Neuroendocrinology Letters Volume 45 No. 1 2024
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Association between COVID-19 vaccination and hair cortisol concentrations.

Yuemin Ding[1,2,3,4*], Yuxuan Xiong[2*], Jingru Zhao[2*], Ying Zhang[2], Qiang Zheng[2], Zulun Gao[2], Liuyi Lu[2], Kai Guo[2], Zhihao Bai[2], Hangdi Chen[2], Jiali Zhang[2], Meiyin Zhong[2], Aiwu Huang[1], Shiwei Duan[2,4], Xiong Zhang[5]

1 Affiliated Hospital, Hangzhou City University, Linan, 311300, China.
2 Hangzhou City University, School of Medicine, Department of Clinical Medicine, Hangzhou, 310015, China.
3 Hangzhou City University, School of Nursing and Health Sciences, Hangzhou, 310015, China.
4 Hangzhou City University, Key Laboratory of Novel Targets and Drug Study for Neural Repair of Zhejiang Province, 310015, China.
5 Zhejiang University School of Medicine, Department of Basic Medical Science, Hangzhou 310058, China.
*Yuemin Ding, Yuxuan Xiong, and Jingru Zhao contributed equally to this work.

Correspondence to: Aiwu Huang, Affiliated Hospital, Hangzhou City University, Linan, 311300, China. E-MAIL: 1525696656@qq.com
Shiwei Duan, Hangzhou City University, School of Medicine, Department of Clinical Medicine, Hangzhou, 310015, China. E-MAIL: duansw@hzcu.edu.cn
Xiong Zhang, Zhejiang University School of Medicine, Department of Basic Medical Science, Hangzhou 310058, China. E-MAIL: xiongzhang@zju.edu.cn

Submitted: 2023-09-06 Accepted: 2023-12-05 Published online: 2024-12-25

Key words: **Cortisol; Stress; COVID-19; Vaccination; Adverse reactions**

Neuroendocrinol Lett 2024; **45**(1):22–30 **PMID:** 38295425 NEL450124A01

Abstract

BACKGROUND: Cortisol is a steroid hormone secreted mainly by the adrenal cortex and is associated with chronic stress levels in the body. Hair cortisol concentration (HCC) is a reliable index to assess human stress levels. So far, no study has reported whether COVID-19 vaccination is associated with the changes of HCC.

METHODS: Hair samples were collected from 114 college students at Hangzhou City University and Zhejiang University. Among them, 57 cases completed COVID-19 vaccination and others did not. HCCs were measured by the chemiluminescence immunoassay (CLIA). The psychological stress levels were evaluated using the Chinese College Student Psychological Stress Scale (CCSPSS). General information and adverse reactions of the subjects were collected by questionnaire.

RESULTS: Compared with the vaccinated college students, the unvaccinated students had higher HCC levels in both A and B hair segments respectively corresponding older or six weeks before and newer or six weeks after vaccination ($p < 0.05$), reflecting higher stress levels. Besides, the vaccinated group had significantly higher HCCs in segment B compared with segment A ($p < 0.05$). Further analysis showed that the value of ΔHCC ($HCC_{seg.B}$ - $HCC_{seg.A}$) of the vaccinated group was strongly associated with COVID-19 vaccination ($p < 0.05$), but was not associated with age, gender, BMI, CCSPSS score, hormone use, exercise frequency, hair washing frequency, or hair treatment. Finally, the number of self-reported systemic adverse reactions in the vaccinated group was associated with ΔHCC ($p < 0.01$).

CONCLUSION: The COVID-19 vaccination had an impact on the value of HCC,

which might be linked to the occurrence of systemic adverse effects following vaccinations.

Abbreviations:

ACTH	- adrenocorticotropic hormone
ADH	- hypothalamic-pituitary-adrenal
BMI	- body mass index
CCSPSS	- The Chinese College Student Psychological Stress Scale
CLIA	- chemiluminescence immunoassay
CRH	- corticotropin-releasing hormone
HCC	- hair cortisol concentration
HPA	- hypothalamic-pituitary-adrenal axis

INTRODUCTION

Chronic stress, also known as long-term stress, is mainly controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Upon receiving foreign stimuli, the paraventricular nucleus of the hypothalamus secretes antidiuretic hormone (ADH) and corticotropin-releasing hormone (CRH), which act on the anterior pituitary gland to release adrenocorticotropic hormone (ACTH), resulting in increased cortisol concentrations (Stalder *et al.* 2013). These hormones and the HPA axis response are used as reliable stress indicators. A growing body of evidence suggests that maintaining an appropriate level of stress is important to prevent the occurrence of diseases and maintain body health (Dunlavey 2018), which cannot be ignored at different stages of life, especially during the COVID-19 global pandemic in the previous years.

