Relationship Between Cysteine, Interleukin (Il)-2, and Interleukin (Il)-10 in Children with Marasmus Type Malnutrition

Teguh Wahyudi[1, 3*], Anik Puryatni[2, 3], Tinny Endang Hernowati[4]

[1]Biomedical Science Master Study Program, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[2]Pediatric Department, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[3]dr. Saiful Anwar Hospital, Malang, Indonesia
[4]Faculty of Medicine, Brawijaya University, Malang, Indonesia

ABSTRACT

Malnutrition is one of the health issues in developing countries. The most commonly found malnutrition is the marasmus type. Infection in marasmus patient is the main cause of morbidity and mortality in developing countries. In marasmus, there is a decrease in protein level such as cysteine which is one of the glutathione forming components that plays a significant role in immune system. In malnutrition, there is a disturbance of lymphocyte in the form of down-regulation of type 1 cytokine (IL-2 and IFN-γ) and up-regulation of type 2 cytokine (IL-4 and IL-10). IL-2 is needed for the development of regulatory T produced by thymus and for NK cell cytotoxicity which plays a role in infection process, while IL-10 inhibits activation of lymphocyte T so the cellular immunity reaction ends. Several studies about the relationship between cysteine, IL-2, and IL-10 have been done in malnutrition-patients, but there is no study focusing on patients with marasmus. This study is to find out the relationship between cysteine, IL-2, and IL-10 in patients with marasmus type malnutrition. This study was an observational analytic study using cross-sectional design consisting of 20 children with marasmus type malnutrition and 20 well-nourished children treated in Saiful Anwar Hospital Malang. The cysteine, IL-2, and IL-10 level then measured using Elisa method. Normality and the various test were done. The Pearson correlation test was done to find out the relationship between cysteine and IL-2 level, cysteine and IL-10 level, and IL-2 and IL-10. The standard of cysteine and IL-2 level in children with marasmus is significantly lower than the control group, which was 1.616 ± 1.039 vs 3.298 ± 0.519 pg/mL; p = 0.000 dan 12.38±4.94 vs. 16.58 ± 4.80 pg/mL; p = 0.010, respectively. IL-10 in children with marasmus was significantly higher than control group (19.08± 5.93 vs 10.46 ± 3.90 pg/mL; p = 0.000). The cysteine level was positively correlated to the IL-2 level (p = 0.000; r = 0.71), while the cysteine and IL-10, IL-2 and IL-10 was negatively correlated with p = 0.014; r= -0.53 and p=0.037; r= -0.46 in marasmus. There was a positive correlation between the cysteine and IL-2 level and negative correlation between IL-10 and IL-2 also IL-2 and IL-10 in children with marasmus.

Keywords: *serum cysteine level, IL-2 level, IL-10 level, marasmus type malnutrition*

INTRODUCTION

Malnutrition is one of the health issues in the world, especially in developing countries in Asia, Africa, Central America, and South America [1, 2]. RISKESDAS 2013 data shows that the national prevalence of malnutrition in 2013 is 5.7%. There is an increase compared to 2007 (5.4%) and 2010 (4.9%). In East Java alone, there is 19.1% children under the age of 5 with malnutrition with the absolute number of 568.482 from 2.976.344 children under the age of 5 [3]. The most commonly found malnutrition patients are the marasmus type. Arif from Dr. Sutomo Hospital Surabaya finds 47% children with marasmus type malnutrition [4].

Data from WHO shows that 54% morbidity in children under the age of 5 is caused by malnutrition, 19% diarrhea, 19% acute respiratory tract infection, 18% perinatal, 7% measles, 5% malaria, and 32% other causes [5].

