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### High Seroprevalence of Anti-*H. pylori* Antibodies in Patients with Ventilator Associated Pneumonia

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#### ABSTRACT

**Background:** Despite major advances in the management of ventilator-associated pneumonia, its pathogenesis is not clearly known. Recently, the role of gastric colonization has been proposed. We compared the prevalence of *H. pylori* by serology in patients with VAP and in control subjects to determine the role of *H. pylori* and gastric colonization in the pathogenesis of VAP.

**Methods:** 118 intubated and mechanically ventilated patients were included and divided into two groups; 59 subjects with VAP and 59 control patients. Results of the serologic tests, demographic characteristics and time of blood sampling were registered.

**Results:** Mean age in seropositive patients was significantly higher. 71.2% in the VAP group and 61.01% in controls were IgG seropositive (P=0.24). IgM seropositivity was 23.73% versus 8.47% in VAPs and controls, respectively (P=0.024). By increasing the time of intubation, more patients became seropositive for IgM (Pearson's correlation coefficient=0.4, P=0.002).

**Conclusion:** IgM seropositivity and serum level were significantly higher in VAP patients. Also by increasing the duration of intubation and time of sampling, serum levels and seropositivity for IgM increased significantly.

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## Introduction

Ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs 48 hours after the initiation of endotracheal intubation and is clinically suspected when leukocytosis, fever, purulent tracheal secretions and development of new or progressive infiltrate on chest radiograph are seen (1). It is the most common infection in intensive-care-unit (ICU), affecting 6 to 52% of patients (2). Recently, the role of gastric colonization in the pathogenesis of the VAP has been proposed (3). Some studies have shown that stomach is colonized by enteric Gram-negative bacilli (GNB) and increasing the gastric PH is the main predisposing factor (4, 5).

*Helicobacter pylori* (*H. pylori*) is a Gram-negative bacillus which colonizes the stomach. The prevalence of *H. pylori* is up to 80% among adults in developing countries (6). This organism produces the urease enzyme that catalyzes the hydrolysis of urea into ammonia and CO<sub>2</sub> which leads to gastric pH alteration (6). Few studies have demonstrated that *H. pylori* can gain access to the bronchial tree and may cause pneumonia due to GNB (7). Also, recent studies suggest that there might be an association between *H. pylori* infection and respiratory diseases (8, 9). Several pathogenic mechanisms have been proposed including activation of inflammatory mediators by *H. pylori* infection, inhalation of *H. pylori* or its exotoxins into the respiratory tract and genetic predisposition (10, 11).

Despite major advances in the management of VAP patients, the mortality rate is relatively high ranging from 20% to 76% (12, 13). More knowledge about the pathogenesis of VAP, could improve prevention and treatment options. In this study, we aimed to evaluate the prevalence of *H. pylori* by serology in patients with VAP and compare it with control subjects to determine the role of *H. pylori* and gastric colonization in the pathogenesis of VAP.

## Materials and Methods

### Study Design

This case-control, a retrospective study was conducted in Imam Reza Hospital (Tehran, Iran), a referral care university teaching hospital, from August 2014 to November 2015.

### Patient selection

118 patients who were intubated and mechanically ventilated in ICU at least 48 hours were enrolled in the study. All patients aged 18 to 75 years old.

The case group has consisted of 59 patients with VAP. The diagnosis was made based on the presence of at least 3 from 4 major clinical criteria including leukocytosis, fever, purulent tracheal secretions and development of new or progressive infiltrate on chest radiograph.

There were also 59 controls who were matched with cases in gender and age to decrease the effect of confounding factors.

### Sampling

All patients (cases and controls) underwent blood sampling for assessment of anti-*H. pylori* IgM and IgG serologic tests with enzyme-linked immunosorbent assay (ELISA) (Monobind Inc, Lake Forest, California). When the serum level of IgM antibodies against *H. pylori* was  $\geq 20$ , between 10 and 20 and  $\leq 10$  U/ml, the result was considered as positive, equivocal and negative, respectively. Also, for IgG antibodies these serum levels were  $\geq 12$ , between 8 and 12 and  $\leq 8$  respectively. Equivocal results were excluded from further analysis. Sample analysis was done by technicians who were unaware of the patient's condition. Results of the serologic tests, demographic characteristics of the patients and time of blood sampling (days after intubation) were registered in Data entry form.

### Ethical consideration

Informed consent was obtained from all subjects prior to the study. Patients' information remained confidential. All aspects of this study were approved by the Institutional Review Board of the AJA University of Medical Sciences.

### Statistical analysis

Analysis was performed using the SPSS ver.18 (SPSS Inc., Chicago, USA). For analysis the difference between groups,  $\chi^2$ -test and Independent Samples T-Test were used to compare proportions and continuous variables, respectively. A P-value<0.05 was considered statistically significant.

### Result

Demographic characteristics of the participants are shown in table 1. There was no significant difference in age or gender distribution and intubated days between the groups ( $p>0.05$ ).

From all 118 participants, 66.1% (78 patients) and 16.1% (19 patients) were seropositive for IgG and IgM, respectively. Mean age in seropositive patients was significantly higher than others (53.59 versus 38.45 for IgG<sup>+</sup> and IgG<sup>-</sup> subjects, respectively,  $P<0.001$  and 60.74 versus 46.1 for IgM<sup>+</sup> and IgM<sup>-</sup> subjects, respectively,  $P<0.001$ ). 71.2% in the VAP group and 61.01% in controls were IgG seropositive which the difference was not significant ( $P=0.24$ ). Also, the serum level of IgG was not significantly different between the groups ( $P=0.16$ ). More information is shown in table 2.