Cortisol, an effector hormone of the HPA axis, plays a dual role in the physiological function of the body. On the one hand, it is involved in many basic physiological processes, such as lipid and glucose metabolism, blood pressure regulation, inflammation, and immune response, which helps the body adapt flexibly to various environments (Sapolsky R M 2000). On the other hand, long-term exposure to high cortisol concentrations is a risk factor for psychiatric disorders such as depression (Dettenborn *et al.* 2012) and post-traumatic stress disorder (Luo *et al.* 2012), and is also closely associated with chronic cardiovascular diseases such as hypertension (Bautista *et al.* 2019). Therefore, it is very important to accurately and effectively evaluate the level of chronic stress to prevent stress diseases.

It has been suggested that analysis of hair cortisol concentration (HCC) is a better tool for the measurement of long-term exposure to stress and assessment of the HPA axis response (Russell *et al.* 2012) since the commonly used measurements of cortisol in blood, saliva, and urine have yielded variable results (Koumantarou Malisiova *et al.* 2021). Cortisol content in body fluids is affected by the circadian rhythm of hormone secretion and is related to the mental state of the subject at the time of sampling; thus, it can only reflect the instantaneous stress level. Under long-term stress, circulating cortisol can spread through the bloodstream into the hair roots and accumulate near the scalp (Lee *et al.* 2015). Hair growth occurs at a rate of approximately 1 cm/month. It can be considered that the cortisol content of hair grown in a specific period reflects the individual's chronic stress level at that time, and can effectively indicate the long-term reactivity of the HPA axis. HCC has been used by many researchers as an observational marker of stress level, and has been widely used to assess the stress level of various status of subjects, such as women during pregnancy (Vuppaladhadiam *et al.* 2021), students at the beginning of school (Groeneveld *et al.* 2013), and participants who suffered from circadian rhythm disorder (El Mlili *et al.* 2021).

In the past few years, researchers around the world have evaluated the psychosomatic effects of the COVID-19 pandemic on people of different occupations, genders, and ages by measuring the levels of HCC, and have taken some necessary interventions accordingly. During the COVID-19 pandemic, healthcare workers were on the frontlines and under enormous pressure. Studies have shown that due to the direct involvement in the care, diagnosis, and treatment of patients, healthcare workers' stress and burnout symptom index increased, and their HCCs increased compared with that before the pandemic. Data from 372 healthcare workers in Canada showed that the HCC of this population was 3.1 nmol/L before the pandemic from November 2019 to March 2020 and increased by 0.6 nmol/L after the outbreak of the pandemic from March 2020 to June 2020 (Marcil *et al.* 2022). The results of another study in Argentina showed that the HCC levels of 40% of 234 healthcare workers were outside of the healthy reference range (Ibar *et al.* 2021). In addition, a Slovak study followed 57 nurses who had hair samples tested for HCC levels in the spring of 2020 during the first wave of the COVID-19 pandemic (Rajcani *et al.* 2021b) and in the autumn of 2020 during the second wave (Rajcani *et al.* 2021a). The data showed that the HCCs were higher than before in the hair segments of both spring and autumn corresponding to the time of two waves of pandemic.

Through sampling of different populations, several studies have shown that HCC levels were higher among family members, especially mothers and children, during the epidemic. A study from the Middle East starting in 2019 and ending in November 2020 showed that HCC levels rose by 0.17 nmol/L in mothers and 0.06 nmol/L in children after the epidemic. These data suggested that mothers and children were less psychologically resilient in the face of the epidemic (Hastings *et al.* 2021). Experimental data from the Midwest, USA, between August and November of 2020 suggested that mother HCC was significantly associated with child HCC, with a correlation coefficient of 0.40, especially in young children (Perry *et al.* 2022). A study conducted in Germany from late 2019 to August 2020 found that individuals with higher neuroticism and extraversion,

Tab. 1. Sociodemographic characteristics of the population with both hair samples and questionnaire data (n = 114)

