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Demographic, clinical characteristics and psychosocial factors associated with salivary cortisol as a stress biomarker in children with leukemia

Características demográficas, clínicas y factores psicosociales asociados al cortisol salival como biomarcador de estrés en niños con leucemia

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SUMMARY

Objective: *This study aims to determine the relationship among demographic, clinical characteristics, and psychosocial factors related to salivary cortisol in children with leukemia.* **Methods:** *This research was a descriptive study with a cross-sectional design. The sample was children with leukemia in Dr. Sardjito General Hospital Yogyakarta from January to February 2018. The sample size was 30 respondents who had met the inclusion criteria, was aged 6-18 years, did not experience an emergency condition, and did not eat and drink 30 minutes before the saliva sample was taken. Salivary samples were assessed for free cortisol levels using an ELISA kit 96 wells DES661.* **Results:** *The salivary cortisol level was in the range of 0.19 ng/dL to 16.67 ng/dL. Most of the respondents showed a high level of stress in the category of severe stress (70 %) with the Mean ± SD (of 5.2665±3.3430 ng/dL). There was no relationship between demographic and clinical characteristic factors with cortisol levels in children with leukemia (p> 0.05).* **Conclusion:** *Although this study showed that there is no relationship between salivary cortisol levels and demographic factors as well as clinical characteristics, the results indicate that there was a link between psychosocial factors and increasing salivary cortisol levels in children with leukemia. Thus, the results of this study can be the basis for hospitals and health workers to provide care strategies and interventions to help children who are at risk of experiencing psychosocial problems.*

Keywords: *Biomarkers, cortisol, leukemia, pediatrics, stress.*

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RESUMEN

Objetivo: *El objetivo de este estudio es determinar la relación entre las características demográficas, clínicas y los factores psicosociales relacionados con el cortisol salival en niños con leucemia.* **Métodos:** *Esta investigación fue un estudio descriptivo con un diseño transversal. La muestra fueron niños con leucemia en el Dr. Sardjito General Hospital Yogyakarta durante el periodo de enero a febrero de 2018. El tamaño de la muestra fue de 30 encuestados que cumplieron con los criterios de inclusión, con edad entre 6 y 18 años, que no experimentaron una condición de emergencia y no comieron ni bebieron 30 minutos antes de la toma de la muestra de saliva. Las muestras de saliva se cuantificaron para cortisol libre utilizando un kit ELISA de 96 pocillos DES661.* **Resultados:** *El nivel de cortisol salival estuvo en el rango de 0,19 ng/dL a 16,67 ng/dL. La mayoría de los encuestados mostró un alto nivel de estrés en la categoría de estrés severo (70 %) con la Media ± DE (5.2665±3.3430 ng/dL). No hubo relación entre los factores característicos demográficos y clínicos con los niveles de cortisol en niños con leucemia (p> 0,05).* **Conclusión:** *Aunque este estudio no proporcionó evidencia de una relación entre los niveles salivales de cortisol y los factores demográficos, así como las características clínicas, los resultados indicaron que había un vínculo entre los factores psicosociales y el aumento de los niveles salivales de cortisol en niños con leucemia. Por lo tanto, los resultados pueden ser la base para que los hospitales y los trabajadores de la salud brinden estrategias de atención e intervenciones para ayudar a los niños que están en riesgo de experimentar problemas psicosociales.*

Palabras clave: *Biomarcadores, cortisol, leucemia, pediatría, estrés.*

INTRODUCTION

Cancer is the leading cause of death in children worldwide, and its incidence tends to increase yearly (1). Cancer with the highest incidence in children is Leukemia (2,3). Leukemia in children is a major threat to public health worldwide, including in developing countries such as Indonesia. Based on research data in 15 regencies and cities in Indonesia in 2011, it was reported that Leukemia was the most common cancer as the cause of death in children aged 0-18 years (4). Acute Lymphoblastic Leukemia (ALL) is the largest contributor to childhood leukemia, with approximately 75 % of cases (4,5).

Over the last few years, technological advances have made significant and continuous improvements in the treatment of children with cancer, which, of course, has an impact on their survival (6,7). However, it is undeniable that being diagnosed and treated for cancer can make children stress and potentially traumatized (8). Children with Leukemia will undergo a series of treatment and diagnostic procedures within a sufficient period (9). This prolonged treatment process predisposes children to a variety of painful invasive procedures (10) and it can result in children having long-term morbidity, which in turn can increase mortality in children with cancer, especially Leukemia (11).

