



Self-collection of hair samples during the COVID-19 pandemic

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ABSTRACT

Purpose: Due to the COVID-19 Pandemic, public health restrictions were implemented that required study protocol revisions for our collection of hair cortisol samples from in-person to self-collection. The purpose is to provide descriptions of the protocol transition process from in-person hair cortisol sample collection to participant self-collection using written and video instructions as well as the acceptability and feasibility of this transition.

Main results: The protocol transition required revisions to the written instructions with a link to a newly developed video for hair sample self-collection. There was little difference in the initial participant agreement to provide a hair sample between in-person (79/114; 69.3%) versus self-collection (254/417; 60.9%) protocol methods. Some participants were initially hesitant to provide a self-collected hair sample but commented that self-collection was easier than they anticipated which supports the acceptability of this protocol change. However, regarding feasibility, 16.8% of participant self-collected hair samples were not received by study staff despite reminders (14.3%) or there was an issue with mail delivery or return (2.5%).

Major conclusions: The transition of our hair sample collection protocol from in-person to self-collection was acceptable and feasible. Providing instructions for hair sample self-collection in a variety of formats (oral, written, video) helped to decrease uncertainty and hesitancy regarding the process and promote agreement among participants. In addition, consistent follow-up communication was key to timely receipt of the hair samples from participants.

1. Introduction

Emotional and physical stress, whether acute or chronic, have significant negative effects on individual health and quality of life. Cortisol is a steroid hormone secreted in response to stress [1]. Historically, saliva, blood, and urine samples have been collected to measure cortisol levels. However, cortisol levels have been shown to fluctuate throughout the day, with a slight increase in the morning and decrease in the evening [2]. In addition, measurement differences in cortisol level were apparent when saliva, blood, and urine samples were collected with drastic variations by timing of sample collection [3].

To address these practical challenges in assessing cortisol levels, Cirimele et al. identified ten corticosteroids, including cortisol in human hair [4]. Hair cortisol analysis captures the long-term cortisol level thus eliminating the need for multiple samples of saliva, urine or serum [5]. Subsequently, hair collection was adopted as a non-invasive, cost-effective, and objective method of measuring cortisol levels in humans [5]. Specifically, hair cortisol concentrations reflect a retrospective

measurement of hypothalamic-pituitary-adrenal (HPA) function over time, similar to hemoglobin A1C representing glycemic control [6]. High cortisol levels have been found in hair samples of individuals with trauma, severe chronic pain, major depressive disorder and are particularly relevant in the assessment of chronic stress [7].

Due to the COVID-19 pandemic, the demand for safe and effective sampling techniques have increased making hair cortisol sample collection an ideal option. Human hair grows approximately 1 cm per month, therefore, analysis of hair cortisol levels can provide a historical timeline of stress levels [8]. Hair sample collection is advantageous because samples are easily stored at room temperature and shipped via a postal service to a testing laboratory [8].

Our original study protocol required a trained research assistant to complete four in-person data collection visits in a private place of the participant's choosing (e. g., home or public library). This visit included a hair sample collection by the research assistant. Due to the COVID-19 Pandemic, public health restrictions were implemented that necessitated protocol revisions; elimination of in-person visits and transition to

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all remote operations. Therefore, our hair sample collection protocols had to be altered to enhance participant and research staff safety yet maintain standardized sample collection techniques.

While there is a plethora of research articles demonstrating the validity of hair cortisol sampling as a quantitative measure of stress, there is a scarcity of articles providing sample self-collection details or a self-collection protocol. For instance, Castillo-Mancilla et al. mentioned that hair sample self-collection kits with written instructions and a demo video were mailed to each participant [9]. Ouellet-Morin et al. reported that hair sample self-collection and return of such samples via the postal service is a reliable assessment of chronic cortisol secretion that was confirmed by analysis of samples collected by a research assistant [10]. Although these articles described self-collection techniques, none provided detailed, step-by-step written or video hair self-collection instructions nor was the acceptability and feasibility of hair sample self-collection addressed.

The purpose of this article is to provide descriptions of the protocol transition process from in-person hair cortisol sample collection to participant self-collection using written and video instructions as well as the acceptability and feasibility of this transition.

