UNIVERSITAS CAROLINA PRAGENSIS

First Faculty of Medicine, Prague, Czech Republic

DETECTION OF HELICOBACTER PYLORI IN OROPHARYNGEAL PATHOLOGIES

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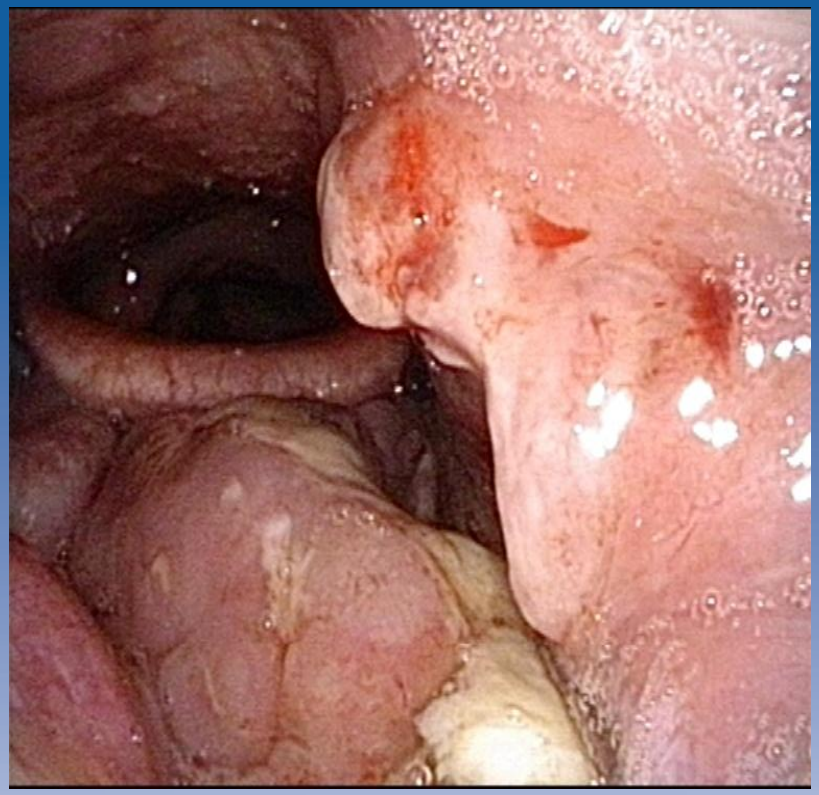
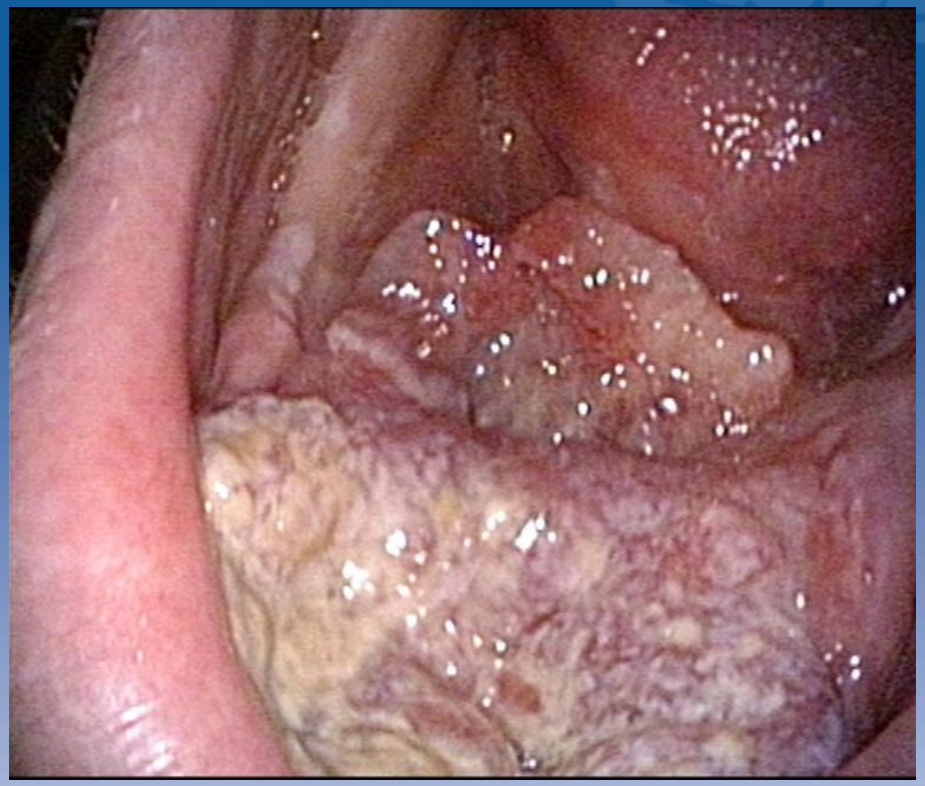
INTRODUCTION

Increasing rate of oropharyngeal malignancies in recent years in certain countries forced investigators to study possible role of chronic infections in development and progression of cancer in oropharyngeal area.



