Original Article http://mjiri.tums.ac.ir
Medical Journal of the Islamic Republic of Iran, Vol. 27, No. 2, Spring, May 2013, pp. 77- 82

Searching the H. pylori; serology & PCR in children with adenoid hypertrophy and rhino sinusitis: a cross sectional study, Tehran, Iran

Mohammad Farhadi[1], Samileh Noorbakhsh[2], Azardokht Tabatabaei[3]

Research Center of Pediatric Infectious Diseases and ENT and Head &Neck Research Center, Tehran University of Medical Sciences, Tehran, Iran.

Received: 14 June 2012 Revised: 6 October 2012 Accepted: 18 November 2012

Abstract

Background: The adenoid tissue may act as a reservoir for bacteria.Goal of study was to evaluate the role of H.pylori infection (PCR and serology) in children with adenoid hypertrophy and rhino sinusitis.

Methods: a cross- sectional study had done upon 53 children (Mean age 8 ±1.9 years) with adenoid surgery in ENT and Pediatric Department of Rasul Akram Hospital (2008-2010). Of 53 cases with adenoid surgery, 40 cases had rhino sinusitis (in sinus CT scan). The resected adenoid tissues (n=40) centrifuged and homogenized and its DNAs extracted and searched for H. pylori- DNAs by qualitative PCR. Serum H. pylori antibodies (IgG &IgA ELISA) calculated quantitatively. Chi square values ($p<0.05$) calculated for all categorical variables. The agreement between serologic test and PCR was assessed by the calculation of kappa statistic.

Results: Positive PCR for H. pylori detected in 15%of cases; regardless of sex and age of cases. Positive IgA: 17.5% (7/40) and IgG: 20% (8/40) respectively. All cases with positive H .pylori- IgG were female; $p=0.003$ and meaningful differences in mean age of cases observed for positive IgA ($p=0.001$) and IgG ($p=0.01$). Poor agreement observed between positive PCR and serum IgG&IgA (Fisher's Exact test=0.3; 0.5).

Conclusion: Positive PCR in adenoid tissue (15%) was very close to positive serum IgA (15%) but without any agreement for each case. The H .pylori infection may have a relative role at least in 15% of children with adenoid surgery. Chronic sinusitis and ear infection might be added to infected adenoid tissue as a reservoir for bacteria. The search by specific culture may elucidate better the role of H. pylori infections in both gastric and adenoid tissues. The decision for use of antibiotics to eradicate the H. pylori infection in recurrent or chronic adenotonsillar infections (with rhinosinusitis) before adenoid surgery needs Randomized Control Trial (RCT) studies. Drug of choice for eradication of H. pylori dependent to antibiotic sensitivity test in each country

Keywords: Adenoid tissue; Adenoid surgery; H. pylori; IgA, IgG; PCR.

Introduction

Tonsillar tissue is a component of mucosa-associated lymphoid tissue (MALT), which has evolved to protect vulnerable mucosal surfaces.

Aden tonsillectomy usually performed for obstructive symptoms, recurrent infection and chronic infection is the third indication for surgery (1-2).

Based on studies done on chronic rhinosinusitis (with otitis media) and adenoid hypertrophy in children have one etiologic factor (1-3). Brook et al highlighted the importance of the usual bacterial load in the adenoids in contributing to the etiology of recurrent otitis media, recurrent adenotonsillitis, and obstructive adenoid hypertrophy (4).

1. MD, Professor of ENT, Head &Neck Research Center, Tehran University of Medical Sciences, Tehran, Iran. farhadi@ent-hns.org
2. **(Corresponded author)** MD, Professor of Pediatric Infectious Disease, Research Center of Pediatric Infectious Diseases. Tehran University of Medical Sciences, Tehran, Iran. samileh_noorbakhsh@yahoo.com & s-noorbakhsh@tums.ac.ir
3. MS in microbiology, Research Center of Pediatric Infectious Diseases, Tehran University of Medical Sciences, Tehran, Iran. azardokht_tabatabaei@yahoo.com

Adenotonsillectomy efficacious in reducing the number and severity of subsequent episodes of throat infection for at least 2 year (1-4). The adenoid, which has a central role in the development of secretory otitis media, may act as a reservoir for bacteria causing ear infection and chronic rhinosinusitis (3-4).

H. pylori is a gram negative bacterium and the etiologic agent of some gastrointestinal and extra gastrointestinal diseases. Colonization of H. pylori has been found in dental plaques, saliva, tonsils, sinus mucosa and adenoids (5). Pediatric-based Helicobacter pylori research continues to contribute significantly to our understanding of both clinical and pathophysiological aspects of this infection. Studies from the Middle East report a high H. pylori prevalence and interfamilial transmission within population (6-8).

