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Original Research

Activity of alpha amylase in the tracheobronchial secretions of patients without morbid salivary aspiration

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ABSTRACT:

Background

The normal values of α-amylase in humans is unknown.

Objective

To determine the normal values of α-amylase activity in humans.

Material and methods

From October 2009 to June 2011 we studied 107 patients referenced to thoracic service to be submitted to bronchoscopy.

The patients were positioned in supine position, performed local antisepsis and anesthetized with 2% lidocaine. Thereafter we introduced a needle into catheter by puncturing the cricothyroid membrane using a 14G cateter. Finally we introduced ten milliliters of saline and immediately aspirated with the maximum power of the vacuum system. The samples were sent for α- amylase activity determination by CNPG method.

Results

The activity of α-amylase of tracheal aspirate ranged from 24 to 10.000 IU/l), and a mean 1914IU/L. The levels had no statistical differences according age, sex, race, smoking history and the lung diseases.

Conclusion

We could define the probably physiologic levels of α-amylase activity in humans beings.

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INTRODUCTION

There found no reference in the previously published material on the normal value of α-amylase in tracheobronchial secretions. The amylase in the lungs of humans under normal condition probably has two components—one is from the local production and other from the physiological microaspiration (Nandapalan *et al*., 1995a; Sano *et al*., 1986; Amberson 1937; Huxley *et al*., 1978; Gleeson *et al*., 1997; Clarke *et al*., 1981). Takano in 1938 demonstrated in rabbits that the blood of their right heart had major amylase concentration than the left chambers. The plausible explanation was the passage of amylase from lung circulation to general circulation (Nandapalan *et al*., 1995a). Sano studied the activity of enzymes in lung tissue of humans by histochemical techniques found that the enzyme most frequently presented on the specimens was amylase, principally of salivary type and discovered salivary glands acinus in lung parenchyma (Sano *et al*., 1986). In 1985 Nandapalan studying α-amylase activity in laryngectomized patients without salivary fistula found an α-amylase activity range from 35 to 1025, mean 428, standardad deviation 367, and median of 295 IU/l in tracheobronchial secretions (Amberson 1937). His group considered these values as the normal levels of this enzyme in normal human beings. We disagree with that conclusion because it had long being demonstrated that normal people microaspirates saliva (Huxley *et al*., 1978; Gleeson *et al*., 1997; Clarke *et al*., 1981; Pecora 1959; Morishita *et al*., 2000; Almeida *et al*., 2015). This is the main reason of our work, to define the normal levels of this enzyme in tracheobronchial secretions of people with very low possibility of morbid aspiration, based on known risk factors. There is a possibility that α-amylase could be a marker of tracheobronchial aspiration of saliva and may be considered a tool for assessing aspiration of patients with oropharyngeal dysphagia (Pecora 1959; Morishita *et al*., 2000; Nandapalan *et al*., 1995b). The main application of this tool could be in weaning from mechanical ventilation, allowing us to diagnose oropharyngeal dysphagia with major accuracy than the card test. Which is very subjective and a as rule not used in clinical practice. Currently, an objective tool to diagnose oropharyngeal dysphagia before extubation does not exist. By comparing saliva and tracheobronchial amylase activity, it is possible to diagnose oropharyngeal dysphagia and take measures to reduce saliva secretion and prevent weaning failure, thereby reducing morbidity, costs and mortality. This project was approved by CEP HU UFJF, Number:0129/2009.

The ideal procedure to define physiologic parameters of lung amylase should use healthy volunteers; but this probable would not be approved by the Committee on ethics in human beings, because the procedures have some side effects and a little, but possible, risk of complications.

Objective

To determine the normal levels of α-amylase in tracheobronchial secretions and saliva of patients without the risk of aspiration.