Evidence indicate that chronic diseases, including Leukemia, are the leading causes of stress for children (12,13), and are related to symptoms of anxiety, stress, and depression (14). Symptoms of anxiety, stress, and depression during treatment potentially harm the quality of their life (15). Chronic health conditions are associated with high levels of problems in physical, emotional, developmental, and behavioral problems that decrease children's quality of life (12,13). In fact, children were unable to adapt to trauma due to physical illness suffered in the long term which has consequences for psychiatric disorders such as post-traumatic stress disorder (PTSD) and depression with suicidal ideation or suicide attempts. Furthermore, properly unidentified, and unmanaged stress symptoms can lead to post-therapy stress and suicidal ideation in adulthood (14,16).

It is known that during a stress reaction, the hypothalamus-pituitary-adrenal glands (HPA) axis activity is increased. At the hypothalamic level, the stressor activates Cortico-Releasing Hormone and vasopressin release; these hormones stimulate the anterior lobe of the pituitary gland, which releases ACTH (adrenocorticotropic hormone), which in turn stimulates glucocorticoid (cortisol) synthesis and secretion in adrenal cells. Thus, the assessment of cortisol levels is an alternative to identifying stress responses in children with cancer (17). Serum-free cortisol or

its surrogate, salivary cortisol as opposed to total cortisol concentrations, offers a better reflection of the activation of the HPA axis, being cortisol the gold standard for hormone examination and is considered a biochemical marker for acute and chronic stress (18,19). The concentration of this the hormone in the saliva accounts for 70 % of the non-bound blood cortisol that enters saliva by diffusion through the basolateral membrane of the salivary gland acini. Salivary cortisol correlates highly with free blood cortisol for it is independent of the transport mechanisms and the type, quantity, and low of saliva. While salivary cortisol reflects levels of bioactive-free cortisol, salivary cortisol also correlates closely with serum-free cortisol over 24 hours. The very method of taking salivary samples is simple, standardized, safe, non-invasive, less stressful, and does not cause stress in children during sampling, which can affect serum cortisol concentrations; the method is easy to repeat and does not require any special level of training or equipment (20). Salivary cortisol has been widely used to identify the presence of stress or mental health disorders in cancer patients, especially those in treatment and diagnosis (6). Salivary cortisol concentration reflects exposure to a stressor, however, the relationship between cortisol levels and demographic, clinical, and psychosocial characteristics in children with chronic conditions such as leukemia is still very limited. This study aims to evaluate the relationship between demographic factors, clinical and psychosocial characteristics, to increased salivary cortisol levels in children with leukemia.

METHODS

Study Design

This research was a descriptive study with a cross-sectional design. Was conducted in Dr. Sardjito General Hospital Yogyakarta from January to February 2018. The research received the approval from the ethics committee team and obtaining permission from the research location.

Participants and sample size

The study was limited and focused on children with Leukemia in Dr. Sardjito General Hospital Yogyakarta. The sample size in this study was 30 respondents, who had met the inclusion criteria, including aged 6-18 years, who did not experience an emergency condition and who did not eat and drink 30 minutes before the saliva sampling was taken. In contrast, the exclusion criteria are uncooperative children and parents who are not willing to have their children become respondents. All children who meet the criteria are involved as research samples. All patients in this study received corticosteroid therapy as part of their treatment protocol, so it was assumed that all patients had the same or homogeneous conditions.

Instrument

ELISA kit 96 wells DES661, an enzyme immunoassay for the quantitative determination of free cortisol in saliva, was used to measure salivary cortisol levels. This assay presents a Standard range of detection of 0.1-30 ng/mL and a sensitivity of 0.019 ng/mL. Salivary cortisol concentration was measured at the Clinical Pathology Laboratory, Gadjah Mada University. Cortisol levels reflect stress levels. The categories of salivary cortisol levels included mild stress (0 - <1.5 ng/dL), moderate stress (1.5 - < 2.5 ng/dL), and severe stress (>2.5 ng/dL) (21).

Analysis

Cortisol levels in all respondents were expressed in ng/mL and reported graphically. Data on demographic, clinical characteristics, and categories of salivary cortisol levels were shown as frequency distribution tables. To assess the relationship between demographic factors and clinical characteristics with concentrations of cortisol levels in children with leukemia, the Chi-Square test was used. The results were analyzed using The IBM SPSS Statistics version 26.0.

