated with gastric adenocarcinoma that is known as the second most common fatal cancer and MALT syndrome. Hence WHO recommended *H. pylori* as an actual carcinogen (type I) (1). Almost all infected individuals remain asymptomatic lifelong or have mild gastritis, some of them (about 15%) will become the worse and transform to the acute gastrointestinal symptoms such as gastric and duodenum ulcer that in many cases convert to gastric tumors (4, 5). Some potential virulence factors of *H. pylori*, such as urease, motility, cytovacuolated toxin (VacA) and especially CagA cytotoxin stimulate the inflammation reactions therefore increase the clinical symptoms of laryngeal Papillomatosis (14, 27). Identification of pathological aspects of *H. pylori* in aero-digestive system and papillomatosis looks very important. So far, several reports that have been indicated the isolation of *Helicobacter pylori* from dental plaque or oral cavities were presented different statistics about this. In 2008 a review study analyzed the data from 15 papers that were about *H. pylori* infection and larynx cancer with meta-analysis software, the result demonstrated that HP infection can be a risk factor for larynx cancer (28). In 2005 a study with using the rapid urease test, that was performed on 80 samples from patients with mucosal cell carcinoma of the larynx and 34 control samples from patients with benign laryngeal lesions, showed that 62.5% of the patient specimens and 37.8% of the control samples were positive urease (29). In 2008 a Case-Control study was evaluated the relationship between HP infection and laryngohypopharyngeal carcinoma, During this study, 105 cases of healthy controls (A) and 70 cases of laryngeal carcinoma (B) and 28 cases of hypopharyngeal carcinoma (C) were assessed, the HP positivity was more common in group B and C. So HP was introduced as a risk factor for laryngopharyngeal carcinoma (30). In 1999 a study which determined the anti-HP IgG antibody titers in patients with SCCL showed that HP can be a primary organism or an promoter to SCCL, but it cannot be the main cause of laryngeal cancer certainly (31). In a study conducted in 2008 by Titiz the presence of HP in normal tissues and tumors of the larynx was examined by PCR. The results showed that there is a correlation between the presence of *H. pylori* and development of mucosal cell carcinoma of the larynx (25). Although several studies have identified the presence and relevance of HP with laryngeal disorders but because of low specificity and sensitivity of their methods and low volume of samples were not satisfactory. For example, the result of urease test is affected with some organisms like *Proteus mirabilis*, *Klebsiellapneumoniae*, *Ureaplasmaurealyticum*, *Streptococcus salivarius*, different types of *Haemophilus* and *Corynebacterium*. The quality of culture medium and delivery condition are effective on the outcome of in vitro culture. Whereas false negative result can be seen in UBT experiment because of the treatment by antacid, bismut and antibiotic (32). Serological methods which

are not expensive because of doubting accuracy are limited. Among molecular methods traditional PCR requires the primary devices and its accuracy and specificity is more acceptable than other laboratory methods. In this study PCR was used to detect the *cagA* gene from *Helicobacter pylori*. Simultaneous presence of *Helicobacter pylori-cagA* + strains in samples from the patient's mouth and stomach can reveal the fecal - oral transmission of HP and prove this hypothesis that oral cavity is a reservoir for HP further the role of HP as an agent in laryngeal papillomatosis. However, with molecular typing techniques to demonstrate the equality between two separated species will be require proving this hypothesis. Finally, according to the results of this study, it seems that oral cavity and Laryngeal Papillomatosis are appropriate storage for *Helicobacter pylori*, especially in patients who suffer in upper digestive and respiratory system. Also it can conclude that there is probably a direct correlation between the presence of *Helicobacter pylori* in oral cavity and gastric biopsy in dyspeptic patients. Accordingly, Complementary experiments will be needed to achieve more definitive results to replace oral cavity sampling instead of gastroendoscopy. Or it can be possible the sampling from oral cavity as the primary test to screen for the presence of *H. pylori*. And HP can be a possible cause in Laryngeal Papillomatosis and treatment of HP also is considered in the treatment of patients with Laryngeal Papillomatosis.

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