

## Correlation between Serum hs-CRP and Magnesium Ion with Osteocalcin in Acute Leukemia Patients

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### ABSTRACT

Acute leukemia is a proliferation of immature cells in the bone marrow that affects peripheral blood or other organs. Inflammation has a vital role in cancer pathophysiology. Inflammation in leukemia occurs through two mechanisms: intrinsic and extrinsic. Magnesium deficiency leads to inflammation in acute leukemia patients. Manifestation of inflammation in acute leukemia are abnormalities in musculoskeletal systems such as osteopenia, osteoporosis, osteonecrosis, and pathological fractures. Increased inflammation in the bone remodeling process increases osteoclast (OC) regulation and decreases osteoblast (OB) activity resulting in reduced osteocalcin (OCN) production. Osteocalcin, also known as gamma-carboxy glutamic acid-containing protein or bone Gla-protein, is a small, non-collagen protein associated with the bone matrix. Osteocalcin is known as bone formation. This study aimed to analyze the correlation between hs-CRP, ion Mg and serum OCN levels in acute leukemia. A cross-sectional observational analytic study in acute leukemia subjects was conducted at Clinical Pathology Installation of Dr. Moewardi Hospital, Surakarta from August to September 2020. Normality test of Shapiro-Wilk was used to determine data distribution, Pearson correlation test was used to analyze the correlation strength between variables. From a total of 35 subjects, there was a significant negative correlation between hs-CRP and serum OCN in acute leukemia ( $r = -0.46$ ;  $p = 0.06$ ), but no correlation was found between Mg ion and serum OCN in acute leukemia patients ( $r = 0.09$ ;  $p = 0.957$ ). The mean of hs-CRP, Mg ion, and OCN was  $2.95 \pm 4.95$  mg/dL,  $0.49 \pm 0.05$  mmol/L, and  $16.32 \pm 19.46$  ng/mL, respectively. Advanced research with chronic leukemia population and other variants as needed.

**Keywords:** Acute leukemia, C-reactive protein, osteocalcin, ion magnesium

### INTRODUCTION

Leukemia is one of the most common malignant diseases affecting the world population. Globally, in 2018, leukemia ranked as the fifteenth most common cancer with 309,006 mortalities. Data from the World Health Organization (WHO) in 2018 showed that the most cases were found in East Asia with 101,285 new cases and 78,805 deaths, while the number of hematopoietic malignancies in Indonesia reached 6% to 8% of the total new malignancies per year. Leukemia triggers inflammation through two mechanisms; such as intrinsic and extrinsic.<sup>1-3</sup>

Increased transcription of NFkB in acute leukemia triggers an increase in proinflammatory cytokines and an increase in hs-CRP. Mg ion deficiency in acute leukemia can be caused by inadequate intake, diarrhea, anorexia due to chemotherapy, and tubular dysfunction. Lack of Mg ions can trigger inflammation in acute leukemia patients due to an increase in Ca ion exchange (Mg is a competitive ion with Ca ions), and the accumulation of intracellular

Ca ions increases the risk of inflammation by activating NFkB transcription.<sup>1-3</sup>

Interleukin (IL) 1 $\beta$ , IL-6, IL-10, and Tumor Necrosis Factor (TNF $\alpha$ ), inflammatory proteins, and enzymes are several stimuli that activate inflammation and induce the production of inflammatory cytokines. Inflammatory proteins in the blood include C-Reactive Protein (CRP), haptoglobin, serum amyloid A, fibrinogen, and alpha 1-acid glycoprotein. C-reactive protein is an acute-phase protein, synthesized mainly by the liver based on IL-1 $\beta$  and IL-6 stimuli. The CRP test can be replaced with a High Sensitivity C-Reactive Protein (hs-CRP) test because the hs-CRP test is more sensitive than the CRP test. Inflammation in leukemia is caused by a decrease/deficiency of Mg ions due to diarrhea, chemotherapy, and reduced intake.<sup>4-7</sup>