Variable	Whole sample (n =114)	Vaccinated (n = 57)	Unvaccinated (n = 57)	*p* value
	M±SD/ n(%)	M±SD/ n(%)	M±SD/ n(%)	
Age (years)	20.46±0.48	20.37±0.68	20.69±0.82	0.63
BMI (kg/m^2)	21.45±2.27	21.09±2.26	21.16±2.37	0.79
CCSPSS score	54.26±27.93	56.95±26.88	51.24±27.16	0.17
Get vaccinated				0.02
YES	57 (50%)	57	/	
NO	57 (50%)	/	57	
Gender				0.15
Male	47 (41.23%)	19 (33.33%)	28 (49.12%)	
Female	67 (58.77%)	38 (66.67%)	29 (50.88%)	
Hormone used				0.29
YES	14 (12.28%)	6 (10.52%)	8 (14.04%)	
NO	100 (87.72%)	51 (89.47%)	49 (85.96%)	
Dietary pattern				0.61
High-fat diet	14 (12.28%)	4 (7.02%)	10 (17.54%)	
Low-fat diet	20 (17.54%)	11 (19.30%)	9 (15.79%)	
Standard diet	80 (70.18%)	42 (73.68%)	38 (66.67%)	
Dieting	0 (0%)	0 (0%)	0 (0%)	
Exercise Frequency				0.55
>3 times per week	14 (12.28%)	4 (7.02%)	10 (17.54%)	
1-3 times per week	82 (71.93%)	44 (77.19%)	38 (66.67%)	
Never	18 (15.79%)	9 (15.79%)	9 (15.79%)	
Hair washing frequency				0.42
Every day	26 (22.81%)	8 (14.04%)	18 (31.58%)	
Every 2 days	52 (45.61%)	28 (49.12%)	25 (43.86%)	
Every 3 days	26 (22.81%)	17 (29.82%)	10 (17.54%)	
Every 3+ days	10 (8.77%)	4 (7.02%)	4 (7.202)	
Hair treatment (perm and/or dye)				0.51
YES	14 (12.28%)	6 (10.52%)	8 (14.04%)	
NO	100 (87.72%)	51 (89.47%)	49 (85.96%)	

Note: BMI: body mass index; CCSPSS: the Chinese College Student Psychological Stress Scale.

classified by the Big Five personality traits, tended to have higher HCC levels during the COVID-19 pandemic (Engert *et al.* 2021).

Since the outbreak of the COVID-19 pandemic, local governments have implemented different control measures. Several large surveys have shown the impact of these measures on the mental health of residents. A cross-national study conducted in Austria, Italy, and Germany in spring 2020 investigated the impact of lockdown policies on the psychological status of residents during the COVID-19 pandemic. The results showed that HCC levels decreased during lockdown, with a median of 4.14 pg/mg compared with 4.98 pg/mg before lockdown. The researchers suggested that this change might be related to a reduction in "daily hassles" at home, changes in daylight and/or temperature, and a decrease in physical activity (Feneberg *et al.* 2022).

Other studies investigated the effect of different external factors on human stress levels during the pandemic. A pilot study in Austria evaluated the psychobiological effects of in-person vs. virtual choir singing in children and adolescents before and after the acute phase of the COVID-19 outbreak in 2020. They observed a stronger positive psychobiological effect in the in-person setting

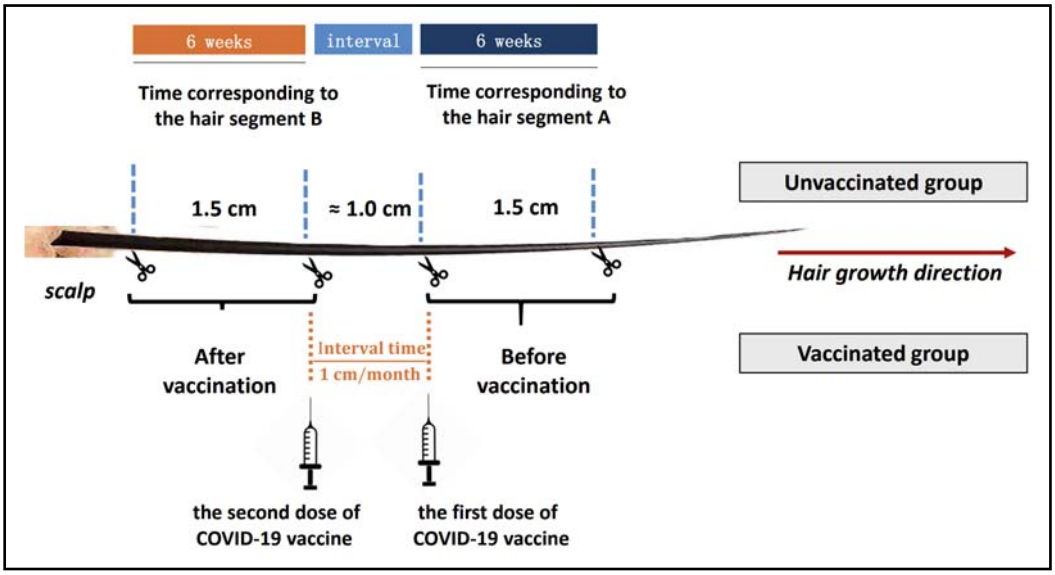

Fig. 1. Illustrative diagram of hair sample collection with two 1.5 cm hair segments in vaccinated and unvaccinated groups. Segment A: hair segments older or six weeks before the first dose of vaccine. Segment B: hair segments newer or six weeks after the last dose of vaccine.

compared to the virtual one. For example, the HCC of the in-person singing group decreased from a median value of 2.12 pg/mg to 1.98 pg/mg (Grebosz-Haring *et al.* 2021). A German research group planned to implement a stepped-care program concerning a series of questionnaires and HCC levels to provide online and face-to-face interventions for adults, adolescents and children. They suggested that the study might help to understand the impact of the COVID-19 pandemic on mental health and to reduce the transition to manifest mental disorders using indicated prevention (Langhammer *et al.* 2021).

