

Determining the effectiveness of stress management program by using hair cortisol concentration

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ABSTRACT

Many studies have shown that healthcare workers are exposed to higher levels of stress than other occupations. However, little research has been conducted on the use of biomarker tools to assess chronic stress and assess the effectiveness of stress management programs for healthcare workers. The aim of this study was to measure the effectiveness of a Stress Management Program by measuring Depression Anxiety Stress Scale (DASS-21) scores and hair cortisol concentration (HCC) levels of healthcare workers in public healthcare facilities. This study was a three-group, quasi-experiment study with pre- and post-study assessment sessions. A total of 119 healthcare workers (28 from Group A, 21 from Group B, and 70 from Group C) were followed for 6 months. Significant favorable intervention effects on DASS-21 scores were found in Group A (Effect size =0.6) as compared to Group B (Effect size=0.2) and Group C (Effect size =0.2) at the end of the program. Time and group interaction effects were examined using the repeated measure ANOVA test in which there was a significant group *time interaction and effect size of 0.2 (p-value <0.01) across all the groups with a reduction in hair cortisol concentrations following the program. The study showed that stress management under healthy communities, building the nation (*KOSPEN PLUS*) program was successful in reducing stress levels, as seen through a decrease in both stress scores and hair cortisol levels. This is the first study in Malaysia to use hair cortisol as a biomarker for stress management, suggesting its reliability.

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1. INTRODUCTION

Chronic stress is a factor that contributes to the development and progression of a wide range of illnesses, as indicated by the findings of several studies [1]–[5]. Chronic stress has been linked to a few adverse health effects, some of which include cardiovascular, metabolic, and behavioural illnesses, in addition to abnormalities in the immune system [6]. These potentially harmful effects of stress are partially mediated by the hormone cortisol, which is generated as the last hormone by the hypothalamic-pituitary-adrenocortical (HPA) axis. Cortisol has a role in the regulation of a wide variety of physiological processes throughout the body. Therefore, fluctuations in cortisol secretion may have the potential to influence metabolism and body composition, which can lead to a variety of health difficulties [7], [8]. Up until quite

recently, most cortisol measurements were taken from either saliva or serum. However, because cortisol levels change throughout the day, using these biomarkers requires a very specific collection schedule [4]. This is because cortisol levels follow a rhythm. In addition, the participants' willingness to cooperate reduced the amount of saliva that was used as a matrix [5]. Short-term stress indication, invasive and costly extraction processes, and storage instability are some of the additional issues that are linked with the process of extracting cortisol from saliva, urine, and serum [9]. Cortisol levels may briefly be measured in the body using several different bodily fluids, including serum, saliva, and urine. The amount of time provided by saliva and serum is only a few minutes, and the amount provided by urine is only 12 to 24 hours. The use of several extraction methods is particularly intrusive for serum that is supplied intravenously. The extraction of serum has the potential to introduce bias into statistical analysis since it is a stressful process that raises cortisol levels. Individuals are required to give their informed permission before having their urine collected over a period of 24 hours [10]. In addition, the storage requirements for each of these three methods are somewhat onerous. Urine must be analysed within 12 to 24 hours, whereas saliva and serum need to be frozen to keep their viability [11].

In addition, the long-term effects of these single observations on cortisol concentrations are not reflected in the acute alterations in cortisol release that have been seen. In the past, the only techniques that were used to identify stress markers in the body were those that included serum, saliva, and urine [9]. Current research on individual cortisol levels is being conducted with the intention of developing a method that is more accurate, such as measuring cortisol levels in hair [12]. However, the procedures and technologies that are utilised in hair cortisol extraction are fundamental, do not involve a great deal of complexity, and may be simply implemented into pre-existing biomonitoring systems [10]. The alterations that were anticipated are reflected in hair cortisol, which also provides several benefits and avoids a number of extraction methods that are not acceptable. A researcher can assess the levels of cortisol in an individual for an extended period by extracting just one hair follicle from the subject's scalp. The length of one month is equal to the amount of hair that has grown in centimetres. Cutting the hair into sections one centimetre wide, commencing from the tip of the hair rather than the root, is a visual representation of the stress that an individual goes through monthly [11], [13].

