



The Use of Oral Urease Activity as an Assessment of COPD Symptoms in Current and Former Smokers

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Abstract

Rationale: Certain bacteria express urease which breaks down urea into ammonia and carbon dioxide and raises the local pH. Urease activity is thought to be important in the mouth as it creates a buffer to help combat the acidic environment associated with dental caries. Dental health has been associated with respiratory illness, and bacteria and inflammation have been proposed as a possible link. The purpose of this study was to evaluate urease activity in the oral cavity of current and former smokers with COPD, and test its utility as a biomarker for chronic daily respiratory symptoms.

Methods: Subjects greater than 40 years old with stage 3 or 4 COPD and at least a 10 pack year smoking history were included into the study. Exclusion criteria included recent exacerbation in the last 4 weeks requiring steroids or antibiotics, or edentulous subjects. A BreathTek test kit traditionally used to diagnose H. pylori infections was used in the oral cavity to isolate and measure urease activity of the mouth only. Urease activity was measured every minute for 5 minutes after an oral rinse with a solution containing ¹³C₂ labeled urea. Subjects reported symptoms of breathlessness, cough, sputum production and wheeze daily into an electronic COPD diary.

Results: 7 former smokers and 7 current smokers were recruited, and they recorded daily COPD symptoms for a mean of 58 (± 22) days. Linear regression demonstrates a significant negative correlation with urease activity at 2 minutes and dependent variables of BORG score ($\beta = -0.26$, $R^2 = 0.42$, $p = 0.01$) and percent of days with wheeze ($\beta = -3.87$, $R^2 = 0.28$, $p = 0.05$). There was also a negative correlation with percent of days with cough ($\beta = -2.97$, $R^2 = 0.18$, $p = .13$) and sputum ($\beta = -2.46$, $R^2 = 0.18$, $p = .21$), but did not reach statistical significance. There was no significant difference in symptoms between current and former smokers using Wilcoxon rank sum testing. Current smoker's median urease activity at 2 minutes of 3.2 (IQR 2.3 – 4.5) was lower than former smoker's median urease activity of 10.7 (IQR 8.1 – 18.3), (Wilcoxon rank sum, p = value 0.002).

Conclusion: Current smokers have decreased urease activity in the mouth, and urease activity negatively correlates with daily COPD symptoms of breathlessness and wheeze. Urease activity may be a potential biomarker to assess symptom severity in patients with COPD.

Background

Urease is a unique bacterial enzyme that hydrolyzes urea releasing CO₂ and NH₃. In the oral cavity urease activity is thought to be important in combating the acidic environment associated with dental caries. However in the lung, some pathogens (eg pseudomonas) use their urease activity to improve survival. The detection of urease activity in the stomach has been used to detect H. pylori in the stomach but there has been little examination of urease activity in the oral cavity or lung.

The oral and lung microbiomes have been shown to be similar in previous studies. Some of the overlap may relate to micro-aspiration. The correlation of urease containing bacteria in the oral cavity and lung has not been studied. Dental health has been associated with respiratory illness, and bacteria and inflammation have been proposed as a possible link.

Purpose

Evaluate urease activity in the oral cavity of current and former smokers with COPD. Correlate the results with chronic daily respiratory symptoms

Methods

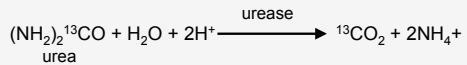
Subjects greater than 40 years old with stage 3 or 4 COPD and at least a 10 pack year smoking history were included into the study.

Subjects with recent COPD exacerbation in the last 4 weeks, or edentulous subjects were excluded.

A BreathTek test kit traditionally used to diagnose H. pylori infections used to measure urease activity in the mouth only.

Urease activity was measured every minute for 5 minutes after an oral rinse with a solution containing ¹³C₂ labeled urea (see equation 1).

Subjects reported symptoms of breathlessness (with BORG score), cough, sputum production and wheeze into an electronic COPD daily diary.



Equation 1 – Equation for the breakdown of urea by urease, which is utilized by the H. pylori BreathTek kit used by our subjects. ¹³CO₂ is liberated by this reaction and measured to determine urease activity.

Results

30 subjects were recruited (see Table 1). 2 former smokers dropped out of study shortly after beginning to record symptoms so their symptom data were not included for analysis

Daily symptoms were recorded on average for 64.3 ± 14.3 days, with 93% ± 10% compliance.

