ORIGINAL RESEARCH

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Hair Cortisol as a Biomarker of Stress in the 2011 Libyan War

Abstract

Purpose: There is a substantial body of research that utilizes saliva cortisol levels to examine wartime stress; however, there is a paucity of literature that utilizes hair cortisol levels, which allows for long-term assessment of chronic stress, to investigate the stress of war. The present study aimed to evaluate changes in hair cortisol concentrations before, during, and after the 2011 Libyan war.

Methods: This study examined hair cortisol concentrations of young adult women who were living in Tripoli, Libya during the 2011 war. The participants were recruited at the campus of Tripoli University. Participants needed to have at least 24 cm of hair and to have resided in Tripoli before, during and after the 2011 Libyan war. Hair was sectioned to reflect 3 month windows of cortisol exposure corresponding to periods before, during and after the war. Hair cortisol concentrations were quantified using a modified salivary ELISA test. The women were also asked to complete the Perceived Stress Scale pertaining to the post-war period.

Results: Median hair cortisol concentrations in the post-war period (226.11 ng/g; range 122.95-519.85 ng/g) were significantly higher than both the pre-war (180.07 ng/g; 47.13-937.85 ng/g) and wartime (186.65 ng/g; 62.97-771.79 ng/g) periods ($P < 0.05$). The mean PSS score (24) was in the range of "much higher than the mean" for this test and the vast majority of participants were either in the "much higher than the mean" or "slightly higher than the mean" ranges.

Hair cortisol determination suggests that in Tripoli, the post-war period appears to have been more stressful than the war itself. This is consistent with the fact that during the war the civilian participants were not directly involved with warfare, nor were they targeted by the international coalition fighting Gaddafi. In contrast, the post-war period was characterized by chaos and total lack of authority, with the participants exposed to injury, lack of food and destruction.

Conclusion: This study documents the utility of hair cortisol levels to retrospectively assess stress before, during, and after an armed conflict.

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War is one of the most potent sources of physiological and psychological stress, and the adverse physical and psychological traumas of war have been well documented [1]. The devastating effects of wartime stress have been measured with the use of questionnaires [2-3], physical and psychological assessments [4-5], and with the use of biomarkers [6]. The most commonly used stress biomarker is cortisol [6-7]. To date, much of the research that has utilized cortisol levels to evaluate wartime stress has focused on either acute battle-related stress and soldiers' experiences [8-9], or on the development of post-traumatic stress disorder (PTSD) in severely traumatized victims [10-13]. Less research has been conducted on the use of cortisol levels to assess the stress of war in people who were non-combatants and those who were not directly victimized. Moreover, the existing literature utilizes almost exclusively saliva and plasma to assay cortisol levels, thus providing useful acute stress information, but not necessarily depicting the nature of the chronic stress leading up to, during and following war.

Hair cortisol analysis is now at the forefront of chronic stress research [6, 14-15]. Hair grows at an average rate of 1 cm/month [16], incorporating cortisol into the hair shaft as it grows; thus, hair segments are capable of providing months or even years of information on cortisol secretion. Hair's ability to retrospectively examine chronic stress makes it ideally suited to use in the context of war, when it would otherwise be unethical or impractical to measure cortisol. Presently, only one study has measured hair cortisol concentrations (HCC) in a wartime setting [17]. The study examined survivors of the Ugandan civil war, and found that those with PTSD had significantly elevated HCC when compared with the control group.

On February 17, 2011, a civil conflict erupted in Libya. There were anti-government protests, and a rebel coalition was formed with the objective of overthrowing the Gaddafi regime. NATO forces intervened under the United Nations Security Council resolution 1973 adopted on March 17, 2011. Tripoli, the capital city of Libya, and a stronghold of the Gaddafi regime, fell in August 20, 2011, marking the end of the civil war and of the Gaddafi regime. Prior to this period, Tripoli had a long period of civil stability and had been relatively insulated from military conflict.

The present study aimed to evaluate changes in HCC as a biomarker of population stress among noncombatants during the 2011 Libyan war.

Methods

Ethical considerations and participants

The study was approved by The Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) and by the Libyan National Committee for Bioethics and Biosafety (LNCBB).

Recruitment took place in September 2012, one year after the end of the civil war, at the campus of Tripoli University. Interested participants were informed of the purpose of the study and written consent was obtained. To be eligible, participants were required to have had a minimum of 24 cm of hair, and to have resided in Tripoli before, during and after the 2011 war. Exclusion criteria included Cushing's Syndrome, the use of parenteral glucocorticoids or topical glucocorticoids within the last 24 months, pregnancy in the last 24 months, chronic systemic disorders (e.g., renal failure) that may affect the clearance of cortisol, and unwillingness or inability to provide a 24 cm hair sample. Given the substantial length of hair that was needed, this study was only able to recruit women for the study, as most Libyan men maintain short hair.

