

## Low Level of Vitamin D Increased Dendritic Cell Maturation and Expression of Interferon- $\gamma$ and Interleukin-4 in Systemic Lupus Erythematosus

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**Abstract:** Vitamin D has been reported contribute to the regulation of immune responses by inhibiting DC maturation, T cell-dependent DC activation, reduce the secretion of Th1 and Th2 cytokines and induce the development of regulatory T cells. The aimed of this study to determine the association of vitamin D level with DC maturation, IFN- $\gamma$  and IL-4 cytokine expression in Indonesian SLE patients.

The study was conducted in fifty four active disease SLE patients (SLEDAI score >5). Twenty three healthy volunteers serves as controls. The level of vitamin D (25(OH)D3) was assayed by ELISA. The expression of CD11c+/CD86/CD40/CD83, IL-4 and IFN- $\gamma$  molecules were measured by Flowcytometry. Differences levels of vitamin D, the maturation of DCs (moDC, iDC, mDC), the expression of IL-4 and TNF- $\alpha$  between SLE patients and healthy controls were analyzed with independent T test. Correlation of vitamin D level with the differentiation of DC maturation, Th1-Th2 cytokine expression were analyzed by Spearman test. The level of vitamin D in SLE patients was significantly difference compared to healthy controls (35.15 $\pm$ 7.61 ng/mL vs 22.80 $\pm$ 16.23 ng/mL ; p = 0.00). There was no difference in the percentage of moDC between two groups, however, the percentage of iDC significantly increased and the percentage of mDC were slightly higher in SLE patients compared to the healthy controls. The percentage of cells producing IFN- $\gamma$  and IL-4 in SLE patients tend to be higher than those of the healthy controls (9.08  $\pm$  6.52 vs 13.19%  $\pm$  9.44% ; p = 0.062 and 5.05 $\pm$ 8.53 vs 3.35 $\pm$ 5.31 ; p = 0.058). While the ratio of IFN- $\gamma$ /IL-4 in SLE patients were similar in two groups (1.29 and 1.20 ; p = 0.520). There were negative correlation between vitamin D levels and the expression of CD11c+/CD86/CD40/CD83 at iDC and mDC, however, there were no correlation with IFN- $\gamma$  (r = 0.069, p = 0.712) and IL-4 production (r = 0.146, p = 0.433).

Vitamin D (1,25(OH)2D3) serum concentration was significantly lower in Indonesian SLE patients compared with healthy controls. The low concentration was related to higher expression of CD11c+/CD86/CD40/CD83 in iDC cells and higher secretion of Th1 cytokines (IFN- $\gamma$ ) and Th2 cytokines (IL-4).

**Keywords:** vitamin D, CD11c+/CD86/CD40/CD83, IFN- $\gamma$ , IL-4, SLE

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### I. Background

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease that occurs more frequently in the last decade. The etiology and pathogenesis of SLE remains unclear, however evidences suggest that deviation function of T cells, B cells, monocytes and regulatory T cells ultimately lead to systemic inflammation and tissue damages [1, 2]. Although the 10-year life expectancy of SLE patients has risen significantly to 90% in developed countries, a study by Handono showed that life expectancy of SLE patients in Indonesia remains low, 70% within 5-year and 55% with in 10-year [3].

A recent study concluded an association between the onset of autoimmune disease with vitamin D deficiency [4]. A high percentage of these patients have been found to live in continental/temperate climate countries [5]. Vitamin D contributes to the regulation of immune responses in several ways by inhibiting monocyte differentiation into dendritic cells (DC), inhibiting DC maturation or differentiation, and maintaining the condition of immature DC (iDC) [6]. Furthermore, it has been reported that vitamin D inhibits the activation of T cell-dependent DC [7], reduces the secretion of Th1 and Th2 cytokines and induces the development of regulatory T cells (Treg) [8].

It has been known that DC maturation markers are surface molecular expression of CD11c+/CD86/CD40/CD83. Monocytes (moDC) that develop into DC are characterized by the expression of CD11c+ and lower expression of CD86/CD40/CD83 co-stimulatory molecules. However, expression of the molecules will increase in immature DC (iDC) and the highest expression occurred in mature DC (mDC).

