

Cysteine, Malondyaldehyde (MDA) and Glutathione (GSH) Levels in Marasmic Type Malnutrition and Well-Nourished Children in Saiful Anwar Hospital

Dwi Indah Cahyani ^{1,4*}, Anik Puryatni ^{2,5}, Nur Permatasari ³

¹ Department of Pediatrics, Faculty of Medicine, Brawijaya University, Malang, Indonesia

² Department of Nutrition and Metabolic Disease Division-Pediatric, Faculty of Medicine, Brawijaya University, Malang, Indonesia

³ Department of Biomedics, Faculty of Medicine, Brawijaya University, Malang, Indonesia

⁴ Department of Pediatrics, Saiful Anwar Public Hospital, Malang, Indonesia

⁵ Department of Nutrition and Metabolic Disease Division-Pediatric, Saiful Anwar Public Hospital, Malang, Indonesia

ABSTRACT

Micronutrient deficiency in severe malnutrition will reduce antioxidant capacity that needed for oxidative stress defense. Cysteine, a non-essential amino acid, is one of an important component for reduced glutathione (GSH). This study aims to prove the difference between the levels of cysteine, MDA and GSH levels in children with marasmic malnutrition and well-nourished children and prove whether there is a relationship between those parameters. Fiftysix patients participated in this study were grouped into two groups of samples that were marasmic type malnutrition group (28 patients) and control groups that were well nourished group (28 patients). Examination begins with a complete laboratory screening, followed by examination of cysteine, MDA and GSH level. Of the 28 patients included in marasmic type malnutrition group consisting of 15 male patients (53.6%) and 13 female patients (46.4%), while the well-nourished group consisted of 13 male patients (46.4%) and 15 female patients (53.6%). The average age is 54.61±56.35 months in the group of marasmic type malnutrition and 48.25±45.34 months in the well-nourished group. By using the Mann Whitney test, there were significant difference between the levels of cysteine and GSH in marasmic malnutrition and control group ($p=0.000$ and $p=0.000$ respectively). Spearman correlation test between cysteine and GSH levels, cysteine and MDA levels, MDA and GSH levels in marasmic malnutrition children shows no significant correlation ($R = -0.206$, $p = 0.294$; $R = -0.036$, $p = 0.856$; $R = 0.210$, $p = 0.284$ respectively). In well-nourished group Spearman correlation test between cysteine and GSH levels, cysteine and MDA levels, MDA and GSH levels also shows no significant correlation ($R = -0.053$, $p=0.789$; $R = -0.146$, $p = 0.458$; $R = -0.079$, $p = 0.688$ respectively). From this study it can be concluded that there are significant differences of the levels of cysteine and GSH between severe malnutrition groups compared with the control one. But this study shows no significant correlation between the levels of cysteine and GSH levels in marasmic type malnutrition and well-nourished children.

Keywords: *Ganoderma lucidum*, selectivity, colon cancer, COX-2

INTRODUCTION

Marasmic type malnutrition is one of significant health problem in developing countries. It contributes to 300.000 death among under-five children per year in developing countries and 50% mortality of children around the world. Significant morbidity related to im-

pairment of immune response that correlated to susceptibility of serious infection and long-term impact of growth and development [1, 2, 3]. Severe malnutrition related with macronutrient and micronutrient deficiency. Protein deficiency will reduce plasma albumin and antioxidant enzyme in tissue. Small amount of an

*Corresponding author:

Dwi Indah Cahyani
Department of Pediatrics, Faculty of Medicine,
Brawijaya University
Jalan Veteran, Malang, Indonesia 65145
E-mail: mamaracha82@gmail.com