Tonsillar cancer, male patient , age37



Cancer of epiglotis and oral base, male patient, age 61

AIMS OF THE STUDY

- To confirm presence of *H. pylori* in the lymphatic tissue of oropharyngeal area in patients with different oropharyngeal pathology requiring surgical intervention, using different techniques for both direct and indirect diagnostics
- To genotype DNA of H. pylori isolated from peroperative biopsies for presence of *cagA* and variability of *vacA* genes using synthetised TaqMan hybridisation probes
- To confirm infection of each patient by at least one of different diagnostic tests: UBT, gastroscopy, specific antibody detection in blood (ELISA, WB)
- To compare, in cooperative patients undergoing gastroscopy with positive result, genotypes of isolates from both the gastric and oropharyngeal samples

MATERIAL

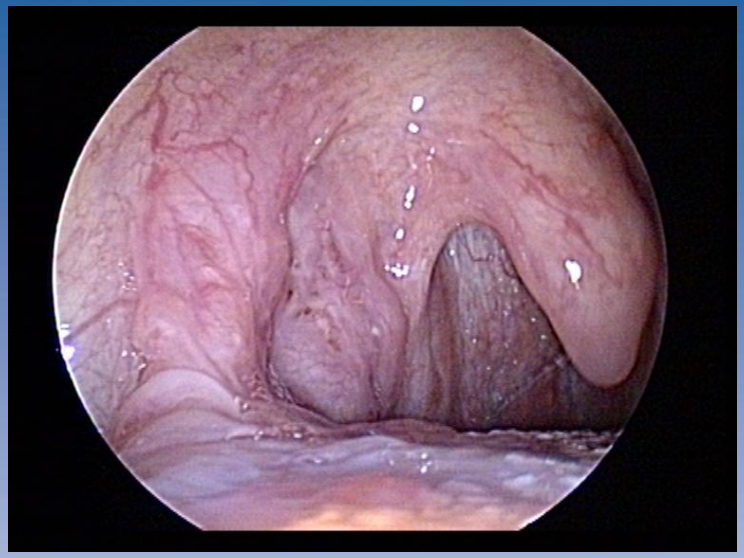
Peroperative biopsies taken from patients with following diagnoses:

Tonsillar cancer - 42

Oropharyngeal cancer - 10

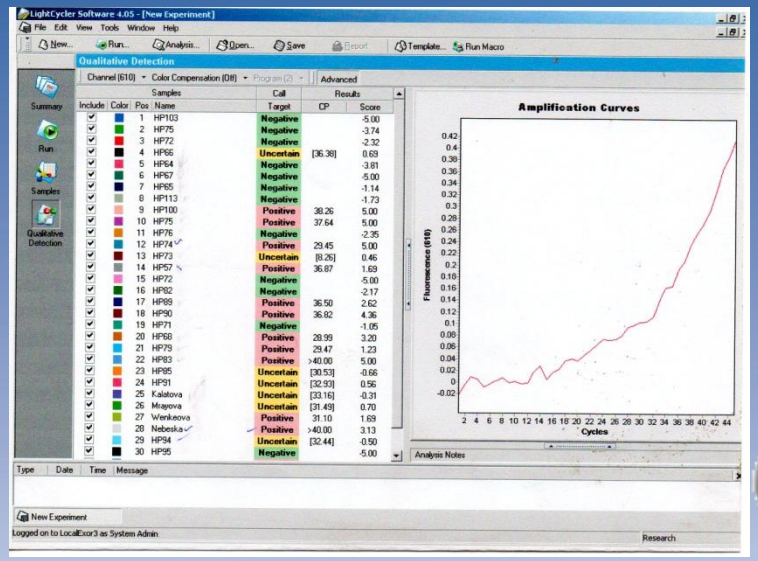
Chronic tonsillitis – 78

SAS



METHODS

- **Sample collection:** biopsies placed directly to Remel M4RT sample collection and transport media. Pre-analytic storage at +4°C (up to 5 days), long-term storage at -20°C
- **DNA Isolation:** Roche MagNA Pure Compact Extractor: MagNA Pure Compact Nucleic Acid Isolation Kit I, Protocol 400-100
- **Real-time PCR:** Instrument Roche LightCycler v.



METHODS

- Primers and TaqMan Probes cagA, vacA s1a, vacA s1b, vacA s2, vacA m1 vacA m2 (vanDoorn et al. 1999) synthetised and optimised by TIB MolBiol Berlin, Germany
- **Urea Breath Test:** in order to confirm H. pylori gastric infection, performed according manufacturer´s instruction in package insert
- **Detection of specific IgM, IgA and IgG:** by ELISA AND IMMUNOBLOT (WB) techniques based on commercial diagnostic tests

RESULTS

Tonsillar Cancer Patients: **Total: 42**

HP not detected	HP detected	cagA detected	cagA not detected
8	34	4	30
19.04 %	80.95 %	0.095 %/*11.76 %*	71.43 %/*88.23 %*

Most frequent genotype: cagA- vacA s1b m2 (8 - 23.53 %)

Oropharyngeal cancer patients: **Total: 10**

HP not detected	HP detected	cagA detected	cagA not detected
4	6	2	4
40 %	60 %	20 % / *33.33 %*	40 %/ *66.66 %*