RESULTS

Overview of cortisol levels in all research respondents

All patients in this study were pediatric leukemia who were receiving corticosteroid therapy. An overview of cortisol levels in all research respondents is shown in Figure 1.

Figure 1 shows that salivary cortisol varied in respondents, and it was in the range of 0.19 ng/dL to 16.67 ng/dL.

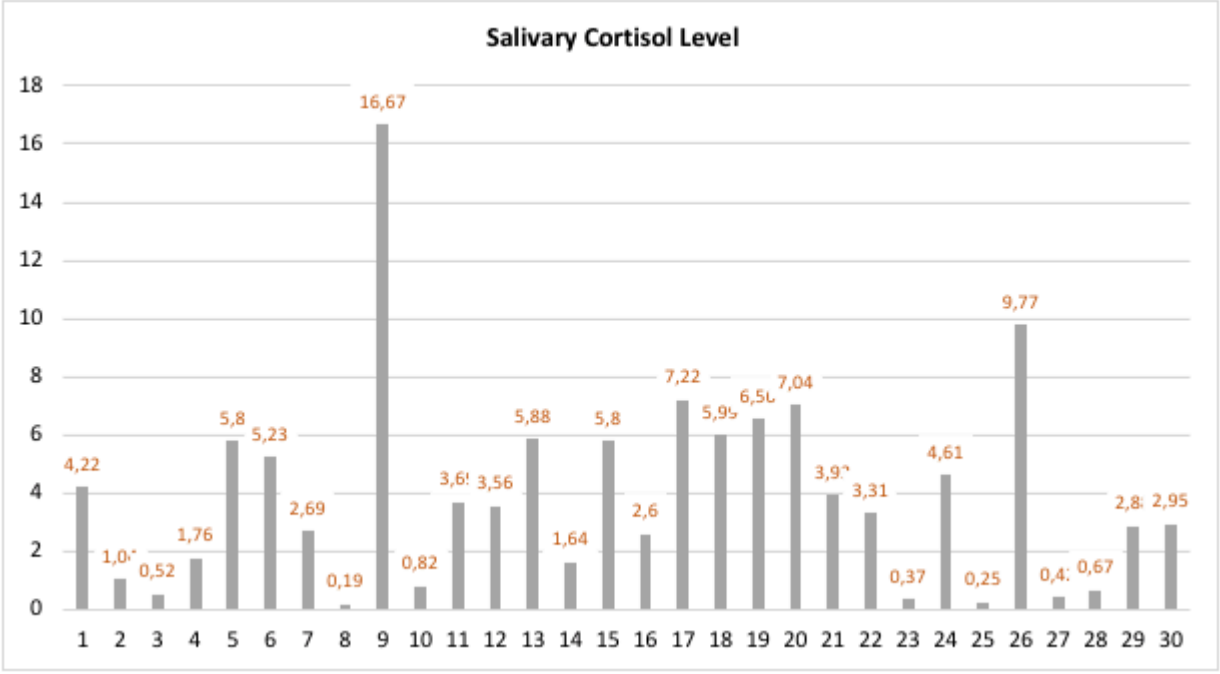

Figure 1. Salivary cortisol level in ng/dL

Characteristics of respondents based on demographics and clinical characteristics

Our results show that most of the respondents (76.7 %) are 6-12 years old and most are male (66.7 %). The clinical characteristics of the respondents indicated that *Acute Lymphocytic Leukemia* (ALL) was the most dominant type of leukemia, which was 90 % of our cases. They are currently undergoing chemotherapy (80 %) and most of which are in the maintenance phase (63.4 %). Anemia was the most common comorbid condition (66.6 %).

An overview of salivary cortisol levels categorized by the stress level

Salivary cortisol levels are categorized into mild stress, moderate stress, and severe stress. Table 2 shows the stress levels of all respondents.

Based on Table 2, the average of the research respondents had cortisol levels that were increased or higher than normal (3.37 ng/dL). Most of the respondents showed a high level of stress in the category of severe stress (70 %) with the Mean ± SD of 5.2665 ± 3.3430 ng/dL.

Table 1. Characteristics of respondents based on clinical characteristics.

