Correlation Between Degrees of Stress and Hair Cortisol Levels in Family of Covid-19 Patients at Universitas Airlangga Hospital

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Abstract

Around 20,3% of hospitalized COVID-19 patients require treatment in the ICU with mortality rate of 40%. The pandemic caused by COVID-19 causes stress for the patient's family members. The degree of stress can be measured using the DASS-42 questionnaire. Cortisol is an indicator of body's response to stress through HPA axis. Hair cortisol levels can reflect HPA activity. This study aims to observe the correlation between stress levels and hair cortisol levels in families of COVID-19 patients. This study was an observational analytic study with a cross-sectional design. There were 23 research subjects who met the inclusion and exclusion criteria. In this study, the DASS-42 questionnaire was utilized which then filled out by the subject and sampling the subject's hair to measure hair cortisol levels on the 1st day of treatment. Data were analyzed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The findings revealed that there was no significant relationship between the degree of stress and hair cortisol levels in the families of COVID-19 patients ($p>0.05$). Hair cortisol levels were within normal range. The degree of stress was not related to hair cortisol levels. Further studies involving multicenters with larger samples and longer observations are needed to confirm the results of this study.

Key Words: COVID-19, Stress, Hair Cortisol, DASS-42

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Introduction

The World Health Organization (WHO) declared coronavirus disease-2019 (COVID-19) as a public health emergency of international concern on 30 January 2020. The corona virus was first identified and formally declared in Indonesia by the President of the Republic of Indonesia on March 2, 2020, and since that time, it has spread to a number of Indonesian provinces. On March 11, 2020, WHO classified COVID-19 a global pandemic due to its rapid spread to over 200 countries. This disease has been responsible for the deaths of more than 1.3 million people and has been related to 52 million confirmed instances of PCR swabs all over the world (Jamil et al., 2020).

An infection caused by COVID-19 might result in mild, moderate, or severe symptoms. The most noticeable clinical symptoms that manifest themselves are fever, cough, chest tightness, and myalgia. Additionally, it may be accompanied by symptoms such as the production of sputum, headache, hemoptysis, and diarrhea. Some of the complications that may arise include acute respiratory distress syndrome (ARDS), anemia, acute heart defects, and secondary infections (Isbaniah & Susanto, 2020).

In addition to having an effect on health workers and patients, COVID-19 also causes stress for the patient's family. There have been a lot of studies done on the effects of COVID-19 on both health personnel and patients, but there has not been any research found on the stress that the patient's family goes through. In point of fact, effective teamwork between healthcare workers and the families of COVID-19 patients was required in order to treat and hopefully cure COVID-19 patients. Many of them were experiencing tremendous mental stress because they were caring for families who have contracted COVID-19. The patient's family must attend to the patient's needs and provide encouragement. They must begin limiting themselves and adopting a healthy lifestyle in order to avoid becoming infected. This situation contributes to their stress levels. Family members were also concerned that COVID-19 patients will be in poor health. The stress felt by the patient's family would be worsened by society's negative stigma. This stigma was undoubtedly problematic since it has the ability to isolate patients and their families with corona virus. Obviously, if the family of a COVID-19 patient experiences stress, the patient's COVID-19 recovery will not be optimized.

Stress indicators can be seen from changes in the behavior of the patient's family. The acute response to stress may manifest as a change in mood (e.g., anxiety, depression, irritability, fatigue); behavior (e.g., social withdrawal, aggressive, tearful, lack of motivation); thoughts (e.g., difficulty concentrating and solving problems); or physical symptoms (e.g., palpitations, nausea, headache). If stress continues, it can cause disorders of the neuroendocrine, cardiovascular, autonomic and immunological systems (Lovibond & Lovibond, 1995)

One subjective measure that was often used to measure stress levels was Depression anxiety stress scales-42 (DASS-42). This measuring instrument was invented by Lovibond & Lovibond (1995) which has been translated into Indonesian and was a set of 3 self-assessment scales designed to measure the emotional state of depression, anxiety, and stress. However, the purpose of this questionnaire was not as a diagnostic tool but as a tool to determine the severity of stressful conditions. In addition to using subjective measuring tools Depression anxiety stress scales-42 (DASS-42), the use of cortisol as a parameter in assessing stress was increasing (Lovibond & Lovibond, 1995). In addition to the DASS questionnaire, there was also the Hamilton Anxiety Rating Scale (HAM-A), but the weaknesses of the HAM-A questionnaire should be filled in by a psychiatrist or someone who has been trained (Bruss et al., 1994; Maier et al., 1988).