	Healthy Control (n = 10)	Former Smoker (n = 10)	Current Smoker (n = 9)
Age	56.1 ± 11	65.2 ± 8.0	58.0 ± 5.4
Male sex – no. (%)	6 (60)	5 (50)	7 (78)
BMI	29.0 ± 7.9	26.7 ± 3.8	28.6 ± 4.6
Black race – no. (%)	3 (30)	9 (90)	5 (56)
FVC (% pred)	93.2 ± 13.2	65 ± 16.9	77.1 ± 12.5
FEV1 (% pred)	94.4 ± 17.6	32.6 ± 12.2	40.7 ± 9.4
MMRC	0.3 ± 1.0	2.7 ± 0.5	2.4 ± 0.9
Pack years	4.2 ± 11.0	46.8 ± 36.4	35.4 ± 24.4
Oxygen Use – no. (%)	0	5 (50)	0
Exacerbations/person in last year	-	1.6	0.8

Table 1 – Baseline Characteristics. All values reported as means ± SD

Oral Urease Activity Of Former and Current Smokers with COPD

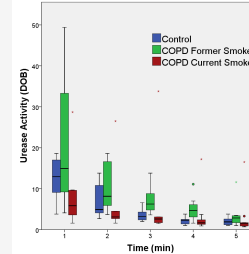


Figure 1 – Oral urease activity over time after oral rinse with ¹³C₂ labeled urea. Data represented as box and whisker plots with median and IQR. Urease activity measured as delta over baseline (DOB), which represents the difference between ¹³CO₂/¹²CO₂ before and after oral rinse with ¹³C₂ labeled urea. Measured with a BreathTek kit.

Min	Urease Activity			Kruskal Wallis	P – Value		
	Healthy Control (n = 10)	Former smoker (n = 10)	Current smoker (n = 10)		Healthy vs Former	Healthy vs Current	Former vs Current
1	13.0 (9.1 – 17)	15.0 (9.2 – 33.3)	5.9 (3.6 – 9.6)	0.04	0.41	0.03	0.028
2	4.9 (4.1 – 10.8)	8.2 (5.9 – 16.6)	3.1 (2.7 – 4.5)	0.008	0.10	0.05	0.005
3	3.3 (2.4 – 4.3)	6.3 (4.8 – 8.8)	2.6 (1.7 – 3.1)	0.002	0.007	0.16	0.003
4	2.3 (1.4 – 2.5)	4.7 (3.1 – 6.1)	1.7 (1.3 – 2.4)	0.01	0.007	0.47	0.013
5	1.9 (1.2 – 2.6)	2.9 (1.7 – 3.4)	1.5 (0.9 – 1.7)	0.18	.20	0.40	0.10

Table 2 – Urease activity in each group over time. Values are represented as median (IQR). Kruskal wallis test performed to detect between group differences. Wilcoxon rank sum test performed to compared individual groups. Given multiple comparisons, p value considered significant at level of < 0.017. Data shows that current smokers have significantly less urease activity than former smokers. Current smokers have less urease activity than healthy controls, but it does not reach statistical significance.

Oral Urease Activity as a Marker of COPD Symptoms

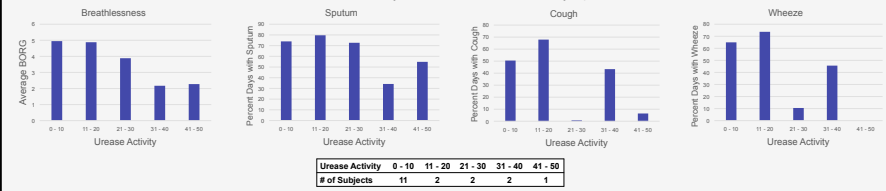


Figure 2 – Urease activity of the oral cavity compared to daily COPD symptoms. Urease activity measured at the first minute after rinse with ¹³C₂ labeled urea. COPD symptoms of breathlessness, sputum, cough, and wheeze assessed with the use of an electronic daily diary. Only 18 COPD subjects participated in the daily symptom monitoring. Bar charts show a trend toward high urease levels correlating with less COPD symptoms. After adjustment for smoking, linear regression demonstrates no significance with oral urease activity and daily symptoms of breathlessness ($\beta = -0.04$, $R^2 = 0.21$, $p = 0.32$), sputum ($\beta = -0.60$, $R^2 = -0.04$, $p = 0.47$), cough ($\beta = -1.10$, $R^2 = 0.08$, $p = 0.27$), or wheeze ($\beta = -1.17$, $R^2 = 0.15$, $p = 0.16$).

Summary

Smokers with COPD have decreased urease activity of the oral cavity. It is significantly lower when compared to former smokers and trends towards significance when compared to healthy controls. The decreased activity could be related to acid/base status of the oral mucosa, a different oral microbiome, or toxic effects of cigarettes. Urease activity of the mouth did not correlate with any daily respiratory symptoms that are common in COPD.

Future Studies

Urease activity will be correlated with the bacteria present (cultures and PCR). The results of this study provide background of urease activity in stable COPD subjects. Future studies are planned to examine urease activity in both the oral cavity and lung (using nebulized ¹³C-urea) in stable COPD subjects and those with exacerbations.