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tioxidant intake in diet (vitamin A, C and E) will also directly reduce antioxidant capacity in malnutrition children.⁴ Another micronutrient deficiency that occurred in malnutrition children are cuprum, zinc, selenium, glutathione and glutathione peroxidase [5, 6]. Prolonged infection or inflammation related with malnutrition produced excessive free radicals. This condition will lead to oxidative stress [7]. Free radicals will promote lipid peroxidation that impact directly to tissue damage such as skin lesion and change of hair color. Then free radicals also act on protein or circulating lipoprotein that will induce sustainable liver injury to fatty liver. Malondyaldehyde (MDA), as one of lipid peroxidation product, then excessively produced. This oxidative stress lead to apoptotic acceleration that worsen patient prognosis.⁸

Cysteine is important source of sulphur compound in human and one of the important precursor of glutathione [9]. Cysteine is one of amino acid that contribute to patomechanism of oxidative stress in malnutrition. Decrease of erythrocyte glutathione synthesis in malnutrition children related with low concentration of cysteine and methionine [10]. Malnourished children with infection has low concentration of cysteine that caused by decrease of proteolysis [11]. Cysteine also regulate redox state of skin and mucous membrane so that related with evidence of hair damage, skin erosion, and intestinal mucous membrane atrophy and mucin depletion [12]. Glutathione (GSH) is a tripeptide that consists of glutamate, cysteine and glycine. It is a potent antioxidant that also contribute for maintaining redox state of the cells [13, 14].

The purpose of this study is to prove the difference between the cysteine, MDA and GSH levels in children with marasmic type malnutrition than well-nourished ones, and verify whether there is a relationship between cysteine, MDA and GSH levels in both of the group.

MATERIALS AND METHODS

Definitions used in this study

The dependent variable in this study is cysteine and GSH levels. Cysteine Levels measured in plasma using Enzyme Immuno Assay (ELISA). Cysteine levels normally expressed in micromole/liter, which for men 43 $\mu\text{mole/liter}$ (average 20 - 91 $\mu\text{mole/liter}$), for women 38 $\mu\text{mole/liter}$ (average 19 - 96 $\mu\text{mole/liter}$) [15]. GSH levels in serum were analyzed by ELISA using a Human Glutathione (GSH) ELISA kit OXIS by units of mg/mL. Glutathione levels normally expressed in mg/dL, which for well-nourished children is 55.56 mg/dL, while for

marasmic type malnutrition is 46.62 mg/dL. Malondyaldehyde levels normally expressed in nmol/mL, which for wellnourished children 1.3 nmol/mL, while for marasmic type malnutrition is 1.9 nmol/mL [16].

Independent variables used in this study is nutritional state that are marasmic type malnutrition and well nourished. Marasmic type malnutrition is defined as the measurement of weight compared to height/body length of less than -3 SD (standard deviation) or less than 70% or more below the average for the reference value of the National Center for Health Statistics, a child with clinical signs of malnutrition such as the xylophone ribs and old man face. Well nourish is defined as the measurement of weight compared to height between 90% to 110% (percentile), or between -2 SD (standard deviation) up to +2 SD for the reference value of the National Center for Health Statistics [3].

Study design and study population

This is an observational and analytic methods using cross sectional design study. The population were all children diagnosed with marasmic type malnutrition. Affordable population were children suffering from marasmic type malnutrition and hospitalized in the pediatric ward General Hospital dr. Saiful Anwar Malang. Samples were children suffering from marasmic type malnutrition of stabilization phase and hospitalized at the General Hospital dr. Saiful Anwar Malang and met the inclusion criteria. All respondents in this study received an explanation of the research and parents are asked to sign the consent (informed consent). The sample size used in this study was calculated using the formula sample calculation [16] and the results minimum number of samples was 28 for each group.

Inclusion and exclusion criteria

This study uses both inclusion and exclusion criteria in the sample and the control. In the sample inclusion criteria used were diagnosed with marasmic type malnutrition, aged between 1 month to 14 years, and allow parents with children enrolled in the study after being given an explanation (informed consent). While exclusion criteria were used in the sample is suffering from an autoimmune disease, kidney disease, liver disease and suffering from malignant disease.