There was a correlation between stress and the onset of premature aging, disability, chronic disease, depression, and a reduction in quality of life. Cortisol, as an indicator of the body's hormonal response to stress, was measured in saliva, urine, or blood samples and was controlled by the hypothalamus-pituitary-adrenal (HPA) axis. Cortisol has a diurnal pattern and therefore the time it was taken was essential. Salivary cortisol levels vary because it has a diurnal cycle, which increases 20-50% within 30-45 minutes after waking up. Salivary cortisol levels will soon fall thereafter. Urinary cortisol levels must also be considered in elderly patients with chronic renal failure because urinary cortisol will experience a reduction. Meanwhile, blood/plasma cortisol describes both free cortisol and bound cortisol. Another weakness of the three sampling methods was the fact that they were cost more. Sampling for saliva, urine, and blood was about $20.00 USD per sample (Wright et al., 2015).

Another alternative indicator of HPA axis activation that has been identified was hair cortisol. The cortisol level in head hair seems to be an indicator that can measure the accumulating HPA activity without limiting the use of additional specimens (saliva, urine, and blood). Hair cortisol in the proximal 3 cm of hair could be used to measure HPA axis activity retrospectively in the past 3 months. The mean amount of cortisol was 20.4 pg/mg and the standard deviation was in 22.7 pg/mg (Wright et al., 2015).

Therefore, measuring head hair cortisol could be a method for determining the stress levels of Covid-19 patient families. Based on the preliminary study and the description above, this study aimed to analyze the correlation between hair cortisol and stress using the Depression anxiety stress scales-42 (DASS-42) in families of COVID-19 patients at Airlangga University Hospital.

Methodology

This study was a paired categorical comparative analytical study with a cross sectional design to analyze differences in blood levels of S100B in

This research was carried out at the ICU of Universitas Airlangga Hospital in October 2021–April 2022. This research was an observational analytical study with a cross-sectional design that aims to analyze the correlation of hair cortisol with stress using the stress scale Depression anxiety in the families of COVID-19 patients at Universitas Airlangga Hospital. Observational research refers to a study in which the researcher only makes observations without intervening with the research subject. Meanwhile, analytics refers to a study that explores how and why health phenomena occur. This study was designed using a cross-sectional design, which was a type of research that emphasizes the time of measurement/observation of independent and dependent variable data in only one measurement. The research instrument used was patient medical record data, form Depression anxiety stress scales 42, and hair cortisol levels.

The study population was the families of COVID-19 patients who were treated in the ICU of Universitas Airlangga Hospital in February-April 2022. Meanwhile, the sample was the families of COVID-19 patients who were treated in the ICU of Universitas Airlangga Hospital who met the inclusion criteria (i.e., patient's family aged 18 to 65 years; the patient's family are spouses, biological children, parents, siblings, relatives who live in the same house; willing and have signed informed consent to participate; and can communicate well, not deaf, not mute, not mentally retarded and not senile) and were willing to be research subjects who did not meet the exclusion criteria (i.e. suffering from cushing disease, ectopic cushing syndrome, adrenal gland tumor based on anamnesis; suffering from Addison's disease, hypopituitarism based on history; consuming drugs regularly containing corticosteroids; there is a life-threatening organ dysfunction; and suffering from mental disorders with drug therapy).

The estimated number of subjects used for correlative analytic studies can be calculated using the following formula (Levy & Lameshow, 2009):

$$n = \left[\frac{z_{a+} z_{\beta}}{\frac{1}{2} \text{In} \left(\frac{1+p}{1-p} \right)} \right]^2 + 3$$

The sample size was calculated using the sample size formula for correlative analytic research as follows:

Information:

Zα = Standard normal distribution value (Table Z) at a certain α, Zα 5% = 1.96

Zβ = Standard normal distribution value (Table Z) at a certain b, Zβ 20% = 0.84

R = Correlation value from previous studies = 0.6 (because no previous research is the same)

Based on this calculation, the sample size in this study was obtained to be 20 subjects.

The sampling technique used was the consecutive sampling technique, namely the sampling technique by specifying subjects who met the research criteria were included in the study in the period February-April 2022 so that the number of samples reached 20.