Mature dendritic cells play a major role as Antigen Presenting Cells (APC) that initiate professional response to cellular immunity and humoral immunity. In addition, mDC plays a role in maintaining peripheral tolerance, which is a mechanism to prevent autoimmunity such as SLE [9].

It has been shown that the deviation in DC function results in increased of T cells activation. The secretion of pro-inflammatory and anti-inflammatory cytokines has been reported to increase, which indicates the abnormalities of T reg functions. This condition is associated with the onset of clinical manifestations of SLE [10, 11].

The clinical manifestation of SLE patients in Indonesia was different from those of Caucasian. Indonesian SLE patients showed a more severe and relatively more patients with anti-dsDNA and photosensitive manifestation [3]. In this study, we aimed therefore to determine the association of vitamin D level, DC maturation markers and Th1 and Th2 cytokine expression in Indonesian SLE patients. The level of vitamin D was considered as normal when the concentration  $>30$  ng/ml [5].

Furthermore, randomly selected ten SLE patients with hypovitamin D and ten healthy controls with normovitamin D, used to analyse the expression of CD11c+/CD86/CD40/ CD83, IL-4 and IFN- $\gamma$  molecules by Flowcytometry (FC).

## II. Research Methods

### Subjects and study design

The study conducted in fifty four female SLE patients age  $31,12\pm 9.99$  (diagnosed according to the American College of Rheumatology Criteria, 1992), in active disease state (SLEDAI score  $>5$ ), and not consuming vitamin D supplements. Patients are gathered from outpatient clinics and inpatient Department of Internal Medicine, Dr Saiful Anwar Hospital Malang, Indonesia. Twenty three healthy female volunteers matched for age  $32.91\pm 5.92$ , and ethnicity serves as controls. This study was approved by the Ethics Committee of the University of Brawijaya, Faculty of Medicine Malang and informed consent was obtained from all participants.

### Sampel preparation

Three milliliters of peripheral venous blood were collected from each participants. Serum was isolated, stored in a  $-80^{\circ}\text{C}$  until used to measure the concentration of vitamin D (25(OH)D3) by ELISA in accordance with the manufacturer's instructions (Cusabio, USA). All samples were assayed in duplicate. The level of vitamin D was considered as normal when the concentration  $>30$  ng/ml [5].

Furthermore, randomly selected ten SLE patients with hypovitamin D and ten healthy controls with normovitamin D, used to analyse the expression of CD11c+/CD86/CD40/ CD83, IL-4 and IFN- $\gamma$  molecules by Flowcytometry (FC).

### Analysis of CD11c+/CD86/CD40/CD83 molecules expression on moDC, iDC and mDC

Peripheral blood mononuclear cells (PBMC) were obtained from twenty milliliters of heparinized peripheral blood by Ficoll-Hypaque density centrifugation (1600 rpm/min for 30 minutes at room temperature). PBMC were then isolated and washed twice in phosphate buffered saline (PBS). Furthermore, PBMC were suspended at a density of  $2 \times 10^6$  cells / ml in Roswell Park Memorial Institute/RPMI media 1640 (Sigma-Aldrich, USA) with L-Glutamine and 10% heat inactivated Fetal Calf Serum (Gibco, USA), supplemented with 100 ug/ml streptomycin, 100 U/ ml penicillin (Sigma-Aldrich, USA). Surface staining were performed with PE anti-human CD11c+ antibody, PerCP anti-human CD86 antibody, FITC anti-human CD83 antibody, FITC anti-human CD40 antibody (all from Biolegend, USA). moDC were recognized as cells expressing CD11c+/CD86/CD40/CD83 on the day 0.

In order to identify iDC, PBMC were cultured in TC plate 96 well, aliquots of  $5 \times 10^5$  cells / well with 150 ml medium II (50 ng / ml GM-SCF (Gibco, USA) and 100 ng/ml IL-4 (Prospec, USA), incubated 6 days of  $37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ . Replaced the medium every 3 days. At the end of day 6, cells were triple stained with monoclonal antibodies for 30 minutes, washed in PBS and the expression of CD11c +/CD86/CD40/CD83 were analyzed by FC.

