

Nasal saline irrigation has no effect on normal olfaction: a prospective randomized trial

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Background: Nasal saline irrigation is a safe treatment for chronic rhinosinusitis; however, its effect on olfaction is unclear. Cyclic adenosine monophosphate (cAMP) is a key second messenger in the mechanism of olfaction and has been shown to be associated with smell function. In animal studies, olfactory cilia may be harvested by simple saline preparations. This study aimed to characterize the effect of nasal saline irrigation on smell function.

Methods: Volunteers with normal olfaction were randomized into a control or irrigation cohort. In the initial appointment, subjects completed a University of Pennsylvania Smell Identification Test (UPSIT) and nasal samples were obtained by 2 methods: the nasal curette and cytobrush. The irrigation cohort performed daily nasal saline irrigations. Both cohorts then returned in 1 week. The UPSIT and nasal cell collection were repeated, and each subject completed a subjective olfactory transition scale. Nasal samples were processed for cAMP levels using a commercial assay.

Results: Thirty-two subjects were enrolled and randomized into each cohort. Control and postirrigation mean UPSIT

scores were 36.8 and 36.7 ($p = 0.48$). No subjects reported a subjective smell loss. Ten pairs of nasal samples were assayed. Using the curette, control and postirrigation cAMP levels were 509 and 490 fmol/(mg/mL), respectively ($p = 0.94$). Using the cytobrush, respective cAMP levels were 424 and 449 fmol/(mg/mL), respectively ($p = 0.94$).

Conclusion: Nasal saline irrigation has no subjective or objective effect on olfaction. It also does not appear to affect cAMP levels, a potential marker of smell function. © 2013 ARS-AAOA, LLC.

Key Words:

CAMP; nasal cell harvesting; nasal curette; nasal cytobrush; olfaction; olfactory cilia; saline irrigation

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Nasal saline irrigation plays an important role in the adjuvant management of chronic rhinosinusitis and allergic rhinitis. Research studies show that nasal saline irrigations can actually improve the symptoms of these two diseases.¹⁻³ Nasal saline irrigations are well tolerated, with

only infrequent mild side effects and extremely rare severe adverse events reported.^{3,4} Despite these infrequent side effects, there are no known clinical studies on the effect of nasal saline irrigation on olfaction. Our interest stemmed from animal studies on olfactory cilia, which are critical to our understanding of human and animal olfactory function. In animal models, cilia may be harvested by hypertonic saline or calcium chloride preparations.^{5,6} This brought into question the potential effects of nasal saline irrigation on human olfactory cilia and hence, olfactory function.

Animal studies examining olfactory cilia show that cyclic adenosine monophosphate (cAMP) is an important second messenger in the mechanism of olfaction.⁷ Clinical studies show that cAMP levels relate to olfactory function and may therefore serve as a potential objective marker of olfaction.⁸

The purpose of this study is to evaluate the effect of nasal saline irrigation on human olfactory function using the University of Pennsylvania Smell Identification Test (UPSIT) and nasal cAMP levels.⁹

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Subjects and methods

This was a prospective, randomized controlled trial approved by the University of Washington Institutional Review Board. Thirty-two healthy volunteers with self-reported normal olfaction were recruited. The inclusion criteria were as follows: (1) healthy individuals with self-reported normal smell function; and (2) age greater than 18 years. Volunteers were excluded from enrollment for any of the following reasons: (1) unable to give informed consent or complete self-administered questionnaires written in English because of cognitive impairment, language barriers, or severe medical conditions; (2) allergy to lidocaine; (3) active sinonasal disease; (4) previous nasal or sinus surgery; (5) currently smoking or using other smoked or inhaled drugs; and (6) pregnant or planning to become pregnant.

We aimed to recruit a total of 32 subjects, 16 in each cohort. This was derived from a power calculation based on the UPSIT using the following parameters: an effect size of 4, standard deviation of 4.1, power of 80%, and $\alpha = 0.05\%$.^{10,11}

Two instruments were used to measure olfactory function: the UPSIT and a transition scale. The UPSIT is a validated 40-item scratch-and-sniff test. The transition scale asks subjects if their smell has improved, worsened, or remained the same.

All subjects were scheduled for 2 appointments. Block randomization with random block sizes was used to generate assignments. The assignments were revealed to the primary author in an envelope during the initial visit. Half of the subjects was assigned to use nasal saline irrigation (240 mL delivered with NeilMed Sinus Rinse kit) once daily for 1 week (NeilMed Pharmaceuticals Inc., Santa Rosa, CA, USA). All subjects randomized into the control cohort had no additional intervention. All subjects then returned for a follow-up appointment 1 week later. During each appointment, subjects completed an UPSIT and underwent nasal cell collection with 2 comfortable methods, the nasal curette (Arlington Scientific, Springville, UT) and cytobrush (Erskine Dental, Marina Del Ray, CA). Both are established methods used to collect nasal specimens for cytologic studies.^{12,13} At follow-up, subjects also completed a subjective transition scale regarding their smell function.