The current study was conducted to compare the changes in hair cortisol in different undergraduate populations with or without the COVID-19 vaccination. We also assessed whether COVID-19 vaccination was related to changes in HCCs of the vaccinated group. Furthermore, other factors such as age, gender, BMI, CCSPSS score, hormone used, exercise frequency, hair washing frequency, and hair treatment were assessed as potential confounders.

MATERIAL AND METHODS

Study participants

Hair samples were collected from 202 college students at Hangzhou City University and Zhejiang University during two peak periods of COVID-19 vaccination in China. Among them, 98 samples were collected between June 2021 and July 2021 and 104 samples were collected between February 2023 and March 2023. All participants provided informed consent before undergoing any study-related procedures or sample processing. After screening by exclusion criteria, 114 samples were selected. The vaccinated group consisted of 57 students who received full vaccination. The inclusion criteria were as follows:(1) age ≥ 18 years, (2) 18.5 < body mass index (BMI) < 30, (3) completed at least two doses of vaccine between March 2021 and March 2023 without a severe systemic allergic reaction, and (4) volunteered to participate in this study and cooperate with hair sample collection. The exclusion criteria were as follows: (1) having endocrine system diseases, (2) actively smoking, (3) pregnancy, and (4) suffering from severe acute infections during this period. The COVID-19 inactivated vaccine (Vero cells) was produced by Beijing Institute of Biological Products Co., Ltd. and Sinovac Life Sciences Co., Ltd. The control group (unvaccinated) consisted of 57 students enrolled in the same period. In addition to the status of completion of the COVID-19 vaccination, the inclusion and exclusion criteria were the same as those in the vaccination group. This study has been reviewed and approved by the Ethics Committee of Hangzhou City University.

General information collection

The general information of the subjects was collected with a questionnaire that included age, sex, BMI, medication, hormones used (such as glucocorticoids for immune system diseases, thyroid hormones for hypothyroidism, and sex hormones), eating habits, exercise frequency, hair washing frequency, hair treatment (perm and/or dye), vaccine producer, self-reported local adverse events (such as induration, itching, pain, redness, swelling), and self-reported systemic adverse events (such as anorexia, constipation, coughing, diarrhea, dyspnoea, fatigue, headache, nausea, vomiting) (Guo *et al.* 2021).

Psychological stress assessment

The Chinese College Student Psychological Stress Scale (CCSPSS) is organized and compiled by the Mental Health Assessment System research group for College Students of the Ministry of Education of China. It is designed for Chinese college students to assess their social and cultural characteristics with high reliability

Tab. 2. Hair cortisol concentration and COVID-19 vaccination in the studied population

Group	Vaccinated group			Unvaccinated group		
	Whole sample	Male	Female	Whole sample	Male	Female
Sample (n)	57	19	38	57	28	29
HCC (pg/mg)						
Seg. A	52.83±6.07$^{\#}$	52.94±5.97	52.80±6.07	57.03±3.68	57.53±4.30	56.81±3.02
Seg. B	53.71±5.18$^{*\#}$	53.67±5.50*	53.81±4.86*	56.20±3.13*	56.71±3.28*	55.97±3.14*
ΔHCC	0.88±0.96$^{\#\#}$	0.73±0.94$^{\#}$	1.01±0.75$^{\#\#}$	-0.83±0.77	-0.82±0.71	-0.84±0.92

Note: HCC: hair cortisol concentration. Data are shown as M ± SD; $^{*}p < 0.05$, HCCs of segment B vs. segment A; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, HCCs or ΔHCC of vaccinated group vs. unvaccinated group at same gender.

and validity (Liang Baoyong 2005). The scale aims to evaluate the level of psychological stress of college students from five dimensions: study, life, development, social interaction, and family, with a total of 85 items. Participants were asked to report life events and daily chores they experienced over some time and to evaluate their "psychological impact." Participants were asked to evaluate the degree of each event according to the intensity and duration of the psychological impact, which was divided into seven grades from minimum to maximum, scoring 1 to 7. The sum of these scores was calculated as the total psychological stress score.

Hair sample collection and laboratory procedures

A total of 80-100 hairs were obtained from the occipital region, as close to the scalp as possible. The root segments of the hair were marked with adhesive tape and stored in envelopes at room temperature. Hair samples were washed with isopropyl alcohol for 2 min and dried at room temperature for 2 days. Considering an average hair growth rate of 1 cm per month, the hair samples were divided into 6-week growth sections before (segment A) and after (segment B) vaccination using scissors and weighed (Fig 1). In the unvaccinated group, two sections of hair samples of 1.5 cm each were clipped accordingly (Segment A: older and Segment B: newer), and the interval distance was calculated according to the average interval time of the vaccinated group. Since the average interval time between two injections of the vaccinated group was 30.2 days, the interval distance of the unvaccinated group was set at 1.0 cm (Fig. 1). Subsequently, hair samples were cut into pieces and cryogenically rotated in 1.5 ml of methanol for 24 hours for cortisol extraction. The supernatant was collected after centrifugation and the methanol was evaporated. When the samples were completely dry, pH 7.4 phosphate-buffered saline (PBS) at a ratio of 1:10 was added. Hair cortisol concentrations (HCCs) was determined using a commercially available chemiluminescence immunoassay kit (IBL-Hamburg, Germany). The cortisol concentration was calculated according to the standard curve and expressed in pg/mg.