To identify mDC, further incubation was required for 2 days. Culture medium was replaced with 150 ml medium III (50 ng / ml GM-CSF (Gibco, USA) with 100 ng/ml IL-4 and 50ng/ml TNF-alpha (all from Prospec, USA). At the end of day 8, cells were triple stained with monoclonal antibodies and the expression of CD11c+/CD86/CD40/ CD83 were analyzed by FC.

### Stimulation of peripheral blood lymphocytes and detection of cytokines.

PBMC were resuspended in 150 ml RPMI 1640 with 10% human AB serum and then preincubated with  $\beta$  mercapto ethanol ( $2 \times 10^6$  cells/well),  $30\mu\text{M}$   $\alpha\text{CD3}$  and  $2\mu\text{l/ml}$  IL-2 for 3 days at  $37^{\circ}\text{C}$  and 5%  $\text{CO}_2$ . Furthermore, PBL were incubated with PMA (2 ml PMA + 8 ml RPMI) for 6-8 hours ionomycin ( $0,7\mu\text{g/ml}$ ) in the presence of brefeldin-A ( $10\mu\text{g/ml}$ ). Cells were labelled with FITC-CD4 and incubated for 20 minutes, fixed

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and incubated for further 30 minutes. Furthermore, intra-cellular staining were performed with monoclonal antibodies : PE-IFN $\gamma$  (BD Bioscience) and PerCP-IL-4, incubated for 20 minutes and were wahsed in Perm/Wash buffer. Finally samples were analyzed by FC. Cytokine expressing CD4+ cells were classified as Th1 (producing IFN $\gamma$ ) and Th2 (producing IL-4).

#### Statistical Analysis

Statistical analysis were performed with SPSS 16 version. Differences levels of vitamin D between SLE patients and the control group were determined by independent t-test. The differences in the differentiation of DCs (moDC, iDC, mDC) and the expression of IL-4 and IFN- $\gamma$  in the two groups were analyzed with independent T test. Correlation of vitamin D level with the differentiation of DC, Th1/Th2 cytokine production were analyzed by Spearman test ; p value <0.05 was considered significant.

### III. Results

#### Characteristics of subjects

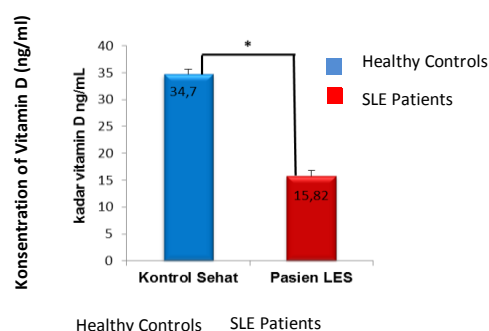
There were no differences of age in the SLE patients and healthy control subject ( $31.12 \pm 9.99$  and  $32.91 \pm 5.92$  years,  $p > 0.05$ ). Nephritis was the most clinical symptom in SLE patients (50 %), followed by arthritis, photosensitivity and hematological abnormalities. The mean level of anti-dsDNA was  $142.57 \pm 79.73$  IU/ml and 70.3% of SLE patients showed high levels of anti-dsDNA (>92 IU/ml). The characteristics of the study subjects are summarized in Table 1.

**Table 1.** The clinical manifestations of SLE patients (N = 54)

The clinical manifestations	N = 54
oral ulcers	27,7%
arthritis	48,1%
malar rash	33,3%
discoïd rash	20,3%
photosensitive	42,5%
nephritis	50%
neurologic disorders	16,6%
hematologic abnormalities	42,5%
anti-dsDNA>92 IU/ml	70,3%

#### Vitamin D levels in SLE patients and healthy control

The mean level of vitamin D in SLE patients was  $22.80 \pm 16.23$  ng/mL (2.1-50.4 ng/mL), with 92.5% of the patients showed hypovitamin D. However, the mean level of vitamin D in healthy controls was  $35.15 \pm 7.61$  ng/mL (18.9-48.30ng/mL) with only four subjects (17.3%) had vitamin D deficiency. There were significant difference between the level of vitamin D in SLE patients and healthy control ( $p = 0.00$ ), (Fig. 1).