It is possible to test hair cortisol concentration (HCC) in an outpatient setting, and patient compliance with sampling instructions is not needed. This is a big advantage of the method, and it is also one of its other advantages. In addition, the ability to build retrospective timelines enables researchers to study patterns of cortisol exposure spanning months to years in the past using only a single hair sample [5], [9]. This is made possible by the ability to generate retrospective timelines. As a result, the method that Raul and colleagues developed in 2004 to measure HCC has the potential to be utilised as a biomarker for stress-related diseases in the context of psychobiological research [14]. This approach may be used to retroactively assess a person's cortisol levels [5], given that an average hair growth rate of 1 centimetre per month is observed. In the beginning, HCC was evaluated to see if it might function as a reliable and precise indication of long-term cortisol levels in clinical settings [9]. Subsequently, the method was applied to stress-related ailments as well as mental issues, although the results were inconsistent [5].

2. METHOD

2.1. Participants

In this study, 300 healthcare workers were screened in a tertiary government hospital, government primary health clinic and a control group, which were grouped into group A, group B, and group C respectively. It was a quasi-experimental, 3-group, pre-post study design used to evaluate the effectiveness of the mental health component under the non-communicable disease (NCD) KOSPEN Plus Program in the Ministry of Health (MOH) Malaysia. All permanently employed healthcare workers were included in the study. Subjects were excluded if they refused to sign the informed consent form or if they presented serious underlying medical or psychological conditions that could interfere with the results of the study (such as Cushing's syndrome, depression, or generalized anxiety disorder) or pregnant mothers. Healthcare workers who had just returned from a long vacation/holiday (>1 months) was also excluded, as the beneficial effects of vacation faded out within one month [15]. Further exclusion criteria were individuals with dyed hair and/or hair length shorter than 3 cm.

Power and sample size were calculated using the PS Software. The total sample size calculated for this pilot trial is 91, based on power of 80%, significance level (α) of 0.05, anticipated difference (d) of 1, standard deviation (s) of 2.4 based on a previous similar study [16] in each group and 1 sample size ratio (m) between the 3 groups. The attrition rate is anticipated at 10%, thus the estimated total sample size is 102. The estimated sample size in each arm of this 3-armed trial is 34. Confidence interval is set at 95% and statistical significance declared at two-tailed p -value <0.05. The participants did not receive any kind of compensation for participating in the study and all of them gave written prior informed consent. This was a pilot study to assess chronic stress with an objective assessment by using HCC as a biomarker among Malaysian healthcare workers.

The present study was officially registered with the National Medical Research Register (NMRR-17-1163-36409) and received approval from the Medical Register and Ethics Committee (MREC), Ministry of Health, Malaysia (KKM.NIH.SEC.P17-1511(17) on December 20, 2017. Helsinki Declaration has been followed for involving human subjects in this study. Prior written informed consent had been taken from the participants who had participated in this study.

2.2. Program

This study serves to meet the stakeholder's interest in the *Komuniti Sihat, Perkasa Negara* or Healthy Community, Empowers the Nation (KOSPEN PLUS) Program, supported by the Occupational Health and Environmental Health Section, Disease Control Division, MOH Malaysia. The KOSPEN Plus Program is an extension of the community based KOSPEN Program to address worker's health in MOH as well as other governmental and non-governmental agencies. The initial KOSPEN program was initiated in July 2013 to address the increasing burden of NCD in Malaysia. This initiative was put forward to transform the public health services to promote the health of Malaysians by enhancing and increasing community participation in public health initiatives, which will be accomplished via the combination of mechanisms already in place within the government, particularly at the grassroots level [17]. Implementation of this initiative was by taking a proactive approach to the formation of operational units around the country, each of which will be staffed by community members who have volunteered their time and energy in the sake of improving their health. The KOSPEN PLUS program is an intervention program for NCD that aims to reduce the amount of behavioural and biological NCD risk factors among workers-employees and employers. It also focuses on modifying aspects of the social and physical environments to promote and encourage the development of modified patterns of behaviour [18].

This study serves to complement the mental health component of the KOSPEN PLUS Program, in accordance with the occupational safety and health (OSHA) Act 1994 (Section 15 & 24). Ultimately when a worker's health is improved, quality of life improves, which reduces disability and healthcare utilization and finally enhances productivity at the workplace. By utilizing the hair cortisol levels as biomarkers of chronic stress in the Mental Health component of KOSPEN PLUS Program, the pre- and post-intervention results serve to support the effectiveness of the mental health interventions in reducing stress and promoting a healthy well-being among healthcare workers [19].

