HHS Public Access

Author manuscript

Dev Psychobiol. Author manuscript; available in PMC 2019 January 01.

Published in final edited form as:

Dev Psychobiol. 2018 January ; 60(1): 118–122. doi:10.1002/dev.21584.

In-Home Salivary Melatonin Collection: Methodology for Children and Adolescents

Belinda N. Mandrell, **Yvonne Avent**, **Breya Walker**, **Megan Loew**, **Brooklee Lightsey Tynes**, and **Valerie McLaughlin Crabtree**

St. Jude Children's Research Hospital, Memphis TN

Abstract

In-home salivary collection quality and adherence to a prescribed collection methodology for evaluation of dim light melatonin onset (DLMO) is unknown in children. Primary aims of this study were to 1) describe a novel family-centered methodology for in-home salivary collection, 2) determine the acceptance and feasibility of this methodology, 3) measure adherence to collection instructions and 4) identify patterns between participants' age and quality of samples collected. After receiving instructional handouts from the study team, families utilized in-home salivary melatonin collection. Participants (N=64) included 39 children (21 female, mean age 9.5 ±1.61 years) and 25 adolescents (11 female, mean age 15.9 ±2.12 years) with craniopharyngioma. Participants were 90% adherent to collection schedule, and 89% of the samples collected were of sufficient quantity and quality, with no differences found between age (child verses adolescent) and melatonin sample quantity and quality. In-home saliva collection provides an acceptable and feasible method to collect salivary melatonin and biomarkers in children and adolescents.

Keywords

Saliva sampling; Melatonin; Biomarker; Circadian Rhythm; Methodology; Craniopharyngioma; Family-centered

INTRODUCTION

Salivary melatonin can serve as a biomarker of circadian rhythm, and collection can be useful in the diagnosis and treatment of circadian rhythm sleep-wake disorders (Keijzer, Smits, Duffy, & Curfs, 2014; Lockley, 2005; Rahman, Kayumov, Tchmoutina, & Shapiro, 2009), as melatonin's secretion is stimulated by the pineal gland (which is regulated by the hypothalamic-pituitary-adrenal axis) and its onset or rise occurs approximately two hours prior to habitual bedtime, and such timing is consistent with normal circadian rhythm

Corresponding Author: Belinda Mandrell, PhD, RN, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Mail Stop 738, Memphis, TN 38105-3678, belinda.mandrell@stjude.org, Phone: (901)595-4209; Fax: (901)595-2866.

Conflict of Interest: The authors (Mandrell, Avent, Walker, Loew, Tynes, Crabtree) have no conflict of interest to disclose.

Author Contributorship: Mandrell designed the method of collection for the study and writing of the manuscript, Avent collected the saliva samples and contributed to writing, Walker completed data analysis, design of tables and writing of manuscript, Loew, Tynes and Crabtree contributed to the design and writing/editing of the manuscript. All authors agreed on final manuscript revisions and accountability for published work.

(Czeisler et al., 2004; Wright, Gronfier, Duffy, & Czeisler, 2005). The dim light melatonin onset (DLMO) is the most often used marker of melatonin rise and is defined as a melatonin range of 3–5 pg/mL in saliva (Carskadon, Labyak, Acebo, & Seifer, 1999; Weber, Schwander, Unger, & Meier, 1997; Wirz-Justice, Werth, Renz, Muller, & Krauchi, 2002).

Although recent studies support in- home saliva collection (Keijzer, Smits, Duffy, & Curfs, 2014; Pullman, Roepke, & Duffy, 2012), many others still rely on in-laboratory collection, which can increase cost as well as research participant burden (Grima, Ponsford, Hilaire, Mansfield, & Rajaratnam, 2016; Paul et al., 2015). Of importance, Keijzer et al., 2014 have noted that no consensus has yet been established in the general field with respect to the best method for sampling salivary melatonin secretion. In particular, very little is known about optimal methods for pediatric patients, particularly those with a high likelihood of sleep-wake disorders. Hanrahan and colleagues (2006) suggested establishing a standardized collection in which methodology, timing and materials are consistent. Methodology must be refined in a user-friendly manner in an effort to make collection successful for both adult and pediatric populations.

