

Review

Helicobacter pylori in Tonsillar and Adenoid Tissue and Its Possible Role in Oropharyngeal Carcinogenesis

(*Helicobacter pylori* / carcinogenesis / oropharyngeal carcinoma / tonsillar tissue / adenoid tissue)

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Abstract. *Helicobacter pylori* is a well-known gastric pathogen. It plays a major role in the pathogenesis of chronic gastritis, duodenal and gastric ulcers, adenocarcinoma and gastric lymphoma. HP infection is one of the most common bacterial infections worldwide. Recently, the oral cavity was proposed as an extragastric reservoir of HP infection. HP was detected by culture and PCR in both dental plaque and saliva. It is supposed that HP infection can cause the same immunological changes in the oropharyngeal mucosa as in gastric mucosa and can also contribute to the progression of oropharyngeal diseases. HP can induce production of different cytokines and regulatory molecules, which are suggested to play a role in carcinogenesis of the oropharynx. Only a few studies have explored the presence of HP in tonsillar and adenoid tissue, where MALT is present similar to the gastric mucosa. The results of these studies were inconsistent. The question of persistence of HP in tonsillar and adenoid tissue and its role in the pathogenesis of oropharyngeal diseases still remains unclear. In this review, recent findings about oral HP are considered. Possibilities of diagnostics of HP in oral specimens are discussed.

Introduction

Helicobacter pylori (HP) is a spiral, microaerophilic, Gram-negative bacterium. Infection by HP has been established as the major cause of chronic gastritis and plays an important role in the pathogenesis of other gastroduodenal diseases such as peptic ulceration, gastric lymphoma, and gastric cancer (Israel and Peek, 2001). HP is considered to be the most common chronic bacterial infection in humans (Cave, 1996). The prevalence has been estimated to range from 40 to 80 % and it varies widely by geographic area, age, race, ethnicity, and socioeconomic status (Bures et al., 2006). However, the way of its transmission remains unknown. It is supposed that humans represent the only reservoir of HP, which is spread from person to person by oral-oral or faecal-oral route (Brown, 2000). When considering these ways of transmission, it is supposed that HP should be present in the oral cavity. HP was detected in both dental plaque and saliva (Kim et al., 2000). As it was described lately, the presence of HP in gastric mucosa is bound to mucosa-associated lymphatic tissue (MALT) (Cammara et al., 1997). The same tissue is located in the oral cavity and pharynx in Waldayer's circuit. For this reason, great interest has recently been focused on detection of HP presence in tonsillar and adenoid tissue.

HP pathogenesis

Immunological changes caused by HP in the stomach mucosa have been explained recently (Tummala et al., 2004). There are no more detailed data about HP's effect in the oropharyngeal mucosa. HP has several mechanisms to elude host defences (Portal-Celhay and Perez-Perez, 2006). It is able to survive the acidic gastric environment by producing the enzyme urease, which metabolizes urea to carbon dioxide and ammonia to buffer the gastric acid. HP moves across gastric mucus and can adhere to epithelial cells using a variety of adhesin-like proteins (Sachs et al., 2003). It has been shown that HP can also survive inside macrophage phagosomes by inhibiting phagosome maturation (Ramarao and Meyer,

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Abbreviations: *cagA* – cytotoxic-associated gene A, CLO – campylobacter-like organism, EGF – epithelial growth factor, HP – *Helicobacter pylori*, MALT – mucosa-associated lymphatic tissue, NK – natural killer, NOS – nitric oxide synthases, PAI – pathogenicity island, PCR – polymerase chain reaction, RUT – rapid urease test, SCC – spinocellular carcinoma, VacA – vacuolating cytotoxin A, TGF – transforming growth factor.

2001). Once adhered to epithelial cells, HP induces a strong immune system response (Crabtree, 1996). This response does not lead to elimination of the bacterium, but causes development of chronic inflammation. HP is not eradicated unless an infected individual is treated with a combination of antibiotics (Portal-Celhay and Perez-Perez, 2006). Chemical products of HP attract cells of the immune system into lamina propria (Blanchard et al., 2004). It was shown that HP can induce maturation and activation of monocyte-derived dendritic cells. This activity is mediated by TLRs (Toll-like receptors) expressed on antigen-presenting cells and leads to promotion of NK and Th1 effector responses (Portal-Celhay and Perez-Perez, 2006). Interferon (IFN)- γ -producing Th1-polarized T cells and activated NK cells have been suggested to play an important role in the development of severe pathologies (Hafsi et al., 2004).