Indeed, H pylori infection has been epidemiologically linked to extra-digestive conditions and disease (8-10).

H pylori infection may be responsible for various endocrine disorders, such as autoimmune thyroid diseases, diabetes mellitus, dyslipidemia, obesity, osteoporosis and primary hyperparathyroidism (8-10).

Seroprevalence to H. pylori infection is high in Iranian population (16-18). At least in one study in Iran, H. pylori detected in 48.2% of adenoid specimens by CLO (Campylobacter-like organism) Rapid Urease Test (16).

Masoodpoor et al discussed the role of H, pylori infection in Iranian children with recurrent abdominal pain (17). Baghaei et al determined the H. pylori virulence by analysis of the cag pathogenicity island isolated from Iranian patients (18).

Childhood hygienic practice and family education status determine the prevalence of H. pylori infection in Iran.19 *Mansour-Ghanaei et al* reported the prevalence of H. pylori Infection among Children in Rasht (north of Iran) (20).

Some studies reported the association between H. pylori infection and upper respiratory diseases (e.g. chronic rhinosinusitis; chronic otitis media; chronic otitis media with effusion) (11-14) but little is known about the true colonization and the localization of these bacteria in the adenoid tissue of children in Iran.

The study goal was searching the H. pylori infection in adenoid tissue and serum IgA, IgG antibodies in children with adenoid surgery.

Methods

A cross- sectional study had done on 53 children with adenoid surgery in ENT and Pediatric Department of Rasul Akram Hospital during 2008-2010 .This study was approved by the Ethical Committee in the ENT and head &Neck Research Center in Tehran University of Medical Sciences. (Ethical Considerations detail in the end of article). Initially, a questionnaire was completed by an authorized physician, followed by complete clinical examinations.

Before surgery, all cases were visited by a pediatric specialist to check for other concomitant disorders (immunodeficiencies, diabetes mellitus, renal/heart failure, etc.). We excluded all cases with proven immunodeficiency, diabetes mellitus, renal failure, patients who had received antibiotics up to 2 weeks before surgery, and cases with known malignancy or other diseases proven in pathological studies.

2 ml Blood samples were centrifuged and the serum stored in a freezer at –20°C for the serological examination.

The study group consisted of 53 children (Mean age = 8 ±1.9 years) with recurrent or chronic adenotonsillar infections candidate for adenoid surgery selected continuously. Of 53 cases with adenoid surgery, 40 cases had rhino sinusitis (in sinus CT scan).

Specific H. pylori antibodies (IgG and IgA) were investigated by ELISA assay in all cases and controls. Using commercial kits (Chemicon-Germany), the results were interpreted quantitatively as recommended by the manufacturer.

During surgery, 1 cm^3 of adenoid tissue resected and put in sterile tubes and then

they were centrifuged, homogenized in the tubes and stored in a freezer at –80°C.

The polymerase chain reaction (PCR) template purification kit (Roche Diagnostics GmbH, Germany) was used for all prepared tissue samples. The contents of the binding column tube were transferred to a new sterile 1.5 ml tube, after which the integrity of the DNA assessed by gel electrophoresis (1% agarose gel).

H. pylori DNAs were identified by qualitative specific PCR primer kits (QIA quickP® QIAGEN; Germany). Diagnostic kits included ready-to-use PCR mix kits, positive and negative controls and other qualified reagents along with an easy to follow protocol to detect the H. pylori genome at as low as10 copies.

H. pylori: primers 93089 and 93261 were selected from consensus regions of the two available cag A gene sequences (GenBank accession no. L11714 and EMBL accession no. 70039) 400 bp product

Cag A gene:

Forward: AAT ACA CCA ACG CCT CCA AG
Reverse: TTG TTG CCG CTT TTG CTC TC

Statistical analysis

The student's t-test was used to determine significant differences in means for continuous variables and the Chi-squared test was used to compare categorical data in cases and controls. P values less than 0.05 were considered to be statistically significant.

The agreement between the serologic test and PCR was assessed by calculation of the kappa statistic. Landis and Koch suggested that if kappa is greater than 0.75, it represents excellent agreement, below 0.40, and poor agreement, between 0.40 0.75, which considered as intermediate to good agreement (21).