Nasal cell collections were performed by the same operator (J.J.L.) using the same protocol at both appointments. The procedure began with anterior rhinoscopy to rule out any nasal pathology. A mixture of Afrin and 4% topical lidocaine was sprayed into each nostril to decongest and anesthetize the nasal mucosa. A small cotton ball soaked with 4% lidocaine was then placed into the anterior nasal cavity for 5 minutes to further anesthetize the mucosa. Samples were obtained either from the middle or inferior turbinate depending on the extent of visualization on anterior rhinoscopy. Each sample was placed on dry ice immediately after collection.

Cytobrush and curette samples were resuspended in 500 μ L of phosphate buffered saline (PBS) with 0.5

TABLE 1. Demographic information

	Control (n = 16)	Irrigation (n = 16)	P
Age, years, mean (range)	34.6 (23–60)	34.8 (23–57)	0.94
Gender, female, n (%)	6 (38)	10 (63)	0.29
Ethnicity, Caucasian, n (%)	13 (81)	9 (56)	0.25

mM isobutylmethylxanthine (IBMX), a general phosphodiesterase (PDE) inhibitor that inhibits most PDEs. The cells were disrupted using a sonicator over two 30-second cycles, interrupted by incubation on ice. After sonication, a 50- μ L aliquot was removed and set aside for determination of protein concentration. Two volumes of cool 100% ethanol were added for protein precipitation. The samples were placed on ice for 10 minutes and then centrifuged at 2000g at 4°C for 10 minutes. The supernatant was transferred to a new Eppendorf tube and allowed to dry overnight at 55°C. The dried supernatant was resuspended in 320 μ L of an assay buffer and cAMP concentrations were determined using an Amersham cAMP Biotrak Enzyme Immunoassay (EIA) 96-well system (catalog number RPN2251, Amersham, Piscataway, NJ).

Protein concentration was determined using the 50- μ L aliquot after sonication. This was determined using a spectrophotometric assay in a BioTek Epoch microplate reader.

All data were maintained in an Excel spreadsheet. All cAMP concentrations (fmol) were standardized with the total protein concentration (mg/mL) of each sample. Statistical analyses were performed using Stata software. A Fisher's test was used to compare demographics. A Wilcoxon test was used to compare UPSIT scores and cAMP levels between cohorts and within the irrigation cohort.

Results

A total of 32 subjects with self-reported normal olfaction were enrolled into the study. Sixteen were randomized into the irrigation cohort and 16 into the control cohort. Due to technical and equipment issues during sample processing, cAMP results are reported for 10 pairs of curette and cytobrush samples. Upon initial screening, three subjects were found to have olfactory dysfunction on the UPSIT, 1 in the control and 2 in the irrigation cohort. Demographic data for each cohort are listed in Table 1.

All 32 subjects returned for their follow-up appointment. All subjects had normal nasal exams and had no olfactory-related comorbidities such as neurodegenerative diseases. There was 1 case of self-limited epistaxis after nasal cell collection. All other subjects tolerated the procedure well and had no complications.

All but 1 subject in the irrigation cohort performed saline irrigations for all 7 days. None of the subjects in the irrigation cohort reported a subjective smell loss on the transition scale. Two subjects in the irrigation cohort reported a subjective improvement in smell.

TABLE 2. Baseline UPSIT scores and cAMP levels for the curette and cytobrush in each cohort

	Control	Preirrigation	<i>p</i>
UPSIT score (n = 16)	36.3 ± 0.5	36.4 ± 0.5	0.96
Curette cAMP (n = 10)	521 ± 33	536 ± 39	0.88
Cytobrush cAMP (n = 10)	456 ± 42	523 ± 50	0.36

Values are mean ± SEM.
cAMP = cyclic adenosine monophosphate; SEM = standard error of the mean;
UPSIT = University of Pennsylvania Smell Identification Test.

TABLE 3. UPSIT scores and cAMP levels for the curette and cytobrush at 1 week follow-up stratified by each cohort

	Control	Postirrigation	<i>p</i>
UPSIT score (n = 16)	36.8 ± 0.7	36.7 ± 0.4	0.48
Curette cAMP (n = 10)	509 ± 38	490 ± 32	0.94
Cytobrush cAMP (n = 10)	424 ± 36	449 ± 63	0.94

Values are mean ± SEM.
cAMP = cyclic adenosine monophosphate; SEM = standard error of the mean;
UPSIT = University of Pennsylvania Smell Identification Test.

Baseline mean UPSIT scores for the control and irrigation cohorts were 36.3 and 36.4 (*p* = 0.96), respectively (Table 2). Baseline curette mean cAMP levels were 509 and 536, respectively (*p* = 0.88). Baseline cytobrush mean cAMP levels were 424 and 523, respectively (*p* = 0.36).

