Salivary Amylase Level in Bronchoalveolar Fluid as a Marker of Chronic Pulmonary Aspiration in Children

Mutasim Abu-Hasan, MD,¹ Mai Elmallah, MD,¹ Dan Neal, MS,² and James Brookes, MD³

Background: Chronic pulmonary aspiration is a common cause of chronic respiratory symptoms in children. However, there is no gold standard diagnostic test for aspiration. In this study, we explore the diagnostic value of measuring salivary amylase in bronchoalveolar lavage (BAL) fluid as a marker of chronic aspiration in children with different chronic respiratory illnesses.

Methods: Measurements of salivary amylase in BAL fluid were routinely done in patients undergoing flexible bronchoscopy. Patients' demographic and clinical data were extracted from records and reviewed by one of the investigators. Patients were classified into three different groups based on the reviewer's assessment of risk for aspiration. BAL amylase measurements were masked from the reviewer. Multiple regression analysis was used to determine the effect of the patients' clinical variables on BAL amylase.

Results: Sixty-four patients (median age 2 years; range 0–14 years) were included. Indications for bronchoscopy included chronic cough ($n=20$), chronic wheezing ($n=27$), Cystic Fibrosis ($n=6$), recurrent pneumonia ($n=5$), and lung infiltrate in immunocompromised patients ($n=6$). Young age, history of excessive drooling, and wet cough were predictive of high BAL amylase. Thirteen patients were considered at no risk of aspiration, 41 patients were at low risk, and 10 patients were at high risk based on clinical symptoms and other diagnostic tests. No significant differences in BAL amylase levels were found between the three groups. However, when high and low risk groups were combined and compared to the no risk group, there was a significantly higher BAL amylase level in the combined at risk groups (1,722 vs. 307 U/L; $p=0.03$). Receiver operator curve analysis demonstrated that amylase cutoff value of 250 U/L differentiates between the two risk groups with sensitivity of 66.7% and specificity of 69.2%.

Conclusion: Salivary amylase level in BAL can help identify children at risk for chronic pulmonary aspiration.

Introduction

CHRONIC PULMONARY ASPIRATION (CPA) of digested and undigested food is a common problem in children. It causes significant morbidity in affected patients due to chronic respiratory symptoms. These symptoms include chronic cough, wheezing, choking, recurrent pneumonia, and radiological signs of chronic lung injury.^{1–5}

Diagnostic tests for chronic aspiration include swallow study, radionuclide salivograms, fiberoptic-endoscopic evaluation of swallowing (FEES), and lipid laden macrophages in bronchoalveolar lavage (BAL) fluid.^{6–9} Unfortunately, gold standard diagnostic test for CPA does not exist.¹⁰ In this study, we explore the use of measuring amylase in BAL fluid as a diagnostic test for CPA in children.

Besides aspiration of food, CPA also includes the repeated passage of saliva into the airways in a manner sufficient to

cause chronic or recurrent respiratory symptoms. Chronic aspiration of saliva is a recognized form of aspiration in children with neurological impairment.¹¹ Most neurologically impaired children who aspirate saliva do so because of severe swallowing incoordination and absent laryngeal sensation.¹² An evaluation for aspiration of saliva is warranted in these children who continue to have symptoms of aspiration despite cessation of oral feeding and despite adequate treatment of gastroesophageal reflux. A demonstrative case would be a child with neurological impairment who is exclusively fed through a gastrostomy tube and had received a gastric funduplication procedure. Patients at risk for CPA of food due to poor coordination could aspirate saliva concomitantly. Therefore, detection of saliva in the lower airways could serve as a general marker for chronic aspiration in children.

Salivary amylase is produced by salivary glands, particularly by the parotid, contributing up to 20–30% of total

¹Division of Pediatric Pulmonary and Allergy; ²Department of Neurosurgery, College of Medicine; University of Florida, Gainesville, Florida.

³Pediatric Otolaryngology—Head and Neck Surgery, Alberta Children's Hospital, Calgary, Alberta, Canada.

protein in saliva. The majority of the enzyme (80%) is synthesized in the parotids, and the remainder in the submandibular glands.¹³ Amylase is not produced in the tracheobronchial tree or alveoli. Therefore, its presence in the lower airways should be abnormal.