Inflammation in acute leukemia can cause a high risk of fracture, osteopenia, osteoporosis, and osteonecrosis. Osteocalcin (OCN) is a non-collagen protein, which has a close relationship with bone metabolism. Osteocalcin is produced by osteoblasts

(OB), which is known as a biomarker of bone formation. Leukemic cells inhibit OB and bone formation by destroying messenger ribonucleic acid (mRNA) OCN in CD45-bone marrow.<sup>7,8</sup>

Inflammation that occurs in acute leukemia can cause many complications, including disorders of the musculoskeletal system. Research by Yang *et al.* found a positive correlation between CRP levels and bone lesions with a value of  $r=0.6096$ . In addition, CRP levels were positively correlated with levels of Receptor Activator of Nuclear Factor Kappa-Beta Ligand (RANKL). Research Atkinson *et al.* found a correlation between OCN and Mg in acute leukemia patients with a value of  $r=0.54$ . The oral administration of Mg can normalize serum Mg values and serum OCN levels. However, in Indonesia, there has been no research that determines the correlation between serum OCN levels and inflammatory markers of CRP and Mg.<sup>9</sup>

This research was expected to provide information on the importance of serum hs-CRP and Mg ions as a screening to determine the inflammatory process that can cause musculoskeletal system abnormalities in acute leukemia patients and can be used as an alternative to serum OCN measurement in terms of economics and laboratory capabilities.

## METHODS

This study was an observational analytic study with a cross-sectional approach to determine the correlation between levels of hs-CRP, Mg ion, and serum OCN in acute leukemia patients. The research was conducted at the Clinical Pathology Installation of the Regional General Hospital Dr. Moewardi in Surakarta from August to September 2020. The study's target population was patients with acute leukemia (AML and ALL) diagnosed based on the results of Bone Marrow Puncture (BMP) and/or immunophenotyping at the Clinical Pathology Installation of Dr. Moewardi Hospital from August 2020 to September 2020. Classification of acute leukemia in this study was based on the French-American British Cooperative Group (FAB), using a limit of the number of immature cells (blast cells) in the bone marrow of 30%. Patients were taken blood samples for measurement of hs-CRP, Mg ion, and serum OCN. During 2 months, 35 patients with acute leukemia who met the inclusion and exclusion criteria of the study were found. The estimated sample size based on the sample size formula for the correlative analysis research design was as follows:

$$N = \left\{ \frac{Z\alpha + Z\beta}{0.5 \ln [(1+r)/(1-r)]} \right\}^2 + 3$$

$Z\alpha$ : alpha standard deviation

$Z\beta$ : beta standard deviation

$r$ : correlation

Type 1 error 5% oneway hypothesis,  $Z\alpha$  1.64 with 95% confidence level. Type 2 error 10%,  $Z\beta$  1.28. A study by Fatahi *et al.* found a correlation between OCN and Mg with  $r=0.475$ . Therefore, the authors set a correlation value of 0.475. By entering this value into the sample size formula, a minimum sample size of 30 people was obtained. Inclusion criteria in this study were as follows: New acute leukemia patients (AML and ALL) diagnosed based on the results of BMP interpretation and/or immunophenotyping issued by a Clinical Pathologist Specialist at Dr. Moewardi Hospital, children and adults, male and female who agreed to participate in the study by signing informed consent. Exclusion criteria in this study were as follows: history of liver disease {(Serum Glutamic Pyruvic Transaminase (SGPT) > 102 U/L)} obtained from data in medical records, pregnancy and lactation based on anamnesis, bone abnormalities and pathological fractures obtained from the anamnesis, no widespread inflammation found from physical examination, no electrolyte disturbances and kidney disorders found from laboratory results.<sup>7</sup>