HP infection in gastric mucosa is associated with production of both the proinflammatory and immunomodulatory cytokines. Changes in secretion of interleukin (IL)-8, IL-1 β , IL-6, tumour necrosis factor (TNF)- α , TGF- β have been described (Stromberg et al., 2003). These cytokines are produced by both the immune system and epithelial cells. The response of host cells is dependent on production of HP virulence factors (Blanchard et al., 2004). The most important virulence factors, which are associated with gastric pathogenesis, are CagA (cytotoxic-associated gene A) and VacA (vacuolating cytotoxin A).

Genome sequence analysis led to identification of genes encoding these virulence factors grouped in the so-called pathogenicity island (PAI) (Mobley, 1996). HP strains producing CagA are associated with increased risk of severe gastric pathologies compared with CagA-negative strains (Portal-Celhay and Perez-Perez, 2006). Injection of bacterial proteins into the gastric cells by a type IV bacterial secretion system (a multi-molecular complex that mediates translocation of the bacterial factors into the host cell) has been described (Segal et al., 1999; Oliveira et al., 2006). In this way, the CagA protein can get inside the host cells and stimulate cell signalling through interaction with several host proteins. This interaction leads to increased cytokine and regulatory molecule production (Guillemin et al., 2002) and could be related to initiation of tumour transformation (Segal, 1997; Tummala et al., 2004; Hatakeyama, 2006).

VacA is another important HP virulence factor. This bacterial toxin with multiple activities is inserted into the host cell membrane, inducing cytoplasmic vacuolation (Cover and Blaser, 1992). There are differences among HP strains in the structure of VacA (Portal-Celhay and Perez-Perez, 2006). There are two types of signal regions, s1 or s2, and two types of mid-regions, m1 or m2. HP strains with different forms of VacA differ in association with diseases. For example, VacA s1/m1 strains are associated with gastric carcinoma (Miehke et al., 2000).

HP-induced carcinogenesis

HP is a declared type I carcinogen (Logan, 1994). However, the exact mechanism of carcinogenesis has not yet been fully understood. There are three possible ways of HP carcinogenic action:

- 1) HP could act as a direct mutagen: interaction of intracellular signalling molecules and HP CagA may predispose cells to accumulate multiple genetic and epigenetic changes that promote multistep carcinogenesis (Hatakeyama, 2006).
- 2) HP-produced vacuolation cytotoxin can cause immunosuppression by blocking proliferation of T cells (Boncristiano et al., 2003).
- 3) HP can induce cell proliferation by increasing levels of several cytokines and regulatory molecules, which are involved in tumour formation and cell transformation (Konturek et al., 1997; Sakaguchi et al., 1999; Keates et al., 2001; Gobert et al., 2002; Schiemann et al., 2002; Wang et al., 2002). Current information about the regulation mechanism of oropharyngeal epithelial tissue by cytokines and regulatory molecules focus the interest mainly on epithelial growth factor (EGF), TGF and nitric oxide synthases (NOS) (Gallo et al., 1998; Rubin Grandis et al., 1998; Sakaguchi et al., 1999; Gobert et al., 2002; Schiemann et al., 2002).

The hypothesis about direct and indirect influence of HP in pathogenesis of oropharyngeal spinocellular carcinoma came from these data.

Methods of HP detection in the oral cavity and pharynx

When detecting HP in the oral cavity, there is a need to use invasive tests. Non-invasive tests like the urea breath test (UBT), stool antigen test, or serology antibody test cannot show the presence of HP directly in the oral cavity. In gastric specimens, histological identification of HP can be performed. Several staining methods are in use. These include e.g. haematoxylin and eosin, modified Giemsa, Warthin Starry, Gimenez, and Genta (Rotimi et al., 2000). These staining methods provide low specificity in the case of oral samples, where other bacterial strains are often found (Dowsett and Kowolik, 2003). Some authors used immunohistochemistry to detect oral HP. They found no positivity in oral specimens (Di Bonaventura et al., 2000; Skinner et al., 2001; Uygur-Bayramicli et al., 2002). Detection of HP in the oral cavity has often used the CLO (campylobacter-like organism) test or RUT (rapid urease test), which is based on detection of urease production by HP (Qureshi et al., 1992). This test provides a suitable detection method for use on gastric samples. The oral cavity is residence to some other urease-producing species, including *Streptococcus* spp., *Haemophilus* spp., and *Actinomyces* spp. The potential for false-positive results is considerable. Therefore, this test alone cannot be recommended for

definitive identification of oral HP (Dowsett and Kowolik, 2003).