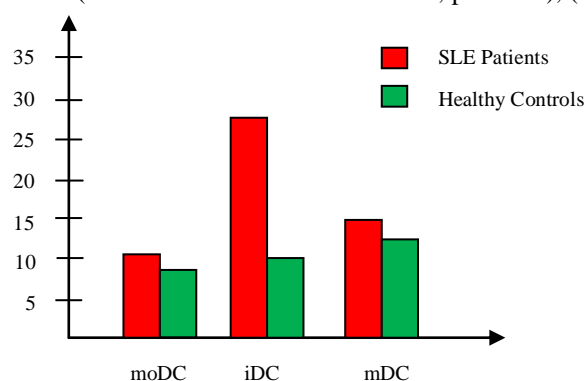


**Figure 1:**The mean level of vitamin D in healthy control and SLE patients. Significant differences are marked with an asterisk (\*  $p < 0.05$ ). Levels of serum vitamin D (25(OH)D3) was assayed by ELISA. Results expressed as mean  $\pm$  SD.

#### Differentiation of DC (moDC, iDC, mDC), expressing CD11c+/CD86/CD40 molecules in SLE patients and healthy control

Our results indicated that the percentage of moDC (cells expressing CD11c+/CD86/CD40 molecules on the day 0) in SLE patients did not differ to the healthy controls ( $10.41 \pm 7.52\%$  vs  $8.32 \pm 4.05\%$ ,  $p = 0.261$ ). Interestingly, the percentage of iDC (cells expressing CD11c+/CD86/CD40 molecules on the day 6) in SLE patients significantly increased then the healthy control ( $28.52 \pm 9.76\%$  vs  $9.51 \pm 5.63\%$ ,  $p = 0.043$ ). However, the

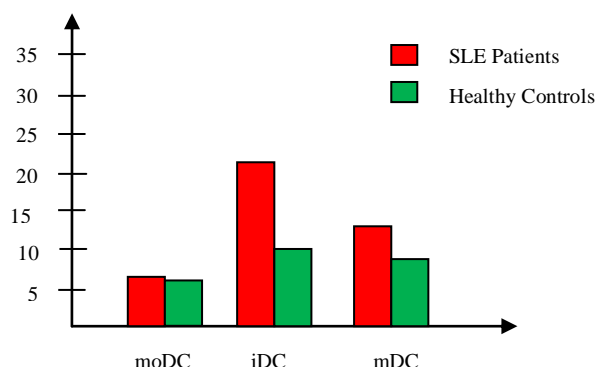
percentage of mDC (cells expressing CD11c+/CD86/CD40 on the day 8) in SLE patients were slightly higher compared with the healthy controls ( $14.93 \pm 7.83\%$  and  $12.97 \pm 4.38\%$  ;  $p = 0.229$ ), (Fig 2).



**Figure 2:** The percentage of DC expressing CD11c+/CD86/CD40 in healthy controls and SLE patients. moDC were analyzed on day 0, iDC analyzed on the day 6 and mDC analyzed on the day 8. Extracellular staining with PE-anti-CD11c+, PerCP-anti-CD86 and FITC anti-CD40 antibodies.

**Differentiation of DC (moDC, iDC and mDC) expressing CD11c+/CD86/CD83 molecules in SLE patients and healthy controls**

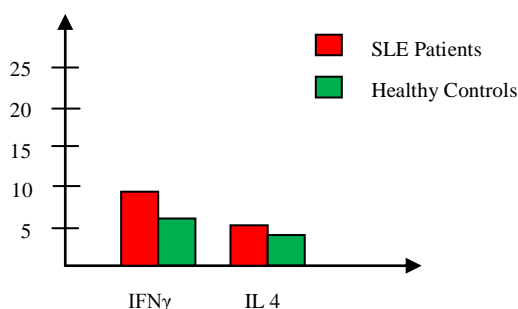
The percentage of moDC (cells expressing CD11c+/CD86/CD83 on the day 0) were similar between SLE patients and healthy controls ( $6.73 \pm 5.43\%$  vs  $6.23 \pm 4.01\%$ ,  $p > 0.05$ ). While the number of iDC (cells expressing CD11c+/CD86/CD83 on the day 6) were significantly higher in SLE patients than healthy controls ( $21.96 \pm 4.94\%$  vs  $9.69 \pm 5.78\%$ ,  $p = 0.021$ ). The percentage of mDC (cells expressing CD11c+/CD86/CD83 on the day 8) were also similar between two groups ( $20.59 \pm 9.55\%$  and  $12.54 \pm 5.85\%$ ), with only trend towards a higher percentage in the SLE patients ( $p = 0.258$ ) (Fig 3).