Table 1. General data of patients involved in the study

		Frequency	Percentage	Mean	P Value	S. Deviation
Race	White	71	66%			
	Non-White	31	29%		0.159	
	Missing	5	5%			
Sex	Male	73	68%		0.139	
	Female	34	32%			
Smoking	Yes	71	66%			
	No	33	31%		0.222	
	Missing	3	3%			
Age (Years)				48.7		14.4

Table 2. Comparison of α-amylase activity according to the demographyc data

		Mean	S.Deviation	P Value
Sex	Male	2041IU/L	1603IU/L	0.389
	Female	1573IU/L		
Race	White	1735IU/L	2199 IU/L	0.388
	Non-White	2177	2169IU/l	
Smoking	Yes	1812IU/L	2467IU/L	0.85
	No	1907IU/L		
Age(Years)		48.5 Y	14.8Y	0.99

IU/L= International unit per liter, Y= Years, S. deviation= Standard deviation

MATERIAL AND METHODS

From October 2009 to June 2011, we prospectively evaluated 107 patients without clinical signals of hypersecretion who underwent transtracheal puncture before undergoing bronchoscopy. Inclusion criteria were patients without hypersecretion in the tracheobronchial tree, and who were referred to the Thoracic Surgery Service for diagnostic flexible bronchoscopy. Patients with any risk factors for aspiration owing to neurologic or muscle degenerative disease, acute cerebral ischemic event, and surgery or radiotherapy of the cervical region, age higher than 65 years were not included in this study.

All patients were asked to lie in a supine position with light cervical hyperextension. The antisepsis was performed at the anterior cervical region using a 70% alcoholic solution. All patients underwent sedation and local anesthesia.

Sedation and analgesia was administered intravenously with a combination of diazepam and meperidine to achieve a sedation level of 2–3 in the Ramsay scale. The local anesthesia comprised the skin, subcutaneous, and cricothyroid membrane levels and was performed using 0.5 to 1 ml of 2% lidocaine solution (Xylestesin[R])

We performed the puncture of the cricothyroid membrane with a 25/7G needle, and 10 ml of lidocaine (2%) without a vasoconstrictor was injected into the tracheobronchial tree. Lidocaine was allowed to take effect by waiting for two minutes and allowing it spreading into the tracheobronchial tree. This technique of anesthesia eliminates the cough reflex and leaves no free lidocaine in the major airways.

The transtracheal puncture was then performed using an intravenous catheter passing into a 14G needle (BioCat[R]-são Paulo-Brazil) (Pecora 1959; Morishita *et al*., 2000; Pecora 1970; Pratter and Irwin 1979; Almeida *et al*., 2015). After the puncture, the needle was positioned about 45°in the cranial-caudal direction. With the catheter introduced 5–10 cm in the tracheal lumen, we performed an aspiration with the vacuum system at maximum power. In the event of no retrieval of secretion, the patient was confirmed as non-hypersecretory. For such cases, we proceeded with the infusion of 10ml saline solution, and immediately the catheter was connected to the vacuum system, through which the specimen was aspirated.

The vacuum system was a conventional system

Table 3. α-Amylase activity in tracheobronchial and saliva secretions of normal humans beings

	Mean	Range	95% CI
TBcheal	1914	24 -10.000	(1436-239IU/L)
Saliva	225362	137543- 313181	(137543-313181)

TBcheal= Tracheobronchial; CI= Confidence interval

Table 4. Comparison between the present and Nanadapalan data

	Almeida	Nandapalan
N.Pts	107	16
A.TB(Mean)	1914IU/L	428IU/L
A.Sal(Mean)	22.536IU/L	28143IU/L
Range A.TB	24-10.000IU/L	35-1125IU/L
AIndex (Range)	0.0- 5.09	0,00- 0,03
Aindex (Mean)	0.10	0.0148

N.PTS= Number of patients, A.TB= Amylase tracheobronchial, A.Sal= Amylase salivary, Aindex= Amylase index

with a pressure from 30 to 40 cm Hg. When the volume of the aspirate was at least 100 µl, it was collected as a sample of saliva (bottle 1) and the aspirate (bottle 2).