2.3. Measurements

2.3.1. Research assessment

Evaluation of the self-administered Depression Anxiety Stress Scales 21 (DASS-21) which is a shorter version of Lovibond and Lovibond's 42-item self-report that measures depressive, anxious, and stressful states. The questionnaire consists of three scales: the DASS-Depression Scale (alpha coefficient of 0.81), the DASS-Anxiety Scale (alpha coefficient of 0.85), and the DASS-Stress Scale (alpha coefficient of 0.85) [20]. The Malay translation of DASS-21 [21] was shown to have excellent concurrent reliability and criterion-related validity. The reliability of the questionnaire was conducted in a similar population in Malaysia in a previous study [21]. The Cronbach's alpha value for the Malay version of the DASS-21 stress subscale was 0.79 [22]. For this particular study topic, the Malay translation of the DASS-21 was utilised. The stress subscale of the DASS-21 [22] assessed the difficulty in relaxing, nervous arousal, and being easily disturbed or agitated, as well as being overreactive or irritable and impatient. Respondents were instructed to use a four-point Likert scale (0: did not apply to me at all, 1: applied to me to some degree, or some of the time, 2: applied to me to a substantial degree, or a good portion of the time, and 3: applied to me very much, or the majority of the time) to assess the degree to which they have experienced over the past 12 months. The overall score on the DASS-21 stress subscale was calculated by summing all the individual values and then multiplying by two anxieties. The more the overall score ratings, the greater the stress experienced.

2.3.2. Biochemical determinants

The levels of cortisol in the participants' hair were evaluated both before (in the pre sample) and after (in the post sample) participation in the program. Hair was clipped closer to the scalp and samples of hair were taken from the posterior vertex, which has been found to have the lowest coefficient of variation [23]. The hair samples of the participants were collected at the commencement of the inaugural session of the programme. Following a duration of six months, the participants' hair samples were collected subsequent to their completion of the programme during the last session. Consequently, there was a six-month interval between the pre-program sample and the post-program sampling. The rate of hair growth, which is around one centimetre per month, allows for the assessment of an individual's cortisol exposure during the preceding six months by analysing the cortisol levels in the first six centimetres of hair nearest to the scalp.

2.3.3. Extraction of cortisol from hair samples

After the samples had been collected, a root segment that was close to the cutting was measured to be between 3 and 6 centimetres in length. After that, the weight of each sample was determined, and in order to conduct an accurate extraction, a minimum of 20 mg of hair was required. The extraction of cortisol was accomplished by first shaking the sample, followed by incubating it for a full 24 hours. The extraction solvent used was methanol. After removing an aliquot of the extract and allowing the solvent to evaporate, the sample was reconstituted and put through Enzyme-linked immunosorbent assay (ELISA) test process. Steps involved from hair collection to analysis by ELISA are as follows [24]: i) Hair sample was collected by using scissors; ii) Hair sample was wrapped in aluminium foils for protection and storage; iii) Hair was washed using 5 ml isopropanol and dried for 12 hours; iv) Hair was grinded and minced finely using surgical scissors for 1-2 mm length, in which the minced hair (minimum 20 mg) was later weighed in glass tubes; iv) Minced hair was poured into glass tubes, and then 1.5 ml of methanol of High-Performance Liquid Chromatography, (HPLC) grade was added in 50 °C temperature for 16 hours with slow rotation 100-200 rpm; vi) The solution was then placed in a 2 ml micro-centrifuge tube and centrifuged for about 120 seconds at approximately 1,000 rpm; vii) Supernatant was removed into new micro-centrifuge and dried at room temperature; viii) The tube that still had some white residue in it was capped and placed in a freezer at a temperature of -80 °C.

Before doing the analysis, the white residue was reconstituted with 250 ul of phosphate buffered saline with a pH8 buffer and swirled until the phosphate and cortisol were both thoroughly dissolved, and cortisol levels was determined using the modified salivary cortisol kit (*Salimetrics*) in accordance to manufacturer's direction. ELISA immunoassay salivary cortisol kit (*Salimetrics*) detects cortisol levels in the range of 0.003-3.0 ng/dl (0.083-82.77 nmo1/L) [25].