Data analysis

SPSS 22.0 was used for statistical analysis. Results are presented as mean (M) ± standard deviation (SD) for continuous variables according to the data distribution. Each independent variable was analyzed separately. Pearson correlation analysis was used for continuous variables and ANOVA was used for categorical variables to compare the differences between groups. Factors influencing HCCs were analyzed using univariate logistic regression.

RESULTS

Sociodemographic characteristics of the samples

The sociodemographic characteristics of the study sample are presented in Table 1. From the information provided in the questionnaires, we collected the general characteristics of the final sample of participants, including health status, medical history, current medication, and lifestyle habits. The CCSPSS scores have also been reported.

COVID-19 vaccination and hair cortisol concentration

Table 2 shows HCCs of college students in vaccinated and unvaccinated groups before (segment A) and after (segment B) vaccination. Variations in HCCs were calculated as ΔHCC ($HCC_{seg.\ B}$ - $HCC_{seg.\ A}$). In the comparison between vaccinated and unvaccinated groups, the HCCs of the unvaccinated group, whether in Segment A or Segment B, were higher than those of the vaccinated group ($p < 0.05$), suggesting that students who did not receive vaccination had a higher level of stress. In addition, it was observed that the unvaccinated group had significantly lower HCCs in segment B compared with segment A ($p < 0.05$), while the vaccinated group had higher HCCs in segment B ($p < 0.05$). These different changes had a direct impact on ΔHCC value. Compared with the unvaccinated groups, the ΔHCC of vaccinated groups increased significantly ($p < 0.05$).

Pearson correlation analysis was carried out to determine the possible impact factors of ΔHCC before and after vaccination. Analysis of variance (ANOVA) was used to compare the differences between the groups

Tab. 3. Univariate logistic regression analysis of factors associated with ΔHCC

Predictor	B	B95% CI	SE B	β	t	*p* value
constant	-0.569	[-1.088,-0.049]	0.247		-2.209	0.031
vaccination	0.743	[0.125,1.387]	0.310	0.359	2.413	0.020

Tab. 4. Correlation between ΔHCC and adverse reactions and vaccine producer

Vaccinated group (n = 57)	Frequency (n)	*p* value
Numbers of Self-reported local adverse events		
0	32	0.804
1	23	
2	2	
Numbers of Self-reported systemic adverse events		
0	26	0.004
1	22	
2	2	
3	4	
4	3	
Vaccine Producer (Inactivated COVID-19 vaccine)		
Sinovac Life Sciences, Beijing	31	0.567
Beijing Institute of Biological Products	26	

of various variables. The results showed a significant difference between the groups with and without COVID-19 vaccination (p = 0.017). However, other factors, such as age (p = 0.63), BMI (p = 0.79), CCSPSS score (p = 0.17), gender (p = 0.15), hormone used (p = 0.29), diet (p = 0.61), exercise frequency (p = 0.55), hair washing frequency (p = 0.42), and hair treatment (p = 0.51), did not significantly affect ΔHCC (Table 1).

To further investigate the influence of the COVID-19 vaccination on ΔHCC, we established a univariate logistic regression model. The data from the logistic regression model are summarised in Table 3. The model showed that COVID-19 vaccination was an independent influencing factor associated with ΔHCC (p < 0.05) with a positive correlation (B = 0.743).

Adverse events and hair cortisol concentration

In the analysis of changes in hair cortisol concentration in the vaccinated group (Table 4), the number of self-reported systemic adverse events (for example, anorexia, constipation, coughing, diarrhea, fever, and fatigue) was significantly correlated with ΔHCC (p < 0.005). However, no evidence showed that ΔHCC was associated with the number of local adverse events (for example, induration, itching, pain, redness, and swelling) or the vaccine producer (Beijing Kexing Zhongwei Biotech Co., LTD., Beijing Institute of Biological Products Co., LTD.).

