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Brief Report

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Effect of Betel (Areca) Nut Chewing on Fractional Exhaled Nitric Oxide: A Pilot Study

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Abstract

Betel (areca) nuts are extensively chewed in many countries. This has been associated with respiratory symptoms. We aimed to determine whether betel nut chewing is associated with acute changes in fractional exhaled nitric oxide, a non-invasive marker of airway inflammation. Betel nut chewing resulted in an immediate significant decline in fractional exhaled nitric oxide levels that persisted for up to 180 minutes. This effect has to be taken into account in epidemiological studies, reference ranges, and patient preparation.

Keywords: Areca; Nitric oxide; Respiratory system; Asthma

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Introduction

Betel (areca) nuts, a fruit of the *Areca catechu* palm tree, are extensively chewed, predominantly in South Asian and Pacific Island countries and India and increasingly in settled immigrant populations in western countries. Between 10% and 20% of the world population are believed to chew betel nut on a regular basis.[1] The betel nut contains a number of alkaloids and betel nut chewing is strongly associated with squamous-cell carcinoma of the oral cavity.[2] Other non-oral conditions associated with betel nut chewing are cardiovascular, metabolic, hepatic, and respiratory. Regarding respiratory conditions, betel nut chewing has been associated with asthma severity,[4] and a significant association between betel nut chewing and asthma has been found.[5]

Fractional exhaled nitric oxide (FENO) levels are routinely used for detecting and monitoring eosinophilic airway inflammation.[6] A number of factors, including cigarette smoking, are known to affect FENO measurement and need to be accounted for.[7] To our knowledge, the effects of betel nut chewing on FENO levels have not previously been determined. The objective of our study was to determine whether betel nut chewing has any effect on FENO measurement.

Materials and Methods

Twenty male Taiwanese were invited to participate in this study. The inclusion criteria were age between 16 and 75 years, and regular (at least once a day for at least one year) betel nut chewing. Those who had a doctor diagnosis of asthma were ex-

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TAKE-HOME MESSAGE

- Betel (areca) nut chewing is common in certain countries.
- We found that betel nut chewing resulted in an immediate significant decline in fractional exhaled nitric oxide levels that lasted for up to at least three hours.
- This has to be taken into consideration in epidemiological studies, reference ranges, and patient preparation in countries where betel nut chewing is prevalent.

cluded from the study. Participants were instructed to abstain from smoking, alcohol, coffee, or spinach intake for at least 4 hours or betel nut chewing for 12 hours prior to FENO testing. All participants gave informed written consent. The study protocol was approved by the Show Chwan Memorial Hospital Ethics Committee.

On the morning of the study, a baseline FENO level was determined for each participant using a portable FENO analyzer according to the manufacturer's instructions (NIOX-MINO®, Taiwan). All measurements were made by a single person, alleviating any intra-observer differences. The subjects then chewed betel nuts wrapped in betel leaves, as is commonly presented and chewed in Taiwan, for 30 minutes. The betel nut preparations were all sourced from one supplier in one batch. Immediately after the 30-minute chewing period, a further FENO measurement was made and then at 30-minute intervals for up to 3 hours. The percentage change from the baseline value in each subject's FENO was calculated. FENO levels were log-normally distributed and the change in FENO was compared with the logged values using the *Student's t* test for paired data. Statisti-