Salivary amylase levels have been studied in the tracheobronchial secretions of seriously ill adults in the intensive care unit and found to correlate with aspiration.¹⁴ However, amylase has not been studied as a tool to diagnose chronic aspiration in children. Therefore, we hypothesized that measuring the amylase content of the BAL is a potential diagnostic test for CPA in children.

Method

The study is a retrospective review of medical records and BAL amylase measurement results in all pediatric patients who underwent diagnostic flexible bronchoscopy and BAL amylase measurement at the University of Iowa between 2004 and 2006. The study was approved by the institution's review board.

Bronchoscopy was performed for different clinical indications, including chronic unexplained cough, chronic wheezing, Cystic Fibrosis (CF), recurrent pneumonia, and immune compromise with lung infiltrate. All procedures were performed under conscious sedation using intravenous (IV) midazolam and IV fentanyl. A flexible bronchoscope was passed through the nose and advanced into the nasal pharynx. Surpraglottic and glottic structures were examined. The bronchoscope was then passed through the vocal cords into the trachea, the main stem bronchi, and main lobar bronchi. BAL was obtained from the lobe that has most pathology based on chest imaging or bronchoscopic findings. In the cases where no obvious pathology existed on radiography or bronchoscopy, the BAL was obtained from the right lower lobe. The BAL was obtained using three aliquot of 1 mL/kg of normal saline. Return fluid was trapped in a sterile container. BAL fluid was then separated for different lab tests, including bacterial culture, viral culture, fungal culture, gram stain, AFB stain, cell count and differential, a quantitative index of lipid laden macrophages, and an amylase assay. BAL amylase level was routinely obtained for all patients.

Amylase measurement in the BAL fluid was conducted by a functional assay using photometric techniques (Roche Diagnostics, Mannheim, Germany). A synthetic glucose polymer (5 ethylidene-G7PNP) is hydrolyzed by amylase present in the patient specimen. This step releases fragments termed "G2PNP," "G3PNP," and "G4PNP." These fragments, in turn are hydrolyzed by α -glucosidase, which is present in the reagent. This process releases glucose and free PNP or p-nitrophenol. Only free PNP is colored, and can be monitored optically at 415 nm. The measurement is kinetic, meaning the reaction rate is determined.¹⁵ This measurement method is the same used for routinely measuring pancreatic amylase in peritoneal and pleural fluid.

Patients' medical records were queried, and the following demographic and clinical information was obtained: age, sex, prematurity, diagnoses, family history, social history, smoke exposure, presenting symptoms, indications for bronchoscopy, other diagnostic tests, and their results.

Patients were divided into three groups based on the clinical determination for the risk of aspiration as follows: patients at high risk for aspiration, patients at low risk for

TABLE 1. CRITERIA USED TO DETERMINE CLINICAL RISK OF CHRONIC PULMONARY ASPIRATION

	No risk	Low risk	High risk
Predisposing conditions	No	Yes	Yes
Abnormal swallow	No	Yes/No	Yes
Positive aspiration test	No	No	Yes

aspiration, and patients at no risk for aspiration (Table 1). BAL amylase measurements were masked from the investigator making the determination. Factors examined included (1) the presence or absence of underlying conditions causing aspiration, (2) evidence of abnormal swallow mechanism, and (3) positive diagnostic tests of aspiration other than BAL amylase. Underlying causes of aspiration included young age (<1 year), developmental delay, seizure disorders, prematurity, gastroesophageal reflux, and craniofacial anomalies. Indications of abnormal swallow mechanism included excessive drooling, oropharyngeal secretions while asleep, choking and cough with feeds, and abnormal swallow mechanism by barium swallow testing (Table 2). Positive diagnostic tests for aspiration included demonstration of aspiration on swallow study and high lipid laden macrophage index >60.¹⁶ Table 1 summarizes the use of the above criteria to determine risk of aspiration.

Statistical analysis

Only patients with available amylase measurements in the BAL fluid were included in the analysis. Analysis of variance was used to compare amylase level between multiple groups, and Student's *t*-test was used to compare the two groups. Logistic regression analysis was performed to determine significant clinical predictors of high amylase level in BAL fluid. Correlation analysis between lipid laden macrophage index and BAL amylase level was also performed. Statistical significance was considered when $p < 0.05$. Since the distribution of amylase level was skewed, all statistical tests were performed on the natural logarithm of amylase to meet distributional assumptions.

