Turkish Journal of Immunology - Open Access
www.turkishimmunology.org

Turk J Immunol 2017; 5(3)89–95

1,25(OH)$_2$D3 Inhibits Endothelial Apoptosis by Neutrophil Extracellular Traps Externalization in Systemic Lupus Erythematosus Patients

1,25(OH)$_2$ D3, Sistemik Lupus Eritematozus Hastalarında Nötrofil Hücre Dışı Tuzakları ile Endotel Apoptozunu Baskılar

Kusworini Handono[1*], Benny Arie Pradana[2], Radhitio Adi Nugroho[2], Dian Hasanah[3], Handono Kalim[3], Agustina Tri Endharti[4], and Fatchiyah[5]

Abstract

Introduction: Systemic Lupus Erythematosus (SLE) is a very complicated autoimmune disease which is characterized by the presence of abnormal neutrophils known as Low Density Granulocytes (LDGs). These LDGs have increased capacity to produce Neutrophil Extracellular Traps (NETs). Vitamin D levels in SLE patients were significantly lower than that of healthy subjects. This study aims to investigate the effects of vitamin D [1,25 (OH)$_2$D3] on NETosis and endothelial cell apoptosis in SLE patients with vitamin D deficiency.

Materials and Methods: Neutrophils of five SLE patients with vitamin D deficiency were treated with four different doses of 1,25 (OH)$_2$D3:0 M as a control, 1x10^{-9} M, 1x10^{-8} M, and 10^{-7}M. Phorbol myristate Acetate (PMA) were given to induce production of NETs. The supernatant obtained from NETs induction was cocultured with Human Umbilical Vein Endodethial Cells (HUVECs). Histone and defensin externalization was investigated by immunofluorescence staining. Endothelial apoptosis was investigated by flow cytometry.

Results: This study shows that neutrophils treated with 1x10^{-8} M of 1,25 (OH)$_2$D3 had significantly lower defensin, histone externalization, and endothelial cell apoptosis compared to those caused by other doses. There was also a positive correlation between histone externalization and endothelial apoptosis.

Conclusion: Vitamin D inhibits endothelial damage by reducing the NETs development.

Keywords: Defensin, endothelial, histone, NETs, 1,25 (OH)$_2$D3, Neutrophil extracellular trap

Öz

Giriş: Sistemik Lupus Eritematozus (SLE), Düşük Yoğunluklu Granülositlerin (DYG) varlığı ile karakterize çok karmaşık bir otoimmün hastalıktır. Bu DYGler, nötrofillerin hücre dışı tuzaklarını (NHT) oluşturma özelliğindedir. D vitamini düzeyleri ise, SLE'li hastalrda normal bireylerdekine göre daha düşük saptanmıştır. Bu çalışmada, Vitamin D [1,25 (OH)$_2$D3] seviyelerinin Vitamin D eksikliği olan SLE hastalarındaki DYG oluşturma endotel hücresi apoptozisi üzerine olan etkisini irdeledik.

Gereçler ve Yöntemler: Beş SLE'li ve D vitamini eksikliği olan hastadan elde edilen nötrofiller, 1x10^{-9} M, 1x10^{-8} M, ve 10^{-7}M 1,25 (OH)$_2$D3 verilen ve kontrol olarak ta hiç D vitamini uygulanmayan gruplara ayrıldı. Bu gruplardaki nötrofillerin NHT oluşturma özelliği irdelendi. NHT'nin arttırılmaya çalışıldığı bu deneylerden elde edilen hücre üstü sıvılar, insan göbek kordonu veni endotel hücreleri üzerine uygulandı. Histon ve defensin çıkarımı immünofloresan boyama tekniği ile araştırıldı. Endotel hücrelerinin apoptozu, akan hücre ölçeri ile değerlendirildi.

Bulgular: Çalışmada, 1x10^{-8} M 1,25 (OH)$_2$D3 uygulanan nötrofillerin diğer gruplara göre daha az defendin ve histon çıkarımı yaptığı ve daha az endotel hücresi apoptozuna neden olduğu saptandı. Aynı zamanda, histon çıkarımı ile endotel hücresi apoptozu arasında bir bağıntı saptandı.

Sonuçlar: D vitamini NHT oluşumunu azaltarak endotel hücresi hasarını azaltır.

