PEPSIN AND AMYLASE IN ORAL AND TRACHEAL SECRETIONS: A PILOT STUDY

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Background
Endotracheal intubation increases the risk for microaspiration of secretions around the tube cuff. Pepsin has been used as a biomarker for gastric aspiration. Amylase is a newer proposed biomarker for aspiration of oral contents.

Objective
To assess the presence of pepsin and amylase in paired oral-tracheal secretions of adult patients treated with mechanical ventilation.

Methods
In this descriptive study, paired samples of oral and tracheal secretions were obtained from adults at baseline and again within 4 hours when a need for endotracheal suctioning was assessed. Assays of pepsin and amylase were processed in a specialty diagnostic laboratory.

Results
The sample consisted of 10 men and 3 women with a median age of 56 years. The majority were intubated with a subglottic suction endotracheal tube (9 patients, 69%), receiving synchronized intermittent mandatory ventilation (10 patients; 77%), and receiving enteral feedings (11 patients; 85%) through a tube distally placed in the stomach (8 patients; 67%). Pepsin was present in oral secretions of 9 patients (69%), and in tracheal specimens of 7 patients (54%) at one or both sampling times. Amylase was detected in all patients’ oral secretions and in tracheal secretions of 5 patients (38%) at one or both sampling times.

Conclusions
Many patients had pepsin, amylase, or both in tracheal aspirates. Pepsin was more commonly detected than was amylase. Although the relationship of this finding to long-term outcomes was not assessed, findings indicate that microaspiration of oral and gastric secretions occurs frequently. (American Journal of Critical Care. 2014;23:334-338)
In this pilot study, we investigated pepsin and amylase as indicators of microaspiration to test the laboratory methods and determine the incidence of microaspiration.

Methods

Design

A descriptive design was used. The study was approved by the appropriate institutional review board. Each patient’s legal proxy provided consent for the patient’s participation.

Setting and Sample

The study was conducted in the critical care units of a large tertiary-care hospital in the Southeastern United States. A convenience sample of adult (>18 years old) patients who were orally intubated and treated with traditional mechanical ventilation was enrolled. Because the primary intent of the study was to test the laboratory methods and gather pilot data related to incidence of microaspiration, no power analysis was performed.

Procedures

Demographic data were obtained from the medical records. After enrollment, paired specimens of tracheal and oral secretions were obtained at baseline. The tracheal aspirate was obtained by using a closed-system suction device (Trach Care, Kimberly Clark). Sterile physiological saline without preservatives (5 mL) was used to flush tracheal secretions into the trap. Oral secretions were collected into a trap via an oropharyngeal suction catheter (Sage Products). The procedures were repeated 1 to 4 hours later when cues for endotracheal tube suctioning were detected. Each patient thus had 2 paired specimens. Specimens were placed on ice, transported to the specialty laboratory, and frozen to -20ºC until analysis. Assays were run in batches, and the protocol specified keeping the specimens frozen until analysis.

Pepsin was measured in a proteolytic enzyme assay with casein labeled with fluorescein isothiocyanate.9-11 A value of 6.5 ng/mL or greater was considered positive for aspiration of gastric contents because of the analytic sensitivity of the lower detection limit of the method. 

Amylase was measured by using the Stanbio α-Amylase LiquiColor reagent kit modified for a microplate reader. Increase in absorbance is measured via a spectrophotometer. A value of 0.6 μmol/min per milliliter (approximately 1305 IU/L) was considered positive for aspiration of oral contents.

Critical ill patients treated with mechanical ventilation are at increased risk for microaspiration of secretions, which may include oral contents, gastric contents, or both, around the cuff of the endotracheal tube.1 Oral secretions are laden with bacteria, increasing the risk for infection in the event of aspiration. Aspiration of gastric contents is associated with inflammation and infection. Microaspiration goes largely undetected until problems associated with inflammation and infection occur.1 Recently, biomarkers of microaspiration in tracheal secretions have been investigated.4,5 Pepsin is widely used to detect aspiration of gastric contents; however, specimens must be obtained within a short period after aspiration occurs because pepsin breaks down quickly.1 Aspiration of oral secretions has been typically assessed by culturing tracheal aspirates, which is costly and time consuming. Researchers1,5-8 have proposed that amylase, normally present in the mouth, could be used as a biomarker for aspiration of oral contents.

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and were receiving synchronized intermittent mandatory ventilation (10 patients; 77%). Median ventilator settings were fraction of inspired oxygen 0.35, tidal volume 700 mL, positive end-expiratory pressure 5 cm H2O, and pressure support 10 cm H2O. Nearly half of the patients (6; 46%) had a trauma diagnosis; the other patients had a variety of conditions. All patients received H2-blocking agents. Twelve patients (92%) had feeding tubes; 8 tubes were placed in the stomach and 4 were postpyloric. Enteral feedings were administered to 11 patients (85%) at rates ranging from 35 to 65 mL/h. Median backrest elevation was 30°, and median pressure in the cuff of the endotracheal tube was 24 cm H2O. The second set of specimens was obtained a median of 2 hours after collection of the baseline specimens.

The Table gives data on the presence of biomarkers in paired secretions. Pepsin was present in the oral secretions of 9 patients (69%) at one or was considered positive for aspiration; this value was the lowest that could be detected with this method. The amylase could be of salivary or pancreatic origin; however, because the patients were in critical care units and the samples were obtained from the mouth and trachea, amylase from a pancreatic source is unlikely. Values were converted to international units per liter.