Data and specimen collection

Age, weight and height, alcohol consumption, smoking status, marital status and occupation data were collected from each participant. The women were also asked to complete the Perceived Stress Scale (PSS), a 10 item questionnaire that assesses perceived stress over the previous month. This tool had been validated in Arabic [18]. A single hair sample was obtained from the vertex posterior of the head from each participant. Participants were not sweating at the time of collection, and gloves were worn while obtaining the sample. Hair samples were stored at room temperature until analysis. All women reported regular hair washing procedures.

Hair Analysis

Three, 3 cm segments, were obtained from each hair sample collected, with a 1 cm/month average growth rate assumed. The pre-war, war and post-war periods were respectively defined as November 2010–January 2011, June–August 2011 and June–August 2012 (Figure 1). Hair cortisol analysis was performed using a salivary cortisol immunoassay (Alpco Diagnostics, Salem, NH, USA) widely used by our laboratory [19]. Cortisol concentrations were adjusted to mass and expressed as nanograms of cortisol per gram of hair. The intra-assay and inter-day coefficients of variation were determined to be 5.05% and 8.05%, respectively. The results of this ELISA hair analysis

FIGURE 1. Schematic of hair segments analyzed, and their temporal relationship to the Libyan civil war.

FIGURE 2. Median hair cortisol concentrations in the post-war period are significantly higher than both the pre-war and war periods (* $P < 0.05$).

correlated strongly with those of the LC-MS-MS method for hair cortisol (14a) ($r^2 = 0.89$) [20-21].

Data Analysis

The distribution of the pre-war, war, and post-war hair cortisol concentrations were assessed using the Shapiro Wilk normality test. The data were not normally distributed, and thus a Friedman test was used with a *post hoc* Dunn's Multiple Comparison test. Correlations between continuous variables were tested using Spearman's rank order correlations.

Results

Thirty-nine women agreed to participate in the study and met the inclusion criteria. All participants were non-smokers, non-alcohol drinking Libyan women. The median age of the participants was 23 years (range 19-42 years). The mean body mass index (BMI) was 23.1 ± 4.8 kg/m². Thirty eight participants (97%) were single and 82.1% were university students, the rest being university staff. None experienced either direct physical body harm to themselves or to their families, and none of their homes were damaged by warfare.

Median hair cortisol concentrations in the post-war period (226.11 ng/g; 122.95-519.85 ng/g) were statistically higher than pre-war (180.07 ng/g; 47.13-937.85 ng/g) and wartime (186.65 ng/g; 62.97-771.79 ng/g) periods ($P < 0.05$) (Figure 2).

The mean post-war PSS scores were 24 ± 6.2 , and ranged from 11 to 33. According to the PSS test definitions [18], the mean score (23.8) was in the range of "much higher than the mean" (21 and up). In fact 26 participants were in the "much higher than the mean range" (21 and up), seven were in the range of "slightly higher than the mean" (16-20), only two were in the range of the mean (12-15) and two were in the range of "slightly lower than the mean" (8-11). There was no correlation between post-war hair cortisol levels and PSS scores. Similarly there were no correlations between post-war hair cortisol concentrations with either age or BMI.

Discussion

To our knowledge, this is the first study to utilize hair cortisol analysis to assess chronic stress of war among non-combatants.

This study population included healthy, young non-smoking, non-alcohol drinking women, who lived in Tripoli during the Libyan war in 2011 and not directly affected by the hostility and trauma. Perhaps surprisingly, our data suggest that stress was higher in the post-war period than either before or during the war. A closer look at the events may, in fact, explain these findings. Before the uprising, Tripoli was a safe city, as the dictator and the core of the government resided there. When the uprising first started in the eastern part of the country, Tripoli remained relatively calm as any unrest in Tripoli itself was brutally crushed; however, citizens experienced increasing isolation with lack of internet connection, traveling outside of the city becoming dangerous, and there were shortages in gasoline and electricity [22]. When NATO forces started bombing military centers of the regime in Tripoli, the citizens were assured that NATO would not target civilian centers and, indeed, this was the case. In fact, the sounds of bombing were greeted by many citizens with joy, as they signified damage to the Gaddafi regime. After the regime fell in October 2011, there was a deep sense of chaos, with no police presence or public services, and with abundant weapons in most homes and on the streets. Most of our volunteers were female students facing postwar safety issues and unclear future (such as academic uncertainty at their university). These concerns undoubtedly induced chronic stress, possibly at levels higher than the pre-war and war experiences, resulting in statistically higher hair cortisol concentrations. Our results corroborate those of the only similar study to date where Steudte and colleagues showed increased hair cortisol in post-war Ugandan patients with PTSD [17].