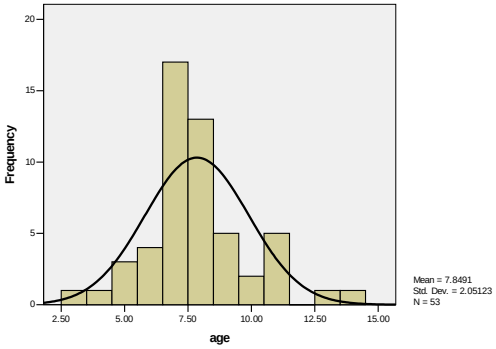

Fig. 1. Age distribution in years with adenoidectomized children.

Results

Demographic results

50% of cases were male and 50% female with the age of between 3-9 years and mean of 7.05±1.3 years (Fig.1).

Positive PCR obtained from tissues of 15% (6/40) of cases without sex prediction (male/female= 2/4; p=0.6).Positive results for serum H .pylori- IgA and IgG were 17.5% (7/40) and 20% (8/40) respectively. All of the cases with positive H .pylori-IgG were female (male/female =0/8; p=0.003) but it was not true for cases with positive H .pylori- IgA (male/female =3/4; p=1).Mean age of the cases had no significant differences between positive and negative PCR (7.3 ±1.3y vs. 7 ±1.3y p=0.5), but meaningful differences between cases with positive and negative resulted for H .pylori- IgA (7.3±0.2 y vs 5.5± 0.8y vs.; p=0.001); and IgG (7.3±0.9y vs 6± 0.2 y; p=0.01) was observed .(Table 1)

Poor agreement observed between the positive PCR and serum IgG antibody (actual agreement=66.6%; fisher test=0.3; Kappa= -0.20) in cases (Table. 2)

Poor agreement observed between PCR results and serum IgA antibody (actual

Table 1. Age and sex distribution in cases with positive and negative results.

Variable results Test	Age: Mean ±SD years Positive / negative	Male/female ratio Positive results
H. pylori-PCR	7.3 ±1.3y vs. 7 ±1.3y; p=0.5	male/female =2/4; p=0.6
H. pylori-IgA	7.3±0.2 y;vs 5.5± 0.8y p=0.001	male/female =3/4; p=1
H. pylori-IgG	7.3±0.9y vs 6± 0.2 y vs;p=0.01	male/female =0/8; p=0.003

P value <0.05 considered statistically significant.

Table 2. Correlation between PCR and IgG antibody in cases.

H. pylori PCR	H. pylori – IgA		Total
	Positive	Negative	Fisher test=0.5; Kappa=-0.19
Positive	0	6	6
Negativ	7	27	34
Total	7	33	40

P value <0.05 considered statistically significant.

Table 3. Correlation between PCR results and IgA antibody.

H. pylori PCR	H. pylori – IgG		Total
	Positive	Negative	fisher test=0.3; Kappa= -0.20
Positive	0	6	6
Negative	8	26	34
Total	8	32	40

P value <0.05 considered statistically significant.

agreement=67.5%; Fisher test=0.5; Kappa=-0.19) in cases (Table 3).

Discussion

Positive H. pylori found in adenoid tissues of 15% (6/40) children with adenoid surgery and was very close to positive IgA serologic results (17.5%) in younger cases with adenoid surgery (5.5± 0.8y ; p=0.001). But positive was not related (poor agreement) to positive IgA in each case.

Therefore, both tests confirmed the role of H. pylori infections in cases with adenoid hypertrophy. Moreover, serological studies could differentiated the real infection (positive PCR in adenoid tissue) from contamination of adenoid tissue during surgery with oral cavity in studied cases. We also found that cases with recent infection (IgA) were younger than those with past infection.

In contrast to IgA, positive serum IgG (past immunity) in 20% of studied cases had poor agreement with positive PCR in adenoid tissues (Kappa= -19; -0.20) and of cases with positive IgG (6 y vs. 7.3y) was close to cases with positive PCR (6 y vs. 7.3 years).

Serologic studies in children with adenoid surgery were very close to previous studies in cases with rhinosinusitis (20). Recent infection (positive IgA) were 15%; 13% in adenoid and rhinosinusitis cases respectively. But past immunity (positive IgG) was higher in children with adenoid surgery in comparison with rhinosinusitis cases (20 % vs. 11%). (20). The higher age for cases with adenoid surgery (7.5 vs. 4.2 years) could explain these differences.

H. pylori infection is a leading worldwide infectious disease since it affects more than half of the world population and causes chronic gastritis, peptic ulcer disease and gastric malignancies (7-8) Studies from the Middle East report a high H. pylori prevalence and interfamilial transmission (7-8).

The infection elicits a chronic cellular inflammatory response in the gastric mucosa. The effects of the local inflammation may not be confined solely to the digestive tract but may spread to involve extra-intestinal tissues and/or organs (7).