DISCUSSION

During the COVID-19 pandemic, there has been a significant negative impact on the mental and physical health status of the general population (Rajkumar 2020; Salari *et al.* 2020). At the same time, the implementations of the lockdown and travel restriction policies might affect the stress level in the body (Droit-Volet *et al.* 2020; Rehman *et al.* 2022). Chronic stress levels can be assessed by measuring cortisol in the hair. So far, quite a few studies have been conducted comparing the changes in HCC before and after a wave of COVID-19 pandemic (O'Byrne *et al.* 2020; Ibar *et al.* 2021; Rajcani *et al.* 2021b; Rajcani *et al.* 2021a; Hastings *et al.* 2021; Perry *et al.* 2022). However, no study has reported whether COVID-19 vaccination is associated with the changes in HCC reflecting stress levels in the body.

In the present study, the difference in HCC levels between vaccinated and unvaccinated groups of college students was compared and the change in HCC levels before and after COVID-19 vaccination was determined. The results showed that the hair cortisol concentration of college students who chose not to receive a COVID-19 vaccine during the corresponding time course was higher than that of the vaccinated group, indicating a higher level of chronic stress in unvaccinated students. To our knowledge, this is the first study to compare HCC as a stress biomarker between vaccinated and unvaccinated college students. We speculate that students who chose not to be vaccinated despite the fact that most people around them chose to be vaccinated might have a strong sense of psychological insecurity, and they were the people who bear greater psychological pressure during the pandemic, resulting in relatively higher stress levels.

Furthermore, we found that the HCC of segment B was lower than that of segment A in the unvaccinated group, which may be due to increased stress

level of the students at the beginning of the semester (February 2021 or September 2022 indicated by segments A). In the middle period of the semester (May 2021 and December 2022 indicated by segments B), as students adapted to campus life, the stress level and cortisol concentration decreased. School-related stressors may affect students' well-being and significantly impact their physiological and psychological health (Schmid-Zalaudek *et al.* 2021). Stetler and colleagues have reported that cortisol levels in hair samples of 56 undergraduates were significantly higher during the academic term compared to the summer break (Stetler & Guinn. 2020). Our findings suggest that school-related stressors are greater at the beginning of the semester, and then decrease gradually due to adaptation to campus life.

On the contrary, we found that the HCC of segment B was significantly higher than that of segment A in the vaccinated group, indicating an increased stress level of the students after they received vaccination. To determine why HCCs in the vaccinated group increased, we conducted a Pearson correlation analysis on the possible impact factors. It was found that COVID-19 vaccination, but not age, gender, BMI, CCSPSS score, hormone used, exercise frequency, hair washing frequency, or hair treatment, had a significant effect on the changes in HCC in college students.

It is noteworthy that CCSPSS contains a large number of life events that may occur in a specific period, which can be considered to reflect the psychological stress levels of the subjects. In this study, there was no significant correlation between the CCSPSS score and ΔHCC, excluding the impact of psychological stress caused by life events. Of the variables mentioned above, COVID-19 vaccination was the only factor that had an impact. This was further confirmed by univariate logistic regression analysis. Univariate analysis revealed that vaccination was significantly correlated with ΔHCC, suggesting that vaccination was an independent factor affecting ΔHCC.

Thus, excluding other possible impact factors, changes in the HCC of college students were likely caused by COVID-19 vaccination alone. The change in stress level reflected by ΔHCC was caused by psychological stress after the injections, which might be related to nervousness, anxiety, panic, and others. This could also be a physiological stress response caused by the inactivated vaccine itself. In the vaccinated group, one unanticipated result was that the degree of change in HCC was found to be significantly associated with the number of self-reported systemic adverse reactions. However, no differences were observed between ΔHCC and the number of self-reported local adverse reactions or vaccine producers. These results indicate that COVID-19 vaccination causes physiological stimulation in the participants at the level of stress. However, whether the HPA axis and chronic stress are involved in the occurrence of adverse reactions after vaccination remains unclear. In addition, it should be noted that although the HCC of segment B increased compared to that of segment A in the vaccinated group, it was still much lower than that of the unvaccinated group. This suggests that the change in chronic stress caused by COVID-19 vaccination is far from causing stressful diseases; thus, it is safe.

Our results were supported by an online survey from January to April 2021 in China designed to explore stress levels related to COVID-19 vaccination. The authors found higher stress scores were associated with COVID-19 vaccination in younger participants, having lower education levels, having a history of chronic diseases, mistrusting vaccine efficacy, and experiencing of vaccine allergy events (Zheng *et al.* 2021). Interestingly, a recent study in Jordan found that the anxiety and stress caused by vaccination were related to the psychological state of respondents before vaccination. Individuals with normal levels of anxiety before vaccination experienced a significant rise in anxiety and stress symptoms after they received their first dose of the COVID-19 vaccine. On the contrary, anxiety significantly dropped after vaccination among anxious individuals (Al-Amer *et al.* 2022).