Subjects were selected consecutively, whereas the patient's identity and medical history were collected when filling informed consent at the Clinical Pathology Laboratory Installation. This study received the approval of the biomedical research ethics committee at the Dr. Moewardi Hospital and the patient's consent. A total of 3 mL venous blood was taken with a tube without anticoagulant/with a gel separator to measure serum levels of hs-CRP, Mg ions, and OCN. Blood samples without anticoagulant/with gel separator were centrifuged for 10-15 minutes at a speed of 5000-6000 rpm. The serum formed was separated and put into an aliquot. Serum samples were stored in a refrigerator at -80 C until sample analysis was carried out. hs-CRP levels were measured in this study using the SEKISUI reagent kit from The SEKISUI Chemical Co.Ltd USA and the control reagent kit of Proline Trulab CRP hs level 1. Mg ions were measured in this study using a reagent kit from Nova Biomedical 8+ with an

ion-selective electrode method. (ISE). OCN levels were measured in this study using a reagent kit from Elecsys N-MID OCN Roche diagnostic. The reagent control kit of PreciControl Varia was also used. Serum OCN levels were measured in this study using the sandwich Electrochemiluminescence Immunoassay (ECLIA) method.

This study has received approval from the Health Research Ethics Committee of the Dr. Moewardi Hospital, Surakarta, with number No: 962/V11/HREC/2020.

## RESULTS AND DISCUSSIONS

This study involved 35 subjects with acute leukemia who were diagnosed based on BMP and/or immunophenotyping results by a Clinical Pathologist Specialist at the Clinical Pathology Installation of the Dr. Moewardi Hospital. Table 1 showed that the total research subjects in this study consisted of 14 (40%) female patients and 21 (60%) male patients. The overall patient age ranged from a median of 9 (3-67) years. The patient's general

average hemoglobin (Hb) level was  $8.97 \pm 0.85$  g/dL. The leukocyte levels for all patients ranged from a median of 18.7 (11.6-33.1)  $10^3/\mu\text{L}$ . The mean total platelet level of the patients was  $90.63 \pm 21.36$   $10^3/\mu\text{L}$ . Serum glutamic pyruvic transaminase with a mean of  $33.83 \pm 10.39$  U/L was also found. The patient's overall creatinine level had a median of 0.9 (0.6-1.1) mg/dL. The patient's overall sodium had a median of 138.7 (124.2-149) mmol/L. Potassium levels were obtained with a mean of  $3.74 \pm 0.54$  mmol/L. Calcium ions in all patients with a median of 1.03 (0.5-1.3) mmol/L were also reported.

The characteristics of the research variables are shown in Table 2. The characteristics of the study were differentiated based on the total study population, AML and ALL. The mean of hs-CRP in all patients was  $2.95 \pm 4.95$  that of AML patients, the mean was  $3.12 \pm 5.88$ , and that of ALL patients was  $2.84 \pm 4.38$ . The average Mg ion level in all patients was  $0.49 \pm 0.05$ , in AML patients was  $0.51 \pm 0.41$  and in ALL patients was  $0.49 \pm 0.54$ . The mean of OCN serum levels in all patients was  $16.32 \pm 19.46$ , in AML patients was  $16.84 \pm 23.96$  and in ALL patients was  $15.54 \pm 16.72$ .

**Table 1.** Characteristics of research subjects

Parameter	Total	Mean $\pm$ SD	Median (min-max)
Age (years) **			9 (3-67)
<b>Gender (%)</b>			
Male	60(%)		
Female	40(%)		
<b>Diagnosis (%)</b>			
AML	40 (%)		
ALL	60(%)		
Hb (g/dL) *		$8.97 \pm 0.85$	
Leukocyte ( $10^3/\mu\text{L}$ ) **			18.7 (11.6-33.1)
Platelet ( $10^3/\mu\text{L}$ ) *		$90.63 \pm 21.36$	
SGPT (U/L) *		$33.83 \pm 10.39$	
Creatinine (mg/dL) **			0.9 (0.6-1.1)
Sodium (mmol/L) **			138.7 (124.2-149)
Potassium (mmol/L) *		$3.74 \pm 0.54$	
Calcium ion (mmol/L) **			1.03 (0.5-1.3)

Note: AML: Acute Myeloid Leukemia; ALL: Acute Lymphoblastic Leukemia; Hb: Hemoglobin; SGPT; Serum Glutamic Pyruvic Transaminase; SD: Standard Deviation; min: minimum; maks: maximum; mg: milligram; mmol/L: millimoles per liter; ng/mL: nanogram per milliliter.