The lack of correlation between hair cortisol and psychological questionnaires such as the PSS is not surprising. PSS is valid in reflecting stress only up to one month prior to the measurements [23], while in our case the PSS was completed up to a year after the time reflected in the most recent post war hair samples. It is highly likely that Libyan citizens experiencing bodily harm to them or to close family and friends, and those whose homes and communities were destroyed, had higher levels of hair cortisol. The homogeneity of our cohort, having experienced the conflict and the postwar hardship as non-combatants, has allowed us to focus on the anxiety and stress experienced by the general population and the effectiveness of this biomarker.

Several limitations of this study need to be acknowledged. Firstly, no men were included. The study design included only one time to sample hair, and this design precluded the recruitment of men as no men were encountered with 24 cm of hair. Future studies using hair analysis to investigate long-term

stresses over the course of war will necessitate multiple collections to resolve this issue. Additionally, a control group was not included in this study; therefore we are unable to conclude if the hair cortisol concentrations in a population undergoing a civil conflict were higher or lower than in a control-matched population with a stable government or how our results may differ from those where government was both stable and non-repressive. Lastly, it could be argued that the higher hair cortisol concentration in the post-war period may have been due to this segment's close proximity to the scalp. Hair that is closest to the scalp has had less time exposure to the environment, and Kirschbaum *et al.* have argued that hair cortisol is leached from the hair as the duration of environmental exposure increases [24]. Based on this reasoning, one would expect the highest concentrations of cortisol in the segments most proximal to the scalp, and the lowest in the segments most distal to the scalp. The vast majority of human and animal studies have found that the hair of healthy controls does not show an overall decline in hair cortisol concentrations while moving further away from the scalp. In our lab, Thomson *et al.*, recruiting nine healthy control subjects with hair lengths ranging from 10-14 cm, could not confirm the results of Kirschbaum's study [25]. Hair samples were segmented into 1 cm sections showing no significant differences in cortisol concentration along the length of the shaft (mean for all sections 147 ± 46 ng/g). Similarly, Manenschijn *et al.* in the Netherlands could not show time-dependent changes [26]: a group of 28 women provided hair samples at least 18 cm in length and these were then segmented into six 3 cm sections. No significant differences in hair cortisol concentration were observed in consecutive segments ($P=0.249$). Adding strong evidence that cortisol does not naturally vary in distribution along the length of the hair shaft, studies using both rhesus macaques [27] and dogs [28] found no significant differences between the most proximal half and the most distal half of the hair shaft.

The question of stability of cortisol over longer durations of time has also been effectively addressed by a study performed by Webb and colleagues in our lab, who obtained hair samples ranging in length from 6 to 21 cm from 10 ancient Peruvian mummies, dating from AD550-1532 [29]. Segmental analysis was performed on the hair samples provided, and while variation was observed from segment to segment, proximal and distal segments were not significantly different. The mean cortisol concentration determined was 281 ± 35 ng/g, similar to those measured in the same laboratory in healthy volunteers in 2010, demonstrating that cortisol levels are not markedly depleted, even in hair that is 500-1500 years old.

It is very unlikely that hair cortisol, which was stable in the women who participated in our study over the pre-war and war period, abruptly changed post-war because of hair demise, as the only study suggesting that changes occur over time, documented a change every month [6].

In summary, this study documents the utility of changes in hair cortisol over time. We suggest that hair cortisol levels might prove useful as a biomarker of chronic stress during and after an armed conflict although more studies are needed in order to validate our results from Libya and the similar results from Uganda [17].