*Corresponding author:
Teguh Wahyudi
Biomedical Science Master Study Program, Faculty of Medicine, Brawijaya University
Jalan Veteran, Malang 65145, Indonesia
E-mail: tgoeh.wahyoedi@gmail.com

Table 1. Subject characteristics

Subject characteristics		*Patient (n=20)*	*Control (n=20)*
Mean age (months)		53.35 ± 35.38	64.15 ± 39.54
Age distribution	< 5 years old	13	11
	5-10 years old	5	6
	10-15 years old	2	3
Sex	Boys	12	11
	Girls	8	9
Diagnosis	Pneumonia	8	7
	Acute respiratory tract infection	2	6
	Diarrhea	4	3
	Acyanotic CHD	6	1
	Dengue fever	0	3
Hemoglobin (g/dL)		10.07±0.94	12.49±1.10
Albumin (g/dL)		3.20±0.27	3.84±0.34
Leukocyte (g/dL)		11.74±4.01	11.95±4.24
Ureum (mg/dL)		25.76±11.57	22.59±9.99
Creatinin (mg/dL)		0.61±0.24	0.46±0.20
SGOT (U/L)		28.25±8.42	23.60±8.63
SGPT (U/L)		29.95±9.87	21.45±9.26

Malnutrition can affect immune response mediated by T-cell [6]. Ozkan *et al.* has related the lymphocyte function disturbance in malnutrition children with down-regulation of type 1 cytokine such as IL-2 and IFN-γ and marked by the up-regulation of type 2 cytokine such as IL-4 and IL-10 [7]. IL-2 and IL-10 plays a significant role in cell biology. IL-2 is needed for the development of regulatory T produced by thymus and for NK cell cytotoxicity which plays a role in infection process [8]. IL-10 inhibits the activation of T lymphocyte, so the cellular immunity reaction ends. Therefore, IL-10 is assumed to have suppressive effect for the immune function [9].

Cysteine (Cys) is a nonesensial sulfur amino acid (SAA) which plays a significant role in cell function. Cys is used for protein and glutathione (GSH) synthesis. The GSH and GSH disulfide (GSSG) system play a significant role in determining the balance of intracellular redox and antioxidant function [10]. In lymphocyte, glutathione is involved in the initiation and development of cellular activation. Intracellular glutathione depletion can inhibit the lymphocyte activation [11].

Several studies about cysteine, IL-2, and IL-10 level in children with malnutrition have been done. Ranjit shows that there is a decrease in IL-1, IL-2, and IFN-γ in malnutrition [12]. Badaloo *et al.* show that the serum cysteine level in kwashiorkor type malnutrition is low [13]. Until today, there is no study which specifically discuss the relationship between cysteine, IL-2, and IL-10 level in marasmus type malnutrition.

MATERIALS AND METHODS

This study was an observational analytic study using cross-sectional design in June 2015 at the pediatric department of Saiful Anwar Hospital Malang under the approval of the ethical committee.

Clinical characteristic of subjects

This study consisted of 2 groups, marasmus patient group and control group. The inclusion criteria were children diagnosed with marasmus type malnutrition treated in dr.Saiful Anwar Hospital Malang, aged 1 to 15 years old, and had been given consent from parents after informed consent. The exclusion criteria are children with marasmus type malnutrition with renal disturbance, hepatic disturbance, malignancy, and autoimmune disease such as Systemic Lupus Erythematosus, Nephrotic Syndrome, etc.

Blood samples

Two cc of peripheral blood sample was taken for the ELISA assessment. The blood was taken using vacutainer EDTA tube, stored in the temperature of 4o C and immediately sent to the Biomedical Laboratorium of Brawijaya University for the ELISA assessment.

Measurement of Cysteine, IL-2, and IL-10 Level

Laboratory examination in both groups was done to measure the level of cysteine, IL-2, and IL-10 using ELISA technique. Cysteine measurement used Creative Diagnostics Human Cysteine ELISA kit cat No. DEIA5203 in ng/mL.

Statistical analysis

Normality test and the various test was done. After that, it was analyzed using unpaired t-test (normal distribution) or Mann-Whitney test (abnormal distribution), Pearson correlation test, multivariate analysis, and path analysis test with a confidence interval of using SPSS 17 system.