The high viscosity of both fluids was responsible for the negative results in 19 patients. After diluting the samples, this problem was solved. After these procedures the patients were submitted to video-naso-laringo-bronchoscopy and evaluated for morbid aspiration. In case of positivity for aspiration the patient should be excluded from the study. This fact never had occurred.

Activity of α-amylase was measured using α-2-chloro-p-nitrophenyl-α-maltotrioside1, 4 which was hydrolised by α-amylase releasing 2-chloro p nitrophenol that could be measured by photometry. We used LabMax 240 (Labtest ®-Amylase CNPG liquiform, Lagoa Santa, Minas Gerais, Brazil) for the measuring of α-amylase in both sample fluids.

SPSS software version 13 for Windows was used to compile the survey data. We used, KS text for normality evaluation, c-squared test for dycotomic versus dycotomic variables, Student T test for means versus dycotomic, Pearson correlation for analyzing the relationship between salivary and tracheobronchial amylase, descriptive statistics and Kruskal-Wallis test to compare amylase activity among the various radiological diagnoses . The study project was approved by the Ethics and Research Commission at the Juiz de Fora Federal University (number: 0129/2009), Minas Gerais, Brazil. All patients signed an informed consent term.

RESULTS

In table 1, the data of participating patients have been provided. There were no difference among the parameters analyzed.

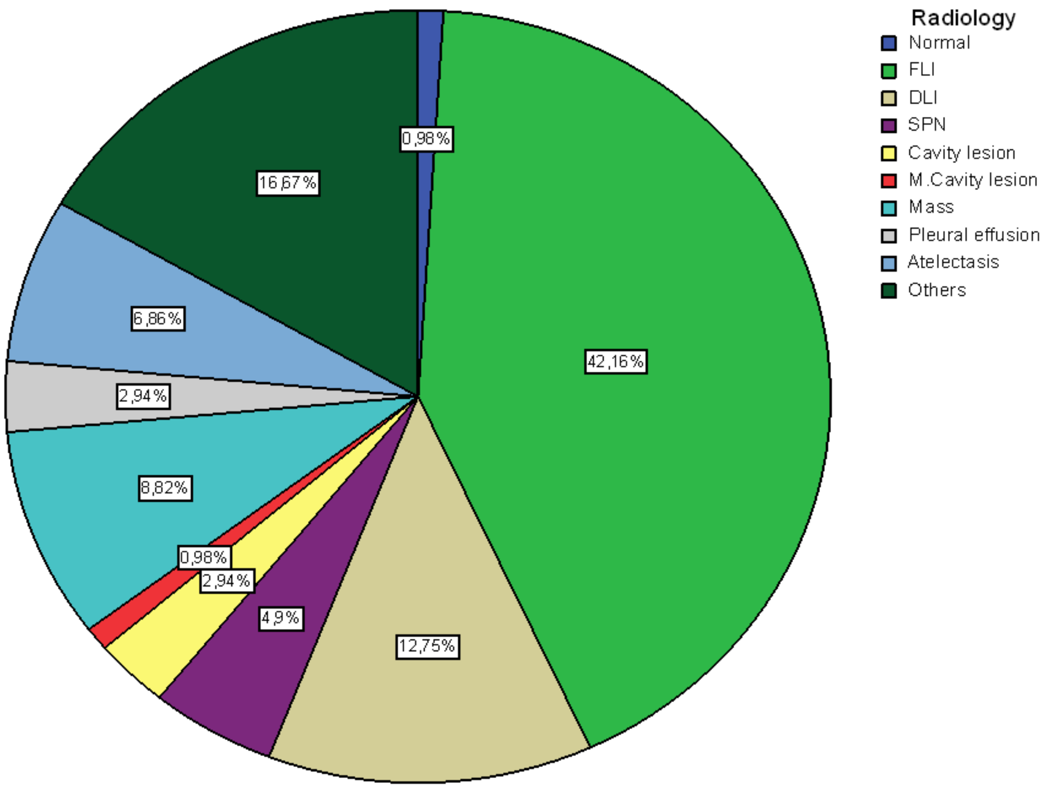
Table 2 contains the values of α-amylase activity in tracheobronchial secretions according to the