Anahtar Kelimeler: Defensin, endotel hücresi, histon, nötrofilin hücre dışı tuzağı, 1,25 (OH)$_2$D3, sistemik lupus eritematozus

[1]Department of Clinical Pathology, Faculty of Medicine Brawijaya University/ Saiful Anwar General Hospital, Malang, Indonesia
[2]Biomedical Sciences, Master's Program, Faculty of Medicine Brawijaya University, Malang, Indonesia
[3]Department of Internal Medicine, Faculty of Medicine Brawijaya University, Saiful Anwar General Hospital, Malang, Indonesia
[4]Department of Parasitology, Faculty of Medicine, Brawijaya University, Malang, Indonesia
[5]Department of Biology, Faculty of Mathematics and Natural Science, Brawijaya University, Malang, Indonesia

Correspondence:
Kusworini Handono, MD, PhD
Universitas Brawijaya, Jalan Veteran, Malang, East Java, 65145
E-mail: dr.kusworini@gmail.com
Phone: +62341551611

Received: Jun 23, 2017
Accepted: Nov 02, 2017

doi: 10.25002/tji.2017.623

Introduction

Systemic Lupus Erythematosus (SLE) is a complicated autoimmune disease characterized by the involvement of multi organ damage. The five-year survival rate of 108 SLE patients in the Cipto Mangunkusumo Hospital in Indonesia was 88%.[1] SLE is Neutrophil extracellular traps characterized by functional changes of neutrophils.[2] A subset of neutrophils have

Turk J Immunol 2017; 5(3):89–95

been identified from SLE patients, called as Low Density Granulocytes (LDGs).[3] These LDGs have increased capacity to secrete (NETs). NETs are secreted during an uncommon process called NETosis that has a contribution in the SLE pathogenesis.[4] NETosis releases antimicrobial substances such as Neutrophil Elastase (NE), Human Neutrophil Protein (defensin/HNP), Myeloperoxidase (MPO), chromatins, DNA, and histone which forms the NETs structure. SLE patients' neutrophils are also capable to secrete NETs.[5] NETs involve in triggering auto-antibody production and complement activation that cause vasculitis and augment clinical manifestation.[6,7] Defensins are antimicrobial peptides that play a role during NETosis through interaction with platelets and exert a cytotoxic effect[8] which leads to endothelial cell damage.[9,10] Histone is a structural protein with various modification mechanisms and has the ability to cause a pathological condition in cells. Circulating histones can cause *in vivo* endothelial damage, increase microvascular permeability, activate the coagulation process, and increase the secretion of IL-6.[11]

A recent study demonstrated the correlation between vitamin D deficiency and autoimmune disease severity.[12] Another study showed that high percentage of SLE patients tend to be vitamin D deficient.[13] Our earlier studies concluded that vitamin D in SLE subjects were lower than healthy subjects.[14] In this present study, we assess the effect of vitamin D on NETosis and endothelial apoptosis.

Methods

Patient Selection and Sample Preparation

Ethic Committee of the medical faculty of Brawijaya University approved this study. Subjects have been given orally and written research information; the consent letter was signed by the participant or the participant's representative. The revised American College of Rheumotology (ACR) criteria for SLE was used for inclusion criteria. Lupus participants were recruited from outpatient rheumatology clinic of Saiful Anwar General Hospital.

The neutrophils were collected from 5 cases newly diagnosed as active SLE (Mex-SLEDAI >5) female patients. The characteristics of the subjects can be seen in Table 1. The serum was isolated and vitamin D concentration was analyzed by ELISA (NovaTein Bio, USA). The umbilical cord was taken from cesarean section at the department of obstetrics, Saiful Anwar General Hospital, Malang.

Table 1. Characteristics of SLE subjects

Characteristics	Means ± SD
Age (years)	26.40 ± 6.77
Duration of Illness (years)	4.4 ± 2.4
Anti-ds-DNA antibody level (IU/ml)	167.42 ± 7.86
Vitamin D serum level (ng/ml)	21.60 ± 4.74
Medication	Methylprednisolone, Omeprazole, Calcium, Diclofenac Sodium, Folic Acid

Neutrophil Isolation

The collected blood was placed over the Polymorphprep (Axis shield, AS) and then centrifuged at 1400 RPM. A layer of neutrophils was harvested and purified with erythrocyte lysis buffer.[15] Neutrophils were confirmed by CD10 and CD14 double positive; purity was >95%.[5]

Neutrophils Culture

Neutrophils were placed in poly-L-lysine coverslip and RPMI 1640 media (Sigma-Aldrich, USA)[5]; they were incubated at 37°C for 15 minutes, 5% CO_2. Then, the isolated neutrophils were treated for 24 hours with $1x10^{-9}$ M, $1x10^{-8}$ M, and 10^{-7}M of 1.25 $(OH)_2D_3$ (Sigma).