Receiver-operating characteristic (ROC) curve analysis was constructed to determine the amylase cutoff value with highest true positive (TP) and lowest false positive (FP) rates. Seventeen BAL amylase measurements were randomly

TABLE 2. COMPARING SYMPTOMS AND DIAGNOSES BETWEEN AT RISK AND NO RISK GROUPS

	No aspiration risk group (n/total)	Aspiration risk group (n/total)	p-Value
Night time cough	2/13	27/51	0.02
Cough with feeds	0/13	12/51	0.05
Wheezing	5/13	23/51	0.46
Excessive drooling	0/13	16/51	0.02
Cystic fibrosis	6/13	0/51	0.00
Immunocompromised	4/13	2/51	0.01
Neurological impairment	0/13	4/51	0.39
Seizure disorder	0/13	4/51	0.39
Gastroesophageal reflux	0/13	2/49	0.63

obtained from the 25th percentile to the 90th percentile of all amylase/log (amylase) measurements and used as possible cutoff values. True positive and false positive rates were calculated for each possible cutoff value using clinical risk of aspiration as the gold standard. The true positive rate at each cutoff value is defined as the number of patients with BAL-amylase that is higher than the cutoff value divided by number of patients at risk for aspiration. False positive rate is defined as number of patients with BAL amylase level that is higher than the cutoff value divided by number of patients with no risk for aspiration. The cutoff value with the highest TP rate and lowest FP rate was then identified.

Results

A total of 64 patients (45 males and 18 females) were included in the study. Median age was 2 years (range 0–14 years). Indications for bronchoscopy included chronic unexplained cough ($n=20$), chronic unexplained wheezing ($n=27$), CF ($n=6$), recurrent pneumonia ($n=5$), and immunocompromised with lung infiltrate ($n=6$).

Based on the investigator's assessment of aspiration, 13 patients (median age 5 years; range 0–14 years) were considered to be at no risk, 41 patients (median age 2 years; range 0–14 years) were at low risk, and 10 patients (median age 2 years; range 0–9 years) were at high risk for aspiration.

No significant differences in BAL amylase levels existed between the three clinically identified risk of aspiration groups. However, when both high and low risk groups were combined into one group and compared to the no risk group, there was a significantly higher mean log(amylase) level in the at risk groups combined compared to the no risk group (mean raw amylase 1,722 and 307 U/L respectively; $p=0.03$; Fig. 1). ROC analysis demonstrated that amylase cutoff value of 250 U/L (Log Amylase 2.4) differentiates between the two risk groups and is associated with true positive rate (sensitivity) of 66.7% and false positive rate (1-specificity) of 30.8% (Fig. 2).

Young age, history of excessive drooling, and history of wet cough were predictive of high BAL amylase (Table 2). There was a significant negative correlation between BAL amylase and age ($p=0.002$; Fig. 3). Also, subjects who drooled excessively had significantly higher amylase levels ($p=0.005$), as did subjects who had a wet cough ($p=0.02$; Fig. 4).

FIG. 1. BAL amylase level in patients who are at risk for chronic aspiration vs patients at no risk.

FIG. 2. Receiver-operator curve of difference BAL amylase levels using clinical risk as gold standard. BAL amylase log of 2.4 has the highest TP and lowest FP rate.

BAL amylase was significantly higher in patients with positive BAL gram stain (1,857 vs. 479 U/L; $p=0.014$) and patients with positive BAL culture (1,632 vs. 84 U/L; $p=0.049$) after excluding CF patients. Also, BAL amylase was significantly higher in patients with positive BAL oral flora culture (1,803 vs. 270 U/L; $p=0.004$). There was no correlation between amylase level and airway inflammation as indicated by BAL white cell count and neutrophil percentage.

Lipid laden macrophage index was not significantly different between at risk and the no risk groups (32 vs. 47 respectively; $p=0.6$). No significant association between LMMI and BAL amylase was found either.

Discussion

In this study, we evaluated amylase levels in the BAL fluid as a diagnostic test for chronic aspiration in children. BAL amylase was obtained in a number of pediatric patients undergoing bronchoscopy and BAL during a 2 year period. Indication for procedure differed between patients. BAL amylase was found to be significantly higher in the group of patients considered at risk of aspiration based on clinical

FIG. 3. Graph showing significant correlation between patient's age and BAL amylase level.