RESULTS AND DISCUSSION

There were 40 subjects in this study. The subjects were divided into two groups, 20 children in marasmus group and 20 children in the control group. The mean age of marasmus and control group were 53.35 ± 35.38 months and 64.15 ± 39.54 months respectively with the youngest age of 15 months and the oldest age of 12 years and ten months in the marasmus group. The youngest age in the control group was 16 months, and the oldest age was 13 years old. There was 12 boys and eight girls in the marasmus group while there was 11 boys and nine girls in the control group. The most commonly found disease in this study was pneumonia The sample characteristics were explained in Table 1.

Before the data analysis was done, the normality test was done. In this study, the data distribution was normal in both groups. After that, the unpaired t test was done.

Table 2 showed the significant difference in cysteine level between the marasmus and control group (p = 0.000). Table 3 showed that IL-2 had significance value of 0.010 ($p<0.05$), therefore it could be concluded that there was a significant difference in IL-2 between marasmus and control group. Table 4 showed that IL-10 had significance value of 0.000 ($p < 0.05$), thus it could be concluded that there was a significant difference in IL-10 level between marasmus and control group.

The Pearson correlation test showed that the cysteine level was positively correlated with IL-2 level (p: 0.000; r = 0.71), therefore, it could be concluded that there was a significant relationship between cysteine and IL-2 level, with positive correlation which means that the lower the cysteine level, the lower the IL-2 level, and vice versa.

The Pearson correlation test showed that the cysteine level was negatively correlated with IL-10 level (p: 0.014; r= -0.53), therefore, it could be concluded that there was a significant relationship between cysteine and IL-10 level, with negative correlation which means that the low the cysteine level result in high IL-2 level and vice versa.

The Pearson correlation test showed that the IL-2 level was negatively correlated with IL-10 level (p: 0.014; r= -0.53), therefore, it could be concluded that there was a significant relationship between IL-2 and IL-10 level, with negative correlation which means that low IL-2 level result in high IL-10 level and vice versa.

From the data characteristics, the age range was 15 months to 13 years old. From 20 marasmus samples, 13 (13/20) children were aged under five years old. This age group was consistent with the epidemiology of malnutrition, where marasmus mostly occurred to children under the age of 5, especially in Asia [14].

According to sex, there were more boys with malnutrition than girls (12 boys and eight girls). This data was consistent with the study by Kandala *et al.* in Congo and Hirani in Pakistan which stated that malnutrition was more commonly found in boys [14].

According to the albumin level, there was a difference between serum albumin degree in sample and control group, where the mean albumin level in marasmus group was 3.20 and 3.84 in the control group. This was consistent with study by Muller *et al.* which showed that there was an increase of albumin deficiency degree in malnutrition1. A study by Avram *et al.* stated that serum albumin could be used as an indicator of nutrition for malnutrition patients [16]. A review by Gupta and Lis also stated that among the most common tool to assess nutrition status was serum albumin level [17]. Low albumin level in malnutrition was caused by an imbalance of intake and need of protein.

According to hemoglobin level, there was a difference of hemoglobin level in sample and control group.

Table 2. Comparison of mean cysteine level in marasmus and control group

Nutrition status	*Mean cysteine level (pg/mL)*	*±SD*	*p-value*
Well nourished	3.298	0.519	0.000
Marasmus type malnutrition	1.616	1.039	

Note: *p-value < 0.05* means there was a significant difference and *p-value > 0.05* means there was no significant difference.

Table 3. Comparison of mean IL-2 level in marasmus and control group

Nutrition status	*Mean IL-2 level (pg/mL)*	*±SD*	*p-value*
Well nourished	16.58	4.80	0.010
Marasmus type malnutrition	12.38	4.94	

Note: *p-value < 0.05* means there was a significant difference and *p-value > 0.05* means there was no significant difference.

