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To Spear or Not to Spear: Comparison of Saliva Collection Methods

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Abstract

The eye spear, or an absorbent sponge-like material, has been proposed as a useful method of obtaining repeated saliva samples from infants and young children for cortisol determination. This brief report examines possible interference effects of different types of eye spears under conditions of relatively high and low cortisol levels, with or without the use of oral stimulant, and using two common assays. In Study 1, one type of eye spear was compared to passively collected drool using two different assays (EIA, DELFIA), across high and low concentrations of cortisol. No differences were found between methods for either assay or cortisol level, indicating that the spears are potentially a viable method of collecting saliva. Study 2 compared three other types of absorbent eye spears to passive drooling under the presence or absence of oral stimulant use. This study revealed that the degree of interference varied as a function of the specific type of eye spear that was employed; stimulant use had no effect. Taken together, the results raise important considerations to take into account when selecting collection materials and procedures in the measurement of salivary cortisol.

Keywords

cortisol

Introduction

The measurement of salivary cortisol as a method of assessing hypothalamic-pituitary-adrenocortical (HPA) axis activation is effective and noninvasive, making it attractive to researchers interested in stress responses across the lifespan. However, researchers who collect saliva from infants and toddlers anecdotally report having difficulty with saliva collection because these young participants are often unwilling or unable to actively participate in saliva expectoration, problems exacerbated when multiple samples are solicited. In addition to difficulties in participation, researchers must ensure that the tools of collection do not interfere with the cortisol assay. For example, previous work has demonstrated that cotton dental rolls affect cortisol assays to varying degrees depending on the type of cotton and the type of assay. Presumably, the cortisol molecule can adhere to the cotton and/or vegetable steroids can leach from the cotton to interfere with the assessment (Shirtcliff, Granger, Schwartz, & Curran, 2000). Also, flavored drink crystals or candy used to stimulate saliva flow can alter cortisol assessment when used in sufficient quantities or

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with particular assays (Gordon, Peloso, Auker, & Dozier, 2005; Talge, Donzella, Kryzer, Gierens, & Gunnar, 2005). Thus, there is an ongoing quest to find a method of obtaining saliva that is well tolerated by young children and does not interfere with the validity of the cortisol assay.

Recently, “eye spears”, small pads of various absorbent materials attached to plastic sticks (~7 cm), have been introduced as a collection option. The fast action of these sponge-like devices was originally designed to absorb tears during medical procedures. However, after being held in the presence of saliva in the mouth for approximately one minute, one to two eye spears absorb a volume of saliva sufficient for analysis. One cellulose-based example of these devices are BD Ophthalmology “Visispears” (product #581089), marketed by Salimetrics as “Sorbettes” (product #5029). deWeerth, Jansen, Vos, Maitimu & Lentjes (2007) found that these devices have adequate cortisol recovery when samples are assayed using an in-house radioimmunoassay.

The goal of this brief report is to describe two methodological studies that examined whether the use of eye spears interfered with salivary cortisol determination using assays and stimulant conditions that mirror those used in studies of infants and young children.

Method

Study 1: Viability of the Sorbette eye spear under two assay methods

This study examined the use of Sorbette eye spears as a collection technique using either an enzyme immunoassay (EIA) or dissociation enhanced lanthanide fluoroimmunoassay (DELFIA) assay. Two pools of approximately 60 mL of saliva were obtained by combining approximately 2 mL samples provided by laboratory staff either within 10 min of morning awakening (AM pool) or between 8-9 pm (PM pool). Early morning and late evening collections were used to allow testing of Sorbette eye spear assay interference at both high (AM) and low (PM) cortisol concentrations. Some volunteers provided only a morning or evening sample, and some provided both; as a result, time of day was not amenable to a within-subjects analysis. This guided our decision to pool the samples within time of day to form uniform high or low cortisol pools. After thorough mixing, each saliva pool was aliquoted into 120 samples. Samples were refrigerated for approximately 2 weeks prior to being assayed. Studies attest to the stability of the cortisol molecule, with levels remaining unaffected following several days of *no* refrigeration (Clements & Parker, 1998). Thus, we believe our storage procedure did not compromise the validity of our findings.

Half of the samples from each 2(AM/PM) by 2 (Sorbette, clear) group were then assayed in either the EIA method used in the assay kit sold by Salimetrics and the DELFIA method used by the Biochemisches Labor at the Universitaet Trier. One evening DELFIA sample was lost to error. Assays were performed in duplicate, with duplicates within 10% of each other. Inter- and intra- coefficients of variation were 2.7% and 10.9%, and at or less than 6.7% and 9.0%, respectively for the EIA and DELFIA assays.