**Figure 3:** The percentage of DC expressing CD11c+/CD86/CD83 in healthy controls and SLE patients. moDC were analyzed at the day-0, iDC were analyzed at day-6, mDC were analyzed at day-8. Extracellular staining with PE anti-CD11c+, PerCP anti-CD86 and FITC anti-CD83 antibodies.

**Differences of IFN $\gamma$  and IL-4 expression and IFN $\gamma$ /IL-4 ratio in SLE patients and healthy controls**

The results of this study showed the percentage of Th1 cells producing IFN- $\gamma$  in SLE patients tend to be higher than those of the healthy controls ( $9.08 \pm 6.52$  vs  $13.19\% \pm 9.44\%$  ;  $p = 0.062$ ). The percentage of Th2 cells producing IL-4 in SLE patients also tend to be higher than in healthy control ( $5.05 \pm 8.53$  vs  $3.35 \pm 5.31$  ;  $p = 0.058$ ). While the ratio of IFN- $\gamma$ /IL-4 in SLE patients were similar in two groups (1.29 and 1.20 ;  $p = 0.520$ ), (Fig 4).



**Figure 4:** The percentage of Th1 and Th2 cells producing IFN- $\gamma$  and IL-4 in SLE patients and healthy controls.

**The correlation of serum level of Vitamin D with DC differentiation and IFN $\gamma$ /IL4 expression**

The result of our study noticed negative correlation between vitamin D levels and the expression of CD11c+/CD86/CD40/CD83 at iDC and mDC (Table 2). However, there were no correlation of vitamin D levels and IFN- $\gamma$  ( $r = 0.069$ ,  $p = 0.712$ ) and IL-4 production ( $r = 0.146$ ,  $p = 0.433$ ).

**Table 2.** The correlation of Vitamin D levels and DC differentiation

Differentiation of dendritic cell	Expression	<i>p</i>	Pearson correlation ( <i>r</i> )
iDC	CD11c+/CD86/CD40	0.163	- 0.507
	CD11c+/CD86/CD83	0.466	-0.280
mDC	CD11c+/CD86/CD40	0.254	-0.425
	CD11c+/CD86/CD83	0.563	0.223

**IV. Discussion**

**SLE patients had lower Vitamin D level than healthy controls**

We found significant differences the level of vitamin D between SLE patients and healthy controls group. SLE patients tend to have lower vitamin D levels than the healthy controls. Kim, et al reported that level of serum 25(OH)D in Korean SLE patients were significantly lower than in normal controls and the risk of the vitamin D deficiency increased 4.6 times in SLE patients [12]. Wozniacka et al also reported that concentration of serum 25(OH)D in SLE patients significantly decreased during the summer season compared to the control group [13]. Cohort studied by Toloza et al showed there were vitamin D insufficiency in female SLE patients, with the prevalence of 66.7% sub optimal levels of 25(OH)D, and 17.9 % deficiency [14].

It still cannot be determined whether the lower levels of vitamin D was caused by or an effect of SLE. Hypovitamin D may be the cause of SLE, as supported by evidence that vitamin D has a role in the regulation of the immune system that can prevent autoimmunity [15], thus low levels of vitamin D may disrupt the regulation of the immune system. The involvement of vitamin D in the immune response demonstrates evidence that 1) the vitamin D receptors (VDR) are expressed in various of immune system cells, including monocytes, macrophages, activated T and B cells [16]. 2) Vitamin 1,25 (OH)2D3 can regulate the activity of T cells either directly or indirectly through modulation of DC as APC [4]. 3) When monocytes differentiate into iDC, vitamin 1,25 (OH)2D3 has a role in improving the ability to capture and bind the microbial antigens that enhance the immune response initiation [17]. 4). Vitamin D deficiency leads to the loss of immune response and tolerance, and add vitamin D invitro that can restore the abnormal immune regulation of SLE patients [7]. Further evidence suggests that SLE patients have higher risks of hypovitamin D, this is likely due to the manifestation of photosensitivity, causing SLE patients to avoid direct exposure to sunlight. Other predisposition factors include the use of sunscreens, the effect of corticosteroid and chloroquine therapy, renal impairment and the presence of antibodies anti-vitamin D [7].