Inclusion criteria for the control is classified in well-nourished children, aged between 1 month to 14 years, and allow parents with children enrolled in the study after being given an explanation (informed consent). While exclusion criteria on the controls is suffering from

Table 1. Characteristics of sample

Characteristics of sample		Marasmic Type Malnutrition (n = 28)	Control (n = 28)
Average of age (months)		54.61 ± 56.35	48.25 ± 45.34
Distribution of age	< 5 years	17	17
	5-10 years	5	9
	> 10 years	6	2
Gender	Male	15	13
	Female	13	15
Initial diagnosis	Pneumonia	11	3
	Tuberculosis	7	0
	HIV	2	0
	Dengue fever	1	14
	Urinary tract infection	1	3
	Meningoencephalitis/encephalitis	2	2
	Diarrhea	1	2
	Endocarditis	1	0
	Cellulitis	1	1
	Tonsillopharyngitis	1	3

an autoimmune disease, kidney disease, liver disease and suffering from malignant disease.

Ethical consideration

The study was approved by the research ethics committee of the Medical Faculty of Brawijaya University-dr. Saiful Anwar hospital. (Ethical Clearance no. 400/109/K.3/302/2016). Informed consent was obtained from mothers or legal guardians of all children.

Methods laboratory tests

Examination of Cysteine level. Cysteine level examination using Creative Diagnostic Human ELISA kit cat No. DEIA5203 (ng/mL). The sample used in the form of plasma and can be stored longer at -20°C.

Examination of GSH level. Glutathione level examination using Human GSH ELISA kit cat No 21040 (mg/dL). The sample used in the form of plasma and can be stored longer at -20°C.

Examination of MDA level. Malondialdehyde level examination using Bioxytech MDA-586 cat No 21044 (nmol/mL). The sample used in the form of plasma and can be stored longer at -20°C.

Statistical analysis

Initially the data cysteine, MDA and GSH levels were performed normality test (to determine the normality of data) by using the Kolmogorov-Smirnov test and test variant (to determine the variant of data). If the distribution of data is normal and variants of data are

same, then used the unpaired t test. If data is abnormal, data will be transformed. If the new variable transformation results are normally distributed, we used the unpaired t test. If the new variable transformation result is not normally distributed, the Mann-Whitney test was used. Pearson correlation test was used to examine the relationship between cysteine levels and GSH levels. Data were analyzed using a 95% confidence level ($\alpha = 0.05$). All data were analyzed using the software *Statistical Package for the Social Sciences (SPSS 17.0)* for Windows.

RESULTS AND DISCUSSION

Baseline characteristic of sample

This is a cross-sectional study involving 56 samples that divided into two groups, namely marasmic type malnutrition group of 28 people and a control group of 28 people. Characteristics of the sample are shown in Table 1.

The average of age in marasmic type malnutrition the group is 54.61 ± 56.35 and the control group 48.25 ± 45.34. The lowest age of the marasmic type malnutrition group is 1 months and the highest age is 13 years old. The lowest age in the control group was 1 months and the highest age is 12 years old. Marasmic type malnutrition group consisted of 15 boys and 13 girls, while the control group consisted of 13 boys and 15 girls. Majority infection in marasmic type malnutrition group was pneumonia and tuberculosis, while in the control group dengue fever was predominant. Laboratory data

Table 2. Laboratory data

Description	Marasmic Type	
	Malnutrition (n = 28)	Control (n = 28)
Haemoglobine (g/dL)	10.41 ± 3.29	11.34 ± 2.43
Leucocyte (10 ³ /μL)	10.75 ± 4.64	11.46 ± 7.87
Albumine (g/dL)	2.95 ± 0.46	3.57 ± 0.40
SGOT/AST (U/L)	31.89 ± 10.60	31.89 ± 7.22
SGPT/ALT (U/L)	19.82 ± 8.97	23.21 ± 8.62
Ureum (mg/dL)	25.46 ± 8.13	21.34 ± 12.21
Creatinine (mg/dL)	0.35 ± 0.11	0.37 ± 0.20