2.3.4. Statistical methods

The analysis was carried out using the intention-to-treat (ITT) methodology, which involved using the last observation carried forward method. For descriptive analysis, the frequency distribution, measure of central tendencies, and measure of distribution were analysed. The continuously dispersed data that follow a normal distribution are shown here in the form of mean values together with the corresponding standard deviations analysis was conducted based on intention-to-treat (ITT) whereby the last observation carried forward method was done. For descriptive analysis, the frequency distribution, measure of central tendencies and measure of distribution produced. The normally distributed continuous data presented in the form of mean values with the corresponding standard deviations. A CONSORT checklist was used to improve the quality of the research [25] as in Figure 1.

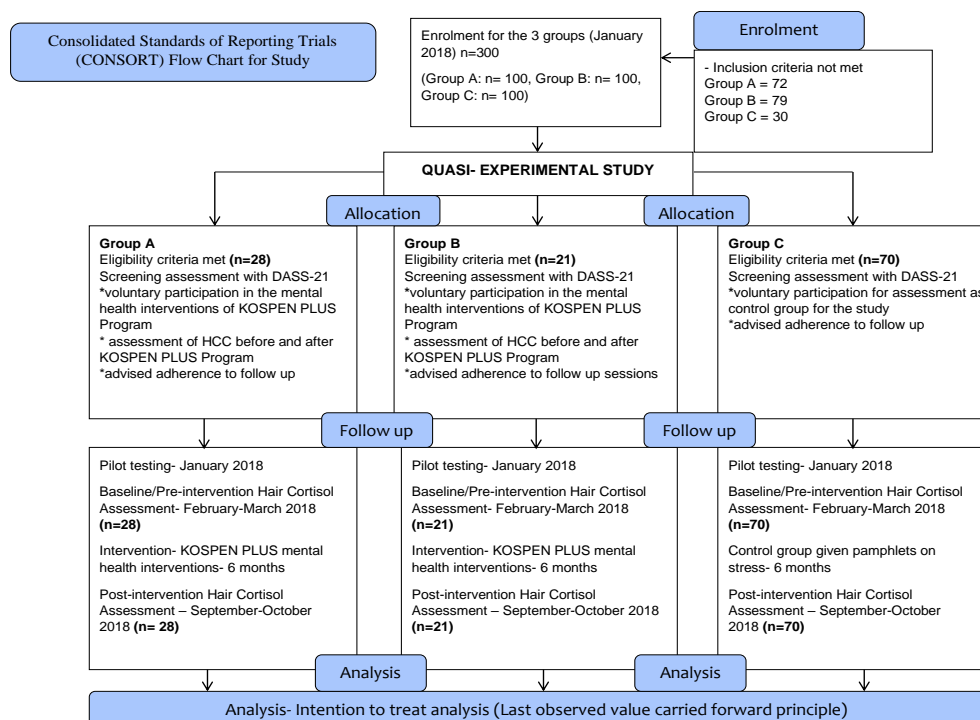


Figure 1. CONSORT diagram of the study

To normalise the data, we first performed normality tests (kurtosis and skewness) and then, based on the results of the transformations, the distribution of the variables was evaluated. A t-test with an independent sample was used to assess the baseline characteristics of the respondents in the three different groups. As a standardised way to measure the degree of change, the effect size, often known as Cohen's d, was computed [26]. The paired sample t-test was utilised to investigate how effectively the research treatments were implemented in each of the groups. In the analysis, this approach was utilised to compare the three groups' mean responses throughout the course of time against one another. It was determined to be statistically significant if the p-value was less than 0.05. For data analysis, the Statistical Package for the Social Sciences (SPSS: version 23.0) was utilised.

3. RESULTS AND DISCUSSION

A total of 28 participants in group A, 21 participants in group B, and 70 people in group C participated in the study and satisfied the inclusion criteria. To analyse the details of the intervention's effect, an intention-to-treat analysis was utilized. Table 1 displays the demographic and socioeconomic features. At baseline, all three groups were homogeneous ($p > 0.05$). There were 119 participants, of which 104 (87.4%) were female and 15 (12.6%) were male. All individuals who attended both sessions were measured for cortisol levels in their hair. One of the limitations of this study might be the small sample size and the fact that the majority of participants were female, given that the relationships between psychosocial stress and endocrine activity may differ between genders [27], [28].