2. Methods

Our original data collection protocol incorporated details from past literature [11] such as the best collection location on the head for the hair sample, how much hair to collect as well as how to label and package the hair sample for storage to assure fidelity; the extent to which research assistant team members comply with the study protocol [12]. All research assistants were trained on the hair collection protocol and every tenth sample was examined for adherence to the protocol before being placed in storage. While past literature provided important details of the hair sample collection for our original protocol, we did not find a comprehensive guide to promote consistency and fidelity during the COVID-19 Pandemic transition to a self-collection protocol.

We also searched online for videos that demonstrated hair self-collection procedures. Some videos were primarily for purposes of drug screening. Sharing such videos could create confusion and become a major deterrent in the acceptability of hair sample collection. Other videos were not adopted because the hair collection site on the head or the procedure varied significantly from our protocol. Therefore, written and video instructions were developed by our study team to demonstrate appropriate hair sample self-collection technique.

Participants were mailed a hair sample collection kit at each time-point they agreed to self-collect a hair sample. The hair sample collection supplies included a 12-inch piece of foil, painter's tape, hair sample envelope, hair care survey, and a self-addressed, stamped return envelope. Participants needed scissors and a tablet/computer (for video viewing) and if desired, a hair clip and comb.

2.1. Hair sample collection protocol

The required amount of hair needed for the sample varies in the literature from 30 to 150 strands [13,14]. Our protocol included the amount of hair mentioned most consistently in the literature (50 strands) and also mentioned the size to approximate half the diameter of a pencil eraser for ease of understanding. The posterior vertex of the head is the best location to obtain the hair sample because it has less cortisol level variation (15.5% versus 30.5%) when compared to other areas [5].

Verbal hair sample self-collection instructions were given to each participant at enrollment. At each time point, comprehensive, easy to understand written instructions (Table 1) with a video instruction link

Table 1
Written hair sample collection instructions.

Table 1: Written Hair Sample Collection Instructions

Read all instructions before collecting hair

- Collect hair from target area on the back of the head (Figure 2)
- Cut sample as close as possible to the scalp
- Make sure you have all necessary supplies



Figure 2: Image of target area for taking hair sample.

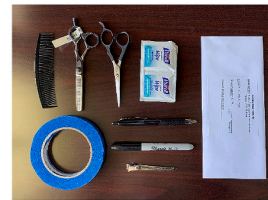


Figure 3: Image of supplies needed for taking hair sample

Supplies Needed for Hair Sample Collection (Figure 3):

IN YOUR PACKET YOU WILL FIND:

- Aluminum foil
- Business envelope
- BLUE Painters tape (holds hair in place on foil)

YOU WILL NEED TO USE YOUR OWN:

- Scissors
- Comb (for parting hair) *OPTIONAL
- Hair clips (to hold down hair as needed) *OPTIONAL

Preparing and Collecting Hair Sample

- Before collecting hair sample, clean scissors with alcohol wipe or very hot water
- Ask a family member if they can assist you, if not you can collect hair sample yourself
- Separate a thin layer of hair (about half the size of a pencil eraser) at the target area of the head (Figure 4)
- Clip down other hair so it is out of the way
- Cut single strand as close to the part/scalp as possible
- Hold the cut ends closely together
- Lay the sample flat on the aluminum foil with cut ends next to where "scalp" is written (Figure 5)
 - Place one strip of tape 1 inch from the scalp/cut end
 - If hair is long enough, place another piece of tap 1-1.5 inches down from the first piece of tape
- Fold foil to ensure hair will not fall out and to fit into envelope
- Place foil inside the business envelope along with the completed Hair Cortisol Questionnaire