The currently accepted gold standard for the diagnosis of gastric HP is culture (Makristathis et al., 2004). Cultured strains of HP provide a high amount of material for molecular diagnostic methods. Culture of HP from the oral cavity has met with limited success (Dowsett and Kowolik, 2003). Tissue specimens must be transported to the laboratory in special transport medium immediately after collection. HP requires a micro-aerophilic environment, supplemented media, and up to 7-day incubation for growth. Under these conditions, overgrowth by other oral species often appears. Direct inhibition of HP by oral species *in vitro* has also been reported (Ishihara et al., 1997). Transformation of HP into unculturable, coccoid form in the unfavourable environment was described (Shahamat et al., 2004). This could also result in the failure to detect this bacterium in oral samples by conventional culture techniques (Dowsett and Kowolik, 2003).

Molecular methods such as polymerase chain reaction (PCR) permit amplification of a HP-specific region of DNA (Song et al., 1999). Even though PCR allows rapid detection of small numbers of bacteria, the results of studies that used PCR for detecting oral HP were highly variable, with a detection rate ranging between 0–90 % (Dowsett and Kowolik, 2003). The lack of uniformity of laboratory procedures can play a role in the reported inconsistencies. Different primers were used, e.g. those based on the urease genes, 16S ribosomal RNA genes, a gene encoding a specific 26-K protein, and randomly selected DNA fragments. The specificity and sensitivity differ among the primers (Song et al., 1999). PCR genotyping makes it possible to distinguish different HP strains and their carriage of genes encoding virulence factors (Pavlik et al., 2007).

The discrepancy of PCR results published recently shows the importance of finding a suitable PCR assay. Tissue sample collection and especially immediate immersion into proper transport medium is essential for successful test results (Pavlik et al., 2007).

It has to be considered that PCR allows detection of a low number of bacteria or non-viable bacteria, which cannot influence progress of the diseases.

HP in oropharyngeal lymphatic tissue

Several studies have explored the presence of HP in the oral cavity using different methods. HP has been detected in both dental plaque and saliva. It is suggested that the oral cavity and pharynx can be an extragastric reservoir for HP infection (Riggio and Lennon, 1999; Song et al., 2000; Allaker et al., 2002; Karczewska et al., 2002). Only a few studies have explored HP in tonsillar and adenoid tissue. The results of these studies were inconsistent. Minocha et al. (1997) first stressed the importance of tonsillar tissue for HP colonization. They found a history of tonsillectomy to be associated with decreased prevalence of gastric colonization by

HP. Nevertheless, the hypothesis that HP can colonize tonsillar and adenoid tissue has not been well confirmed yet (Table 1).

Unver et al. (2001) found HP in adenoid tissue, and Khademi and Imanieh (2005) found HP in tonsillar and adenoid tissue. These authors used only the CLO test and did not use any other method to prove that they really found HP. Yilmaz et al. (2004) investigated tonsillar and adenoid tissue samples. They did not find any tissue sample positive for HP by the CLO test. Skinner et al. (2001) investigated tonsillar tissue samples by the CLO test and immunocytochemistry. They did not find any specimen positive.

Uygur-Bayramicli et al. (2002) investigated tonsillar tissue specimens by histology and immunohistochemistry. They did not find any specimen positive, either. Pitkaranta et al. (2005) investigated adenoid tissue and middle ear fluid by culture. All adenoid tissue specimens and middle ear fluid samples were negative for HP by culture.

Di Bonaventura et al. (2000) investigated 72 bilateral tonsillar swabs for HP by culture and immunohistochemistry. All cultures of tonsillar swabs were negative for HP. In another study (Di Bonaventura, 2001) these authors used PCR for investigation of tonsillar swabs and biopsy specimens with no evidence of HP presence.

Cirak et al. (2003) and Bulut et al. (2006) found HP in tonsillar and adenoid tissue by using PCR. They found HP strains positive for the *cagA* gene. Bitar et al. (2005) investigated adenoid tissue samples by RUT, histology and nested PCR. They found positivity by RUT and histology, but no positivity by nested PCR. In their next study (Bitar et al., 2006) these authors investigated middle ear fluids and adenoid tissue samples using culture, RUT and PCR. All middle ear fluids were negative. In adenoids they found positivity by RUT, but none by PCR. Yilmaz et al. (2004) found HP in middle ear effusions and in one adenoid tissue sample using PCR with a 23S ribosomal RNA primer set. Yilmaz et al. (2006) found HP in middle ear fluid aspirates and promontorium mucosa samples by culture and PCR.