There was no significant difference in UPSIT scores (36.8 for control, 36.7 for postirrigation) between the 2 cohorts at 1 week follow-up (Table 3). Mean cAMP levels were not significantly different between the 2 cohorts. Curette mean cAMP levels were 509 and 490 for the control and irrigation cohorts, respectively (*p* = 0.94). Cytobrush mean cAMP levels were 424 and 449, respectively (*p* = 0.94). These cAMP results are also illustrated in Figure 1.

When comparing preirrigation and postirrigation values within the irrigation cohort (Table 4), mean UPSIT scores

TABLE 4. UPSIT scores and cAMP levels using the curette and cytobrush within the irrigation cohort

	Preirrigation	Postirrigation	<i>p</i>
UPSIT score (n = 16)	36.4 ± 0.5	36.7 ± 0.4	0.52
Curette cAMP (n = 10)	536 ± 39	490 ± 32	0.11
Cytobrush cAMP (n = 10)	523 ± 50	449 ± 63	0.09

Values are mean ± SEM.
cAMP = cyclic adenosine monophosphate; SEM = standard error of the mean;
UPSIT = University of Pennsylvania Smell Identification Test.

were 36.4 and 36.7, respectively (*p* = 0.52). Using the curette, mean cAMP levels were 536 and 490, respectively (*p* = 0.11). Using the cytobrush, mean cAMP levels were 523 and 449, respectively (*p* = 0.09).

Discussion

This is the first known prospective randomized trial showing that short-term nasal saline irrigation has no detrimental effects on olfaction. There was no subjective smell loss in subjects after daily irrigation. In fact, 2 subjects experienced a subjective improvement in smell function. The primary measure of smell function in this study, the UPSIT score, remained essentially unchanged after 1 week of daily nasal saline irrigation. There was no significant difference in cAMP levels between each cohort or within the irrigation cohort.

Randomization appeared to be adequate. The 2 cohorts had comparable demographics and there was no statistically significant difference in baseline UPSIT scores and cAMP levels.

Reported side effects of nasal saline irrigation in the literature include ear fullness, nasal discomfort, and epistaxis.^{1,2,14} There have been no known links or reports of olfactory dysfunction, and the results of this study confirm the safety of nasal saline irrigation.

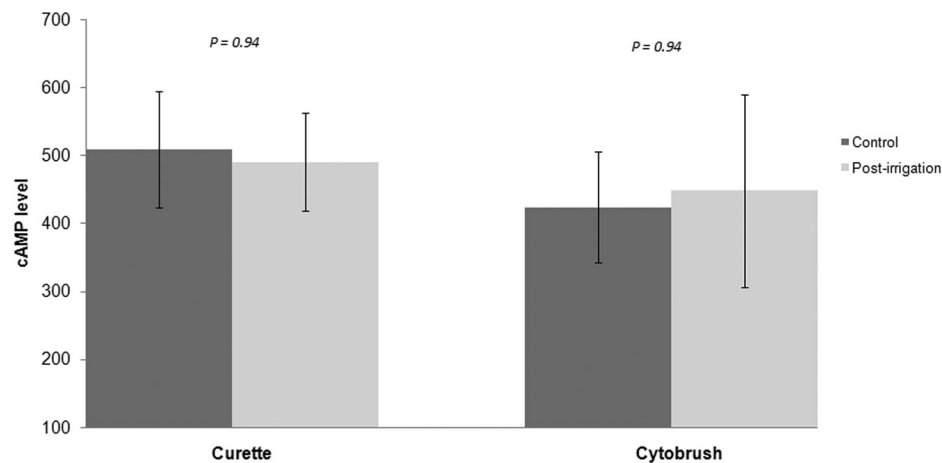


FIGURE 1. Bar graph comparing control and irrigation cohort cAMP levels stratified by curette and cytobrush collections. The error bars indicate 95% confidence intervals. cAMP = cyclic adenosine monophosphate.

The downward trend of cAMP levels within the irrigation cohort is likely insignificant. It has been shown that even partial resection of the superior turbinate does not reliably affect olfaction.¹⁵ Perhaps even if nasal saline is able to displace olfactory cilia, it does not appear to cause a significant clinical change.

At this time, there are no validated methods to collect nasal samples to determine cAMP levels. The methods used in this study determine intracellular cAMP levels and are undergoing further investigation in a separate study. Olfactory cilia have been identified as inferior as the anterior middle turbinate, but their presence on the inferior turbinate is still unclear.¹⁶ The clinical significance and quantification of cAMP levels in olfactory function is still an emerging concept and requires more investigation.

The main strength of this study is its prospective, randomized design. Other strengths include a zero dropout rate and

an adequate age range in each cohort. The primary weaknesses of this study are small sample size, limited power to detect a small difference in UPSIT scores, and limited duration of nasal saline irrigation. Other potential shortcomings include lack of blinding and single institution design.

Conclusion

In conclusion, although olfactory cilia can be harvested by saline preparations in animal models, it does not appear to have any subjective or clinically significant effect on human olfaction with short-term use. 

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