Table 4. Comparison of mean IL-10 level in marasmus and control group

Nutrition status	*Mean kadar IL-10 (pg/mL)*	*±SD*	*p-value*
Well nourished	10.46	3.90	0.000
Marasmus type malnutrition	19.08	5.93	

Note: *p-value < 0.05* means there was a significant difference and *p-value > 0.05* means there was no significant difference.

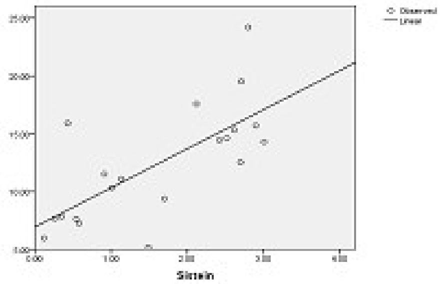

Figure 1. Relationship between cysteine and IL-2 level in children with marasmus. There was a significant positive correlation between cysteine and IL-2 level in children with marasmus

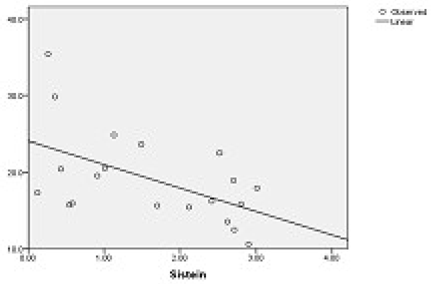

Figure 2. Relationship between cysteine and IL-10 level in children with marasmus. There was a significant negative correlation between cysteine and IL-10 level in children with marasmus

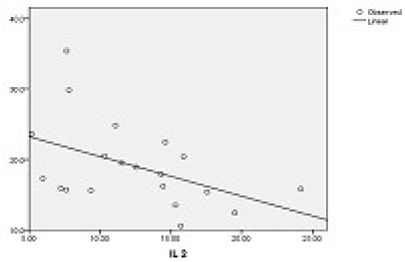

Figure 3. Relationship between IL-2 and IL-10 level in children with marasmus. There was a significant negative correlation between IL-2 and IL-10 level in children with marasmus

In malnutrition group, the average hemoglobin level was 10.07, while the mean level in the control group was 12.49. This result was consistent with a study by Bhoit R, which showed that 73% children with malnutrition had anemia [18]. A study by Yang *et al.* showed that there was a close relationship between malnutrition and anemia in China. Factors suspected were breastfeeding for more than six months and several social-demographic factors [19]. SGOT and SGPT level in this study were higher in patients with malnutrition compared to control group, but both were still with in normal range. This was consistent with study was done by Chrowdury *et al.* which obtained a result that there was an increase of SGOT and SGPT in patients with malnutrition [20]. In this study, the samples were limited to patients with normal hepatic function marked by normal SGOT and SGPT value.

In this study, the ureua and creatinine level of the sample and control group were between normal range with higher ureua and creatinine level in children with malnutrition. This result was not consistent with the study by Hary *et al.* which showed that serum creatinine was decreased in patients with malnutrition [21]. In this study, there was a sample limitation in patients with normal renal function because renal disturbance could be a confounding factor.

There was a significant difference in cysteine level in patients with malnutrition compared to a well-nourished patient. This result was consistent with a study by Jahoor *et al.* which showed that there was a decrease of cysteine level in malnutrition [22]. Cysteine is one of the amino acid needed by the body. In newborns, cysteine was considered as a semi-essential amino acid which indicated that external supply of cysteine was needed because of the low plasma cysteine and protein synthesis [23].

This study showed that the IL-2 level was significantly lower in patients with malnutrition compared to the control group and the IL-10 level was considerably higher in marasmus patients compared to control group. This result was caused by lymphocyte function disturbance in children with malnutrition, down-regulation of type-1 cytokine, and up-regulation of type 2 cytokine such as IL-107. A study by Fock *et al.* evaluated the plasma serum IL-10 level in the rat model with malnutrition. The IL-10 level in circulation increased in an animal with malnutrition compared to control group. This change was partially responsible for the immunodeficiency observed in rat with malnutrition [8].