For pediatric patients, it is particularly critical to identify strategies to promote adherence in both the children and their parents, and this has yet to be established. Specifically, a lack of adherence to collection instructions and missing time points of collection may create a need for increased attention during analysis (Wren, Shirtcliff, & Drury, 2015). Roth et al (2017) recently described the collection of a one-time in-home saliva sample and assessed feasibility by protocol acceptance, completion, instruction adherence and sufficiency of samples. The in-home saliva sample was missing more often than saliva samples collected at the study visit sites, with variability in-home morning sample ranging from 1.3% to 19.8% missing. Home collection may also have age-related complications of timing and adherence particularly within the pediatric population (e.g., crying, refusal). In a recent study by Smith and Dougherty (2014), parent-reported adherence with salivary cortisol collection procedures was compared to the electronic monitoring device (MEMS TrackCap), an objective measurement of adherence, over a two day period consisting of eight sample collections. Adherence was defined as collection of each of the samples within the established time window. While parents reported an overall adherence rate of 83%, objectively overall adherence was 68.8%. Of note, with more required samples, there was a decrease in adherence to sample collection. Furthermore, children of parents who were non-adherent to the collection protocol actually had higher observed waking cortisol when compared to children of adherent parents (Smith & Dougherty, 2014). For pediatric patients, it is particularly critical to identify strategies to promote adherence in both the children and their parents, when multiple collection time points are requested. To our knowledge, no published reports have described the use of developmentally appropriate educational materials to promote adherence to at-home saliva sampling instructions in children, particularly children with high risk of daytime sleepiness and circadian rhythm disturbance.

In an effort to refine a standardized collection process among pediatric patients with high risk of daytime sleepiness and circadian rhythm disturbance, the present study was designed to 1) pilot salivary collection instructions to obtain melatonin samples, 2) determine the acceptability and feasibility of an in-home method of salivary sampling, 3) measure parent-

reported adherence to the necessary collection schedule, and 4) identify any patterns between participants' age and quality of samples collected. The current study proposed that a structured methodology would be acceptable and feasible for in-home salivary melatonin collection, with less acceptable quantity in the younger age group.

METHODS

Participants

Sixty-four patients (7 – 20 years of age; 50% female) who consented/assented to the institutional craniopharyngioma protocol were eligible for salivary melatonin collection. All agreed to participate. Patients and their parents were given instructions on the overnight in-home saliva collection and storage methods for the determination of DLMO. The collection of salivary samples for melatonin was approved by the Institutional Review Board under children's research categories 45CFR46.405 and 21CFR50.52 (FWA00004775).

Procedure

Salivary melatonin samples were collected by the passive drool method, in which the participant allows saliva to pool in his/her mouth and then drools (rather than spits) through a straw into the collection tube (See Figure 1 in supplemental materials for patient and family instructions). Saliva samples were collected according to the participant's reported habitual or typical bedtime, in an effort to capture DLMO. As part of the circadian rhythm assessment patients wore Micromini Sleep Watch ® (Ambulatory Monitoring Inc., Ardsley, NY) five consecutive days including the evening of melatonin collection, marking the time to bed with the device event button. To assure rise in melatonin was captured, patients and parents were instructed to begin collection hourly (three, two and one hour) before bedtime. To document continued melatonin rise, saliva was collected again at bedtime, and parents awakened their children to collect a final sample one hour after bedtime. Saliva collection tubes were color coded according to the collection time (three, two, one hour before bedtime, bedtime and one hour after bedtime) (Figure 2 in supplemental materials). After each hourly collection, the color coded tube was placed in a labeled box in the family's freezer. The following morning, participants and parents returned the box with samples to the study nurse. The samples were then stored in a −80° freezer until shipped for analysis to be processed by Salimetrics®.