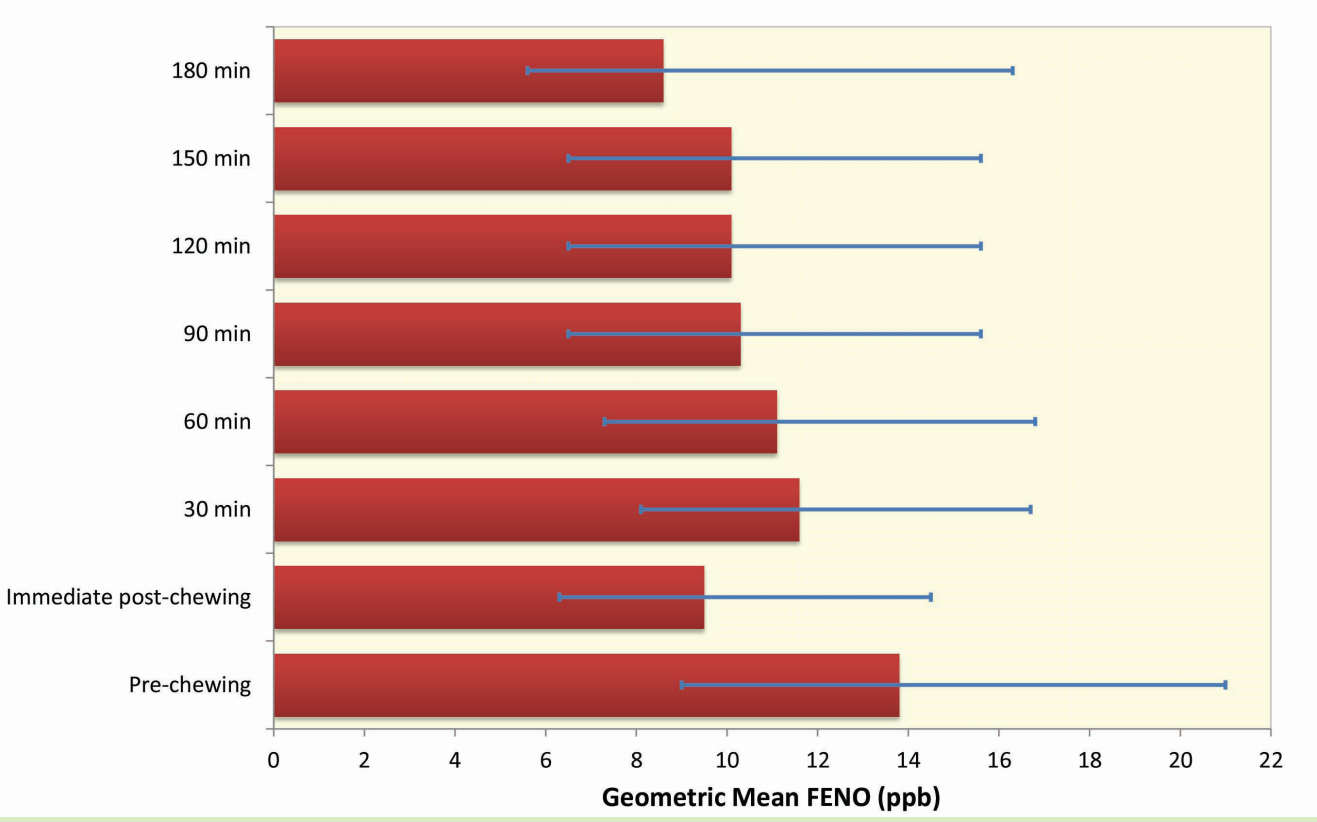

Figure 1: Geometric mean of FENO levels at different times. Error bars represent the 95% CI of the mean.

cal analysis was carried out in R v2.15.2. A p value <0.05 was considered statistically significant.

Results

The subjects had a mean age of 46.9 (range 23 to 67) years. There was a significant initial drop in FENO immediately post-chewing (Fig 1). The level remained lower than the baseline value at subsequent time points for up to 3 hours. Percentage reductions in FENO post betel nut chewing are shown in Table 1.

Table 1: Changes from pre-chewing level in FENO over time after betel nut chewing

Time	Mean (range) %change	Difference in logged values	p value
Post-chewing	30.7 (-73.5 to 37.5)	0.366	0.003
30 min	15.4 (-67.6 to 140.0)	0.168	0.16
60 min	19.3 (-56.7 to 58.3)	0.214	0.048
90 min	25.4 (-68.8 to 58.3)	0.293	0.008
120 min	26.8 (-70.6 to 58.3)	0.312	0.008
150 min	26.8 (-72.2 to 58.3)	0.312	0.012
180 min	37.4 (-72.2 to 58.3)	0.468	0.002

Discussion

We found that betel nut chewing generally resulted in an immediate significant fall in FENO levels that persist for up to at least three hours. Implications are that recent betel nut chewing has to be taken into account when conducting epidemiological studies in countries where betel nut chewing is prevalent. As the FENO levels were still significantly lower at the 3-hour post-chewing period, the last time point in our study, further studies are required to determine how long the FENO reduction effects of betel nut chewing lasts for. Our results cannot be compared to other studies, as we believe our study is the first to show the effects of betel nut chewing on FENO.

The changes in FENO levels were not all one-directional (Table 1), as FENO levels at some time points increased, rather than decreased, compared to pre-chewing levels. However, the overall effect of betel nut chewing was a significant decline in FENO levels at most time points that has implications for deriving population-based reference ranges.

A limitation of our study was that FENO measurements were not made in duplicate, as recommended for clinical studies. However, in normal clinical practice, single measurements of FENO are generally made; a recent study shows excellent repeatability (average difference of -1.61 ppb for repeated measurements) for the NIOX-MINO®, supporting the manufacturer's claim for single breath testing.[8]

A possible explanation for our findings could be a change in the saliva pH due to betel nut chewing. Previous studies with alkaline mouth washes show an immediate decline in FENO, which is most likely due to bacteriostatic effects, thereby reducing the NO produced in the mouth.[9,10] Arecoline is the main alkaloid in betel nuts and is present in high levels in saliva of betel nut chewers.[11] However, the reduction in FENO after mouth washing is temporary (about 10 min) and is not prolonged, unlike post-betel nut chewing. Further studies are required to determine pH changes in saliva after betel nut chewing and how long these possible changes last.

Another possible mechanism for the decrease in FENO levels post-betel nut chewing might be a decrease in inducible nitric oxide synthase (iNOS) mRNA transcription, similar to what has been shown to be the cause for the decrease in FENO due to cigarette smoking.[11] Recently Wang, *et al*, found a significant association between asthma and betel nut chewing in Taiwan.[5]

They also found that arecoline, one of the main alkaloids in betel nuts, is associated with eotaxin-1 release. Tobacco smoking has also been shown to be associated with elevated eotaxin levels.[12] Therefore, the effect of betel nut chewing on lowering FENO levels, as evidenced in our study, might be through this mechanism, although it has been argued that enzyme regulation takes time and is not immediate.

In conclusion, we found that betel nut chewing significantly reduced FENO levels that may have to be taken into consideration in epidemiological studies, reference ranges, and patient preparation in countries where betel nut chewing is prevalent.

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