Table 3. Cysteine, MDA and GSH levels in marasmic type malnutrition and control group

Groups	Marasmic type malnutrition (n = 28)	Control (n = 28)	p-value
Cysteine (ng/mL)	68.56 ± 18.20	99.10 ± 16.51	0.00
MDA (nmol/mL)	23.792 ± 4.21	4.06 ± 4.16	0.00
GSH (mg/dL)	614.78 ± 183.82	919.30 ± 122.55	0.00

for the characteristics of the study sample are shown in Table 2.

According to the test of Shapiro-Wilk, cysteine, MDA and GSH levels in the two groups of samples obtained p-value less than 0.05, so it can be concluded that the data in this study were not normally distributed. Different test conducted later in the levels of cysteine, MDA and GSH of the two groups using Mann-Whitney U test, and produce significant differences between groups of marasmic type malnutrition with the control group ($p = 0.000$, $p = 0.000$, $p = 0.000$ respectively) (Table 3).

Based on Pearson correlation results obtained in patients with marasmic type malnutrition cysteine levels not significantly correlated with GSH levels ($p = 0.294$; $r = 0.206$), cysteine levels not significantly correlated with MDA levels ($p = 0.856$; $r = -0.036$) and also MDA levels not significantly correlated with GSH levels ($p = 0.284$; $r = 0.210$) (Figure 1-3). Thus it can be concluded that there is no significant correlation between the cysteine and GSH levels, cysteine and MDA levels and also MDA and GSH levels in marasmic type malnutrition group

Based on Pearson correlation results obtained in well-nourished patients cysteine levels not significantly correlated with GSH levels ($p = 0.789$; $r = -0.053$), cysteine levels not significantly correlated with MDA levels ($p = 0.458$; $r = -0.146$) and also MDA levels not significantly correlated with GSH levels ($p = 0.688$; $r = -0.079$)

(Figure 4-6). Thus it can be concluded that there is no significant correlation between the cysteine and GSH levels, cysteine and MDA levels and also MDA and GSH levels in the well-nourished group.

This study involved 56 samples that divided into two groups, namely marasmic type malnutrition group (28 samples) and a control group (28 samples). Group of marasmic type malnutrition characterized by the presence of clinical symptoms like wasted, xylophone ribs, changes in hair color and hair that is easily removed, skin dermatoses, baggy pant, by measurement of anthropometric status where weight/height < -3 SD or percentage of ideal body weight < 70%. Both marasmic type malnutrition group and the control group receiving treatment in hospital, with predominant concomitant diseases in marasmic type group was pneumonia and lung tuberculosis and dengue fever in the control group.

Sample characteristics revealed that the age range is 1 month to 13 years old. From 28 samples of marasmic type malnutrition-patients, as many as 17 (17/28) samples aged < 5 years. This is consistent with epidemiological data of malnutrition in the world, where the incidence of malnutrition, especially in Asian countries aged less than 5 years [17].

By sex or gender of the sample, it showed that number of the male is greater than the female patient. The prevalence of children who suffer from malnutrition based on gender is different. The study conducted by Rahman et al. (2010) explain that the incidence of malnutrition is greater in male, as well as research that has been done by Hirani (2012) in Pakistan [17, 18]. While the research conducted by Jamro et al. (2012) in Pakistan and Kuntari (2013) get different results where female is greater than male [19, 20].

Based on the levels of hemoglobin, in this study there was no difference between marasmic malnutrition and control groups. This is not in accordance with previous studies, where research conducted by Bhoite and Lyer (2011) found 73% of children with malnutrition suffer from anemia [21]. Research conducted by Frempong et al. (2012) also mentions that the hemoglobin of malnutrition significantly lower than patients with good nutritional status, despite conditions of severe malnutrition has been undergoing rehabilitation nutrition [22]. Meanwhile, research conducted by Yang et al. (2012) found a close relationship between malnutrition and anemia in China [23]. The discrepancy of that studies might be related to chronic hypovolemia in marasmic type malnutrition and chronicity of the malnutrition that influence the level of macronutrient and micronu-

trient deficiency [24, 25].