The recommended quantity of saliva was 225 μL for adequate quantity provided for the assay, with each saliva sample test quantity 100μL. Each sample of saliva underwent separate analyses, with the average concentration of melatonin reported. The saliva assay has melatonin sensitivity detection as low as 1.37pg/mL. Any sample with a quantity of less than 100μL was reported as insufficient quantity.

For patients and families to be successful with the described saliva collection, education was paramount. Each family received individual education regarding the process with a study nurse and was given a child-friendly instruction book developed by the study team (available upon request). The instruction booklet described the seven step collection process in a child-friendly manner with simple sentences and pictures.

Data Analysis

Participant demographics were summarized by descriptive statistics. Fisher's Exact Test was used to examine the relationship between age and adherence as defined by 1) missing samples and 2) samples with insufficient quantity of saliva. A one-way ANOVA was conducted to assess adherence to specified collection times by age group and adherence of one hour between each sample collection time point. To determine whether age group means for sample collections deviated from the 60 minute time requirement a one sample t-test was performed. All statistical tests used a two-sided significance level of $p<0.05$. Statistical analyses were conducted using SAS Version 9.4 (SAS, 2014).

RESULTS

This study sample consisted of 64 participants including 39 children (21 female, mean age 9.5 ±1.61 years) and 25 adolescents (11 female, mean age 15.9 ±2.12 years). Majority of participants identified as White (67%) followed by 19% Black and 14% Other. Salivary melatonin was collected over 5 hours, beginning at three, two, and one hour before bedtime, at bedtime and one hour after bedtime. Each saliva sample was determined to be sufficient or non-sufficient for melatonin detection based on quantity of saliva. There was no significant difference ($p=0.21$) between age groups (child versus adolescent) on quantity of saliva (sufficient versus non-sufficient) collected across all time-points, with 11 of 39 children and 3 of 25 adolescents having one non-sufficient sample. There was no significant difference ($p=0.46$) found between age group (child vs. adolescent) and the number of missing samples with 7 of 39 children (17.9%) and 2 of 25 adolescents (8%) having at least one missing sample. There was no significant association ($p=0.89$) between the sample collection time point and missing sample, with only 8–6% of samples missing across time points. Furthermore, there was no significant association ($p=0.93$) between the sample time point and the quantity of the saliva (sufficient versus non-sufficient) collected, with only 9–14% of the samples non-sufficient across time points. (Table 1)

Patients were required to collect each sample within one hour of the previous sample. To determine if patients and parents adhered to timing, data were derived from the recorded salivary collection log maintained by the patient/parent. Table 2 describes the average minutes between collections of samples by age group. There was no significant difference between the parent-reported 60 minute collection time between samples by age group and sample time point collected. We also examined the accordance between the reported melatonin bedtime sample (sample 4) and the recorded actigraph bedtime. There was a positive correlation between time of collection for sample 4 and the median actigraph reported bedtime ($p=0.0046$, $r=0.36123$).

The null result of missing samples and sufficiency of saliva collected by age group (child versus adolescent) is relevant to the overall interpretation of our proposed salivary collection methodology. Therefore we conducted a post-hoc power analysis with the program G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) to determine if our sample proportions had adequate power to detect differences between age groups. With low power achieved (Power= 0.34), logistic regression bootstrap with replacement was performed (Efron & Tibshirani, 1993). Bootstrapped confidence intervals (95%) were computed using SAS 9.4. Regression

coefficients divided by the standard deviation yields the critical ratios of the bootstrap. Values within ±1.96 range are not statistically significant (Efron & Tibshirani, 1993). Results of 1000 resamples for each dependent variable by age group revealed no change in conclusions drawn (Table 3).