**Different numbers of moDC, iDC and mDC expressing CD11c+/CD86/CD40/CD83 in SLE patients with hypovitamin D and healthy control**

Dendritic cells are professional APCs that initiate innate and adaptive immune responses, in particular play an important role in the regulation of T cell activation and proliferation [18]. Dendritic cells also play a role in inducing immune tolerance both central and peripheral [19]. The ability of DCs to express co-stimulatory molecules (CD40/CD83) determine their capacity as either cells that maintain tolerance or activator cells. Generally, tolerance is initiated when DC still in immature condition, whereas the initiation of the adaptive immune response requires an effective signal of mDC [18]. Thus it can be concluded that DC expresses CD40/CD83 is highest when in the mature condition. These studied looked differences in the molecular expression of CD11c+ and CD86/CD40/CD83 co-stimulator molecules between moDC, iDC and mDC in the control group and SLE patients with hypovitamin D. Analysis of surface markers CD40 and CD83 are performed separately due to equipment limitations.

These results indicated that the expression of CD11c+/CD86/CD40/83 iDC SLE patients were significantly higher than the control group. This elevated expression of CD11c+/CD86/CD40/CD83 in iDC cells, suggesting that DC has been able to activate T cells even in an immature state. This can be seen as an unfavorable situation, as there will be an expected increase in T cell activation.

Increased expression of CD86 is associated with increased DC migration to lymphoid organs, thus stimulating T cells to increase expression of CD40, which is a ligand for the activation of T cells, demonstrating the ability of DC as a highly efficient APC. Decker et al reports similar results that SLE patients, in particular those in iDC stages showing, "Over-express" of CD86 [20]. Furthermore, Nie et al also reports the increased

expression of CD40 and CD86 in SLE patients in iDC stages [21]. This could explain how peripheral tolerance ability of SLE patients may be impaired and lead to T cell auto-reactivity.

Expression of CD83 is a cell surface marker, mostly found in mDC cells. Function of CD83 molecules has not been clearly defined; however CD83 plays an important role during cells interaction. CD83 enhances the capacity of DC to induce an immune response. Ding et al finds that mDC SLE patients show a high expression of CD80 and CD86 accompanied by increased ability to activate T cells, although the expression of CD83 it self decreases (18). This notion is also supported by Crispin and Varella's reasearch, in which the SLE serum contains several factors capable of inducing phenotypic changes and function of dendritic cells, particularly in active SLE patients [10].

In addition to vitamin D, other factors affecting the increased expression of co stimulatory molecules on DC are a) the presence of infection b) the presence of soluble factors (antibodies or acute phase protein) in the serum of SLE patients that can trigger DCI maturation and c) increase the apoptotic cells in SLE, induces maturation of DC. The latest data shows that apoptotic blebs induce maturation of myeloid DC [11]. DC, a professional APC serves as the main target of active metabolite of vitamin D, 1,25(OH)2D3. The known effects of 1,25 (OH)2D3 inhibits monocyte differentiation into DCs and inhibits DC maturation process [7], and also further maintains it in a state of immaturity (6), in addition 1,25 (OH)2D3 also promotes DC to apoptosis. This results in the inhibitory effect of T cell activation and induces alloreactive T cells into hyporesponsive ones [22].