References

1. Levy BS, Sidel VW. Adverse health consequences of the Iraq War. *Lancet* 2013; 381, 949-958.
2. Stammel N, Neuner F, Bottche M, Knaevelsrud C. Construction of a questionnaire for readiness to reconcile in victims of human rights violations. *Eur J Psychotraumatol*. 2012; doi: 10.3402/ejpt.v3i0.15785
3. Matanov A, Giacco D, Bogic M, Ajdukovic D, Franciskovic T, Galeazzi GM, Kucukalic A, Lecic-Tosevski D, Morina N, Popovski M, Schützwohl M, Priebe S. 2013. Subjective quality of life in war-affected populations. *BMC Public Health* 2013;13, 624.
4. Betancourt TS, Newnham EA, McBain R, Brennan RT, 2013. Post-traumatic stress symptoms among former child soldiers in Sierra Leone: follow-up study. *Br J Psychiatry* 2013; 203; 196-202.
5. Hinton DE, Hinton AL, Eng KT, Choung S. PTSD and key somatic complaints and cultural syndromes among rural Cambodians: the results of a needs assessment survey. *Med Anthropol Q*. 2012; 26, 383-407.
6. Russell E, Kore NG, Rieder M, Van Uum S. Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology*. 2012; 37, 589-601.
7. Inder WJ, Dimeski G, Russell A. 2012. Measurement of salivary cortisol in 2012 – laboratory techniques and clinical implications. *Clin Endocrinol*. 2012; 77, 645-651.
8. Taylor MK, Sausen KP, Mujica-Parodi LR, Potterat EG, Yanagi MA, Kim H. . Neurophysiologic methods to measure stress during survival, evasion, resistance, and escape training. *Aviat Space Environ Med*. 2007; 78, B224-B230.
9. Li X, Huang WX, Lu JM, Yang G, Ma FL, Lan YT, Meng JH, Dou JT. Experimental study on neuroendocrinological and immunological characteristics of the military-trained artillerymen. *Chin Med J* 2012;125, 1292-1296.
10. Gola H, Engler H, Maggie S, Adenauer H, Riether C, Kolassa S, Elbert T, Kolassa IT. Victims of rape show increased cortisol responses to trauma reminders: A study in individuals with war- and torture-related PTSD. *Psychoneuroendocrinology* 2012; 37, 213-220.
11. Mihaljevic S, Vuksan-Cusa B, Marcinko D, Koic E, Kusevic Z, Jakovljevic M. 2011. Spiritual well-being, cortisol, and suicidality in Croatian war veterans suffering from PTSD. *J Relig Health*. 2011; 50, 464-473.
12. Yehuda R, Golier JA, Bierer LM, Mikhno A, Prathett LC, Burton CL, Makotkine I, Devanand DP, Pradhaban G, Harvey PD, Mann JJ. Hydrocortisone responsiveness in Gulf War veterans with PTSD: effects on ACTH, declarative memory hippocampal [(18)F]FDG uptake on PET. *Psychiatry Res*. 2010; 184, 117-127.
13. Masoudzadeh A, Modanloo KM, Ajami A, Azizi A. 2012. Evaluation of cortisol level and cell-mediated immunity response changes in individuals with post-traumatic stress disorder as a consequence of war. *Med Glas*. 2012;9, 218-222.
14. Meyer JS, Novak MA. Minireview: Hair cortisol: a novel biomarker of hypothalamic-pituitary-adrenocortical activity. *Endocrinology*. 2012; 153, 4120-4127.
15. Stalder T, Kirschbaum C. 2012. Analysis of cortisol in hair—state of the art and future directions. *Brain Behav Immun*. 2012;26, 1019-1029.
16. Wennig R. 2000. Potential problems with the interpretation of hair analysis results. *Forensic Sci Int*. 2000;107, 5-12.
17. Steudte S, Kolassa IT, Stalder T, Pfeiffer A, Kirschbaum C, Elbert T. 2011. Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD. *Psychoneuroendocrinology* 2011;36, 1193-1200.
18. Almadi T, Cathers I, Hamdan Mansour AM, Chow CM. An Arabic version of the perceived stress scale: translation and validation study. *Int J Nurs Stud*. 2012; 49, 84-89.
19. Sauvé, B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. 2007. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Invest Med*. 2007;30: E183-E191.
20. Russell E, Kirschbaum C, Laudenslager ML, Stalder T, de Rijke Y, van Rossum EFC, Van Uum S, Koren G. Toward Standardization of Hair Cortisol Measurement; Results of the First International Inter-laboratory Round Robin. 2014; *Ther Drug Monit*. 2017, November 10, PMID 235387254.
21. Gao W, Stalder T, Foley P, Rauh M, Deng H, Kirschbaum C. Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2013 ;928:1-8.
22. 2013 UNHCR country operations profile – Libya. In *UN Refugee Agency*. Retrieved August 2, 2013, from <http://www.unhcr.org/pages/49e485f36.html>.
23. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. *Ann NY Acad Sci*. 1998; 840, 33-44.
24. Kirschbaum C, Tietze A, Skoluda N, Dettenborn L. . Hair as a retrospective calendar of cortisol production-Increased cortisol incorporation into hair in the third trimester of pregnancy. *Psychoneuroendocrinology* 2009;34: 32-37.

25. Thomson S, Koren G, Fraser LA, Rieder M, Friedman TC, Van Uum SH. Hair analysis provides a historical record of cortisol levels in Cushing syndrome. *Clin Endocrinol Diabetes*. 2010; 118, 133-138.
26. Manenschijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. *Steroids* 2011;76: 1032-1036.
27. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol*. 2006;147: 255-261.
28. Bennett A, Hayssen V. Measuring cortisol in hair and saliva from dogs: coat color and pigment differences. *Domest Anim Endocrinol*. 2010; 39: 171-180.
29. Webb E., Thomson S, Nelson A, White C, Koren G, Rieder M, Van Uum S. Assessing individual systemic stress through cortisol analysis of archaeological hair. *J Archeol Sci* 2011; 37: 807-812.