Table 1. Demographic characteristics of the study participants

Characteristics		n (%)
Age (years)	20-29	39 (32.8)
	30-39	67 (56.3)
	40-49	10 (8.4)
	>50	3 (2.5)
Gender	Male	104 (87.4)
	Female	15 (12.6)
Race	Malay	110 (92.4)
	Chinese	3 (2.5)
	Indian	1 (0.8)
	Others	5 (4.2)
Occupation	Doctor	5 (4.2)
	Medical assistant	14 (11.8)
	Medical management	8 (6.7)
	Nurse	77 (64.7)
	Attendant	15 (12.6)

3.1. Effectiveness of NCD KOSPEN Plus program for mental health

Tables 2 and 3 display the participants' pre- and post-NCD KOSPEN Programme DASS-21 scores, HCC levels, and corresponding effect sizes. Hair cortisol from the last session was considerably lower in those participants who completed the programme than hair cortisol from the first session in all groups. This was statistically significant with moderate effect size in the DASS-21 scores and small effect size in the HCC levels among the respective groups.

Table 2. Pre and post KOSPEN Plus program DASS-21 scores among the 3 groups

Group	Pre-program total score [Mean (SD)]	Post program total score [Mean (SD)]	p-value	Effect size
Group A	46.2 (0.17)	34.9 (0.15)	<0.05 (0.009)	0.6
Group B	42.0 (17.8)	38.0 (15.3)	<0.01	0.5
Group C	11.5 (7.6)	9.2 (6.1)	<0.01	0.5

Table 3. Pre and post KOSPEN Plus program HCC scores among the 3 groups

Group	Pre-program total score [Mean (SD)]	Post program total score [Mean (SD)]	p-value	Effect size
Group A	0.29 (19.4)	0.26 (18.1)	<0.01	0.2
Group B	0.22 (0.12)	0.21 (0.14)	<0.01	0.2
Group C	0.05 (0.15)	0.04 (0.13)	<0.01	0.2

4. DISSCUSSION

The purpose of this study was to examine the effectiveness of the NCD KOSPEN Plus program for stress management among government healthcare personnel, as well as the use of HCC as an objective biomarker of stress. Depression, anxiety, and stress, as well as cortisol levels in hair samples, were among the factors to be adjusted. A comparison was made between three study groups, a group of healthcare workers from a tertiary hospital who participated in the stress management program, a group of healthcare workers from a primary healthcare clinic, and a control group with normal stress levels, who were evaluated both before and after the program. The findings of the study indicated that the NCD KOSPEN PLUS program might be successful, as both the DASS-21 and hair cortisol levels decreased. The treatment was effective in reducing chronic stress over time with a moderate to small effect size.

As a result of reduced activation of the HPA axis, there was a decrease in HCC levels following intervention, as measured by the biomarker for chronic stress. This decrease in cortisol levels has beneficial health effects. Some writers have acknowledged the necessity and use of evaluating cortisol levels in hair to determine the success of treatments and discovering lower levels following the intervention [27], [28]. Despite being a pilot study and first of its kind to examine the effectiveness of a stress management program using a validated biomarker to assess chronic stress among Malaysian healthcare workers, the results are markedly significant as they demonstrate the effectiveness of the mental health component of the NCD KOSPEN PLUS program. The healthy healthcare staff were also successful in decreasing DASS-21 score levels at the respective research locations due to the awareness established by this program.

Hair cortisol level is a potential indicator for assessing chronic stress exposure, particularly in larger studies among younger populations where response burden to in-depth interviews of psychological stress can be difficult. Hair cortisol has the potential to be a valuable tool in research among diverse and urban populations due to its cost-effectiveness, ease of acquisition and transportation, non-invasiveness compared to serum sampling, lack of reactivity or bias from the assessment method, and feasibility in large cohort studies, making it a preferable alternative to extensive standardised psychological surveys or interviews. This is potentially useful in studies of more vulnerable populations (e.g., low socioeconomic status) where chronic stress is postulated to disproportionately contribute towards the development of chronic diseases [29], [30]. Thus, hair cortisol may serve as an important indicator to consider in research examining resiliency factors that are postulated to buffer the impact of stress on various health outcomes [31].