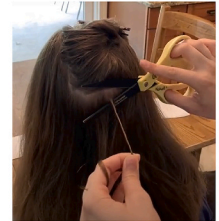


Figure 4: Image demonstrating collecting a hair sample

HOW TO PLACE THE HAIR ON THE ALUMINUM FOIL

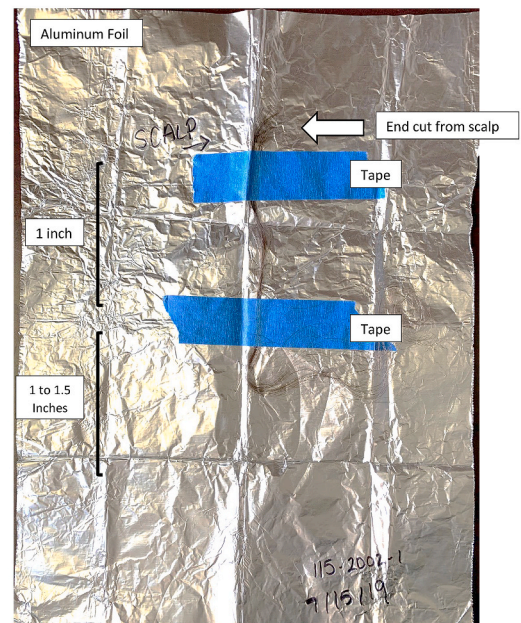


Figure 5: Image of hair sample on aluminum foil



Fig. 1. QR code for hair self-collection video link.

(Fig. 1) were sent to participants to collect a hair sample. Instructions included that after the target area on the back of the head is located (Fig. 2) and collection supplies assembled (Fig. 3), scissors are used to cut a sample of approximately 50 strands of hair as close to the scalp as possible (Fig. 4). The hair sample is then placed on a pre-labeled piece of aluminum foil labeled with the participant's identification number and location for the proximal end of the hair sample (proximal to the scalp) marked. Participants were instructed to anchor the hair sample to the



Fig. 4. Image demonstrating collecting a hair sample.



Fig. 2. Image of target area for hair sample.



Fig. 3. Supplies needed for hair sample collection.

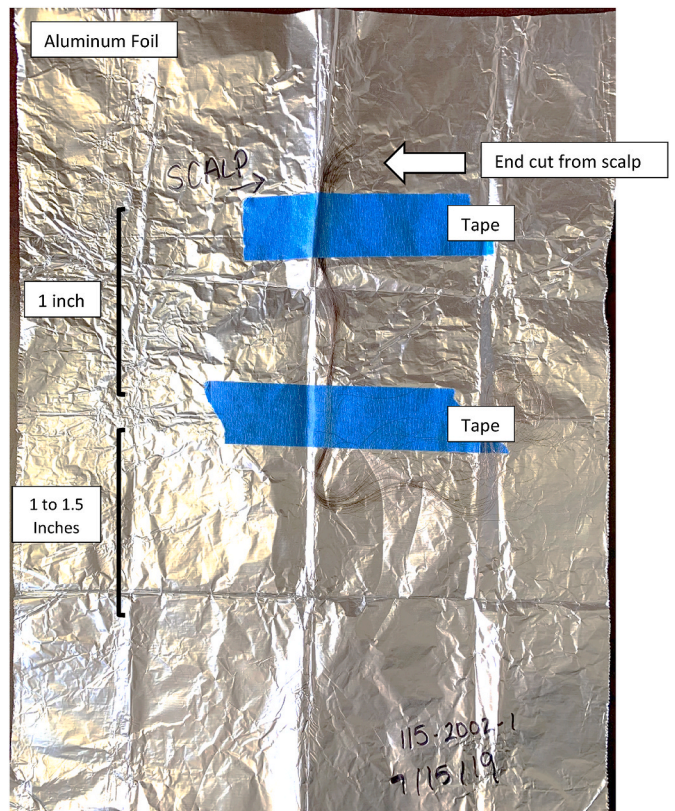


Fig. 5. Image of hair sample on aluminum foil.

foil using the enclosed piece of painter's tape 1–2" from the proximal end of the sample (Fig. 5). The foil is then folded over to enclose the sample in a sealed "pouch" and placed in a standard business envelope with information pre-marked (i.e., participant study ID number,

collection date, data collection time point if longitudinal study) along with the completed hair questionnaire. Participants were instructed to return the hair sample in the self-addressed, stamped envelope provided.