Table 5. Multivariability analysis : Amylase Tracheal versus radiology

Radiology		N	Mean rank	P Value
Amilase por PTC	Normal	1	34.00	
	FLI	35	44.71	
	DLI	13	40.31	
	SPN	3	53.50	
	Cavity lesion	2	73.50	
	M.Cavity lesion	1	7.50	0.69
	Mass	7	29.57	
	Pleural effusion	2	35.50	
	Atelectasis	7	48.57	
	Others	13	39.54	
	Total	84		

FLI= Focal lung infiltrate, DLI= Diffuse lung infiltrate, SPN= Solitaty pulmonary Nodule, M. cavity lesions= Multiple cavity lesion

Radiological diagnoses



FLI= Focal lung infiltrate, DLI= Diffuse lung infiltratre, SPN=Solitary Pulmonary Nodule.

demographic data. Race, sex, age and smoking history did not have influenced the results.

Table 3 shows the normal values of tracheobronchial and saliva α-amylase activity

Table 4 shows the comparison between the present, experimental data and Nandapalan`s data. We have significant differences between and Nandapalan's data which implies that the difference should be due physiologic microaspiration.

Table 5 shows the radiological diagnoses of patients and amylase activity

We did not find influence of the disease in the levels of amylase activity and the radiological diagnose

Figure 1 shows the Receiver Operating characteristic Curve. The cut-off of 0.1, called amylase index, was the best cut-off level I at discrimination between physiologic and abnormal amylase activity

Figure 2 shows the Radiological diagnose of patients and amylase activity. There was no statistically difference among the various radiological findings.

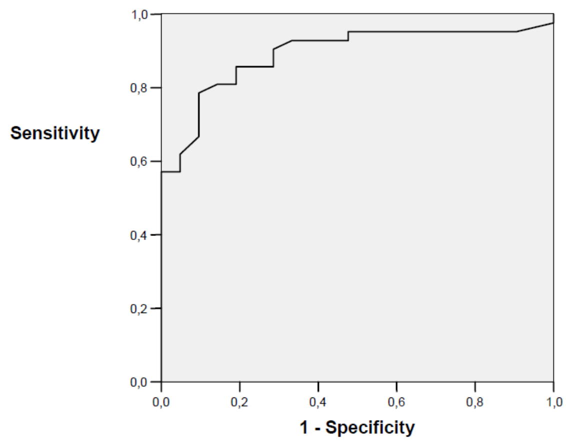
DISCUSSION

The α-amylase activity in the human tracheobronchial tree seems to have two origins.

One is from local production and the other is from physiological microaspiration (Amberson 1937; Huxley *et al*., 1978; Gleeson *et al*., 1997; Clarke *et al*., 1981). Nandapalan in 1995 published two works on this—one on laryngectomized patients and the other on tracheotomized patients. It was shown that the human lung produces amylases and thought that this should be the normal value of α-amylase activity in humans (Amberson 1937).

We disagree with this information because we did know that physiological microaspiration occurs (Huxley *et al*., 1978; Gleeson *et al*., 1997; Clarke *et al*.,

ROC Curve
Relation of Tracheal and Saliva Amylase



Area under the curve: 0.892

1981). Therefore, evaluating patients without the signs of morbid aspiration to obtain normal values of α-amylase in the lungs need to be performed. This is important because α-amylase could be an useful tool in the diagnosis of morbid aspiration (Pecora 1959; Morishita *et al.*, 2000; Nandapalan *et al.*, 1995b; Almeida *et al.*, 2015).

