Observational Study

Medicine®

OPEN

Cystic fibrosis respiratory tract salt concentration

An Exploratory Cohort Study

Simon Grandjean Lapierre, MD[a], Michael Phelippeau, MD[a], Cyrine Hakimi, Master Degree[a], Quentin Didier, Master Degree[a], Martine Reynaud-Gaubert, MD, PhD[b], Jean-Christophe Dubus, MD, PhD[c], Michel Drancourt, MD PhD[a,*]

Abstract

In cystic fibrosis patients, electrolytic and osmolality imbalance secondary to cystic fibrosis transmembrane conductance regulator mutations may impact on mucoid secretion accumulation and secondary colonization by opportunistic pathogens such as nontuberculous mycobacteria.

We performed a noninvasive exploratory prospective controlled clinical study comparing sputum salinity and acid-base characteristics of cystic fibrosis and noncystic fibrosis control patients. A total of 57 patients and 62 controls were included.

Sputum salt concentrations were 10.5 g/L (95% CI: 7.7–13.3) in patients and 7.4 g/L (95% CI: 5.9–8.9) in aged-matched controls, a difference that was found to be statistically significant ($P < .05$). No difference in pH was observed between patients and controls.

These differences in respiratory secretions salt concentrations could influence host-pathogen interactions in the context of cystic fibrosis respiratory infections. We propose to include respiratory secretion salt measurement as a routine analysis on cystic fibrosis patients' sputum submitted for bacterial culture.

Abbreviations: ASL = airway surface liquid, CF = cystic fibrosis, CFTR = cystic fibrosis transmembrane conductance regulator.

Keywords: cystic fibrosis, respiratory infections

1. Introduction

Cystic fibrosis (CF) is the most frequent genetically acquired disease in the northern hemisphere.[1] Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which codes for a cyclic AMP-regulated Cl^- channel, results in impaired CFTR protein production and loss of chloride and bicarbonate excretion in the airways.[2] Secondary electrolytic and acid-base imbalances are thought to be responsible for mucoid bronchial secretions and colonization by opportunistic pathogens although implicated mechanisms remain unclear.[3]

Airway surface liquid (ASL) salinity and acid-base environment and their exact relationship with mucus production and microbial colonisation are poorly understood. Some epithelial Na^+ channels which are implicated in airway surface liquid volume regulation have been shown to be pH sensitive making these ASL determinants interconnected.[4] Nasal voltage has also repeatedly been used as a proxy for measuring the CFTR ion channel activity in the respiratory tract.[5,6] Whether CF individuals ASL has a higher sodium chloride concentration compared to normal subjects remains controversial. In fact, bronchial epithelial cells in vitro experimental models and animal studies yield discordant results.[5,7,8] Similarly, experimental in vitro models, animal and human studies evaluated whether ASL is more acidic in CF.[8–11] This topic also remains without consensus. Nevertheless, respiratory secretions NaCl concentration and acidic environment were proposed to be responsible for immune defense inhibition and increased pathogen susceptibility.[9,12,13] Also, host-adaptation of nontuberculous mycobacteria which frequently colonize and infect cystic fibrosis patients' airways have been shown to correlate with environmental salt concentrations.[14]

How ASL salinity and acid-base characteristics translate in CF respiratory mucoid environment in vivo remains unknown. Prospective experimental data on the measurement of electrolytic and acid-base characteristics in the airways of CF patients have not been reported. These data could provide grounds for a better understanding of host-pathogen interactions in the context of CF respiratory infections. We performed a noninvasive exploratory prospective controlled clinical study comparing sputum salinity and acid-base characteristics of CF and non-CF control patients.

2. Materials and methods

2.1. Clinical specimens

Sputum specimens submitted to our routine diagnostic laboratory for microbiological analysis were prospectively collected in November 2014. Pediatric and adult CF patients actively followed at the Cystic Fibrosis Reference Center in Marseille

Editor: Fu-Tsai Chung.

This study was supported by URMITE, IHU Méditerranée Infection, Marseille, France.

The authors declare no conflicts of interest.

[a] Aix-Marseille Université, URMITE Méditerranée Infection, [b] Center de Resource et de Compétences de la Mucoviscidose adulte; équipe de Transplantation pulmonaire, CHU Hôpital Nord, URMITE Aix-Marseille Université, [c] Center de Resource et de Compétences de la Mucoviscidose pédiatrique CHU Hôpital la Timone, Marseille, France.