Variable	Characteristics	(n = 30)	Percentage (%)
Age	School-age (6-12)	23	76.7
	Teenagers (13-18)	7	23.3
Sex	Male	20	66.7
	Female	10	33.3
Types of Leukemia	ALL	27	90
	AML	3	10
	CML	0	0
Type of treatment	Chemotherapy	24	80
	Transfusion	2	6.6
	BMP	4	13.4
Chemotherapy Phase	Induction Phase	7	23.3
	Consolidation phase	3	10
	Maintenance phase	19	63.4
	Relapse	1	3.3
Comorbidities	Anemia	18	60.0
	Anemia and bell's palsy	1	3.3
	Anemia and fever	1	3.3
	Sepsis	1	3.3
	No comorbidities	9	30.0

ALL (Acute Lymphoblastic Leukemia)
AML (Acute Myeloid Leukemia)
CML (Chronic Myeloid Leukemia)
BMP (Bone morphogenetic protein signaling)

Table 2. Salivary cortisol levels are categorized by the stress level

Cortisol level (ng/mL) (Mean ± SD)	n (%)	Stress Category
0.5835 ± 0.2764	7 (23.3)	Mild stress
1.6989 ± 0.0876	2 (6.7)	Moderate stress
5.2665 ± 3.3430	21 (70.0)	Severe stress

The Correlation between demographics and clinical characteristics with stress levels

The relationship between demographic factors and clinical characteristics with stress levels is shown in Table 3.

Table 3 shows that most children aged 6-12 years experience severe stress (53.3 %) and males experienced the most acute stress (50 %). Meanwhile, children who experience severe or chronic stress are with an elementary school level of education (43.3 %). There was no significant relationship between demographic factors and salivary cortisol levels in children with leukemia ($p>0.05$). Most of the children with type ALL have severe stress (43.3 %) and chemotherapy is the type of treatment that has severe stress a 53.3 %. Children 43 % are in the maintenance phase generating severe stress. Meanwhile, almost all children have comorbidities that indicate severe stress (40 %). It was found that there was no relationship between clinical characteristics with salivary cortisol levels in children with leukemia ($p>0.05$).

Table 3. Correlation of demographic factors and clinical characteristics with stress levels

Variable	Characteristics	Salivary cortisol levels category						Total	p-value
		Mild Stress		Moderate Stress		Severe Stress			
		n	(%)	n	(%)	n	(%)		
Age	School-age (6-12)	5	(16.7)	2	(6.7)	16	(53.3)	23	0.698
	Teenagers (13-18)	2	(6.7)	0	(0.0)	5	(16.7)	7	
Sex	Male	3	(10.0)	2	(6.7)	15	(50.0)	20	0.223
	Female	4	(13.3)	0	(0.0)	6	(20.0)	10	
Types of Leukemia	ALL	6	(20)	2	(6.7)	19	(43.3)	27	0.86
	AML	1	(3,3)	1	(3.3)	1	(3.3)	3	
	CML	0	(0.0)	0	(0.0)	0	(0.0)	0	
Undergoing treatment	Chemotherapy	6	(20.0)	2	(6.7)	16	(53.3)	24	0.603
	Transfusion	1	(3.3)	0	(0.0)	1	(3.3)	2	
	Bone Marrow Puncture	0	(0.0)	0	(0.0)	4	(13.3)	4	
Chemotherapy Phase	Induction Phase	2	(6.7)	0	(0.0)	4	(23.3)	7	0.887
	Consolidation phase	1	(3.3)	0	(0.0)	2	(6.7)	3	
	Maintenance phase	4	(13.3)	2	(6.7)	13	(43.3)	19	
	Relapse	0	(0.0)	0	(0.0)	1	(3.3)	1	
Comorbidities	Anemia	5	(16.7)	1	(3.3)	12	(40.0)	18	0.987
	Anemia and bell's Palsy	0	(0.0)	0	(0.0)	1	(3.3)	1	
	Anemia and fever	0	(0.0)	0	(0.0)	1	(3.3)	1	
	Sepsis	0	(0.0)	0	(0.0)	1	(3.3)	1	
	No comorbidities	2	(6.7)	1	(3.3)	6	(20.0)	9	

DISCUSSION

Leukemia is a chronic disease that can cause severe stress to the sufferer. Chronic stress and anxiety can significantly impact self-esteem, relationships with friends and family, and overall quality of life. Biomarkers of stress and anxiety may be clinically useful to detect psychological distress, particularly in those who may not express their concerns to others. Severe stress has been identified as influencing hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, increasing cortisol levels (22). Cortisol is a major mediator of the effects of the HPA axis during psychological stress (23). An accessible biomarker, salivary cortisol, can be used to measure hypothalamic-pituitary-adrenal (HPA) axis activity. As a biomarker of stress, cortisol can be identified through body fluids. Saliva is the most frequently used diagnostic medium to assess cortisol levels because it is easier and less traumatic in children and the use of salivary cortisol as a stress biomarker has been used for the last 20 years (24).