Indeed, H pylori infection has been epidemiologically linked to extra-digestive conditions and disease (8-10). Afifi et al reported an increased incidence of the iron deficiency anemia among H pylori positive children in India (10).

H .pylori infection varies between countries and often within a country. The positive H. pylori in present study were lower than Khadem et al study in Shiraz (south of Iran). Khadem et al reported the H. pylori infection (positive urease test) in adenoid tissues of 48.2% cases (age= 3 -43 years) (16).

Saffari et al studied H. pylori antibodies in Shiraz population (south of Iran). 28.3%

of population between 20-40 years and 32% 41-80 years had positive H. pylori-IgG. Positive H. pylori–IgA observed in16.7% and 53.5% respectively (15).

H. pylori infection may occured in 15% of our studied cases before 9 years and increases to up 48% in older ages in Khadem et al study (16).

15% of studied persons infected after 7th years. The higher age for studied cases with adenoid surgery in Khademi study could explain this difference (16).

Masoodpoor et al discussed the role of H, pylori infection in Iranian children with recurrent abdominal pain (17). Baghaei et al determined the H. pylori virulence by analysis of the cag pathogenicity island isolated from Iranian patients (18).

Nouraie et al study showed the childhood hygienic practice and family education status determines the prevalence of H. pylori infection in Iran (19).

Mansour-Ghanaei et al reported the prevalence of H. pylori Infection among Children in Rasht (north of Iran) 20 .They found no significant difference between the rate of H. pylori infection and individuals' ages, gender or socioeconomic levels.The source of drinking water had a role in transmission of *H. pylori* and it Transmission can be minimized with the use of boiled or mineral water (20).

All aforementioned data showed that initial H. pylori infection occurs at an early age (near 6 years) in Iran but prevalence of infection increases with age. The infection will increase to 30 % in 2th and 53.5% after 4th decade of life (15). Other studies reported the association between H. pylori infection and upper respiratory diseases (11-14).

A possible role of H. pylori in chronic rhinosinusitis has been reported by Ozdek (11).

H. pylori isolated from middle ear of the patients with chronic otitis media by Rapid urease test and 14C urea breathe test (12).

Skinner et al investigated the Helicobacter pylori and tonsillectomy (13).

Agirdir reported the role of H. pylori in patients with chronic otitis media with effusion (14).

We concluded that adenoid tissue may act as a reservoir for H .pylori infection just like other bacteria. These findings suggest that the detection and eradication of H pylori infection should be considered in the work-up of Iranian children before adenoid surgery; especially in those with sinusitis and chronic ear infection.

Mourad-Baars et al recent probiotic trials have not shown a benefit for H. pylori eradication in children, while sequential therapy remains an attractive therapeutic eradication strategy in children, which requires validation in different geographic regions (7).

The use of suitable antibiotics at least 2 weeks before adenoid surgery would be helpful in some patients (1-4). Response to eradication therapy needs to be further studies.

Limitations of the study

The small number of cases with rhinosinusitis was the main limitation. The search of H. pylori by specific culture or PCR simultaneously in both gastric and adenoeid tissue may elucidate the role of H. pylori infection in producing the adenoid hypertrophy.

Conclusion

Positive PCR in adenoid tissue (15%) was very close to positive serum IgA (15%) but without any agreement in each case. The H .pylori infection may had a relative role in at least 15% of children with adenoid surgery. Chronic sinusitis and ear infection might be added to infected adenoid tissue as a reservoir for bacteria. The search by specific culture may elucidate the role of H. pylori infections in both gastric and adenoid tissues.The decision for use of antibiotics to eradicate the H. pylori infection in recurrent or chronic adenotonsillar infections (with rhinosinusitis) before adenoid surgery needs RCT studies. Drug of choice for eradication of H. pylori depend to antibiotic sensitivity test in each country.

Acknowledgments

This study was supported by the "Research Center of Pediatric Infectious Diseases" and "ENT and head &Neck Research Center "in Tehran University of Medical Sciences. We thank Research Center of Cellular and Molecular Biology in "Tehran University of Medical Sciences.

References

1. Tuncer U, Aydogan B, Soylu L, Simsek M, C.Kucukcan. A Chronic rhinosinusitis and adenoid hypertrophy in children. Am J Otolaryngol. 2004 Jan-Feb; 25(1):5-10.

2. Shatz A. Indications and outcomes of adenoidectomy in infancy. Ann Otol Rhinol Laryngol. 2004 Oct; 113(10):835-8.

3. Bernstein J M, Dryja D, Murphy TF. Molecular typing of paired bacterial isolates from the adenoid and lateral wall of the nose in children undergoing adenoidectomy: implications in acute rhinosinusitis Otolaryngol Head Neck Surg. 2001 Dec; 125 (6):593-7.