Neutrophil Extracellular Traps (NETs) Production

Neutrophils were also seeded in 24 poly-L-lysine-free wells for the production of NETs. For 4 hours, 50 nM PMA was given to neutrophil culture. Then, the wells were washed with RPMI. NETs were collected after centrifugation of well contents for 5 minutes at 650 RPM. Collected NETs were stored at -20°C for the next assay.[16]

HUVECs Culture

Endothelial cells were obtained from human umbilical cord. Collagenase solution was inserted into the umbilical vein. The solution obtained from previous process was then centrifuged at 1600 RPM. Isolated HUVECs were cultured in 24 gelatin coated wells until confluence.[15,16] The purity of endothelial cells (more than 99%) was confirmed by FACS with CD146 (Biolegend), endothelial specific marker.[17]

NETs Immunofluorescence Assay

The neutrophil culture was induced with PMA (Sigma) at 20 nM to produce NETs and then incubated for two

hours. It was then rinsed using ice-cold PBS, and fixed for 15 minutes using paraformaldehyde. The cells were colored using mouse anti-human histone (1:100), goat anti-human HRP (1:250), and rabbit anti-human elastase (1:350) at 37°C for 60 minutes. Then, fixed cells were incubated again for 60 minutes with secondary FITC donkey anti-rabbit (1:500), Rodhamin donkey anti-mouse (1:100) or Rodhamin donkey anti-goat (1:100). Nuclear DNA was stained using Hoechst 33342 (1:100). Prolong Antifade Reagent (Invitrogen) were mounted to coverslips and then analyzed using Olympus microscope.[5] Color intensity of staining was quantified using image RGB analysis from three to four fields (x40). Externalization of histone and defensin at NETs (colocalization of neutrophil elastase and the DNA) was calculated from the red intensity divided by total intensity.

Endothelial Cell Death Assay

HUVECs in 24 well plates were cocultured with 200 µl supernatant contained NETs followed by incubation for 16 hours. Apoptosis was measured using flowcytometry (FACScalibur) after stained with AnnexinV-PI markers. Cells with AnnexinV^{+} was regarded as apoptosis of HUVECs.[5,17]

Statistical Analysis

Data were analyzed using SPSS 16.0. Multiple comparisons were analyzed using ANOVA (One Way Analysis of Variance) with Tukey post tests ($p<0.05$). The relationship between histone and defensin externalization with apoptosis of endothelial cells was measured using Pearson's correlation test.

Results

Effect of vitamin D on the Histone externalization

Vitamin D [1.25 $(OH)_2$D3] reduced the externalization of histone as seen in Figure 1. The difference was statistically significant between P2 and P3 compared with P0 (19.38±6.16% vs 27.89±4.85% p=0.008, and 20.31±0.82% vs 27.89±4.85% p=0.016) (Figure 2). However, the difference between P1 and P0 (22.39±1.39%