Research Procedure

The research was carried out by preparing a research proposal and validation by the research supervisor. Then a research proposal was submitted to obtain ethical feasibility in the Universitas Airlangga Hospital, Surabaya. The research was then continued by collecting data through medical records, filling out the Depression anxiety stress scales-42 form, and taking hair cortisol. Afterward, the research data was processed and presented.

The source of hair shaft cortisol comes from circulation in the medullary region of the hair core. Cortisol, which was obtained from scalp hair, was a lipophilic substance derived from a vascular supply that irritates hair shaft follicle cells. Cortisol, which comes from the surface of the hair, mixes with sweat, hence cortisol was obtained by washing the hair first and grind it. The method of taking hair cortisol through washing, grinding and extraction would get free cortisol that was not bound to steroids. A 3 cm long hair sample was taken from the posterior scalp because of the lower variation of cortisol compared to other areas.

Hair cortisol can be obtained through the standard method (finely cut). Keep a look that the materials were prepared by positioning the hair cutting supplies (aluminum foil, envelope, salon scissors and fine-toothed comb, rubbing alcohol, masking tape, permanent marker, non-latex gloves [optional], and bobby pins). Scissors and comb should be cleaned with rubbing alcohol before taking a sample. Cut out an 8 x 10 inch piece of aluminum foil to use for the sample. At this stage, non-latex gloves were required. Split the hair using the comb horizontally along the posterior vertex of the scalp between the tips of the ears. Take the clip and remove the hair from the top section, then place another clip at the bottom so that a 5 x 10 cm rectangle appears between them. The cut part of the hair should be located on the posterior vertex 1-3 cm long. Grasp about 30 - 50 strands of hair on the right side of the rectangle, then gently pull the hair away from the scalp in a rolling motion between fingers. The standard technique involves clipping the hair as close to the scalp as possible. Collect the sample as close to the scalp as feasible, but avoid cutting into the scalp (Wright et al., 2018).

In the standard method, the hair was cut with special scissors (ROBOZ RS-5853; Gaithersburg, MD) so that it could cut the hair finely into a powder form and then weighed with an analytical balance (Mettler-Toledo AL54, Greifensee, Switzerland). Then, hair should be extracted with 1 ml of methanol and incubated at 52oC for 15 hours and rotated at 200 rpm followed by 1 ml of acetone which was rotated at 200 rpm for 5 minutes at room temperature. This extraction step was repeated 2 times and the supernatant was stored in an explosion-proof refrigerator at 4oC. The dry residue was dissolved in phosphate saline buffer according to the sample weight (eg 350μL for every 50mg hair) (Shi et al., 2021; Slominski et al., 2015).

Operational Framework

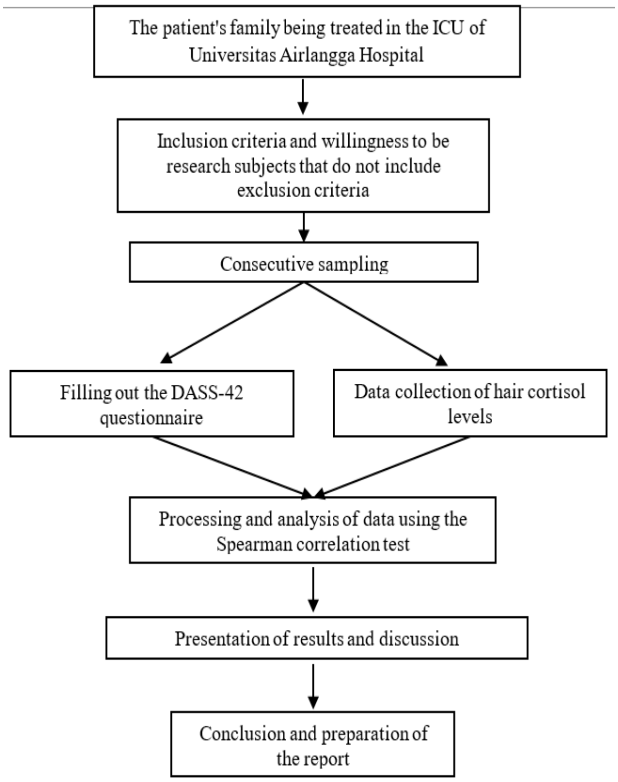

Fig 1: Operational Framework

The collected data was processed using IBM SPSS Statistics 21 software. Data processing was followed by analysis using statistical tests. The statistical test used was the Spearman correlation test, the reason for using the statistical test was adjusted to the research objectives in looking at the relationship between the 2 variables that were ratio and ordinal data scales.