This study had some limitations. First of all, we could not recruit more volunteers to provide stronger evidence of our findings. Due to the limitations in the number of research participants and sample size, it cannot be concluded that the COVID-19 vaccination has caused widespread changes in stress levels in the whole population. Its impact on the level of stress over a wider range needs to be studied further. In addition, this study only accounted for the number of adverse events reported by the participants themselves and did not measure the specific types and the degree of adverse reactions, which might have affected the accuracy of the evaluation of adverse reactions after vaccination. Therefore, whether the changes in HCC values reported by us had clinical significance needs to be investigated more accurately in the later stage. Moreover, psychiatric disorders such as anxiety or depression, and other confounding variables like zinc and vitamin supplements were not measured among the participants, which might also affect cortisol levels before and after the vaccination period. Finally, the literature showed that there are seasonal variation levels that affect serum cortisol (Hadlow NC *et al.* 2014). Because our samples were collected over four seasons, they might not be appropriate to reflect seasonal variation.

CONCLUSION

Unvaccinated college students had higher levels of HCC, reflecting higher levels of chronic stress. However, the COVID-19 vaccination in vaccinated students can also affect HCC levels and is associated with self-reported systemic adverse reactions. Therefore, it is important to conduct a thorough analysis of stress levels in the

population after vaccination, as well as a comprehensive study of potential adverse effects, to alleviate concerns about vaccine safety. This would be a critical step in managing pandemics.

FUNDING

The study was supported by the Basic Public Welfare Research Project of Zhejiang Province (No. LGD19H090008) and the 2021 Student Research Training Program of Hangzhou City University (No. XZ202104075).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENT

The authors would like to thank all the participants for their time. A special thanks to Dr. Naru Zhang for her comments and suggestions on the manuscript.