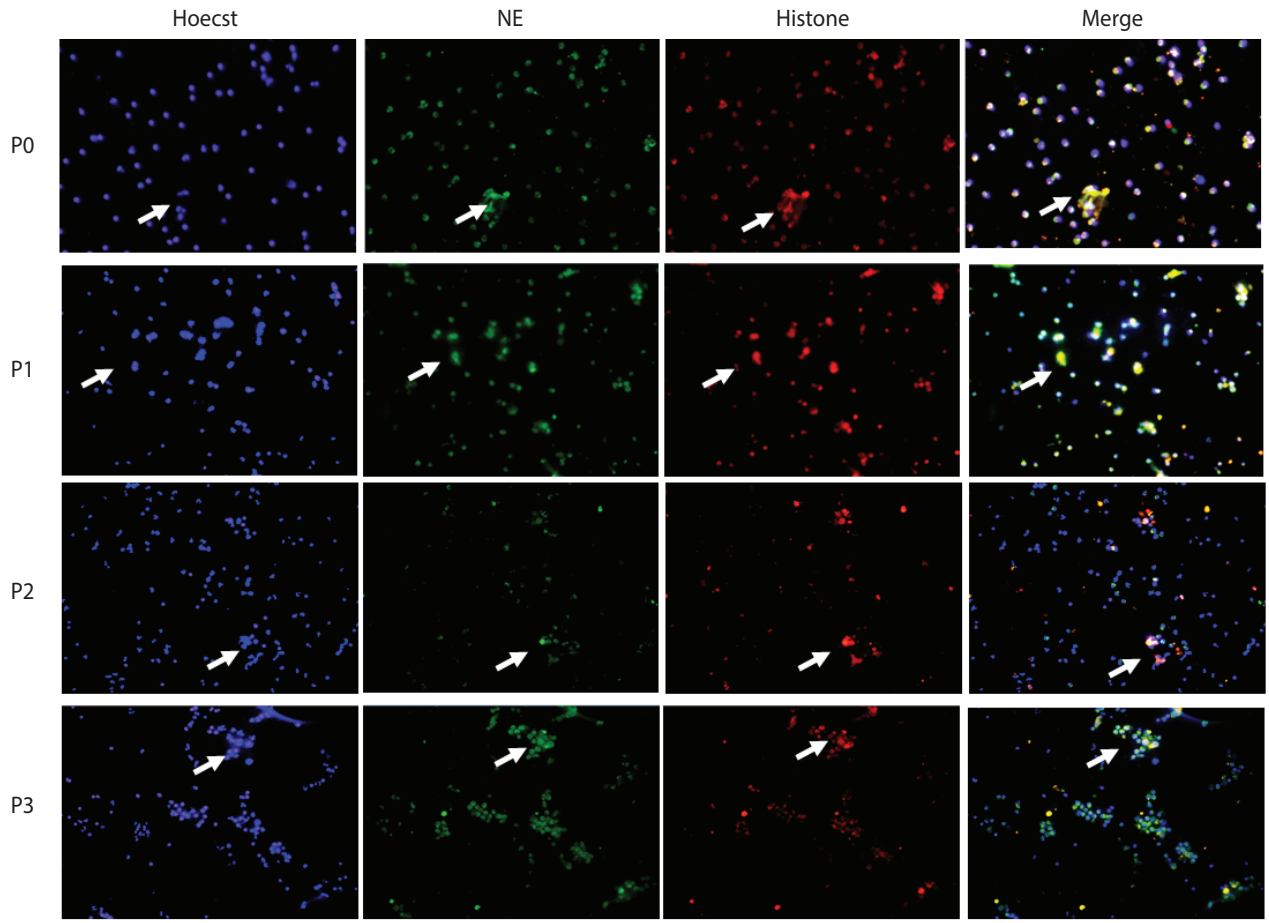

Figure 1. NETs with histone components from lupus Neutrophils during NETosis. Representative images of P0($1x10^{-9}$M), P1($1x10^{-8}$M), P2($1x10^{-7}$M), and P3/100 nM. Cells were colored for identification of nuclear DNA *(blue)*, histone *(red)*, and neutrophil elastase *(green)*.