Results and Discussion

From this study, the number of research samples that met the inclusion criteria was 23 subjects (100,0%). The characteristics of the research subjects presented in table 2 as follows.

Table 2: Characteristics of Research Subjects

Characteristics	N (%) 23 (100,0%)	Mean ± SD
Gender		
Man	15 (65,2%)	
Woman	8 (34,8%)	
Last education		
Bachelor	10 (43,5%)	
Diploma	2 (8,7%)	
Senior High School	9 (39,1%)	
Elementary School	2 (8,7%)	

Age (years)		37,91 ± 11,417
<30	7 (30,4%)	
30-40	8 (34,8%)	
41-50	5 (21,8%)	
>50	3 (13,0%)	
Work		
Doesn't work	5 (21,7%)	
Private	13 (56,5%)	
Civil Servant	2 (8,7%)	
Student	2 (8,7%)	
Builder	1 (4,4%)	
Relationship with patient		
Spouse (husband and wife)	6 (26,2%)	
Parent	3 (13,0)	
Child	11 (47,8%)	
Brothers / Relatives	3 (13,0%)	

For age characteristics, the mean ± SD was 37,91 ± 11,417 years. As for gender characteristics, 65,2% were men.

Stress Degree Data

Research subjects who met the inclusion and exclusion criteria then filled out the DASS-42 questionnaire. All research subjects filled out a complete questionnaire. Data description of research variables were shown in table 3. DASS-42 severity levels were categorized into 5 groups, namely normal, mild, moderate, severe and very severe severity. In addition, each category has its own score range which was divided into 3 categories, namely depression, anxiety and stress.

Table 3: Stress Degree Data

Severity	**Variable**		
	D	A	S
Normal	17 (73,9%)	16 (69,6%)	18 (78,3%)
Mild	2 (8,7%)	0	0
Moderate	3 (13,0%)	3 (13,0%)	4 (17,3%)
Severe	1 (4,4%)	1 (4,4%)	0
Very Severe	0	3 (13,0%)	1 (4,4%)

As many as 73,9% of normal patients were in the depression category, 69,6% of normal patients were in the anxiety category, and 78,3% of normal patients were in the stress category. Furthermore, statistical analysis was carried out for each of the DASS-42 categories, with non-parametric statistical tests, as shown in table 4.

Table 4: DASS-42 Category Data

Variable	P value
D (Depression)	0,018
A (Anxiety)	0,008
S (Stress)	0,030

Hair Cortisol Level Data

Table 5: Hair Cortisol Levels Descriptive

Variable	Mean ± SD	P value
Cortisol	0,68283 ± 0,146509	0,834

The source of cortisol was obtained from the hair of the research subjects which were then measured for cortisol levels using the Human Cortisol Elisa Kit. According to table 5, a descriptive data on hair cortisol levels showed that the data were normally distributed with a p value = 0,834.

Correlation Between Degrees of Stress and Hair Cortisol Levels

Table 6: Correlation of Degrees of Stress with Hair Cortisol Levels

Cortisol Level Correlation	Correlation Coefficient (r Spearman)	P value
Depression	0,299	0,165
Anxiety	0,138	0,530
Stress	-0,028	0,898

Based on the results of statistical tests, there was no significant correlation between the degree of stress and hair cortisol levels. For the Depression category, there was a positive correlation with weak correlation strength (r=0,299), as well as for the Anxiety category (r=0,138). Meanwhile, for the stress category, a negative correlation was obtained with very weak correlation strength (r = -0,028).

Characteristics of Research Subjects

The research involving families of COVID-19 patients who were treated at Universitas Airlangga Hospital aimed to analyze the correlation between hair cortisol levels and stress levels using DASS-42 in families of COVID-19 patients. After screening with inclusion and exclusion criteria, 23 research subjects were obtained. Afterward, measuring hair cortisol levels and filling out questionnaires was carried out for the patient's family.

From the research data, 65,2% of the subjects were men. The age distribution of research subjects was mostly in the age range of 30-40 years as much as 34,8%. For the characteristics of the level of education, 43,5% of the research subjects were undergraduate graduates, while for the characteristics of the type of work, 56,5% of the research subjects worked in the private sector. Most of the relationship between research subjects and COVID-19 patients were children of patients with 47,8%.

The COVID-19 pandemic has had an impact on both adults and children. Stress and uncertainty due to COVID-19 can affect parents and children so that they feel a physical and psychological burden. In addition, it also causes mental health disorders, including anxiety disorders and depression. The population most at risk of mental health disorders, one of which was COVID-19 patients and their families (Shigemura et al., 2020).