Demographic factors on cortisol levels in children with leukemia

Leukemia can occur in infants, toddlers, children, and adults. Our present results indicate that respondents were dominated by children aged 6-12 years. Based on a review of all types of cancer in children under 18 years of age diagnosed at Dr. Sardjito Hospital Yogyakarta, it was shown that for the last ten years (2000-2009), the peak age of children diagnosed with Leukemia was in the age of infants and toddlers (0-5 years), followed by the age of children (6-12 years) and a gradual and progressive decline in early adolescence (25). However, other studies mention that the incidence of cancer in children is high in infants and toddlers (0-4 years), decreases during childhood, then reappears in adolescents (>14 years) (26).

Our data indicate that there was no relationship between age and children's cortisol levels. This is in line with previous research (27), and contrary to previous research which explains that age affects cortisol levels (28). It was shown that cortisol levels increases with age, being cortisol levels in adolescents higher than in children (29). Older children are prone to prolonged adrenal insufficiency, whereas younger children show lower cortisol values, resulting in a faster recovery (30).

The gender variable has a striking difference, in fact, the number of male respondents was higher than females. This is in accordance with data from the 2015 global burden of cancer, which showed that the incidence of Leukemia in males was higher than in females (31). Surveys on the incidence of Leukemia worldwide also show that the percentage of Leukemia in men reaches 5.6 %, while in women is 2.3 % (32,33). The results of this study indicate that the gender factor is not related to cortisol levels. This is not in accordance with several previous studies that reported that men showed a more significant increase in cortisol response than women, where the peak response for both men and women occurred 15 minutes after exposure to stress (34). Other studies also suggest that girls have higher cortisol, stronger circadian rhythms, and more significant developmental influence in adolescence (35). Cortisol levels were positively associated with increased pain tolerance in boys and increased pain sensitivity in girls (36).

Clinical characteristic factors on cortisol levels in children with leukemia

Based on the type of Leukemia, Acute Lymphoblastic Leukemia (ALL) is the predominant type of Leukemia among respondents when compared to other types of childhood leukemia. The incidence of Acute Lymphoblastic Leukemia (ALL) reaches 86 % of cancers in children. In Japan, about 500 children are diagnosed with Acute Lymphoblastic Leukemia every year (37). This is also in line with the results of a study that reported that Acute Lymphoblastic Leukemia occurs five times more often than Acute Myeloid Leukemia (AML) and accounts for about three-quarters of all childhood leukemia diagnoses (7,38). Our present results indicate that the type of Leukemia has no relationship with cortisol levels. Literature explaining the relationship between the type of leukemia and salivary cortisol levels in children with leukemia has not been found.

In this study, all respondents showed high salivary cortisol values, but there was no relationship between the type of treatment they underwent and the salivary cortisol values. Most of the respondents who showed severe stress were respondents who underwent chemotherapy. Chemotherapy is one of the therapies undertaken by children diagnosed with leukemia (39). Treatment using corticosteroids plays an important role in Leukemia patients, especially in patients with acute lymphoblastic leukemia (ALL) (40). Children with acute lymphoblastic leukemia (ALL) usually receive multidrug agents during chemotherapy. Chemotherapy for leukemia patients, especially Acute Lymphoblastic Leukemia (ALL) can directly impact children's emotional functioning, one of which is the effect of using systemic corticosteroids (41). In this study, all respondents received corticosteroid therapy as part of their therapy protocol. Increasing cortisol salivary levels in the respondents of this study can be associated with the use of these drugs. Although the use of these drugs is important in the process of treating Leukemia patients, the use of corticosteroids can cause suppression of the hypothalamus-adrenal-pituitary axis (HPA) (22). A systematic review and meta-analysis (42) also stated that all patients receiving corticosteroid therapy were at risk for adrenal insufficiency. Adrenal insufficiency is manifested by impaired cortisol response (43).

All respondents in this study were in the process of chemotherapy, and most of them were in the maintenance phase. The data show that there was no relationship between the chemotherapy phase and the increase in salivary cortisol values, thus it can be assumed that each stage in the chemotherapy process can increase cortisol levels. This increase in salivary cortisol begins when the patient is in the chemotherapy induction phase (40). The maintenance phase of chemotherapy is one of the most effective in reducing relapse and has contributed significantly to the excellent outcome of childhood acute lymphoblastic leukemia (ALL). In the

maintenance phase of chemotherapy, patients receive low-intensity treatment, but even with low-intensity treatment, drug toxicity is quite common (44).