IgM seropositivity was 23.73% versus 8.47% in VAPs and controls, respectively which was significant ( $P=0.024$ ). Also, there was a significant difference in serum level of IgM between groups ( $P=0.041$ ).

In all patients, intubation days were higher in seropositive patients for both IgG and IgM but the difference was only significant in IgM positive patients ( $6 \pm 2.42$  for IgG<sup>+</sup> vs.  $5.3 \pm 1.6$  for IgG<sup>-</sup>,

$P=0.1$  and  $6.89 \pm 2.26$  for IgM<sup>+</sup> vs.  $5.55 \pm 2.13$  for IgM<sup>-</sup>,  $P=0.014$ ). Also, intubation days for IgM positive patients were significantly higher only in the VAP group ( $7.07 \pm 2.34$  vs.  $5.42 \pm 2.08$  for IgM<sup>+</sup> and IgM<sup>-</sup> patients, respectively,  $P=0.014$ ).

For understanding the relationship between IgM results and intubation days, a bivariate correlation was used. Analysis of the results in VAP group showed that there is the direct and significant relationship between IgM results and intubation days (Pearson's correlation coefficient=0.4,  $P=0.002$ ), in other words by increasing the time of intubation, more patients became seropositive for IgM.

### Discussion

There is no consensus about the role of gastric colonization in the pathogenesis of VAP. Also there are numerable studies about *H. pylori* as the most common pathogen which colonizes the stomach and its association with VAP. In the recent study, the prevalence of seropositive patients for *H. pylori* was evaluated and compared between two groups of patients including cases and controls to determine the role of *H. pylori* in the pathogenesis of VAP.

The mean age of IgM and IgG-seropositive patients was significantly higher. In developed countries *H. pylori* infection is more prevalent in childhood and adolescent, so many adults are seropositive for IgG and the risk of infection is more in older ages. These findings are consistent with several studies which found older patients have a higher IgG level (14-17).

Although IgG and IgM seropositivity and serum level were higher in VAP patients only IgM results were significant. Also by increasing the duration of intubation and time of sampling, serum levels and seropositivity for IgM increased significantly. These findings demonstrate that there are higher rates of acute and chronic *H. pylori* infection in VAP patients. Higher IgG levels could be explained by older ages and probably more comorbidity in VAP patients, but there is no explanation for higher IgM levels

**Table 1.** Demographic characteristics of the participants.

Parameter	VAP	No VAP	P-value
Age	50.34 ± 14.72 <sup>a</sup>	46.58 ± 15.42	0.18
Gender	37 males 22 females	41 males 18 females	0.43
Days intubated	5.81 ± 2.24 (2-12) <sup>b</sup>	5.71 ± 2.17 (2-13)	0.8

a: Mean ± Standard Deviation

b: range (minimum-maximum)

**Table 2.** Detailed information about serology results in cases and controls.

parameter		VAP	No VAP	P-value
IgG	seropositivity	42 (71.2%)	34 (61.01%)	0.24
	serum level (u/ml)	52.31	41.1	0.16
IgM	seropositivity	14 (23.73%)	5 (8.47%)	0.024
	serum level (u/ml)	22.17	14.31	0.041

because it increases in acute infection. It is unlikely to consider IgM rise due to acute primary *H. pylori* infection because of patient's age and condition. They have hospitalized in ICU and *H. pylori* transmission is not a possible route of infection. To explain these findings there is 3 possible hypothesis: 1) Several studies have demonstrated the association between increasing gastric pH and GNB colonization (4, 5). In intubated patients, rates of gastroesophageal reflux are higher (18, 19); so it is possible that in VAP patients, *H. pylori* colonizes the stomach and cause GNB colonization by gastric PH alteration. Then, gastroesophageal reflux and oropharyngeal colonization of GNB occurs and aspiration of colonized bacteria might cause VAP in these patients. This stomach-pharynx-lower respiratory tract infection route has been shown in many studies (4, 7, 20). 2) Gastroesophageal reflux and aspiration of *H. pylori* could help it reach to tracheobronchial tree. It made the environment susceptible for GNB colonization and development of VAP. Also it is possible that *H. pylori* cause VAP primarily by Inflammation of the respiratory membrane by its proteins. Mitz

et al. (7) study supports this hypothesis but, more studies with a precise microbiologic assessment to determine the exact origin of the pathogens are needed. 3) Another possible hypothesis is activation of systemic inflammatory mediators by *H. pylori* proteins or inhalation of *H. pylori* or its exotoxins into the respiratory tract and development of VAP. Studies which have been assessed the association between *H. pylori* infection and respiratory diseases will support this idea (8-11). Future studies are needed to evaluate systemic or respiratory inflammatory factors involved in the pathogenesis of VAP and their association with *H. pylori* infection.

## Conclusion

IgM seropositivity and serum level were significantly higher in VAP patients. Also by increasing the duration of intubation and time of sampling, serum levels and seropositivity for IgM increased significantly. *H. pylori* infection, subsequent GNB colonization and aspiration to a tracheobronchial tree or systemic inflammation are possible causes. Further studies with larger

sample sizes, detailed and precise laboratory and microbiologic assessment are needed to evaluate the possible role of *H. pylori* in the pathogenesis of VAP.

### Conflict of interest

The authors declare that they have no conflict of interest.

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