In this study, the cysteine level had a significant relationship with IL-2 and IL-10 level. Cysteine is one of the important precursors for the production of glutathione protecting cells from free radicals in the human body and other organisms. The GSH content in T cell was proved important in its response toward mitogenic stimulation. The lack of GSH in T cell was involved in the pathogenesis of AIDS and many general immunodeficiencies, including malnutrition, therefore, cysteine could increase the T cell function [24]. This result was consistent with previous study about the efffect of sulfur amino acid in HIV infection treated with

NAC (market product of cysteine) for seven months compared to placebo which showed that NAC caused an increase of immunologic function in the form of the rise of T helper 1 [25]. Giordani *et al.* also stated that N-Acetylcysteine (NAC) increased the production of T helper 1 which produced cytokine such as IL-2 and conversely, NAC decreased the activation of T-dependent B cell which produced cytokine such as IL-10 [26]. IL-10 inhibited the activation of T lymphocyte, so the cellular immunity ended. Therefore, it was assumed that IL-10 had a suppressive effect to the immune function [10]. That suppressive effect probably played a role in the infection risk of children with malnutrition.

This study showed that there was significant and negatively correlated relationship between IL-2 and IL-10 level in patients with marasmus type malnutrition. This was consistent with a review by Geng *et al.*, where Treg cells depended to IL-2 in inhibiting T cell proliferation. The Treg cell was immunosuppressive toward many immune cells thus, it could inhibit the proliferation of T helper 2. The decrease of IL-2 reduced the excess Treg cell from type 2 T helper, therefore, it increased the level of IL-10 [9]. Suppressive effect of IL-10 on monocyte and cytokine synthesis by T helper 1 cells was assumed because IL-10 had a general suppressive effect to the immune function. Recently, IL-10 was used in a preclinic study to evaluate its potency as an immunosuppressive agent for several diseases such as infection, transplantation, and cancer [10].

CONCLUSIONS

The cysteine and IL-2 level in marasmus patients were significantly lower than the control group while the IL-10 level was higher in marasmus patients. There was a significant positive correlation between cysteine and IL-2 level and also a negative correlation between cysteine and IL-10 also IL-2 and IL-10 in patients with marasmus.