Most frequent genotype: cagA- vacAs1b m2 (5 - 83.33 %)

Summarised Data: **Total: 52** cagA- vacAs1bm2 (13 - 32.5 %)

HP not detected	HP detected	cagA detected	cagA not detected
12	40	6	34
23.07%	76,93 %	11.54 %/ *15.00 %*	65.38 %/*85.00 %*

RESULTS

Chronic Tonsillitis Patients: Total: 78

HP not detected	HP detected	cagA detected	cagA not detected
13	65	14	51
16.66 %	83.34 %	17.95 %/*21.54 %*	65.39 %/*78.46 %*

Most frequent genotype: cagA- vacAs1b m1 (20), cagA- vacAs2 m1 (7)

SAS – originaly chosen as Control Group Total: 29

HP not detected	HP detected	cagA detected	cagA not detected
5	24	19	5
17.24 %	82.76 %	65.57 %/*79.16 %*	17,24 %/*20.84 %*

Most frequent genotype: cagA – vacAs1a m2 (4), cagA+vacAs1a m2 (4)

RESULTS

- In summer 2012 a new commercial test for detection of Helicobacter pylori – Bioron RealLine Fla-Format was launched on the market. We have used it to confirm our results
- Testing of all our genotyped isolates showed a 100% agreement in results
- Total samples: 130 + 29 n= 159

GENOTYPING Rt- PCR	BIORON Fla FORMAT rt-PCR	
	POSITIVE	NEGATIVE
POSITIVE	129	0
NEGATIVE	0	30

- This supports evidence of H. pylori presence in tissue of oropharyngeal malignancies

RESULTS

- **Genotyping H. pylori strains obtained from different body systems – stomach and oropharynx:**
- **First 6 patients have completed both the oropharynx peroperative biopsy and GIT biopsy**
- **Hypothesis to be confirmed: Individuals are colonised by the same bacteria strain**
- **Result: in 3 patients, genotyping of cagA and vacA genes showed identical result.**
- **In 3 patients comparison showed difference in both genes tested**
- **Eventhough no strong data on the issue exist yet, we are able to conclude, H. pylori strains may be different.**

DISCUSSION

- **H. pylori is a declared type I carcinogen (IARC 1994), Supposed 3 ways of its carcinogenic action are:**
- **Direct mutagen action (interaction of intracellular signaling molecules and CagA may predispose cells to accumulate genetic changes promoting multistep carcinogenesis – Hatakeyama 2006)**
- **VacA production causes immunosupression by blocking T cell proliferation (Boncristiano et al. 2003)**
- **Cell proliferation induction by cytokins and regulatory molecules involved in cell transformation and tumor formation – TGF, EGF and NOS (Konturek 1997, Gallo 1998, Sakaguchi 1999, Keates 2001, Gobert 2002, Schiemann 2002, Wang 2002)**

Di Bonaventura	2001	75	tonsillar swabs and biopsies	PCR	0 (0 %)
Cirak	2003	23	tonsillar and adenoid tissue	PCR (16S ribosomal RNA, CagA)	7 (30 %) H. pylori positive, 5 out of (71 %) cagA+
Yilmaz et. al.	2005	38	adenoid tissue, mesotitis secretion	PCR (23S ribosomal RNA)	12 (67 %)positives in mesotitis , 1 (5 %) in adenoid tissue
Bitar	2005	25	adenoid tissue	nested PCR (UreA)	0 (0 %) positives in nested PCR
Bulut	2006	71	tonsillar and adenoid tissue	PCR (CagA - glmM gene)	29 (24,6 %) H. pylori positive, 17 out of (58,6 %) CagA +
Bitar	2006	28	adenoid tissue	PCR (ureC-gene)	0 (0 %)PCR positive
Yilmaz et. al.	2006	22	adenoid and tonsillar tissue, mesotitis secretion, promotorium mucose	PCR (16S RNA)	mesotitis secretion 7 PCR+, promotorium 7 PCR+, adenoid tissue 14 (64 %) PCR+, tonsilar tissue 14 (64 %) PCR+
Eyigor et. al.	2009	55	35 adenoid tissues, 20 tonsills	PCR (glmM gene)	0 % PCR positives
Vilarinho et. al.	2010	62	tonsillar and adenoid tissue	PCR - DNA (vacA gene)	0 PCR positives
Abdel-Monem	2011	20	tonsillar and adenoid tissue	PCR (ureC-gene)	5 (16,6 %) PCR positives

CONCLUSIONS

- **Our study provides data supporting hypothesis, that Helicobacter pylori participates in oropharyngeal pathology more than it has been supposed**
- **Long-term colonisation of tonsillar tissue by H. pylori lacking CagA protein, concidered to be responsible for GIT pathology, may lead to development of chronic inflammation and alteration of immune system mechanisms resulting in uncontrolled formation of tonsillar neoplasias**
- **Eventhough detailed recognition of this process requires further investigation, prevention through eradication of so called non.pathogenic strains of the bacteria by antibiotics may help to reduce incidence of oropharyngeal neoplasias**

THANK YOU

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