Within 3–5 days of mailing the hair sample collection kit, participants were contacted to confirm delivery of supplies. After 10 days of the kit delivery, participants were contacted and reminded to mail the sample to the study office. Additional contacts were made if the participant did not respond. At each contact, the research staff asked the participant if they had any questions regarding the hair sample collection. In addition, tracking labels were placed on mailed hair collection kits.

2.2. Sample shipment considerations

All hair samples should be stored together in a dry place at room temperature until the study conclusion then shipped together via postal service to a testing laboratory. Each laboratory has preferred delivery instructions, however use of plastic zipper storage bags with sample number labels help to maintain order and keep samples dry during shipment.

2.3. Safety and informed consent considerations

While hair sample self-collection is simple and straightforward, there are inherent risks. The informed consent process should mention the rare risk of a minor scalp laceration and potential associated infection during hair sample collection. Assistance from a mature family member should minimize such risks.

2.4. Hair cortisol laboratory values

While there are a variety of values reported, typically hair cortisol normal values range from 15 to 80 pg/mg hair; high values are between 90 and 150 pg/mg. Median values have ranged from 46.1 to 55 pg/mg [5,15]. Gonzalez et al. reported cortisol levels as high as 182–520 pg/mg hair in high stress participants [15]. Some researchers reported no differences in dyed versus non-dyed hair while others found lower hair cortisol levels in those who used hair dye, washed their hair daily or were female and higher levels in Black compared to other racial groups [3,15].

2.5. Analysis for acceptability and feasibility

Acceptability of the protocol transition was assessed by analyzing participant comments (positive or negative) regarding the hair self-collection experience and any difference in the percentage of participants who declined to provide a hair sample (in-person versus self-collection). Feasibility of the protocol transition was assessed by analyzing the number of returned self-collected hair samples.

3. Results

Hair samples were collected in person from July 2019 to March 2020. All in-person interviews and enrollment of new participants were suspended in March 2020 due to the COVID-19 Pandemic. During the suspension of in-person interviews, survey data collection via REDCap for enrolled participants continued. However, hair sample collection was suspended until a suitable protocol modification could be developed. The IRB approved virtual protocol modifications in May 2020 and enrollment and data collection resumed. Overall, there were 531 potential hair collection time points. Timepoints where participants withdrew from the study, were unable to be contacted, no longer eligible, outside of the acceptable time range, and those timepoints between suspension of in-person collection and self-collection protocol development were excluded from the total potential number.

Hair cortisol samples were collected in person via research assistants

($n = 79$; 27%) and self-collected ($n = 214$; 73%). Every tenth hair sample examined for adherence to the protocol was found to be accurate with a sufficient hair sample. Before hair sample self-collection modifications were approved, 79 (69.3%) participants agreed to in-person hair sample collection and 35 (30.7%) participants declined. Following the protocol modifications, 254 (60.9%) participants agreed to self-collection of hair samples, while 163 (39.1%) participants declined.

When participants were asked about their comfort in providing a hair sample, there were three main responses: an emphatic yes (participants were eager to help), immediate no (expressed reasons mentioned below), or hesitation. For those who expressed interest and hesitation, the research assistant explained the written instructions. Following the explanation, the research assistant again asked participants about their comfort with providing a sample. The general response was that the process sounded easier than expected, and participants agreed to provide a sample. The returned hair samples were primarily found to be accurately collected, aside from a few outliers (hair clippings from a haircut in a plastic bag). The only adjustment made was assuring that the sample collection date from the hair questionnaire was marked on the outside of the envelope.

The most common reasons for declining ($n = 203$) hair sample self-collection included mailing complications (package was returned to sender/package was lost; $n = 6$), hair constraints (no hair/thin hair/hair styles such as braids and dreadlocks/hair conditions such as alopecia; $n = 15$), personal decision to not provide hair samples (upcoming family photo/concerns regarding DNA collection/in the hospital/new baby/contracted COVID-19; $n = 148$), and the participant did not return a hair sample after agreeing to do so ($n = 34$).

4. Discussion

Transitioning from an in-person to a self-collection hair sample protocol for cortisol determination had several benefits. First, self-collection methods save time and costs compared to in-person visits. Another benefit of the self-collection method was it allowed us to continue collecting hair samples despite the COVID-19 pandemic. The pandemic posed many challenges to research, but concomitantly opened doors to new innovations such as the development of virtual methods due to public health restrictions. The final benefit was it enhanced participants' sense of comfort and privacy derived from autonomous sample collection or if needed, the assistance of a friend or family member at a place of their choosing.