A study examining the impact of the COVID-19 pandemic on the community stated that global pressure during the pandemic resulted in various issue, one of which was fear and anxiety that occurred to oneself and those closest to them, changes in sleeping and eating patterns, feelings of depression and difficulty concentrating, boredom and stress. A study in the era of the COVID-19 pandemic was conducted on parents, which aimed to describe changes in parents' stress levels before and during the pandemic. From the results of the study, it was found that there was an increase in stress in parents by 71.1% of all research subjects. The most common causes of stress were changes to family routines, and concerns about COVID-19 (Adams et al., 2021).

Stress Degree Data

Based on filling out the DASS-42 questionnaire by research subjects, severity data were obtained which were categorized into 5, namely normal, mild, moderate, severe and very severe. A total of 4 research subjects (17,3%) experienced moderate stress, and 1 research subject (4,4%) experienced very severe stress.

Families of COVID-19 patients with ARDS were burdened with a greater psychological stress, including PTSD, anxiety, and depression. Limiting or reducing patient visiting hours, which causes changes in the everyday lives of patients and their families, has become one of the processes that might have a psychological impact on the families of COVID-19 patients. If allowed to visit, the family was required to use personal protective equipment which can be a barrier to effective communication between the patient and his family. In addition, contact between doctors who treat patients and the patient's family was also reduced, which causes communication to not work effectively. Families often get information about patients by telephone because families were not allowed to visit the patients (Azoulay et al., 2022).

The DASS-42 was an instrument that can be utilized to assess mental health conditions such as anxiety, stress, and depression. The purpose of this questionnaire was to determine the severity of the stressful condition. A study on stress levels was conducted on COVID-19 patients using the DASS-42 scale which showed that 97% of research subjects experienced symptoms of stress, of which 84.9% experienced severe and very severe stress during treatment (Zandifar et al., 2020).

The DASS-42 questionnaire also refers to a self-report questionnaire with a Likert-style answer choice, so it could bias the research results, including if the subject does not answer honestly, especially to questions that was sensitive. There was a tendency for research subjects to answer according to socially accepted norms. Another factor that contributes to bias would be the tendency to answer yes or no, replying at the extreme scale of all questions; variances in question interpretation; the presence of the researcher when the subject completed the questionnaire; and flexible offline questionnaires (McDonald et al., 2008). The DASS-42 questionnaire was not used to provide a clinical diagnosis, but rather to characterize symptoms or emotional states throughout the previous week. A combination of questionnaire-based assessments and direct examination of study subjects may provide a more comprehensive picture of the subject's psychological condition.

Hair Cortisol Level Data

Hair hormone cortisol levels in this study were ranged from 0,368 – 1,028 ng/ml, with an average value of 0,68283 ng/ml. Hair cortisol samples were obtained by cutting 3 cm from the posterior of the scalp because of the lower variation of cortisol compared to other areas. Cortisol in the hair could be used to assess stress in the families of COVID-19 patients as cortisol levels in hair might change as a result of stress (Wright et al., 2015). A study that examined changes in hair cortisol levels showed that changes in cortisol levels were related to the stress experienced, so that it could be concluded that the use of examining hair cortisol levels as an appropriate biomarker in evaluating stressful events (Wright et al., 2015). COVID-19 was considered to have a negative effects on mental health, including symptoms of anxiety, depression, and post-traumatic stress disorder (Ibar et al., 2021).

In this study, data on hair cortisol levels were normally distributed, so a parametric test was used to analyze the data. Based on the results of statistical analysis tests, it was found that there was a significant difference ($p<0.001$) in the hair cortisol levels in all research subjects. A study that analyzed changes in hair cortisol levels before and after the COVID-19 pandemic, showed an increase in hair cortisol levels by 29% (Marcil et al., 2022). In this study there was no comparative data for hair cortisol levels, because hair cortisol samples were only taken once, namely when the patient's family received an explanation regarding the initial condition of the patient who started receiving ICU care. Hair cortisol levels may be used as a retrospective cortisol marker, which can describe chronic stress events. HPA axis retrospective activity in the past 3 months was measured 3 cm proximal to the hair. Hair grows at a rate of 1cm every month. Each 1 cm represents HPA axis activity for 1 month, hair cortisol analysis offers the possibility of long-term HPA axis activity, and for comparing multiple hair segments, including segments before a stressful event occurs. Hair cortisol levels described levels of free or bound cortisol and was not affected by oral contraceptive drugs (Staufenbiel et al., 2013).