4. Brook I, Shah K. Bacteriology of adenoids and tonsils in children with recurrent adenotonsillitis. Ann Otol Rhinol Laryngol. 2001 Sep; 110(9):844-8.

5.Cirak MY, Ozdek A, Yilmaz D, Bayiz U, Samim E, Turet S. Detection of H. pylori and its CagA gene in tonsil and adenoid tissues by PCR. Arch Otolaryngol Head Neck Surg. 2003 Nov; 129(11):1225-9.

6. Stasi R, Sarpatwari A, Jodi B. Segal, John Osborn, Maria Laura Evangelista, Nichola Cooper, Drew Provan, Adrian Newland, Sergio Amadori, and James B. Effects of eradication of Helicobacter pylori infection in patients with immune thrombocytopenic purpura: a systematic review. Blood. 2009; 113 (6): 1231-1240.

7. Mourad-Baars P, Hussey S, Jones NL. Helicobacter pylori Infection and Childhood.Helicobacter 2010; 15.S1:53-9.

8. Papamichael KX, Papaioannou G, Karga H, Roussos A, Mantzaris GJ. Helicobacter pylori infection and endocrine disorders: Is there a link? World J Gastroenterol 2009:14; 15(22): 2701–2707.

9. Fujiya M, Kohgo Y. Novel perspectives in probiotic treatment: the efficacy and unveiled mechanisms of the physiological functions Clinical Journal of Gastroenterology 2010: 3(3):117-127.

10. Afifi RAR, Kamal Ali D, Shaheen IAM.A Localized Case–Control Study of Extra-Gastric Manifestations of Helicobacter pylori Infection in Children. Indian J of Ped. 2011;78(4): 418-422.

11. Ozdek A, Cirak MY, Samim E, Bayiz U, Safak MA, Turet S. A possible role of Helicobacter pylori in chronic rhinosinusitis: a preliminary report Laryngoscope. 2003 Apr; 113(4):679-82.

12. Dagli M, Eryilmaz A, Uzun A, Kayhan B, Karabulut H. Investigation of Helicobacter pylori in the middle ear of the patients with chronic otitis media by CLO test and 14C urea breathe test. Otol Neurotol. 2006 Sep; 27(6):871-3.

13. Skinner LJ, Winter DC, Curran AJ, Barnes C, Kennedy S, Maguire AJ, Charles DA, Timon CI, Burns HP. Helicobacter pylori and tonsillectomy. Clin Otolaryngol. 2001 Dec; 26(6):505-9.

14. Agirdir BV, Bozova S, Derin AT, Turhan M.Chronic otitis media with effusion and H. pylori. Int J Pediatr Otorhinolaryngol. 2006 May; 70(5):829-34.

15. Saffari M, Motavalii MA, Fazeli A. Immunoblot Assay in Determination of Serum Antibody Profile of H. pylori Infection. Iran J Med Sci 2003; 28 (2):90-93.

16. Khadem B. Imanieh B. Gandomi, F. Yeganeh N,Nik nejad.Investigation of H Pylori Colonization in Adenotonsillectomy Specimens by Means of Rapid Urease (CLO) Test. Iran J Med Sci 2005; 30(3): 138-140.

17. Masoodpoor N, Darakhshan, Sheikhvatan M. Helicobacter pylori infection in Iranian children with recurrent abdominal pain. Trop Gastroenterol. 2008; 29 (4):221-3.

18.Baghaei K, Shokrzadeh L, Jafari F, Dabiri H, Yamaoka Y, Bolfion M, Zojaji H, Aslani M, Zali MR. Determination of Helicobacter pylori virulence by analysis of the cag pathogenicity island isolated from Iranian patients. Digestive and Liver Disease 2009:41(9): 634-638.

19. Nouraie M, Latifi-Navid S, Rezvan H, Radmard AR, Maghsudlu M, Zaer-Rezaii H, Amini S, Siavoshi F, Malekzadeh R Childhood hygienic practice and family education status determine the prevalence of H. pylori infection in Iran. Helicobacter.2009 Feb; 14(1):40-6.

20. Mansour-Ghanaei F, Yousefi Mashhour M, Joukar F, Sedigh M, Bagher-Zadeh AH, Jafarshad R. Prevalence of Helicobacter Pylori Infection among Children in Rasht, Northern Iran. Middle East Journal of Digestive Diseases, 2009; 1(2):23-9

21. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics.1977; 33:159 –174.