Turk J Immunol 2017; 5(3):89–95

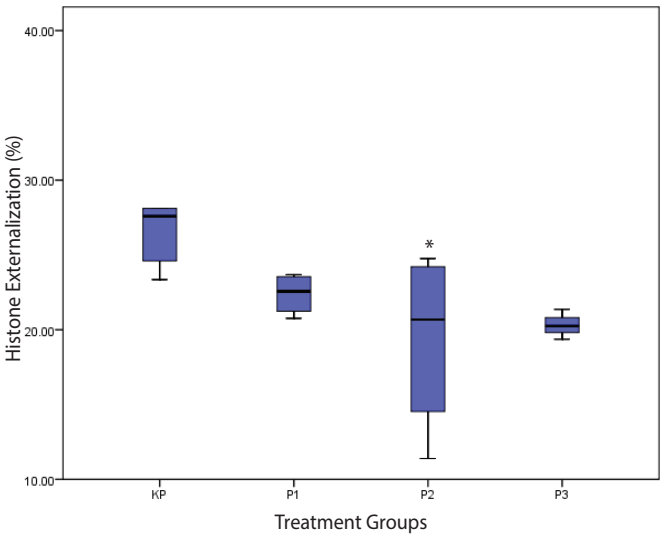

Figure 2. Percentage of NETs with histone components in control group and treatment groups with different doses of 1,25$(OH)_2$D3. There were decreased percentage of NETs with histone at P2 and P3. P2 was the lowest. *The difference was statistically significant between P2 and P3 compared with P0 (19.38±6.16% vs 27.89±4.85% p=0.008, and 20.31±0.82% vs 27.89±4.85% p=0.016)*

vs 27.89±4.85%; p=0.066) or P2 and P3 (19.38±6.16% vs 20.31±0.82%; p=0.751) was not statistically significant. This showed that $1x10^{-7}$M of 1.25 $(OH)_2$D3 was not more effective than $1x10^{-8}$ M 1.25 $(OH)_2$D3 to decrease histone externalization.

Effect of Vitamin D on the Defensin Externalization

Vitamin D [1.25 $(OH)_2$D3] treatments reduced defensin externalization as seen in Figure 3. There were significant differences between P1, P2, and P3 compared with P0 (23.025±5.054 vs 32.12±1.625, p=0.011; 24.232±3.804 vs 32.12±1.625, p=0.023; 24.48±5.560 vs 32.12±1.625, p=0.027) (Figure 4). This result indicated that the treatment of $1x10^{-9}$M, $1x10^{-8}$ M, and $1x10^{-7}$ M of vitamin D [1.25 $(OH)_2$D3] could reduce defensin externalization.