The results of the study showed that SLE patients with hypovitamin D had iDC higher than healthy controls, and there was negative correlation between vitamin D levels and differentiation state of DC especially the expression of CD11c+/CD86/CD40/CD83 in iDC and mDC stages. Several in vitro studies have reported that 1,25(OH)2D3 is negatively correlated with expression of CD40, CD80, CD86 co-stimulatory molecules, inhibit the production of IL-12 and increase the production of IL-10, resulting in a reduced T cell activation DC derived from monocyte-derived dendritic cells (MDDCs) in human peripheral blood or in mouse bone marrow derived dendritic cells, both showing consistent results [17]. Furthermore, Mathieu and Adorini show significant results in therapy 1,25 (OH) 2D3 both in vitro and in vivo that reduces the expression of MHC class II, co-stimulatory molecules (CD80, CD86, CD40) and lower the maturation process of dendritic cells (CD83) [23].

### **Differences in number of Th1 and Th2 cells expressing IFN $\gamma$ and IL-4 in SLE patients and healthy controls**

The results of this study indicate that the percentage of Th1 producing IFN- $\gamma$  cells and the Th2 producing IL-4 cells tend to be higher in SLE patients than those in the healthy control group. It has been understood that SLE is a prototype of autoimmune disease with the dominance of Th2 activity by hyperactivity B cell. However, the result of this study shows that activity Th1 cells increases, this is likely due to compensation by excessive Th1 cells that inhibits the activity of Th2 cells, which subsequently leads to chronic systemic inflammation (4, 24, 25). These results are consistent with Sayed et al, who demonstrate that IFN- $\gamma$  levels are significantly higher in SLE patients compared with the control group (26). Similarly, the ratio of IFN- $\gamma$  / IL-10, IFN- $\gamma$  / IL4, TNF / IL-10, and TNF/IL-4, indicates the dominant participation of Th1 more than Th2. Active metabolite of vitamin D, 1,25-dihydroxy vitamin D (1,25 (OH) 2D3), can alter T cell compartment to be more anti-inflammatory and in a state regulated by Th1 and Th17 cells that inhibits and increases Th2 and Treg cells (26). In a case where vitamin D levels are low, the function will be impaired there by inhibition of T cells tend to be inflammatory.

## **V. Conclusion**

Vitamin D (1,25(OH)2D3) serum concentration is significantly lower in Indonesian SLE patients compared with healthy controls. The low concentration was related to higher expression of CD11c+/CD86/CD40/CD83 in iDC cells and higher secretion of Th1 cytokines (IFN- $\gamma$ ) and Th2 cytokines (IL-4). However, the ratio of the cytokines was not significantly different between SLE patients and healthy controls. The use of vitamin D in SLE patients needs to be examined further.

### **Conflict of Interests**

No conflict of interest has been declared by the authors

## **Reference**

- [1] Shakra, M.A. Do improved survival rates of patients with systemic lupus erythematosus reflect a global trend?. *J Rheumatol* 2008; 35:1906-1908.
- [2] Jianxin LU., Bonnie, K.W., Cheukchun, S. Up date on the role T cell subset in the pathogenesis of systemic Lupus Erythematosus. *J Chinese Clin Medic* 2009; 4(7): 400-409.
- [3] Handono, K. HLA klas II dan kerentanan genetik terhadap LES di Indonesia. *Acta Med Ind* 2001; 32:11-15.
- [4] Toubi, E., and Y. Shoenfeld. The role of vitamin D in regulating immune responses. *IMAJ* 2010; 12:174-175.
- [5] Costenbader, K.H., Feskanich, D., Garcia, E.B., Holmes, M., Karison, E.W. Vitamin D intake and risk of systemic lupus erythematosus and rheumatoid arthritis in women. *Ann Rheum Dis* 2008; 67(4):530-535.