REFERENCES

1 Al-Amer R, Malak MZ, Burqan HMR, Stanculescu E, Nalubega S, Alkhamees AA, et al. (2022). Emotional Reaction to the First Dose of COVID-19 Vaccine: Postvaccination Decline in Anxiety and Stress among Anxious Individuals and Increase among Individuals with Normal Prevaccination Anxiety Levels. J Pers Med. **12**.
2 Bautista LE, Bajwa PK, Shafer MM, Malecki KMC, Mcwilliams CA, Palloni A (2019). The relationship between chronic stress, hair cortisol and hypertension. Int J Cardiol Hypertens. **2**: 100012.
3 Dettenborn L, Muhtz C, Skoluda N, Stalder T, Steudte S, Hinkelmann K, et al. (2012). Introducing a novel method to assess cumulative steroid concentrations: increased hair cortisol concentrations over 6 months in medicated patients with depression. Stress. **15**: 348–353.
4 Droit-Volet S, Gil S, Martinelli N, Andant N, Clinchamps M, Parreira L, et al. (2020). Time and Covid-19 stress in the lockdown situation: Time free, <<Dying>> of boredom and sadness. PLoS One. **15**: e0236465.
5 Dunlavey CJ (2018). Introduction to the Hypothalamic-Pituitary-Adrenal Axis: Healthy and Dysregulated Stress Responses, Developmental Stress and Neurodegeneration. J Undergrad Neurosci Educ. **16**: R59–R60.
6 El Mlili N, Ahabrach H, Cauli O (2021). Hair Cortisol Concentration as a Biomarker of Sleep Quality and Related Disorders. Life (Basel). **11**.
7 Engert V, Blasberg JU, Kohne S, Strauss B, Rosendahl J (2021). Resilience and personality as predictors of the biological stress load during the first wave of the Covid-19 pandemic in Germany. Transl Psychiatry. **11**: 443.
8 Feneberg AC, Forbes PaG, Piperno G, Pronizius E, Stijovic A, Skoluda N, et al. (2022). Diurnal dynamics of stress and mood during COVID-19 lockdown: a large multinational ecological momentary assessment study. Proc Biol Sci. **289**: 20212480.
9 Grebosz-Haring K, Schuchter-Wiegand AK, Feneberg AC, Skoluda N, Nater UM, Schutz S, et al. (2021). The Psychological and Biological Impact of "In-Person" vs. "Virtual" Choir Singing in Children and Adolescents: A Pilot Study Before and After the Acute Phase of the COVID-19 Outbreak in Austria. Front Psychol. **12**: 773227.
10 Groeneveld MG, Vermeer HJ, Linting M, Noppe G, Van Rossum EF, Van IMH (2013). Children's hair cortisol as a biomarker of stress at school entry. Stress. **16**: 711–715.
11 Guo W, Duan K, Zhang Y, Yuan Z, Zhang YB, Wang Z, et al. (2021). Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18 years or older: A randomized, double-blind, placebo-controlled, phase 1/2 trial. EClinicalMedicine. **38**: 101010.
12 Hadlow NC, Brown S, Wardrop R, Henley D (2014). The effects of season, daylight saving and time of sunrise on serum cortisol in a large population. Chronobiol Int. **31**: 243–51.
13 Hastings PD, Partington LC, Dajani R, Von Suchodoletz A (2021). Adrenocortical and psychosocial responses of families in Jordan to the COVID-19 pandemic. Child Dev. **92**: e798–e816.
14 Ibar C, Fortuna F, Gonzalez D, Jamardo J, Jacobsen D, Pugliese L, et al. (2021). Evaluation of stress, burnout and hair cortisol levels in health workers at a University Hospital during COVID-19 pandemic. Psychoneuroendocrinology. **128**: 105213.
15 Koumantarou Malisiova E, Mourikis I, Darviri C, Nicolaides NC, Zervas IM, Papageorgiou C, et al. (2021). Hair cortisol concentrations in mental disorders: A systematic review. Physiol Behav. **229**: 113244.
16 Langhammer T, Hilbert K, Praxl B, Kirschbaum C, Ertle A, Asbrand J, et al. (2021). Mental health trajectories of individuals and families following the COVID-19 pandemic: Study protocol of a longitudinal investigation and prevention program. Ment Health Prev. **24**: 200221.
17 Lee DY, Kim E, Choi MH (2015). Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. BMB Rep. **48**: 209–216.
18 Liang Baoyong HZ (2005). Development of Chinese College Student Psycholohical Stress Scale (In Chinese). Studies of Psychology and Behavior. **3**: 81–87.
19 Luo H, Hu X, Liu X, Ma X, Guo W, Qiu C, et al. (2012). Hair cortisol level as a biomarker for altered hypothalamic-pituitary-adrenal activity in female adolescents with posttraumatic stress disorder after the 2008 Wenchuan earthquake. Biol Psychiatry. **72**: 65–69.
20 Marcil MJ, Cyr S, Marin MF, Rosa C, Tardif JC, Guay S, et al. (2022). Hair cortisol change at COVID-19 pandemic onset predicts burnout among health personnel. Psychoneuroendocrinology. **138**: 105645.
21 O'byrne ML, Faerber JA, Katcoff H, Frank DB, Davidson A, Giglia TM, et al. (2020). Variation in the use of pulmonary vasodilators in children and adolescents with pulmonary hypertension: a study using data from the MarketScan(R) insurance claims database. Pulm Circ. **10**: 2045894020933083.
22 Perry NB, Donzella B, Troy MF, Barnes AJ (2022). Mother and child hair cortisol during the COVID-19 pandemic: Associations among physiological stress, pandemic-related behaviors, and child emotional-behavioral health. Psychoneuroendocrinology. **137**: 105656.
23 Rajcani J, Vytykacova S, Solarikova P, Brezina I (2021a). A follow-up to the study: Stress and hair cortisol concentrations in nurses during the first wave of the COVID-19 pandemic. Psychoneuroendocrinology. **133**: 105434.
24 Rajcani J, Vytykacova S, Solarikova P, Brezina I (2021b). Stress and hair cortisol concentrations in nurses during the first wave of the COVID-19 pandemic. Psychoneuroendocrinology. **129**: 105245.
25 Rajkumar RP (2020). COVID-19 and mental health: A review of the existing literature. Asian J Psychiatr. **52**: 102066.
26 Rehman U, Yildirim M, Shahnawaz MG (2022). A longitudinal study of depression, anxiety, and stress among Indians during COVID-19 pandemic. Psychol Health Med. **28**: 60-68..
27 Russell E, Koren G, Rieder M, Van Uum S (2012). Hair cortisol as a biological marker of chronic stress: current status, future directions and unanswered questions. Psychoneuroendocrinology. **37**: 589–601.
28 Salari N, Hosseinian-Far A, Jalali R, Vaisi-Raygani A, Rasoulpoor S, Mohammadi M, et al. (2020). Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. Global Health. **16**: 57.
29 Sapolsky R M RLM, Munck a U (2000). How do glucocorticoids influence stress responses?Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev. **21**: 55–89.

30 Schmid-Zalaudek K, Fredriksen PM, Fabiani E, Fredriksen MV, Brix B, Rossler A, et al. (2021). Children's hair cortisol and hair testosterone concentrations in response to environmental changes: from summer holidays to school. Stress. **24**: 998–1007.
31 Stalder T, Kirschbaum C, Alexander N, Bornstein SR, Gao W, Miller R, et al. (2013). Cortisol in hair and the metabolic syndrome. J Clin Endocrinol Metab. **98**: 2573–2580.
32 Stetler CA, Guinn V (2020). Cumulative cortisol exposure increases during the academic term: Links to performance-related and social-evaluative stressors. Psychoneuroendocrinology. **114**: 104584.
33 Vuppaladhadiam L, Lager J, Fiehn O, Weiss S, Chesney M, Hasdemir B, et al. (2021). Human Placenta Buffers the Fetus from Adverse Effects of Perceived Maternal Stress. Cells. **10**.
34 Zheng YB, Sun J, Liu L, Zhao YM, Yan W, Yuan K, et al. (2021). COVID-19 Vaccine-Related Psychological Stress Among General Public in China. Front Psychiatry. **12**: 774504.