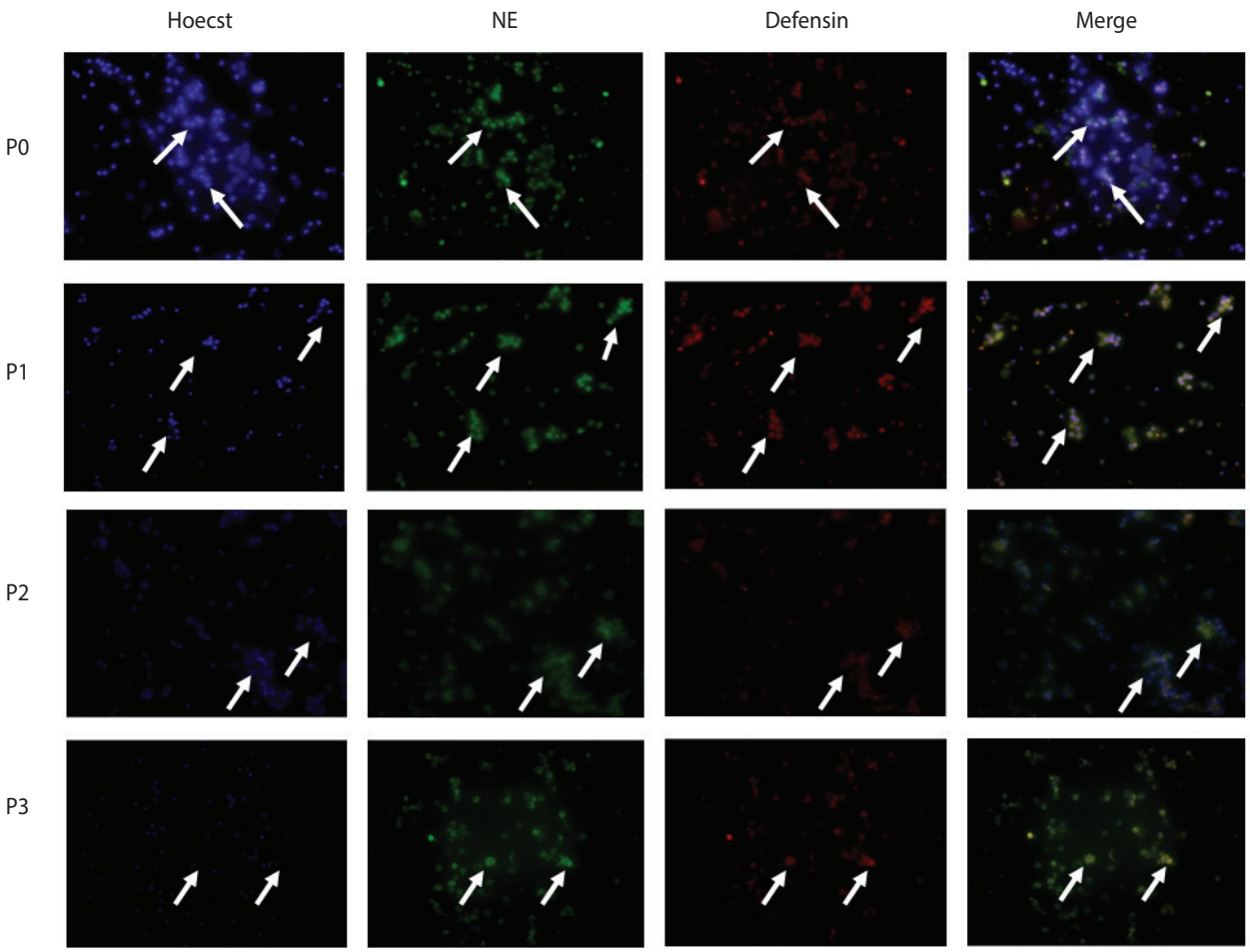

Figure 3. NETs with defensin components from lupus Neutrophils during NETosis. Representative images of P0($1x10^{-9}$M), P1($1x10^{-8}$M), P2($1x10^{-7}$M), Cells were colored for identification of, nuclear DNA *(blue)*, defensin *(red)*, and NE *(green)*.

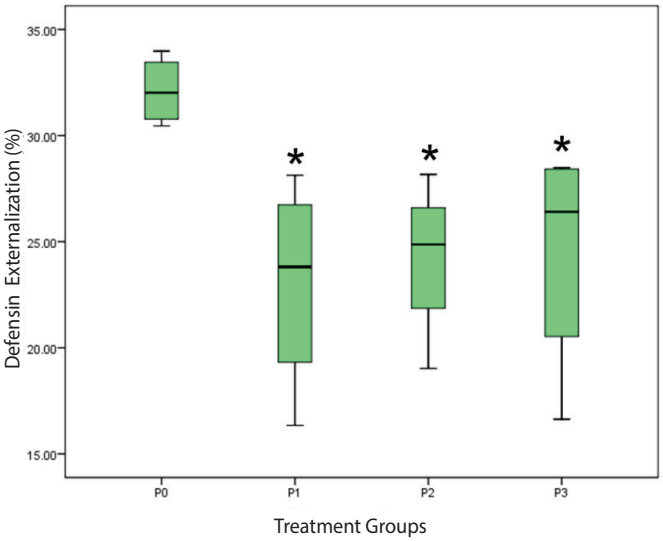

Figure 4. Percentage of NETs with defensin components in control group and treatment groups. There were reduced mean percentage of NETs with defensin components among P2 and P3. *There were significant differences between P1, P2, and P3 compared with P0 (23.025±5.054 vs 32.12±1.625, p=0.011; 24.232±3.804 vs 32.12±1.625, p=0.023; 24.48±5.560 vs 32.12±1.625, p=0.027)*

Effect of Vitamin D on Endothelial Apoptosis

Vitamin D [1.25 $(OH)_2$D3] reduced endothelial apoptosis as seen in Figure 5. The difference was significant between P2 and P0 (3.45±0.68% vs 7.18±1.64%, p=0.002), but was not significant among P1 and P3 in comparison with P0 (6.17±0.18% vs 7.18±1.64%, p=0.256, and 5.53±0.88% vs 7.18±1.64%, p=0.078). This result showed that $1x10^{-8}$ M of vitamin D [1.25 $(OH)_2$D3] was capable to reduce apoptosis.

Furthermore, histone externalization and apoptosis had a moderate positive correlation (r=0.693, p=0.012; the result of correlation can be accepted if value of p from pearson correlation is less than 0.05). However, apoptosis and defensin externalization had a weak positive correlation.