Most of the research respondents suffer from comorbidities such as anemia, fever, sepsis, and Bell's palsy. The most common comorbidity with Leukemia is anemia. Anemia that occurs may be caused by the degree of malignancy of cancer, cancer treatment, blood loss, malnutrition, hemolysis, endocrine disorders, or inflammatory cytokines associated with a chronic disease (45). Comorbidities such as acute and chronic diseases, endocrine, autoimmune diseases, and sepsis significantly affect cortisol levels (46).

Psychosocial factors on cortisol levels in children with leukemia

The standard value of salivary cortisol is 0.5 - 2.16 ng/dL (47). Respondents in this study had an average salivary cortisol level of 3.37 ng/dL. This shows that almost all children with Leukemia have salivary cortisol levels higher than normal values. Cortisol levels are categorized into mild stress, moderate stress, and severe stress. Some respondents showed high cortisol levels (severe stress). This might be caused by several psychosocial factors that occurred during the study.

One child had very high cortisol levels compared to other respondents (16.67 ng/dL). After identification, these respondents experienced a relapse after 1.5 years free from treatment. In addition, during the research process, the respondent's mother cried beside the child who was undergoing chemotherapy. The conditions of relapse and sadness experienced by parents can be exposed to children's stress. Exposure to excessive stress due to this relapse can cause a permanent increase in cortisol regulation (27). An important finding in this study is the impact of uncertainty felt by sufferers and their families, where they think they are between the hope of recovery and death. This uncertainty causes high anxiety and distress for sufferers and their families, which will impact extreme increases in cortisol levels (48,49).

Another respondent had an extreme cortisol level of 9.77 ng/dL. This respondent was a patient who had just been diagnosed with Leukemia and was undergoing the first week of the chemotherapy induction phase. The first week of the induction phase is a leukemia child's first experience undergoing chemotherapy. Diagnosis of the disease and the first hospitalization experience can cause anxiety, fear, and stressful conditions in children. Previous studies have shown that chronic illness and hospitalization are children's leading causes of stress (50), causing fear in children during the first year of treatment (51).

Two children with Leukemia had comorbidities such as Bell's palsy and sepsis and showed relatively high salivary cortisol levels of 7.22 and 7.04 ng/mL, respectively. The diagnosis of Bell's palsy and sepsis resulted in the child suffering from defects in the facial area around the nose and mouth. Elevated high cortisol levels are associated with disability (52). During the study, the respondent's parents said the child felt insecure if the face mask was opened. In addition, when the saliva collection was carried out, the respondent had difficulty expelling saliva. In addition to stressors from the disease and the treatment process, insecurity, and difficulty in salivating due to defects in the nose and mouth can be additional stress exposure.

Children with Leukemia have a high cortisol level, and even some respondents show an extreme cortisol level. We did not find a relationship between demographic factors and clinical characteristics with the concentration of cortisol levels in children with Leukemia. However, based on identification during the study, it was reported that there was a link between psychosocial factors and increasing cortisol levels in children with Leukemia. Extremely elevated cortisol levels may be caused by several conditions, including relapse after 1.5 years of being declared cured of the disease, recently diagnosed with Leukemia, undergoing the first week of the chemotherapy induction phase, having a disability, and having multiple comorbidities.

Present results can be the basis for hospitals and health workers in providing strategies care, and interventions to help children with psychosocial

problems. Interventions carried out include increasing family and peer support, psychological preparation for invasive procedures, use of play therapy, music therapy, clown therapy, and other nonpharmacological interventions.

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Author's contributions

Conceptualization: IH, NN, IK, ZH, NQ, YSA, SZM, TR, Data curation: SZM, ZH, Formal analysis: IH, ZH, Investigation: NN, IK Methodology IH, NN, IK, ZH, SZM, TR, supervision: NN, IK, IH, NQ, YSA, Project administration: IH, TR, Writing– original draft: IH, NN, IK, ZH, SZM, TR, and Writing– review & editing: IH, NN, IK, NQ, YSA, ZH, SZM, TR.

Conflict of interests

The authors have no conflict of interest to declare.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Ethical approval

This study received ethical approval from the Ethical Committee of Medicine and Health, Faculty of Medicine, Public Health and Nursing, Gadjah Mada University, Yogyakarta on 15 December 2017, Number KE/FK/1295/EC/2017.

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