\*Normal distribution (Mean $\pm$ SD)

\*\*Abnormal distribution [median (min-max)]

**Table 2.** Characteristics of research variable

Parameter	Mean $\pm$ Total SD	Mean $\pm$ AML SD	Mean $\pm$ ALL SD
hs-CRP (mg/dL)	$2.95 \pm 4.95$	$3.12 \pm 5.88$	$2.84 \pm 4.38$
Ion Mg (mmol/L)	$0.49 \pm 0.05$	$0.51 \pm 0.41$	$0.49 \pm 0.54$
OCN (ng/mL)	$16.32 \pm 19.46$	$16.84 \pm 23.96$	$15.54 \pm 16.72$

Correlation analysis was carried out to determine the strength of the correlation between serum levels of hs-CRP, Mg ion, and OCN in the total acute leukemia population. The distribution of serum Mg ion data in this study was normally distributed, while the distribution of serum hs-CRP and OCN data in this study was not normally distributed. The distribution of hs-CRP and serum OCN data in this study was normally distributed after data transformation was carried out to normalize the data distribution.<sup>10</sup>

The results of the correlation test showed a negative correlation with moderate and significant strength between serum levels of hs-CRP and OCN in the acute leukemia population ( $r = -0.46$ ;  $p = 0.006$ ); however, there was no significant correlation between serum levels of Mg ions and OCN in the acute leukemia population ( $r = 0.09$ ,  $p = 0.957$ ). The results of the Pearson correlation test of research variables in the acute leukemia population can be seen in Table 3.

**Table 3.** Results of Pearson correlation test for each research variable

	OCN*	
	r	p
hs-CRP *	-0.46	0.006**
Ion Mg	0.09	0.957

Data on the basic characteristics of the subjects in this study showed the age of all patients with a median of 9 (3-67) years. This study was following the research of Ahmad *et al.*, which found the prevalence of acute leukemia of 38%, 17.5%, 15%, 9%, 8.5%, 25%, and 3.75% at the age range of 0-10 years, 11-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, and 61-70 years.<sup>11</sup>

The data on the essential characteristics of the research subjects showed that the subjects in this study consisted of 60% male and 40% female. The results of this study were following data from the American Cancer Society, which found that the incidence of acute leukemia is more common in males than females. In addition, research by Ahmad *et al.* found the prevalence of acute leukemia of 64.5% and 35.5% in males and females, respectively.<sup>11,12</sup>

Acute lymphoid leukemia in this study was found in 60% of subjects, and AML was found in 40% of subjects. The results of this study were in accordance with previous research by Ahmad *et al.*, which saw the most diagnoses of ALL (49.5%) followed by AML (31.25%). Ahmad *et al.* stated that the high incidence of ALL was because the most acute leukemia

survivors with a diagnosis of ALL.<sup>11</sup>

The data on the essential characteristics of the research subjects showed Hb levels with an average of  $8.97 \pm 0.85$  g/dL. The results of this study were not in accordance with the research of Liem *et al.*, which stated that the majority of Hb levels in acute leukemia patients were  $<7$  g/dL at the time of diagnosis. Anemia in leukemia occurs because of the suppression by the leukemia cells against normal blood cells in the bone marrow.<sup>13,14</sup>

The median of leukocytes count of  $18.7 (11.6-33.1) 10^3/\mu\text{L}$  was found in this study. Leukocytosis in acute leukemia is in accordance with the results of Perez *et al.*, who found leukocyte levels  $>14.5 10^3/\mu\text{L}$  in 36.6% of the study population.<sup>15</sup>

The mean platelets count in this study was  $90.63 \pm 21.36 10^3/\mu\text{L}$ . These results were in accordance with the research of Perez *et al.*, which obtained a platelet level of  $150 10^3/\mu\text{L}$ . Thrombocytopenia is one of the symptoms in acute leukemia associated with a poor prognosis due to bleeding that occurs.<sup>15</sup>

