Published in final edited form as:
Ann Allergy Asthma Immunol. 2015 January ; 114(1): 63–64. doi:10.1016/j.anai.2014.09.012.

Comparing Two Methods to Collect Saliva from Children to Analyze Cytokines Related to Allergic Inflammation

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Keywords

Saliva collection methods; Passive drool; Oral swab; Allergic inflammation; Cytokines

Human saliva is a complex fluid, rich in immunological components, which reflects real time systemic concentrations. Advances in biotechnology have enabled us to measure minute concentrations of immunological components such as cytokines in saliva samples with precision and accuracy [1]. There are few data evaluating how the collection method may influence the results in addition to other methodological challenges, resulting in lack of consensus on a universally accepted saliva collection technique[2], especially in children. Specifically, the impact of saliva collection methods on detection and analysis of salivary concentrations of allergic inflammatory cytokines such as the interleukin (IL)-4, IL-5, IL-13, eotaxin 3 (Eo3) and thymic stromal lymphopoietin (TSLP), that mediate allergic inflammation[3] has not been reported. These cytokines play an important role in the development of allergic inflammatory states in increasingly prevalent conditions such as allergic rhinitis (AR), food allergy (FA), asthma (AS), atopic dermatitis (AD), and eosinophilic esophagitis (EoE)[4].

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Declaration: Authors' declare no conflict of interest.

Author Contributions:

1. Conception and design of the study: G.H.; A.O.; C.M.; R.S.; S.D.
2. Data generation: G.H.; S.S.; A.O.; S.D.
3. Analysis and interpretation of the data: G.H.; A.O.; S.S.; C.M.; R.S.; S.D.
4. Preparation or critical revision of the manuscript: G.H.; A.O.; S.S.; C.M.; R.S.; S.D.

The overall goal of this study was to compare two methods of saliva collection in the detection and analysis of salivary concentrations of IL-4, IL-5, IL-13, Eo3 and TSLP in children. Our study was approved by the Baylor Institutional Review Board and informed consent was obtained from the parent and assent, where appropriate, from the child.

We studied 20 children; median age: 14 years (9–17, IQR), female: 14 (64%), Caucasian: 13 (59%). Three (15%) children did not report any allergies and the remaining 17 (85%) children reported one or more of following commonly prevalent allergic conditions: AD (n=1), oral allergy syndrome (n=1), AS (n=2), FA (n=2), AR (n=6) and EoE (n=9). No attempt was made to distinguish salivary cytokine profiles between children with and without allergies in this study.

Participants were *nil per os* for at least 4 hours. They rinsed their mouth with water, and following a 10 min. period, passive drool (PD) was collected followed by saliva collection via an oral swab (OS), in order to minimize the effect of saliva on the flow rate and concentration of cytokines in PD. PD samples were obtained with the head tilted forward while drooling down a 5.5 cm polypropylene Salimetrics Collection Aid® (Salimetrics LLC, State College, PA). Next, the participant placed a Salimetrics Oral Swab® (Salimetrics LLC, State College, PA) in their mouth and gently rolled it side-to-side for approximately 60–90 sec. to saturate the swab, which was then placed in a storage tube that allows saliva to be centrifuged. All samples were collected between 7:30 am and 12:30 pm. Two (9%) participants aged 11 and 14 years were unable to provide PD because they were not able to direct their drool into the collection device. All participants were able to use the OS.

After providing paired saliva samples, all children responded to a short questionnaire assessing their knowledge of salivary diagnostics, asking them to rate their experience in providing each saliva sample in terms of perceived ease of use, acceptability and safety. Only 23% were aware of saliva testing in the surveillance of health or disease. A significantly higher proportion of children preferred the OS over PD sampling (82 % vs. 18 %; $P < 0.05$). Ease (68%) and speed (14%) of providing samples were the most common reasons cited by those who preferred the OS.

Saliva samples were maintained at 4°C and transport ed to the laboratory within 2 hr of collection and were centrifuged at 3,000 rpm for 15 minutes. Supernatants were stored in aliquots and frozen at −80°C. On the day of anal ysis, samples were brought to room temperature, vortexed, and centrifuged for 15 minutes at 3,000 rpm. A magnetic, high sensitivity human multi-analytes profiling bead-based assay kit was used (EMD Millipore, Billerica, MA; lower limit of detection (LLD) for IL-4, IL-5, and IL-13: 0.13 pg/mL, and coefficient of variation (CV) < 10%, <6% and <8% respectively; Eo3 and TSLP: LLD: 3.2 pg/mL, and CV < 9% and <12% respectively). All cytokines were measured on 96-well plates (50 μL saliva/well). The standards and controls were plated on every run using a saliva based sample matrix. Plates were read on the Luminex® 200™ platform (Luminex, Austin, TX) according to the manufacturer's recommendations. Intraassay precision was performed using 10 samples in duplicates and CV was calculated.

IL-4, -5 and -13 were detected in all PD and OS samples. Eo3 and TSLP were undetectable in 3 (17%) PD samples but were detectable in all OS samples. The Wilcoxon-rank sum test revealed that the median concentrations were similar between PD and OS samples (Table); however greater precision was noted in OS compared to PD concentrations. Bland-Altman analysis[5] showed no systematic bias and revealed insufficient agreement between PD and OS, suggesting that PD and OS may not be used interchangeably.

To our knowledge, this is the first study to describe the presence of cytokines related to allergic inflammation in saliva in a pediatric population. Our results suggest that OS provides more precise concentrations and is more acceptable than PD for detection and analysis of cytokines related to allergic inflammation. Furthermore, our data suggest that PD and OS may not be used interchangeably. Our data extend previous findings showing that saliva collection method can affect the salivary concentrations of stress and non-allergic inflammatory markers [6–9].

Strengths to our study included the ability to minimize the effects of external factors such as tooth brushing and recent meals by collecting saliva samples from children who were *nil per os* for an acceptable period of time prior to providing saliva samples. We were successful in collecting saliva samples (both PD and OS) from children as young as 6 years of age.

Limitations to our study include our use of a small convenience sample. In addition, our focus was on cytokines relevant to allergic inflammation. It is possible that either PD or OS may be acceptable for other biomarkers. Presence of eosinophils, eosinophil degranulation products, and/or epithelial cells and their influence on the concentrations of specific cytokines in PD or OS remains unclear.

In summary, in pediatric population, saliva collected by OS appears to offer methodological advantages over PD in analyzing cytokines related to allergic inflammation. Adequately designed studies to evaluate the potential of salivary cytokines as non-invasive, point of care markers to diagnose and/or manage common allergic conditions affecting children are warranted.

Acknowledgements

G.H. is supported by NIH T32 DK007664.

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Table

Salivary concentrations (pg/mL) cytokines associated with allergic inflammation in passive drool (PD) and saliva collected in oral swab (OS).

Cytokine	PD	OS	P value
IL-4	27 (21, 45)*	29 (22, 38)	0.37
IL-5	1.5 (0.8, 3.6)	1.4 (0.9, 2.0)	0.36
IL-13	4.5 (2.5, 10.1)	4.8 (3.1, 5.8)	0.39
Eotaxin 3	52.7 (44.1, 60.3)	45.6 (41.5, 55.2)	0.15
TSLP	7.4 (7.0, 7.8)	7.2 (6.9, 7.4)	0.15

*Median (IQR)