ACKNOWLEDGMENT

-

REFERENCES

1. Muller O, Krawinkel M (2005) Malnutrition and health in developing countries. CMAJ. 2:173.
2. Susanto JC, Mexitalia M, Nasar Sri S (2011) Malnutrisi akut berat dan terapi nutrisi berbasis komunitas. dalam: buku ajar nutrisi pediatrik dan penyakit metabolik. Jakarta: Badan Penerbit IDAI.
3. Kementerian Kesehatan RI (2013) Riset Kesehatan Dasar (RISKESDAS) 2013. Jakarta: Badan penelitian dan pengembangan kesehatan Kemenkes RI
4. Arif S, Indrawati R, Hidayat B, Netty E Pratono (1984) Pola defisiensi protein kalori pada anak di RSUD Sutomo, Buku Abstrak KONIKA VII. Jakarta.
5. Departemen Kesehatan Republik Indonesia Direktorat Jenderal Bina Kesehatan Masyarakat Direktorat Bina Gizi Masyarakat (2011) Buku bagan tatalaksana anak gizi buruk. Departemen Kesehatan RI.
6. Ambrus JL Sr, Ambrus JL Jr (2004) Nutrition and infectious diseases in developing countries and problems of acquired immunodeficiency syndrome. Exp Biol Med (Maywood) (229): 464–72.
7. Fock RA, Borelli P, Nakajima K, *et al* (2008) Protein-energy malnutrition modifies the production of il-10 in response to lipopolysaccharide (LPS) in a murine model. Journal of Nutritional Science and Vitaminology; 54(5): 371-7.
8. Geng X, Zhang R, Yang G, Jiang W, Xu C. 2012. Interleukin-2 and autoimmune disease occurrence and therapy. European Review for Medical and Pharmacological Sciences. 16: 1462-67
9. Kresno SB (2001) Imunologi: Diagnosis dan prosedur laboratorium. Edisi ke-4. Jakarta: Balai Penerbit FKUI.
10. Ozkan H, Olgun N, Saşmaz E, Abacioğlu H, Okuyan M, Cevik N (1993) Nutrition, immunity and infections: T lymphocyte subpopulations in protein--energy malnutrition. J Trop Pediatr (39): 257-60.
11. Dröge W (1993) Cysteine and glutathione deficiency in AIDS patients: a rationale for the treatment with N-acetyl-cysteine. Pharmacology (46): 61-5.
12. Ranjit KC (1997) Nutrition and the immune system: An introduction. Am J Clin Nutr; 66:460S-3S.
13. Badaloo A, Reid M, Forrester T, Heird WC, Jahoor F (2002) Cysteine supplementation improves the erythrocyte glutathione synthesis rate in children with severe edematous malnutrition. Am J Clin Nutr. 76(3): 646-52.
14. Hirani SAA (2012) Malnutrition in young Pakistani children. J Ayub Med Coll Abbottabad. 24:2.
15. Kandala NB, Madungu TP, Emina JB, Nzita KP, Cappuccio FP (2011) Malnutrition among children under the age of five in the democratic republic of congo (DRC): Does geographic location matter? BMC Public Health. 11:261.
16. Avram MA, Chattopadhyay J, Fein PA, Mittman M (2006) Monitoring albumin level as an indicator of nutrition in uremia therapy. Nefrologia I Dializoterapia Polska .10:163-5.
17. Gupta D, Lis CG (2010) Pretreatment serum albumin as a predictor of cancer survival: A systematic review of the epidemiological literature. Nutrition Journal . 9:1-16

18. Bhoite R, Iyer M (2011) Magnitude of Malnutrition and iron deficiency anemia among rural school children: An appraisal. Asian J. Exp Biol. 2:354-62
19. Yang W, Li X, Li Y, Zhang S, Liu L, Wang X, Li W (2012) Anemia, malnutrition and their correlations with socio-demographic characteristics and feeding practices among infants aged 0-18 months in rural areas of Shaanxi province in Northwestern China: A cross-sectional study. BMC Public Health. 12:1127-13.
20. Chowdhury SI, Rahman Z, Haque M, Nahar N, Taher A (2007) Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in different grades of protein energy malnutrition. J Bangladesh Soc Physiol. 2:17-9.
21. Hari P, Bagga A, Mahajan P, Lakshmy R (2007) Effect of malnutrition on serum creatinine and cystatin C levels. Pediatric Nephrology. 22:1757-61.
22. Jahoor F1, Badaloo A, Reid M, Forrester T (2006) Sulfur amino acid metabolism in children with severe childhood undernutrition: Cysteine kinetics. Ajcn.Nutrition.11:1393-9.
23. William L (2005) Amino Acid. Journal of Pediatric Gastroenterology and Nutrition. 41:12-8.
24. Martin SS, Qasim A, Reilly MP (2008) Leptin Resistance. J Am Coll Cardiol. 52:2101-10.
25. Breitkreutz R1, Pittack N, Nebe CT, *et al.* (2000) Improvement of immune functions in HIV infection by sulfur supplementation: two randomized trials. J Mol Med (Berl) (78): 55-62.
26. Giordani L, Quaranta MG, Malorni W, et al. (2002) N-acetylcysteine inhibits the induction of an antigen-specific antibody response down-regulating CD40 and CD27 co-stimulatory molecules. Clin Exp Immunol; 129(2): 254-64.