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- [6] Dusso, A.D., Brown, A.J., Slatopolsky, E. Vitamin D. *Am J Physiol Renal Physiol* 2005; 289:8-28.
- [7] Cutolo, M. Vitamin D and autoimmune rheumatic diseases. *Rheumatol* 2009; 48:210-212.
- [8] Ghoreishi, M., Bach, P., Obst, J., Komba, M., Fleet, J.C., Dutz, J.P. Expansion of antigen-specific regulatory T Cells with the topical vitamin D analog calcipotriol. *J Immunol* 2009; 182: 6071-6078.
- [9] Rossi, M., and W.Y. James. Human dendritic cells: potent antigen-presenting cells at the crossroads to innate and adaptive immunity. *J Immunol* 2005; 175:1373-1381.
- [10] Crispin, J.S., and J.A. Varela. The role myeloid dendritic cells play in the pathogenesis of systemic lupus erythematosus. *Autoimmunity reviews* 2007; 6(7):450-456.
- [11] Fransen, J.H., Vlag, J.V.D., Ruben, J., Adema, G.J., Berden, J.H., Hilbrands, L.B. The role of dendritic cells in the pathogenesis of systemic lupus erythematosus. *Arthritis Research & Therapy* 2010; 12:207.
- [12] Kim, H.A., Koh, B.R., Jeon, J.Y., Kim, K.Y., Suh, C.H. Vitamin D deficiency in Korean patients with systemic lupus erythematosus. *Lupus* 2010; 19:1.
- [13] Wozniacka, Anna McCauliffe, Daniel P. Bogaczewicz, Jaroslaw Lukas zkiwicz, Jacek Kaleta, Beata Sysa-Jedrzejowska, Anna. The relationships between serum vitamin D levels, vitamin D receptor polymorphism, anti-vitamin D autoantibodies, IL-17 and IL-23 levels, and clinical parameters in SLE patients. The 9th International Congress on SLE June 24-27- *Lupus* 2010; 19: 1.
- [14] Toloza, S., D.E.C., Cole, D.D., Gladman, M.B., Urowitz. Vitamin D insufficiency in a large female SLE cohort. *Lupus* 2010; 19:13-19.
- [15] Bonakdar, L., F. Jahanshahifar, A., Gholamrezaei. Vitamin D deficiency and its association with disease activity in new cases of systemic lupus erythematosus. *Lupus* 2011; 20:1155-1160.
- [16] Bikle, D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab* 2009; 94(1):26-34.
- [17] Baeke, F., Takiishi, T., Hannelie, K., Conny, G., Chantal, M. Vitamin D: modulator of the immune system. *Curr Op in Pharmacol* 2010; 10:482-496.
- [18] Ding, D., Mehta, H., McCune, J., Kaplan, M.J. Aberrant phenotype and function of myeloid dendritic cells in systemic lupus erythematosus. *J Immunol* 2006; 177:5878-5889.
- [19] Maddur, M.S., Vani, J., Dimitrov, J.D., Balaji, K.N., Desmazes, S.L., Kaveri, S.V., Bayry, J. Dendritic cells in autoimmune diseases. *The Open Arthritis Journal* 2010; 3:1-7.
- [20] Decker, P., Ina, K., Reinhold, Beate, B., Hans, G.R. Monocyte-derived dendritic cells over-express CD86 in patients with systemic lupus erythematosus. *Rheumatol* 2006; 45 :1087-1095.
- [21] Nie, Y.Z., Mok, M.Y., Chan, G.C.F., Chan, A.W., Jin, O., Kavokondala, S., Lie, A.K.W., Lau, C.S. Phenotypic and functional abnormalities of bone marrow-derived dendritic cells in systemic lupus erythematosus. *Arthritis Research & Therapy* 2010; 12:1-12.
- [22] Penna, G., and L. Adorini.  $1\alpha,25$ -Dihydroxyvitamin D<sub>3</sub> Inhibits Differentiation, Maturation, Activation, and Survival of Dendritic Cells Leading to Impaired Alloreactive T Cell Activation. *J Immunol* 2000; 164:2405-2411.
- [23] Mathieu, C., and L. Adorini. The coming of age of  $1,25$ -dihydroxyvitamin D<sub>3</sub> analogs as immunomodulatory agents. *TRENDS in Molecular Medicine* 2002; 1471:1-6.
- [24] Cantorna M.T and B.D Mahon. Mounting Evidence for Vitamin D as an Environmental Factor Affecting Autoimmune Disease Prevalence. *Exp Biol Med* 2004; 229:1136-1142.
- [25] Adams, J.S., and M. Hewison. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab* 2008; 4(2): 80-90.
- [26] Sayed, M.E., Nofal, E., Mokadem, S.A., Makhzangy, I.A., Gaballah, H., Akl, H. Correlative study of serum Th1/Th2 cytokines level in patients with systemic lupus erythematosus with SLEDAI. *J Egypt Dermatol Online* 2008; 4(1): 1-15.