The relationship between HP infection and gastric tumour pathogenesis has been well described. It is influenced by HP's ability to modify the host immunological response. It is supposed that HP could act in the same way in progression of oropharyngeal tumorigenesis. Some authors tried to identify a correlation between HP and cancers of head and neck (Table 2). Tests which determined serum levels of anti-HP antibodies in patients with head and neck spinocellular carcinoma (HNSCC) brought inconsistent results (Grandis et al., 1997; Aygenc et al., 2001; Rubin et al., 2003; Nurgalieva et al., 2005). Okuda et al. (2000) proved the presence of HP in oral swab samples and oral cancer samples using reverse transcriptase PCR (RT-PCR) and culture. On the other hand, Kanda (2005) found no HNSCC specimen positive using PCR, culture and immunohistochemical analysis. Kizilay et al. (2006) did not find HP in laryngeal SCC and non-neoplastic specimens using haematoxylin and eosin stain or modified Giemsa stain. Akbayir et al.

Table 1. *Helicobacter pylori* in tonsillar and adenoid tissue

Author	Date of Publ.	No. of Subjects	Specimens	Diagnostic Method	Number of Subjects Positive
Di Bonaventura et al.	Oct. 2000	36	tonsillar swabs	culture, immunohistochemistry	0 (0 %)
Di Bonaventura et al.	April 2001	75	tonsillar swabs and biopsy	PCR	0 (0 %)
Unver et al.	Dec. 2001	19	adenoid tissue	CLO test	11 (58 %)
Skinner et al.	July 2001	50	tonsillar tissue	CLO test, immunocytochemistry	0 (0 %) CLO test and immunohistochemistry
Uygur-Bayramicli et al.	March 2002	27	tonsillar tissue	histology, immunohistochemistry	0 (0 %) histology and immunohistochemistry
Yilmaz et al.	Oct. 2003	50	tonsillar and adenoid tissue	CLO test	0 (0 %)
Cirak et al.	Nov. 2003	23	tonsillar and adenoid tissue	PCR (16S ribosomal RNA, <i>cagA</i>)	7 (30 %) positive for HP 5 of them (71 %) positive for the <i>cagA</i> gene
Yilmaz et al.	Dec. 2004	38	adenoid tissue, middle ear effusions	PCR (23S ribosomal RNA)	12 (67 %) in middle ear effusion 1 (5 %) in adenoid tissue
Pitkaranta et al.	Nov. 2004	20	adenoid tissue and middle ear fluid	culture	0 (0 %)
Khademi et al.	Sept. 2005	56	tonsillar and adenoid tissue	CLO test	27 (48 %)
Bitar et al.	May 2005	25	adenoid tissue	RUT, histology and nested PCR (UreA)	21 (84 %) positive by RUT 4 (16 %) positive by histology 0 (0 %) positive by nested PCR
Bulut et al.	April 2006	71	tonsillar and adenoid tissue	PCR (<i>cagA</i> - <i>glmM</i> gene)	29 (24.6 %) positive for HP 17 of them (58.6 %) CagA positive
Bitar et al.	Feb. 2006	28	adenoid tissue and middle ear fluid	culture, RUT, PCR (urease-C, adhesion subunit genes)	0 (0 %) middle ear fluids 10 (77 %) adenoid tissue by RUT 0 (0 %) by PCR
Yilmaz et al.	May 2006	22	middle ear fluid, promontorium mucosa, adenoid and tonsillar tissue	culture, PCR (16S RNA)	middle ear fluids: 2 positive by culture, 7 by mucosa PCR: 1 by culture, 7 by PCR adenoids 11 (50 %) by culture, 14 (64 %) by tonsillar PCR tissue: 12 (55 %) by culture, 14 (64 %) by PCR

(2005) found HP in specimens collected from laryngeal cancers and benign laryngeal disorders by using histopathological methods, but not immunohistochemical methods. Only one study performed PCR genotyping of HP strains in samples collected from the oropharynx. Tonsillar tissue specimens were collected from patients with chronic tonsillitis, obstructive sleep apnea syndrome (OSAS) and tonsillar cancer. The detected HP strains differ from strains found in the stomachs of Czech patients with gastric diseases (Pavlik et al., 2007).