[*] Correspondence: Michel Drancourt, Aix-Marseille Université, Méditerranée Infection, URMITE, UMR CNRS 7278, IRD 198, INSERM 1095, IHU – Méditerranée Infection, 19–21 Boulevard Jean Moulin, 13005 Marseille, France (e-mail: michel.drancourt@mediterrane-infection.fr).

Medicine (2017) 96:47(e8423)

Received: 27 April 2017 / Received in final form: 3 August 2017 / Accepted: 4 October 2017

http://dx.doi.org/10.1097/MD.0000000000008423

were considered for this study. One specimen per individual was included. Specimens were collected in sterile recipients (Gosselin, Hazebrouck, France), stored at 4°C and processed on the day they were received. Specimens containing more than 10 epithelial cells per field at 100x on direct Gram staining were excluded to prevent the inclusion of saliva specimens.[15] Induced sputum specimens obtained through saline solution physiotherapeutic inhalation procedures were excluded to prevent potential specimen contamination. All measurements were blindly performed by trained personnel in our laboratory.

2.2. Salinity and pH measurements

Standardized quantities of 0.5 mL of sputum and 0.5 mL of distilled sterile water were vortexed in an Eppendorf tube. Samples were centrifuged at 10,000x g for 10 minutes. Supernatant was then heated at 95°C for 60 minutes for microorganisms' inactivation and protein precipitation. Following a 5-minute cooling step at room temperature, salinity was measured using a spectrophotometer lens (Digital refractometer PR-100SA, Atago, Tokyo, Japan) according to manufacturer's instructions. Two separate measurements were performed on every sample to assess same sample intermeasurements variability. Recommended quality controls and calibration using deionized water were performed between each measurement. Sample pH was measured using pH test strips (Sigma-Aldrich, Saint-Quentin Fallavier, France) according to manufacturer's instructions. Neutrophils intracellular pH and epithelial cells chloride channels stimulation have been shown to be altered in the context of CF.[16,17] Therefore, a microscopic examination and Gram staining of the supernatant was performed on each sample to confirm the absence of cellular or bacterial material which could interference with the salinity and acidity measurement.

2.3. Statistical analysis

Because of shorter life expectancy of this specific population and the fact that sputum samples are less frequently obtained from non-CF pediatric patients, included CF patients were expected to be significantly younger than controls. Included patients were therefore prospectively and randomly matched in a 1:1 ratio with a same-age CF or non-CF patient. A 5-year age difference was tolerated. Nonparametric Mann-Withney test was used to compare sputum salinity and pH values between CF and non-CF individuals in both the whole study population and the age matched population. Two-tailed unpaired Student *t* test was used to evaluate baseline characteristics differences between CF patients and non-CF controls in the whole population and the matched controls analysis. The impact of sex and age on sputum salinity and pH were respectively evaluated using Student *t* test and linear regression analyses. Statistical analyses were performed by our biostatistics personnel using GraphPad Prism version 7.0 software (https://www.graphpad.com).

2.4. Ethical review

This work was approved by the local ethic committee of IFR48, Faculty of Medicine, under the reference number 07–008. No written consent was required as no additional clinical samples were obtained for the study and results had no impact on patient diagnosis or management. (LOI n° 2004–800 relative à la bioéthique" published in *Journal Officiel de la République Française* on August 6, 2004)

3. Results

3.1. Study population

Sputum specimens were prospectively collected from 57 CF patients and 62 controls. Among these, 54 patients were randomly matched based on their age. Demographic characteristics of the 119 included patients are presented in Table 1. As expected, included CF patients were younger (20.4 +/– 16.0 yr, mean +/– SD) than non-CF controls (49.4 +/– 22.1 yr, mean +/– SD), a difference that was found to be statistically significant ($P < .05$).