Levels of albumin obtained in this study showed differences between groups of subject's marasmic malnutrition than the control group. A study conducted by Muller et al. (2005) showed a decrease of albumin levels in malnutrition [24]. Research conducted by Avram et al. (2006) found that serum albumin can be used as an indicator of a person's nutrition [26]. Gupta and Lis (2010) explains that the most common indicators can be used to assess the nutritional status of a person is the serum albumin. Malnutrition and inflammation will suppress protein synthesis [27]. Low albumin levels in malnutritional condition occurs because of an imbalance between intake and protein needs.

Based on the levels of AST and ALT, this study found no differences between the two groups. A study conducted by Hyder et al. (2013) explain that the levels of AST and ALT increased on condition of viral hepatitis infection, alcohol use, and cirrhosis of the liver [28]. While the research conducted by Crawdury et al. (2007) get different results, which found elevated levels of AST and ALT in marasmic malnutrition patients [29]. In this research that we had excluded patients with liver disease and autoimmune diseases, so there were no differences between the two of groups.

This study also found no significant difference between ureum and creatinine levels of marasmic malnutrition group and the control group. Research conducted by Hary et al. (2007) found that decreased levels of serum creatinine in patients with malnutrition [30]. Impaired renal function as exclusion criteria in this study might be done because renal dysfunction may be one factor confounding the results, which can influence levels of protein in patients.

Cysteine levels in this study showed significant differences between the samples of marasmic malnutrition compared to well-nourished controls. It is the same as research conducted by Jahoor et al. (2012) which explain decreased levels of cysteine in malnutritional condition. It also explained that the condition of malnourished children and infected then cysteine production is slower than the recovery period due to a decrease in protein breakdown [31]. The decline would actually appear larger on the type of kwashiorkor malnutrition [32].

In this study, glutathione level in marasmic type malnutrition group showed a significant difference compared to the control group. This concordance with a study conducted by Une (2013) that showed a decrease of antioxidant capacity in marasmic type malnutrition [33]. The decrease of glutathione level may be caused by

the decrease of synthesis or increase of antioxidant demand. The decrease of synthesis may be caused by amino acid deficiency. Glutathione composed by tripeptide, glutamate, cysteine and glycine that catalyzed by glutathione synthetase [34]. As the antioxidant, glutathione will eliminate reactive oxygen and nitrogen species by direct interaction with reactive species such as ROS, RNS, HO, HOCl, RO, RO₂, ¹O₂ and ONOO⁻ and form thiyl radicals (GS) [35]. Glutathione also act as the antioxidant on detoxification of lipid oxidation product that induced by ROS such as malondialdehyde and 4-hydroxy-2-nonenal [36, 37].

In this study, there was no significant association between cysteine and GSH levels in marasmic type malnutrition patients. Thus can be explained because cysteine was not the only precursor for GSH, the other amino acid such as glycine and glutamate might influence the GSH levels. The other condition might influence the GSH levels, such as level of NADPH that produced during pentose phosphate pathway. These parameters should be considered [35, 38]. According to Figure 1, although not significant, the relationship between cysteine and GSH was negative. This fact might be caused by the potency of cysteine to produce another toxic radical (hydroxyl radical) due to activation of arginase because of reaction between cysteine and Fe. As we know that during acute infection, oxidative hemolysis process will increase so that free Fe will be released to the circulation. The more end product of increment free radical production releasing to the circulation the more GSH consumption occurred [39].

CONCLUSION

Cysteine and GSH levels in marasmic malnutrition patients was significantly lower compared than well-nourished ones. There was no significant relationship between cysteine and GSH levels in marasmic type malnutrition patients.

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