The mean of SGPT levels in this study was  $33.83 \pm 10.39$  U/L. These results followed the research of El-Shafey *et al.*, which found SGPT levels of 21-39 U/L in acute leukemia patients before chemotherapy. The value will increase during the chemotherapy process with SGPT levels of 25-61 U/L. However, the results of this study were not following the research of Abdalla and Akasha, which found an increased SGPT level in acute leukemia patients with levels of  $85.1 \pm 34.8$  U/L.<sup>16,17</sup>

Kidney function in this study was observed in the creatinine test with a median of 0.9 (0.6-1.1) mg/dL. The results of this study were not in accordance with the study of Salman *et al.*, which found an increase in serum creatinine in acute leukemia patients with levels of  $1.3 \pm 0.9$  mg/dL. Contrastingly, a study by Tamil found normal serum creatinine levels in 53% of the study population.<sup>18,19</sup>

The median sodium and calcium ion levels in this study were 138.7 (124.2-149) mmol/L and 1.03 (0.5-1.3) mmol/L, respectively, while the mean of potassium levels was  $3.74 \pm 0.54$  mmol/L. The results of this study were following the research of Sallman *et al.*, which found the sodium ions of  $137 \pm 7.9$  mmol/L, potassium ions of  $3.92 \pm 0.76$  mmol/L, and calcium ions of  $1.94 \pm 0.4$  mmol/L in the acute leukemia population before receiving chemotherapy.<sup>18</sup>

There was a negative correlation between serum hs-CRP and OCN with moderate and significant strength in all acute leukemia patients ( $r = -0.46$ ;  $p = 0.006$ ). Previous research by Gu *et al.* showed the



correlation between hs-CRP and OCN with an r-value of -0.475 in the overweight male population compared to obese. The correlation between serum hs-CRP levels and OCN in acute leukemia can occur due to two processes, extrinsic mechanisms involving immunity and microenvironment factors, and intrinsic mechanisms include genetic disorders and tumor suppression of oncogenes that activate inflammatory pathways. The mutation process in leukemia will trigger an increase in the binding of the chemokine CXCL12 to its receptor CXCR4 and trigger the activation of NFkB. Increased activity of NFkB induces the production of proinflammatory cytokines. C-reactive protein is produced by hepatocytes based on the stimulus of proinflammatory cytokines and increases during the inflammatory process. Inflammation affects the bone remodeling process by increasing bone resorption by increasing osteoclastogenesis and increasing the expression of RANKL and RANK. In acute leukemia, an increase in NFkB transcription triggers an increase in proinflammatory cytokines and hs-CRP. An increase in the proinflammatory cytokines make an essential contribution to bone loss by promoting bone resorption and impairing bone formation.<sup>6,20,21</sup>

Correlation analysis in this study found no correlation ( $r=0.09$ ;  $p=0.957$ ) between serum Mg ions and OCN. The results of this study were in accordance with the study of Salman *et al.*, which found no significant difference between Mg ion levels before and after chemotherapy in acute leukemia patients.<sup>18</sup> Mg ion levels in the body are very dependent on intake. The human body will respond to Mg ion hemostasis when there is a change in Mg ion intake, thereby making it difficult to evaluate the hypomagnesemia condition in each individual.<sup>21</sup>

Analysis of Mg and OCN ion levels is needed in acute leukemia patients to screen the severity of inflammation that can cause musculoskeletal system disorders in acute leukemia patients.<sup>22</sup>

No differentiation of serum OCN based on age and no differentiation of diagnosis of acute leukemia were limitations in this study.

## CONCLUSIONS AND SUGGESTIONS

It was concluded that there was a significant negative correlation with moderate strength between serum levels of hs-CRP and OCN in acute leukemia patients ( $r= -0.46$ ;  $p=0.006$ ), but no correlation was found between serum levels of Mg ions and OCN in acute leukemia patients ( $r=0.09$ ;

$p=0.957$ ).

Therefore, further research was needed to determine the correlation between serum hs-CRP levels and OCN in the acute leukemia population using different study designs such as case-control or cohorts and studies in chronic leukemia populations.

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