3.2. Respiratory secretions salinity

Our salinity measurement protocol was found to be highly reproducible with consistently less than 2% same sample intermeasurements variability. Mean values were therefore used for statistical analysis. Mean sputum salinity was 8.9 g/L (95% CI: 7.3–10.6) in CF patients and 7.6 g/L (95% CI: 6.7–8.5) in controls ($P > .05$). This trend towards higher sputum salt concentrations in cystic fibrosis patients did reach statistical significance ($P > .05$) in the age-matched control group where mean sputum salinity was 10.5 g/L (95% CI: 7.7–13.3) in CF patients and 7.4 g/L (95% CI: 5.9–8.9) in controls (Fig. 1). Correlations between sputum salinity and age are presented in Figure 3. Although linear regression analysis showed no statistically significant correlation between age and salinity values, a trend towards higher salinity in older subjects was observed.

3.3. Respiratory secretions pH

Mean sputum acidity was 6.3 (95% CI: 6.1 – 6.6) in CF patients and 6.1 g/L (95% CI: 5.7 – 6.4) in controls. Analysis of the age-matched populations showed similar results with a mean sputum acidity of 6.4 (95% CI: 5.9 – 6.8) in CF patients and 6.1 g/L (95% CI: 5.5 – 6.6) in aged-matched controls (Fig. 2). This trend towards less acidic values in CF patients was not statistically significant in both the total population and the age-matched

Table 1

Cystic fibrosis and control patients' baseline characteristics.

	Whole population analysis			Age-matched analysis		
	CF patients (N=57)	Controls (N = 62)	*P*	CF patients (N = 27)	Controls (N = 27)	*P*
Age, yr	20.4 +/−16.0	49.4 +/−22.1	< .05	30.9 +/− 17.0	28.4 +/− 14.1	.56
Female sex, %	50.9	33.9	.06	40.7	37.0	.78

*Plus-minus values are means +/– SD. Controls were significantly older than CF patients in the whole population based analysis.

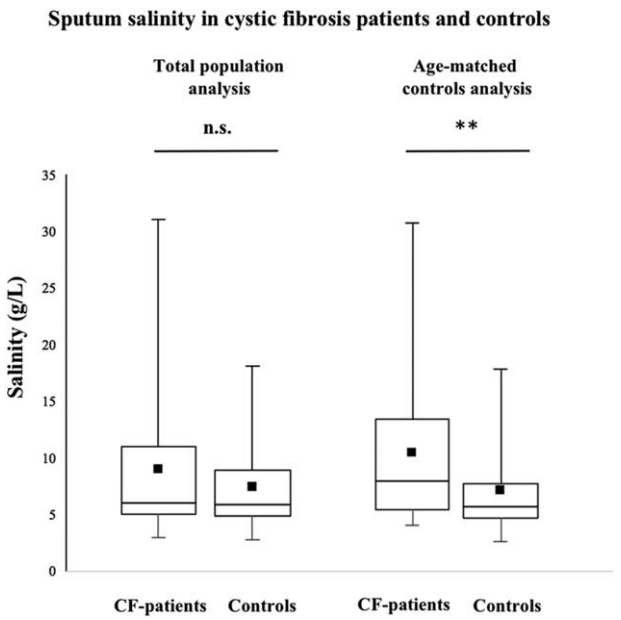

Figure 1. Sputum salinity in cystic fibrosis and noncystic fibrosis patients. n.s., not statistically significant; **, statistically significant.

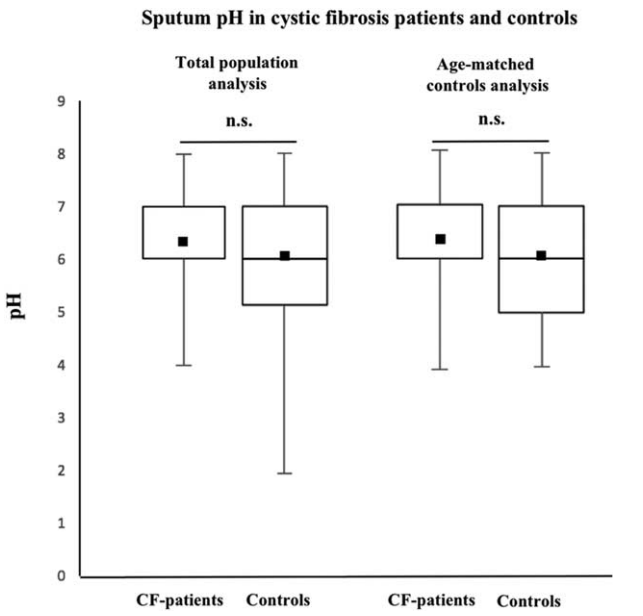

Figure 2. Sputum pH in cystic fibrosis patients and noncystic fibrosis patients n.s., not statistically significant.

population analysis. Correlations between sputum pH and age are presented in Figure 3.