DISCUSSION

The hypothesis that an in-home salivary melatonin collection method would be acceptable and feasible in children and adolescents with craniopharyngioma was supported. All participants in the larger treatment protocol agreed to participate in the saliva collection, and patients and parents were receptive to the education and salivary collection instructions to successfully obtain melatonin samples. The described instructions in collection and storage of saliva appear to be understandable for children and adolescents as well as for their parents without significant missing or insufficient quantity of samples. The individualized educational approach for saliva collection resulted in 92–97% collection percentage across all five samples. Finally, although it was anticipated that children versus adolescents might have more difficulty obtaining all five samples with sufficient quantity to analyze, there was no difference between age groups in missing samples or insufficient samples. Of note, non-standardized qualitative parent reports noted the last time point, one hour after bedtime, as being the most difficult to collect. Although parental report was typically offered spontaneously and not rigorously collected, it is important to note that reasons cited by parents for difficulty in obtaining the final sample included: difficulty in waking the child after sleep for saliva collection; parents not setting an alarm to wake themselves up to assist in saliva collection, and perceived inability to collect adequate quantity due to difficulty arousing the child from sleep. However, this perceived difficulty was not found to impact sample collection or sufficiency of sample.

While we anticipated potential difficulty in collecting samples over five-times, we felt this is important in obtaining multiple samples in determining DLMO. In laboratory settings, this is typically conducted by having samples obtained every two to three hours across a 24 hour period. In a home setting, this can be approximated by determining the participant's usual bedtime and timing hourly samples prior to and just after bedtime. Furthermore, the literature supports hourly sampling as being more affordable and most accurate for patients with suspected circadian rhythm sleep-wake disorders (Molina & Burgess, 2011; Crowley, Suh, Molina, Fogg, Sharkely, & Carskadon, 2016). With children and adolescents, waking-up participants for a sample collection can be challenging; however, parents were able to successfully do so. Furthermore, although passive drooling can be a challenging concept to comprehend, with adequate and child-friendly instruction materials, we were successful in obtaining samples with sufficient quantity in 89% of our sample. The method of passive drool was chosen over swab collection for several reasons. Saliva collected by swab must undergo additional centrifuge process for sample storage, increasing cost and risk of contamination. When low volume may be expected in special circumstances such as developmental age, absorbent devices may introduce error variance in saliva measurement (Harmon, Hibel, Rumyantseva, & Granger, 2007). Our findings indicate that with educational materials targeted toward children and instructions that are simple to understand for parents (e.g., color coded tubes), adequate samples of saliva can be collected at home

across multiple time points. Thus, support is provided for use of in-home sampling, providing a more family-centered approach to data collection that is non-invasive, cost effective, and conducted in the child's home environment.

Although our methodology was acceptable and feasible in our sample, some limitations were present. We focused on parent-reported adherence and did not utilize an objective measurement of adherence, such as Medication Event Monitoring Systems (MEMS) caps. Thus, although we can objectively ascertain the number of samples collected, we cannot ensure that they were collected at the predetermined times, which could affect the reliability of the methodology. However, we did find the actigraph bedtime and reported collection of sample 4 was in accordance. Lastly, acceptability of in-home saliva collection among families of children and adolescents with craniopharyngioma may not generalize to other samples of children. Because these patients are at high risk for circadian rhythm disruption and daytime sleepiness, their families may be more motivated in collection adherence of melatonin for determination of circadian rhythmicity than other families might.

Salivary metabolite collection in pediatrics is of particular interest, as the invasive means of blood collection can serve as a deterrent to research participation. Although previous studies have used in-laboratory settings to ensure adherence to saliva collection techniques, this can be burdensome to participants, particularly children and their families. Finding novel ways of ensuring adequate saliva collection in the home setting provides a non-invasive, cost-effective, palatable means of collecting many hormones that contain salivary metabolites, particularly melatonin and cortisol. Use of child-friendly education materials, including the use of color-coded tubes to aid in appropriate timing of collection, appears to improve adherence to adequate collection. Thus, this approach to in-home collection of saliva may improve the feasibility and acceptability of research projects requiring salivary metabolites in children and adolescents, extending beyond simply melatonin analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: This study was supported by Cancer Center Support Grant (CA21765) from the National Cancer Institute; and ALSAC.