There are several possible causes for the lack of substantial associations between hair cortisol and self-reported subjective stress or stressful life events. First, our sample size was small, lowering our statistical power, and there may have been unidentified variables relating to participants' distinctive traits (e.g., pubertal status). However, the small to moderate relationships (r 's-0.1-2.1) are consistent with previous research [13] and show a low to moderate overlap between self-reported stress indicators and hair cortisol.

Secondly, even though our stress measures were well-validated and temporally coincided with the collected samples, they may not have effectively indicated chronic stress that is ongoing. For instance, life events from the previous three months and stress for the previous thirty days may have recorded events that momentarily altered cortisol levels, but not long enough to affect levels observed in hair. It may be possible to identify whether chronic stressor exposure has a higher long-term effect on HPA activity by a comprehensive evaluation of life events and work-related variables. Prior experience with persistent unemployment [32] bolsters this likelihood.

The findings indicate that perceived stress remained consistent, although HCC levels increased after six months. Since there is no correlation between perceived stress and physiological stress [23], there may be variations in stability based on the type of measure employed. The changes in HCC between the two time periods may have diverse causes. First, the research reveals that cortisol secretion changes throughout the year [13], [33]; like our study, the first stress test was conducted in January and February 2018 and the second in July and August 2018.

Stress is a multidimensional phenomenon that emerges on physiological, cognitive, behavioral, and emotional levels. This idea was somewhat supported by the findings. The findings indicate that perceived stress remained consistent, although HCC levels increased after six months. Since there is no correlation between perceived stress and physiological stress [34], there may be variations in stability based on the type of measure employed. The changes in HCC between the two time periods may have diverse causes. Literature indicates that cortisol secretion changes throughout the year [35]; in the present study, the first stress assessment was conducted in January and the second in June.

In our study, individuals had greater objective stress, but there were no changes in perceived stress, possibly because healthcare workers had trouble identifying stress even while they were experiencing it. In this instance, the healthcare workers may have gotten acclimated to the stressful time; yet their bodies noticed the stress even though they were unable to detect it subjectively. As a result, their cortisol levels were greater during the evaluation despite the absence of reported stress variations, as revealed by our research as stated by the Lazarus model [36], a further factor might be the appraisals of the stressful circumstance and their own resources for coping with such situations. From this perspective, an individual may suffer greater

objective stress (HCC), but his or her subjective assessment may be influenced by other reasons. According to the research, however, the link between objective and subjective stress, as well as the temporal stability of stress levels as measured by various instruments, is unclear. Considering the paucity of studies examining the stability of HCC and felt stress among healthcare professionals, it will be interesting to evaluate the HCC levels temporally at many time intervals in future research.

This study is beneficial for the prevention and promotion of mental health among healthcare professionals despite its limitations since it increases the assessment and understanding of chronic stress within this population. The study increases our knowledge of stress by, among other things, providing reference ranges for HCC among Malaysian healthcare professionals, which do not exist in the existing literature. Thus, due to the increased detection of stress levels and the provision of an efficient program, it will be possible to prevent the onset of stress-related emotional issues [37]–[39].

5. CONCLUSION

This study aimed to explore the impact of a stress management intervention program on stress for healthcare workers. This study adds to the growing body of data, showing persistently increased cortisol levels are directly connected to chronic stress. Our findings confirmed that stress levels decreased quantitatively in relation to hair cortisol levels following an effective stress management program for healthcare workers. Cortisol levels in hair may be beneficial in investigations of psychosocial, behavioral, or mental health prognosis. Unfortunately, the majority of the existing information is derived from small cross-sectional research, limiting the generalizability of the findings. Given that healthcare workers suffer from a high risk of burnout, more research regarding evidence-based effective interventions is warranted to alleviate their psychological distress by incorporating non-invasive bio-monitoring tools like hair cortisol levels. Further longitudinal studies involving larger samples of patients with chronic stress and healthy participants have the potential to enhance our understanding of the effects of chronic cortisol excess on occupational stress and inform the development of more effective preventive and treatment interventions.

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