4.1. Lessons learned

There were many lessons learned during the transition to the self-collection hair sample protocol. First, we found it best to explain hair sample collection to participants during the enrollment process and offer to meet with them via Zoom for support during sample collection if needed. In addition, following completion of the baseline surveys and agreement to provide a hair sample, participants were sent written and video instructions. By taking these steps, we provided multiple avenues for participants to learn the steps in the self-collection hair sample process. We also learned to recommend that participants seek a family member or friend's assistance as needed to facilitate sample collection.

Additional steps were required to facilitate data collection following the transition to a sample self-collection protocol. The lessons learned included the pragmatics of mailing supplies to participants' homes. Timing was particularly important in the self-collection process. For example, hair sample collection kits were immediately mailed to participants following completion of the surveys at each time point to facilitate hair sample collection within the two-week window. In addition, we encouraged participants to obtain the hair sample as soon as they received the collection kit in the mail. Also, we learned that follow-up communication by text or telephone call was essential for timely

collection and return of the sample to the study office. Due to factors such as rural location and/or postal delays, timing was critical in mailing and receiving self-collected hair samples within the two-week window.

Another lesson learned was regarding the acceptability of hair sample self-collection for participants who were initially hesitant. Regardless of a participant's initial response to hair sampling, research assistants explained hair collection instructions. Following the explanation, the research assistant asked participants what questions they had about the process and confirmed their agreement to provide a hair sample. Participants who were initially hesitant and asked questions such as the size of the sample, often agreed after hearing self-collection protocol instructions.

4.2. Key takeaways

There were two key takeaways from the hair sample self-collection protocol implementation process. First, successful hair sample collection required close follow up of participants by research staff. This was accomplished by making 2–5 follow up contacts via the participant's preferred contact method. The second key takeaway was that the hair sample self-collection protocol was acceptable and feasible for our study participants. There was little difference in the initial participant agreement to provide a hair sample between in-person (79/114; 69.3%) versus self-collection (254/417; 60.9%) protocol methods. Some participants were initially hesitant to provide a self-collected hair sample but commented that self-collection was easier than they anticipated which supports the acceptability of this protocol change. However, regarding feasibility, 16.8% of participant self-collected hair samples were not received by study staff because they were not returned despite reminders (14.3%) or there was an issue with mail delivery or return (2.5%). Nonetheless, overall, the new protocol was feasible.

Our findings are consistent with Enge et al. who reported that 78% of participants opted for sample self-collection versus research assistant collection [16]. However, it is unclear how many participants did not return a hair sample via the mail. Furthermore, Enge et al. found little difference in insufficient hair sample size for self-collection (5.3%) versus research assistant collection (1.8%) supporting similar adherence to the protocol [16]. Skoluda et al. reported a significant positive association in cortisol levels when comparing hair samples that were either self-collected or collected by lab technicians on the same or next day, utilizing a collection method that did not rely on having to mail back samples [17]. Additionally, they found that a combination of written and video instructions was more comprehensive than solely written instructions [17].

4.3. Future considerations

Future considerations for hair sample self-collection protocol revision are to include a paper ruler to help appropriate the size and amount of hair needed for the sample. Also, the written instructions should include hair sample size photos to improve sample size approximation. A limitation is that true validation of a cortisol level requires the comparison between the adapted hair sample self-collection protocol and the usual research assistant hair collection protocol. However, past research has validated the reliability of hair self-collection [10].

5. Conclusion

In conclusion, the transition of our hair sample collection protocol from in-person to self-collection was acceptable and feasible. Providing instructions for hair sample self-collection in a variety of formats (oral, written, video) helped to decrease uncertainty and hesitancy regarding the process and promote agreement among participants. While there was a similar initial participant agreement rate, fewer actual samples

were collected due to issues of participants not returning a sample as agreed or mail issues. In addition, consistent follow-up communication was key to timely receipt of the hair samples from participants.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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