The gold standard for diagnosis of aspirations are video fluoroscopy and video endoscopy (Logemann *et al.*, 1998). However, both methods have limitations. Video fluoroscopy is only useful in patients with some degree of cognition to obey the order made by the speech therapy specialist for treating oropharyngeal dysphagia. Video endoscopy has advantages and disadvantages in comparison with video fluoroscopy (Logemann *et al.*, 1998); for example, video endoscopy does not evaluate the oral phase of dysphagia, and it is also not worldwide economically viable.

In addition to highly attractive, such an approach should be stimulated by health managers. Health systems are overloaded with the increasing technological costs in recent decades.

Transtracheal aspiration or puncture (TTA, TTP) has been used widely because of the original publication, although it is not an ideal procedure because transfixing the anterior wall of the trachea, as in the original method carries major risk of haemorragic complications (Pecora 1959; Morishita 2000; Pecora 1970; Pratter and Irwin 1979; Almeida *et al.*, 2015). The recommended name for this technique would be, "transcricothyroid membrane puncture" owing the fact it is an avascularity structure and with less chance of thyroid gland penetration, therefore with less chance of bleeding (Pecora 1970; Pratter and Irwin 1979; Almeida *et al.*, 2015).

The more appropriate term for the technique should be, "transcrycoid puncture (TCP)." However, the descriptor "transcrycoid" does not exist, and all the research on the current subject must use the terms "transtracheal aspiration and puncture." It is worth noting that our pilot study of 33 patients was critical in modifying the classical technique, which had been used exclusively for patients showing large levels of tracheobronchial secretions. Under such conditions, acquiring secretion samples by TTP (TCP), with or without anesthesia of the tracheobronchial tree or intravenous sedation, is technically easier due to the fact that patients with hypersecretions show reduced or nonexistent airway reflexes (Pecora 1959; Morishita *et al.*, 2000; Pecora 1970; Pratter and Irwin 1979).

The current study differs from the previously published ones in various aspects. First, the sample population is inherently unique compared to that used previously, wherein all patients showed large amount of tracheobronchial secretions, and many of them were in poor health state. By contrast, patients of this study mostly were outpatients with good cognition and without any tracheobronchial hypersecretion.

Second, a sedation protocol with the intention of obtaining 2–3 levels in the Ramsay sedation scale was used. In this situation, patients remained conscious and cooperative.

In the present study it was observed that the range of variation of α-amylase in the tracheobronchial tree, using 10 ml of saline and transtracheal puncture was

24–10,000 IU/L (mean 1,914 IU/l; standard error mean, 240 IU/l.). The inference of de double origin of α-amylase in tracheobroncheal secretions is intuitive because the values in laryngectomized patients is very different from our population without the risk of morbid aspiration and with normal airways anatomy and confirmed such data by naso video laryngo bronchoscop (Pecora 1959). The differences between the present study and Nandapalan`s findings is that a contribution of a physiologic microaspiration that occur in most normal people. The absolute value of α-amylase activity from oral cavity has a wide range of variation.

What could differentiate a normal from a morbid aspiration is the innocculum into tracheobronchial tree and due to the large variation of amylase activity in saliva this very important aspect only could be evaluated by indexation of tracheal and saliva amylase (Amylase index). After analyzing many absolute and indexed amylase activity (Tracheal amylase divided by saliva amylase) and using the Receiver Operator Curve we have found the best cut-off point of 0.1 (Ten percent) to distinguish between normal and abnormal saliva aspiration .

Our work has many limitations, such as the sample size and being a single center study. We hope that other researchers could also confirm our data and solve this controversial issue.

CONCLUSION

We have defined the normal values of amylase in tracheobronchial secretions of human beings.

The α-amylase in the human lung has probably two components: one produced locally and the other due to normal physiological aspiration.

List of abbreviations

TTP: Transtracheal puncture
TCP: Transcrycoid puncture
NS: Normal saline
SEM: Standard error mean
UFJF: Federal University of Juiz de Fora- Minas Gerais- Brazil
UFMG: Federal University of Minas Gerais- Belo Horizonte- Brazil

The authors declare no conflict of interest.

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