Correlation between Degrees of Stress and Hair Cortisol Levels

Furthermore, data on the degree of stress and hair cortisol levels were analyzed, to show a correlation between the two. From the results of statistical analysis, it was found that each category in DASS-42 which represented the stress condition of the research subjects did not show a significant correlation with hair cortisol levels. This finding was different with the theory that stimulation from psychological stress would activate the HPA axis thereby increasing the production of the hormone cortisol. This condition may be caused by no significant stress level obtained from the subjects in this study (the majority were within normal stress levels based on DASS-42) and levels of the hair hormone cortisol were lower than the normal range. A study that analyzed the correlation between stress and hair cortisol levels, showed the result that 40% of the population showed an increase in hair cortisol levels (Ibar et al., 2021). Another study showed that 75% of research subjects stated that the COVID-19 pandemic increased stressful events, so that hair cortisol levels also increased during a pandemic, compared to before. This can be interpreted as a relationship between long-term stress and hair cortisol levels. Evidence of increased hair cortisol levels under conditions of chronic stress has been studied previously, for example in shift workers, unemployed, dementia patient nurses, and burnout populations (Rajcani et al., 2021).

In a study conducted to assess the quality of life and mental status in the youth community after the third wave of COVID-19, it was stated that, in the research subjects, there was an increase in quality of life and a decrease in mental status problems compared to the previous wave of COVID-19. This was due to looser restrictions, better school organization, and expectations of vaccination that increase optimism. In addition, the reduced level of psychological problems may be the result of adaptation processes to stressful factors that have already occurred. In Lazarus' transactional theory, stress was defined as a relationship between the individual and the environment that may be influenced by two mediators, 'cognitive judgment' and 'coping'. Whereas the assessment of stressful situations (eg, COVID-19 outbreak) was based on factors such as: expectations, individual dispositions, predictability, and control and influence individual stress reactions, coping includes cognitive and behavioral strategies to manage, tolerate, or reduce stress reactions. The increased rates of mental health problems and decreased quality of life in the first year of COVID-19 could be interpreted as a reaction to stress caused by worries about health and unexpected changes in the social, economic and personal lives of the population. This was consistent with the impact of phases one and two of the epidemic where restrictions on public and private life and shutdowns of health services could increase psychological distress, but also decrease the likelihood of receiving assistance, for example, from the health care system. Phase three was seen as the 'return to normality' phase, during which reordering and rebuilding of services and practices takes place. In this phase, new coping strategies and rules (eg, vaccinations, tests, wearing masks) have been developed and reduce perceived stress as they made situations more predictable and controllable. Hence, this may affect mental health and quality of life in general. Furthermore, the decision to get vaccinated could be interpreted as an individual coping strategy which in turn can increase self-efficacy and lower psychological stress (Hussong et al., 2022).

The findings of this investigation were limited in a number of ways. First, this study only looked at the DASS-42 scores of the patient's family members when the patient was first hospitalized to the intensive care unit, but every centimeter of hair indicated HPA activity for an entire month. Second, in order to validate the findings, an investigation involving other centers was required. Nevertheless, there were a number of positive aspects to this research. This study was the first of its kind to investigate whether or not there is a correlation between the levels of hair cortisol and the levels of stress experienced by the families of COVID-19 patients in Surabaya. The diurnal cycle does not have any effect on the amounts of cortisol found in hair. In addition, the findings of this research have made a contribution to the expansion of information concerning the relationship between levels of cortisol in hair and stress.

Conclusions

Based on statistical analysis and discussion on the correlation between stress levels and hair cortisol levels in families of COVID-19 patients, it was found that 17.3% of families of COVID-19 patients experienced moderate stress and 4.4% experienced very severe stress based on DASS-42. In addition, cortisol levels in the hair were within normal limits. Meanwhile, no relationship was found between the degree of stress and hair cortisol levels in families of COVID-19 patients. 2871

In order to reap the benefits of this study's findings, the researcher suggests conducting follow-up studies involving multicenters to confirm the study's findings, as well as undertaking follow-up research with longer observations and using a cohort or case-control design as well as measuring cortisol levels more than once for comparison. Additionaly, thorough investigation with involving other stress factor variables so as to describe the relationship between the degree of stress and cortisol levels can also be carried out.

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