FIG. 4. BAL amylase in patients with and without history of excessive drooling.

presentation and diagnostic tests other than BAL amylase. These results suggest that BAL amylase can potentially be a valuable diagnostic test for chronic aspiration.

Amylase is secreted by salivary gland and is present in abundance in the oropharyngeal secretions.¹³ In theory, amylase should not be present below vocal cords because of the absence of any amylase secreting glands in the airways. Therefore, any amylase detected in BAL fluid is from the oropharynx. Entry of saliva into the airways below the vocal cords may be due to chronic aspiration of oropharyngeal secretions. However, it could also be due to the acute aspiration of secretions during the bronchoscopy procedure, or due to contamination of bronchoscopy equipment with upper airway secretions. This may explain the high variability of amylase levels obtained in both groups of patients.

To minimize such variability of amylase BAL level further, more strict standardization of the BAL collection is required. Also, measurements of urea level may be needed in the future to adjust for dilution effect and to eliminate variability due to difference in concentrations of retrieved BAL fluid. Further limitations of this study stems from the fact that the BAL measurements were conducted using semi-quantitative functional assay rather than a quantitative detection method. The retrospective design of the study is another limitation. The study does not include normal subjects, and therefore the normal BAL amylase values remain unknown.

In a study of 16 tracheotomized adults who underwent total laryngectomy and were believed to have no communication with the GI tract, Nandapalan *et al.* found that small but variable amounts of amylase were found in their tracheobronchial aspirate (35–1,125 U/L).¹⁷ This study and future similar studies in subjects without known pulmonary symptoms may help define normative values of BAL amylase.

Measurement of BAL amylase as a diagnostic test in pediatric patients has not been previously studied. There is one report of measuring amylase in tracheal aspirate from endotracheal tubes in critically ill adults to evaluate aspiration of secretions.¹⁸ Another report evaluated tracheal aspirate in tracheotomized adults and showed that high amylase level was associated with higher rate of lung infections.¹⁴ Most recently, Weiss *et al.* demonstrated that BAL amylase > 125 U/L was predictive of risk of aspiration and bacterial pneumonia in intubated adult patients.¹⁹

Aspiration of oral secretions is a recognized clinical problem in the pediatric population, especially among children with severe neurological impairment.^{20,21} Some children may have aspiration of saliva with continued respiratory symptoms, even if aspiration from oral feeding and gastroesophageal reflux has been effectively treated. Some of these patients continue to have chronic and recurrent respiratory symptoms after gastric funduplication procedure and despite being exclusively fed through a gastrostomy tube, which strongly suggests that their symptoms are due to aspiration of oral secretions rather than aspiration of digested or undigested food. Moreover, treatment of excessive saliva production in neurologically impaired children results in improvement of respiratory symptoms.^{22–24}

This study showed no association between lipid laden macrophage index and BAL amylase. This lack of correlation could be due to differences in accuracy between the two tests. It also indicates the possibility that different disease processes influence the two tests.

The finding in this study that young age and history of excessive drooling were predictive of high BAL amylase indicates that excessive drooling can be a sign of poor swallow coordination in young children and therefore raises the suspicion for aspiration as a cause of chronic respiratory symptoms.

The radionuclide salivagram is used to try to detect salivary aspiration. A small quantity of radiotracer is placed in the buccal pouch, and serial images are taken until the tracer clears the mouth. Activity in the trachea or bronchi indicates aspiration. Though it has been reported that the salivagram is a sensitive test for salivary aspiration, there was only 26–28% prevalence of positive salivagrams in children suspected of aspiration seen in three retrospective studies.^{25–27} Future studies are needed to compare the salivagram to BAL amylase in diagnosing aspiration of oral secretions.

In conclusion, measurement of amylase in BAL fluid can be helpful in the diagnosis of chronic aspiration and aspiration of oropharyngeal secretions. A standardized method to measure BAL amylase is needed with a comparison to salivagrams. However, in light of the absence of any golden standard test of aspiration, diagnosis of aspiration in children remains largely clinical.

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Author Disclosure Statement

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Address correspondence to:
Mutasim Abu-Hasan, MD
Department of Pediatrics
University of Florida
1600 Archer Road
Gainesville, FL 32606

E-mail: mabuhasan@peds.ufl.edu

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