Data Analysis
Frequencies (categorical data) and descriptive statistics (continuous data) were computed by using IBM SPSS, version 20, software (IBM SPSS).

Results
Data on 10 men and 3 women were collected between June and December 2012. The median age of the patients was 56 years, and the median duration of intubation was 5.5 days. The majority were intubated with a subglottic suction endotracheal tube (9 patients, 69%) and were receiving synchronized intermittent mandatory ventilation (10 patients; 77%). Median ventilator settings were fraction of inspired oxygen 0.35, tidal volume 700 mL, positive end-expiratory pressure 5 cm H2O, and pressure support 10 cm H2O. Nearly half of the patients (6; 46%) had a trauma diagnosis; the other patients had a variety of conditions. All patients received H2-blocking agents. Twelve patients (92%) had feeding tubes; 8 tubes were placed in the stomach and 4 were postpyloric. Enteral feedings were administered to 11 patients (85%) at rates ranging from 35 to 65 mL/h. Median backrest elevation was 30°, and median pressure in the cuff of the endotracheal tube was 24 cm H2O. The second set of specimens was obtained a median of 2 hours after collection of the baseline specimens.

The Table gives data on the presence of biomarkers in paired secretions. Pepsin was present in the oral secretions of 9 patients (69%) at one or
both sampling times. The median at baseline (T1) was 6.9 ng/L (25th-75th percentile [interquartile range, or IQR], 0-27.6 ng/L). The median at the second sampling (T2) was 7.3 ng/L (IQR, 0-38.4 ng/L).

Pepsin was detected in the tracheal secretions of 7 patients (54%) at one or both sampling times; the median was 0 ng/L (IQR, 0-9.6 ng/L) at T1 and 0 ng/L (IQR, 0-14.2 ng/L) at T2. Amylase was detected in oral secretions of all patients at both times (median at T1 was 195 029 IU/L [IQR, 47 767-773 358 IU/L] and median at T2 was 273 305 IU/L [IQR, 104 617-627 628 IU/L]) and in the tracheal secretions of 5 patients (38%) at one or both sampling times (0-6115 IU/L at T1 and 0-5240 IU/L at T2).

In summary, 4 patients (31%) experienced no microaspiration, 4 patients (31%) had only pepsin detected, and 2 patients (15%) had only amylase detected. A total of 3 patients (23%) had both pepsin and amylase present in at least 1 tracheal specimen.

Discussion

The majority of critically ill patients had detectable levels of pepsin in tracheal secretions, despite a median backrest elevation of 30°. Investigators have shown that elevation of the head of the bed is inversely related to the presence of pepsin. The majority of our patients who tested positive for pepsin had feeding tubes and received gastric feedings, which could be factors leading to pepsin positivity in the airway. However, we had 1 patient without a feeding tube whose oral and tracheal secretions also were positive for pepsin, indicating that aspiration can occur in those without enteral feedings. Presence of pepsin varied widely between specimen collections in our study, possibly because of various mechanisms of gastric reflux, which does not occur continuously.

We detected amylase in the oral secretions of all patients and in the tracheal secretions of 5 (38%). The number of patients positive for amylase during the second specimen collection decreased to 4, and the mean value was lower. The reduction may be due to the oropharyngeal suctioning procedure. The suction catheter may be more effective than is the suction swab commonly used at our medical center for removal of oral secretions.

We detected pepsin, amylase, or both in 9 patients despite interventions known to reduce microaspiration (see Table). All patients who aspirated oral or gastric secretions had the head of the bed elevated 30° or higher, and the pressure in the cuff of the endotracheal tube was 20 cm H₂O or greater. Among our sample, 6 patients with aspiration were intubated with a subglottic suction endotracheal tube, which is thought to reduce microaspiration. However, 3 of the 4 patients with a traditional endotracheal tube had documented aspiration.

Our study is limited by the small sample size and data collection at only 2 time points. The patients’ history of gastric reflux was not obtained. Trending of data over time to identify patterns and the relationship to outcomes, such as ventilator-associated pneumonia, are important. However, our primary purpose was to test the laboratory methods and to obtain data for a related clinical trial. The lower limit for detecting amylase with our methods was higher than that reported in other studies. Additional modifications of our laboratory procedures to detect lower levels of amylase are warranted; however, the values we obtained were similar to those reported for patients receiving mechanical ventilation who had risk factors for aspiration and infections.

Our findings are consistent with those of other reports of pepsin and amylase as biomarkers of microaspiration. Dewavrin and colleagues argue that the use of amylase is only moderately accurate in diagnosing microaspiration. However, their definition of microaspiration was the presence of pepsin in the tracheal aspirate. We argue that patients can aspirate secretions from the stomach or the mouth and that both pepsin and amylase may be needed to assess the presence of microaspiration. Aspiration of oral contents without stomach contents may be of clinical importance itself. Furthermore, the known abundance of amylase in the mouth may make it a better biomarker for detecting aspiration of oral contents, given that reflux of stomach contents is only episodic. Knowledge of potential microaspiration may facilitate earlier detection of patients at risk, and additional prevention efforts could be implemented.

FINANCIAL DISCLOSURES

None reported.