4. Discussion

We performed a noninvasive exploratory prospective controlled clinical study comparing sputum salinity and acid-base characteristics of respiratory tract secretions in CF patients and non-CF controls. Salinity and pH measurements were blindly performed using highly reproducible and widely used refractometry protocol and pH strips. Saliva or sodium chloride contaminated specimens were excluded and the potential impact of our centrifugation and heating based protocol on salinity and pH was controlled by rigorously applying a uniform protocol to CF and non-CF specimens. Although broncho-alveolar lavage specimens better reflect lower respiratory tract and ASL environment, they require invasive procedures and are more frequently collected in the context of severe active infection of which the effect on respiratory secretions acidity and salinity are unknown. Also, airway colonization is a process which happens both in the upper and lower respiratory tract. We therefore selected sputum as a proxy for the evaluation of the upper respiratory tract environment since it can be routinely obtained for salinity measurement in a diagnosis perspective.

Our salinity measurements were in accordance with those measured in vivo in CF pig and mouse animal models and in vitro in human bronchial epithelium models.[5,7,8] Our study showed sputum salt concentrations to be significantly higher in CF patients than age matched controls. Higher salinity values in CF animal models had not been previously reported but were described in vitro.[7–9] The pH values range observed in our study did not differ from previous in vitro epithelium cell cultures or in vivo animal studies.[8] Considering host-adaptation of frequent CF respiratory pathogens such as nontuberculous mycobacteria has previously been shown to correlate with environmental salt concentrations,[14] our data provide grounds for a better

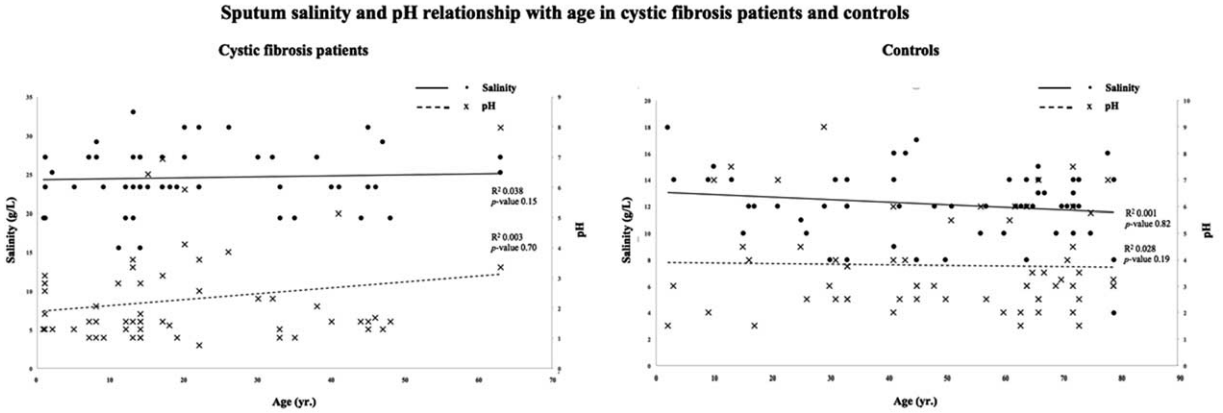

Figure 3. Sputum salinity and pH relation with age in cystic fibrosis and noncystic fibrosis patients.

understanding of host-pathogen interactions in the context of CF respiratory infections.

Hypertonic saline inhalation therapies were shown to allow short term increase in mucoid secretions expectoration and forced expiratory volume values but had no impact on clinical cystic fibrosis associated chronic rhino-sinusitis.[18,19] A Cochrane systematic review further concluded that there is insufficient evidence to support the use of inhaled hypertonic saline as a treatment for cystic fibrosis patients.[20] In fact, our clinical data and previously published in vitro data suggest that sodium chloride inhalation therapies and nebulized medications associated with high sodium load could have an impact on long term airways colonization.[9,12] Whether nebulized sterile isotonic solution has a persistent impact on respiratory secretions saline concentration and microbial colonization warrants further investigation.