References

Carskadon MA, Labyak SE, Acebo C, Seifer R. Intrinsic circadian period of adolescent humans measured in conditions of forced desynchrony. Neurosci Lett. 1999; 260(2):129–132. [PubMed: 10025716]

Crowley SJ, Suh C, Molina TA, Fogg LF, Sharkely KM, Carskadon MA. Estimating the dim light melatonin onset of adolescentw within a 6-hour sampling window: the impact of sampleing rate and threshold method. Sleep Med. 2016; 20:59–66. [PubMed: 27318227]

Czeisler C, Walsh J, Roth T, Schwartz J, Wright K, Dinges D. Modafinil for excessive sleepiness associated with shift work sleep disorder. European Journal of Neurology Supplement. 2004; 11:33–34.

Efron, B., Tibshirani, R. An introduction to the bootstrap. Cox, DR.Hinkley, DV.Reid, N.Rubin, DB., Silverman, BW., editors. Boca Raton (Florida): Chapman & Hall/CRC; 1993.

Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 2007; 39:175–191. [PubMed: 17695343]

Grima NA, Ponsford JL, Hilaire MAS, Mansfield D, Rajaratnam SM. Circadian Melatonin Rhythm Following Traumatic Brain Injury. Neurorehabilitation and neural repair. 2016; 30:972–977. DOI: 10.1177/154598316650279 [PubMed: 27221043]

Hanrahan K, McCarthy A, Kleiber C, Lutgendorf S, Tsalikian E. Strategies for salivary cortisol collection and analysis in research with children. Applied Nursing Research. 2006; 19:95–101. [PubMed: 16728293]

Harmon AG, Hibel LC, Rumyantseva O, Granger DA. Measuring salivary cortisol in studies of child development: watch out-what goes in may not come out of saliva collection devices. Dev Psychobiol. 2007; 49:495–500. [PubMed: 17577235]

Keijzer H, Smits MG, Duffy JF, Curfs LM. Why the dim light melatonin onset (DLMO) should be measured before treatment of patients with circadian rhythm sleep disorders. Sleep Med Rev. 2014; 18(4):333–339. DOI: 10.1016/j.smrv.2013.12.001 [PubMed: 24388969]

Lockley SW. Timed melatonin treatment for delayed sleep phase syndrome: the importance of knowing circadian phase. Sleep. 2005; 28(10):1214–1216. [PubMed: 16295202]

Molina TA, Burgess HJ. Calculating the dim lifht melatonin onset: the impact of threshold and sampling rate. Chronobiol Int. 2011; 28(8):714–718. [PubMed: 21823817]

Paul MA, Love RJ, Hawton A, Brett K, McCreary DR, Arendt J. Sleep deficits in the High Arctic summer in relation to light exposure and behaviour: use of melatonin as a countermeasure. Sleep Med. 2015; 16:406–413. [PubMed: 25747331]

Pullman RE, Roepke SE, Duffy JF. Laboratory validation of an in-home method for assessing circadian phase using dim light melatonin onset (DLMO). Sleep Med. 2012; 13(6):703–706. [PubMed: 22445311]

Rahman SA, Kayumov L, Tchmoutina EA, Shapiro CM. Clinical efficacy of dim light melatonin onset testing in diagnosing delayed sleep phase syndrome. Sleep Med. 2009; 10(5):549–555. DOI: 10.1016/j.sleep.2008.03.020 [PubMed: 18725185]

Roth R, Baxter J, Vehik K, Hopkins D, Killian M, Gesualdo P, et al. The feasibility of salivary sample collection in an international pediatric cohort: the TEDDY study. Dev Psychobiol. 2017; 59:658–667. [PubMed: 28555778]

SAS. SAS 9.4 Output Delivery System: User's Guide. Cary, NC: SAS institute; 2014.