Conclusion

The published data about oropharyngeal HP infection can be summarized as follows:

HP infection is very common in the population. HP is considered to be type I carcinogen and plays an important role in initiation of GIT carcinomas and lymphomas. HP could act as a direct mutagen, an immunosuppressor, and can induce increased production of cytokines and regulatory molecules, which are involved in tumour formation and cell transformation (e.g. EGF, TGF, NOS). It has been described recently that HP can be present in the oral cavity. It was found in dental plaque and saliva. However, the data about HP acting in oropharyngeal carcinogenesis are deficient.

Findings of HP in oropharyngeal lymphatic tissue are remarkable. Published data about the HP presence in tonsils and adenoids are inconsistent. The reported low detection rate could be due to usage of diagnostic tests

Table 2. *Helicobacter pylori* in head and neck cancers

Author	Date of Publ.	No. of Subjects	Specimens	Diagnostic Method	Number of Subjects Positive
Grandis et al.	May 1996	42	21 HNSCC 21 controls without SCC	serology – IgG antibodies	57 % with SCC 62 % controls
Aygenç et al.	Sept. 1999	58	26 laryngeal SCC 32 controls without SCC	serology – IgG antibodies	73 % with SCC 41 % controls
Okuda et al.	Jan. 2000	116	116 gastric and oral samples including 58 oral cancers	RT-PCR, culture	46.6 % gastric samples 12.1 % oral swab samples 100 % oral cancer swabs
Rubin et al.	Feb. 2003	61	6 severe laryngeal dysplasia 5 tonsillar SCC 50 other SCC	serology	38 seropositive (including all tonsillar SCC)
Akbayir et al.	Oct. 2003	100	50 laryngeal cancers 50 benign laryngeal disorders	histopathological and immunohistochemical methods	28 (56 %) SCC 1 benign by histol. 0 % evaluated by immunohist.
Kanda et al.	July 2005	31	31 HNSCC	PCR, culture, immunohistochemical analysis, serology – from urine	21 seropositive 0 PCR, culture, immunohist.
Nurgalieva et al.	Jan. 2005	230	119 laryngeal or pharyngeal SCC 111 controls without SCC	serology – IgG antibodies	32.8 % with SCC 27.0 % controls
Kizilay et al.	Apr. 2006	99	69 laryngeal SCC 30 non-neoplastic controls	histology – HE, modified Giemsa stain	0 %
Pavlik et al.	Dec. 2006	7	3 chronic tonsillitis 3 tonsillar SCC 1 OSAS	serology IgA, IgG, IgM PCR genotyping	2 of 3 chronic tons. serologically, 2 of 3 tons. 3 of 3 SCC and 1 of 1 OSAS by PCR

not sensitive enough for precise HP detection. The group of most accurate tests includes serology antibody tests that prove the presence of specific anti-HP antibodies, a test based on molecular technology (PCR) that proves specific HP DNA, and culture, which proves viable HP cells. CLO or RUT tests and histology, which are often used for detection of gastric HP, are not specific enough for detecting oral HP. Serology antibody tests are both specific and sensitive for HP infection. Nevertheless, these tests are not able to show the exact location of the infection. PCR is the most accurate method for HP detection. Both the sensitivity and the specificity rates are shown to be very high. This method is mostly resistant to the influence of external factors. However, the design of the PCR assay plays a decisive role for successful detection; the false-positive or false-negative rates are shown to be low. PCR is able to show the exact location of the detected infection. On the other hand, PCR is not able to show whether the nucleic acid material came from viable bacterium or just dead cells. Culture seems to be the most specific diagnostic method, but it is very sensitive to the influence of external factors (use of antibiotics, local disinfection, or local anaesthetics) and shows high rates of false-negative results.

Serology antibody tests can be used for screening HP-infected patients. PCR seems to be the most profit-

able method for detection of oropharyngeal HP infection. The negative results of culture should be treated with caution. On the other hand, the positive culture for HP seems to be the most accurate for evaluating the viability and pathological potential of HP strains. The combination of PCR and culture should yield the most valuable results of HP detection in oral specimens. With a combination of these methods, sufficient sensitivity and specificity rates should be achieved and light may be shed on some interesting new facts concerning the role of HP in oropharyngeal pathogenesis.

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