Results

A 2 (assay: DELFIA, EIA) X 2 (treatment: clear, speared) X 2 (time: AM, PM) ANOVA was performed. An expected main effect for time of day was found, $F(1,28) = 3904.97, p < .001, M_{am} = .55, M_{pm} = .10$. A main effect for assay type was also found, $F(1,28) = 93.91, p < .001, M_{EIA} = .30, M_{DELFIA} = .36$. This effect has been seen in the past and was expected (Gunnar, Donzella, & Gustafson, unpublished data). No effect was found for treatment type, $F(1,28) = 2.4, ns, M_{clear} = .32, M_{spear} = .33$, and no interactions were significant. Additionally, cortisol levels of speared and clear samples were highly correlated within

assay type, for EIA, $r(118) = 0.95, p < 0.001$, for DELFIA, $r(117) = 0.99, p < 0.001$. See Figure 1 for a scatter plot of the individual data points.

Study 2: Are all eye spears equal?

A variety of products other than the Sorbettes tested in Study 1 are marketed as “eye spears” and made of different synthetic and natural materials. This study addressed the question of whether other materials sold as eye spears are also acceptable for use in collecting saliva for cortisol determination. Given that some researchers use oral substances to stimulate saliva flow, the different absorbent materials were tested under conditions of stimulant use or none.

Six healthy volunteers passively drooled approximately 300 mL of saliva between 7:00-9:00 AM (to produce relatively high cortisol levels). For each participant, saliva was aliquoted into 6 samples. Three samples were exposed separately to each of the following absorbent materials: Cellulose eye spear (Stradis Medical #9430), Lasik eye spear (Stradis Medical #9427), or PVA eye spear (Stradis Medical #9429). The remaining three samples were left without absorbent treatment, in order to provide within-subject no-treatment comparisons. In addition, a between-subject saliva stimulant condition was created by treating the samples of three participants to .025 g of cherry-flavored KoolAid while the remaining three were left untreated. All samples were refrigerated until assayed with the DELFIA method used by the Biochemisches Labor at the Universitaet Trier, as in Study 1. One Lasik sample was lost to error.

Results

To answer the question of whether a given absorbent material significantly altered cortisol levels, a mixed-measures ANOVA was performed for each of the three absorbent materials: 2 (treatment: absorbent material vs. none) as a within-subject factor X 2 (stimulant: none, Kool-Aid) as a between-subject factor. Individual data points are shown in Figure 2. A main effect of absorbent material was found in each case, with no significant stimulant main effects or interactions, Lasik: $F(1,3) = 15.55, p < .05, M_{\text{absorbent}} = .19, M_{\text{none}} = .42$; PVA: $F(1,4) = 23.15, p < .01, M_{\text{absorbent}} = .21, M_{\text{none}} = .42$; Cellulose: $F(1,4) = 11.45, p < .05, M_{\text{absorbent}} = .56, M_{\text{none}} = .46$. For Lasik and PVA materials, these results held regardless of which one of the three within-subject treatment controls were used; for Cellulose, use of one of the control samples resulted in a non-significant main effect for absorbent type. The three control samples were highly correlated (Spearman's $r_s .94 - 1.00, p < .01$), and did not statistically differ from one another. The correlation between the Cellulose samples and control samples was significant, Spearman's $r(6) = .94, p < .01$, although the correlations associated with the PVA and Lasik samples were not, PVA: Spearman's $r(6) = .77, p = .07$; Lasik: Spearman's $r(5) = .80, p = .10$.

Discussion

The results of Study 1 indicate the Sorbette eye spear did not significantly affect salivary cortisol assessment, regardless of whether the EIA or DELFIA assay technique was employed. Additionally, no interactions with time of day were observed, suggesting that study findings did not vary as a function of cortisol concentration. Furthermore, the high correlation of cortisol levels between Sorbette-speared and clear samples (all r 's $> .95$) suggests minimal interference with respect to the assessment of individual differences. However, the results of Study 2 suggest that these findings cannot be generalized across all types of eye spear products, as each of the three different absorbent devices examined in Study 2 were not equally robust to interference effects.

These studies did not include an exhaustive analysis of all products marketed as “eye spears,” and it should be noted that, with the exception of Sorbettes, these products are intended to collect tears during medical procedures and are not marketed as saliva collection devices. As shown in Study 2, any deviations from the above materials and assay types may cause interference, and it should be noted that if these materials were used to assay other salivary biomarkers, or were used with different assay methods, further testing would be required.

Nonetheless, the small size of these collection matrices may be advantageous, either when sampling from a very small individual such as a premature infant or to enhance young children's compliance with repeated sampling procedures. However, the small size of the eye spears also increases the risk of choking if the spear is inhaled. As always, researchers are cautioned to take particular care to supervise saliva collection and consider evaluating the effects of their saliva collection procedures given of the specific type of matrix or assay that is employed. Despite these provisos, the appropriate eye spear might well be a solution for researchers who wish to obtain quick, repeated measurements of salivary cortisol from young participants.

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Figure 1.

The top panel shows the individual data points for samples treated as clear versus speared for AM, or relatively high cortisol levels. Assay type is denoted by markers to illustrate the main effect of assay on cortisol level. The same is presented in the bottom panel, for PM or relatively low cortisol levels.

Figure 2.

This figure shows the individual data points for each participant, demonstrating the differing influence of various absorbent materials.