Smith VC, Dougherty LR. Noisy spit: Parental noncompliance with child salivary cortisol sampling. Dev Psychobiol. 2014; 56:647–656. [PubMed: 23754778]

Weber J, Schwander J, Unger I, Meier D. A direct ultrasensitive RIA for the determination of melatonin in human saliva: comparison with serum levels. J Sleep Res. 1997; 26(757):b53.

Wirz-Justice A, Werth E, Renz C, Muller S, Krauchi K. No evidence for a phase delay in human circadian rhythms after a single morning melatonin administration. J Pineal Res. 2002; 32(1):1–5. [PubMed: 11841593]

Wren M, Shirtcliff E, Drury S. Not all biofluids are created equal: Chewing over salivary diagnostics and the epigenome. Clinical Therapeutics. 2015; 37(3):529–539. [PubMed: 25778408]

Wright KP, Gronfier C, Duffy JF, Czeisler CA. Intrinsic period and light intensity determine the phase relationship between melatonin and sleep in humans. Journal of Biological Rhythms. 2005; 20(2): 168–177. [PubMed: 15834113]

Table I

Sample Collection Time Point Proportions

	Missing Tubes by Sample					
	Sample 1 N (%)	**Sample 2 N (%)**	**Sample 3 N (%)**	**Sample 4 N (%)**	**Sample 5 N (%)**	**P value**
Tubes present	59 (92.2)	62 (96.9)	60 (93.8)	61 (95.3)	60 (93.8)	0.89
Tubes absent	5 (7.8)	2 (3.1)	4 (6.2)	3 (4.7)	4 (6.2)	
Sufficient	57 (89.1)	58 (90.6)	58 (90.6)	58 (90.6)	55 (85.9)	0.93
Non-Sufficient	7(10.9)	6 (9.4)	6 (9.4)	6 (9.4)	9 (14.1)	

Note: Proportion of saliva samples: present compared to those absent and sufficient compared to those not sufficient

Table II

Salivary Melatonin Data Collection Time

Comparison between sample collection time points					
	Average AYA minutes	Average C minutes	*F statistic (p value)	**T statistic (p value)	
				AYA	C
Sample 1 to Sample 2	60.88	64.94	1.60 (0.21)	0.63 (0.53)	2.01 (0.05)
Sample 2 to Sample 3	59.96	63.22	1.17 (0.28)	−0.16 (0.88)	1.30 (0.20)
Sample 3 to Sample 4	60.16	58.18	0.55 (0.46)	0.37 (0.72)	−0.85 (0.40)
Sample 4 to Sample 5	60.06	64.50	2.18 (0.15)	0.16 (0.87)	1.86 (0.07)

Note:

*the average difference in time between recorded salivary melatonin collection compared between age groups.

AYA= Adolescent/Young Adult C= Child.

**Difference in age group means of sample collection time from 60 minutes

Table III

Bootstrap Confidence Intervals and Regression Coefficients

95% Confidence intervals for 1000 resampling with replacement					
	Lower interval	**Upper intervals**	**Regression coefficient**	**Original OR estimate**	**Critical ratio***
Sufficient saliva data	−0.052	11.77	1.78	2.88	1.78/2.83=0.63
Missing tubes data	−0.012	11.81	1.87	2.52	1.87/3.05=0.61

Note: Logistic regression bootstrap CI intervals for the comparison of age group (i.e., AYA vs. C) on dependent variables (i.e., Sufficiency and Missing tubes) with C being the referent group.

*Regression coefficient divided by standard deviation yields the critical ratio with absolute numbers < 1.96 equating to non-significance.