We identify airways' salt concentrations as a one of many probable determinants of bacterial colonization and subsequent infection in CF patients. Our data support the inclusion of salt concentration measurements as a routine analysis on CF respiratory tract specimens. Our exploratory data need to be validated in different settings and further correlated with microbiologic and clinical data. These future complementary data could justify trials evaluating the impact of isotonic fluids physiotherapeutic interventions on microbial colonization.

Acknowledgments

The authors thank the URMITE, IHU Méditerranée Infection epidemiology and statistics team.

References

[1] Southern KW, Munck A, Pollitt R, et al. A survey of newborn screening for cystic fibrosis in Europe. J Cyst Fibros 2007;6:57–65.
[2] Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. N Engl J Med 2015;372:351–62.
[3] Martiniano SL, Nick JA. Nontuberculous mycobacterial infections in cystic fibrosis. Clin Chest Med 2015;36:101–15.
[4] Garland AL, Walton WG, Coakley RD, et al. Molecular basis for pH-dependent mucosal dehydration in cystic fibrosis airways. Proc Natl Acad Sci U S A 2013;110:15973–8.
[5] Chen JH, Stoltz DA, Karp PH, et al. Loss of anion transport without increased sodium absorption characterizes newborn porcine cystic fibrosis airway epithelia. Cell 2010;143:911–23.
[6] Rowe SM, Clancy JP, Wilschanski M. Nasal potential difference measurements to assess CFTR ion channel activity. Methods Mol Biol 2011;741:69–86.
[7] Zabner J, Smith JJ, Karp PH, et al. Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia in vitro. Mol Cell 1998;2:397–403.
[8] Jayaraman S, Song Y, Vetrivel L, et al. Noninvasive in vivo fluorescence measurement of airway-surface liquid depth, salt concentration, and pH. J Clin Invest 2001;107:317–24.
[9] Pezzulo AA, Tang XX, Hoegger MJ, et al. Reduced airway surface pH impairs bacterial killing in the porcine cystic fibrosis lung. Nature 2012;487:109–13.
[10] Abou Alaiwa MH, Beer AM, Pezzulo AA, et al. Neonates with cystic fibrosis have a reduced nasal liquid pH; a small pilot study. J Cyst Fibros 2014;13:373–7.
[11] McShane D, Davies JC, Davies MG, et al. Airway surface pH in subjects with cystic fibrosis. Eur Respir J 2003;21:37–42.
[12] Smith JJ, Travis SM, Greenberg EP, et al. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell 1996;85:229–36.
[13] Goldman MJ, Anderson GM, Stolzenberg ED, et al. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 1997;88:553–60.
[14] Asmar S, Sassi M, Phelippeau M, et al. Inverse correlation between salt tolerance and host-adaptation in mycobacteria. BMC Res Notes 2016;9:249.
[15] Sharp SE, et al. Sharp SE Cumitech 7B, Lower respiratory tract infections. Coordinating ed. Washington, DC: ASM Press; 2004.
[16] Coakley RJ, Taggart C, Canny G, et al. Altered intracellular pH regulation in neutrophils from patients with cystic fibrosis. Am J Physiol Lung Cell Mol Physiol 2000;279:L66–74.
[17] Hauber HP, Manoukian JJ, Nguyen LH, et al. Increased expression of interleukin-9, interleukin-9 receptor, and the calcium-activated chloride channel hCLCA1 in the upper airways of patients with cystic fibrosis. Laryngoscope 2003;113:1037–42.
[18] Robinson M, Hemming AL, Regnis JA, et al. Effect of increasing doses of hypertonic saline on mucociliary clearance in patients with cystic fibrosis. Thorax 1997;52:900–3.
[19] Mainz JG, Schumacher U, Schadlich K, et al. Sino nasal inhalation of isotonic versus hypertonic saline (6.0%) in CF patients with chronic rhinosinusitis: results of a multicenter, prospective, randomized, double-blind, controlled trial. J Cyst Fibros 2016;15:e57–66.
[20] Wark P, McDonald VM. Nebulised hypertonic saline for cystic fibrosis. Cochrane Database Syst Rev 2009;2:CD001506.