Discussion

Many studies have revealed that production of NET is a crucial process in the alteration and presentation of auto-antigen to the immune system.[18,19] Increased NET formation may have an important role in autoimmune diseases.[14,19] SLE patients neutrophils have capability to secrete NETs with abundant amount of neutrophil proteins that have important roles in autoimmunity induction. NETs itself is composed of histone, DNA, MPO, NE, defensing, and other proteins.[14,18]

Our study revealed that histone externalization was decreased by 1.25 $(OH)_2$D3 treatment in $1x10^{-8}$ M and $1x10^{-7}$ M doses. A study by Hirsch et al. revealed that vitamin D induced 5-LOX gene and reduced expression of COX-2 gene which resulted in decreasing production of inflammatory mediators.[15] In another study, vitamin D was able to inhibit transcription of gp91 Phox. Gp91 Phox is a component of Phox (NADPH oxidase) which is a key enzyme in NETosis.[20,21] 1.25 $(OH)_2$D3 was also able to inhibit PI3K/AKT/mTOR pathway.[20–22] McInturff et al. (2012) found that inhibition of the mTOR pathway decreased NETs formation.[23]

Our study showed that defensin externalization during NETosis was decreased by 1.25 $(OH)_2$D3 treatment. A study by Kim et al. (2009) showed that treatment with calcipotriol (a vitamin D analog) decreased expression of defensins. Vitamin D improved antimicrobial peptide externalization efficiency that regulates the innate immunity. Reduced expressions of defensin were probably caused directly by the effect of calcipotriol.[24] Another mechanism that was probably related to the reduced defensin externalization is CYP24. The abnormal CYP24 regulation was detected in many autoimmune diseases, such as multiple sclerosis.[25]

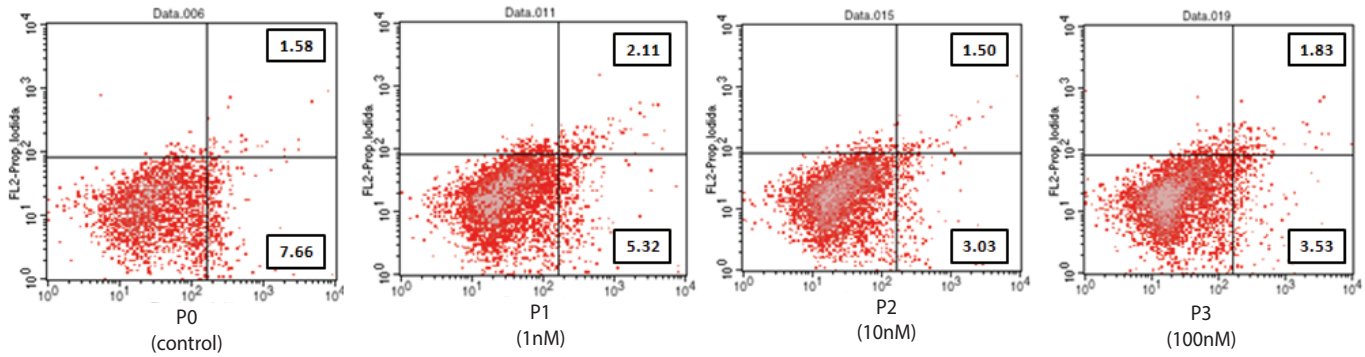

Figure 5. Vitamin D [1,25$(OH)_2$D3] reduced endothelial apoptosis. Percentage of endothelial apoptosis was presented at right quadrant.

In our study, we also found that vitamin D reduced apoptosis of endothelial cells. NETs can induce cell death via several components, one of which is a histone. Circulating histones *in vivo* can cause endothelial damage.[26] In a previous study by Abrams et al. (2011), CRP was able to prevent endothelial damage through the inhibition of histone integration on the membrane and thus prevent the influx of Ca^{2+}. In this study by Saffardzeh et al. (2012), histones and MPO were able to cause damage to the alveolar epithelial and endothelial cells.[16] Histone probably caused apoptosis due to its potential to integrate with the plasma membrane and induce calcium influx. The increased influx of calcium into the mitochondria will promote alteration of its permeability. This will induce cytochrome C activation, followed by the apoptosome formation.[27,28]

There are several mechanisms in which defensin may induce apoptosis. NETs contain auto-antigens such as MPO, PR3, LL37, and defensin. These auto-antigens can be recognized by Anti Neutrophil Cytoplasmic Antibodies (ANCA). ANCAs interact with their antigen and cause neutrophil activation. This activation leads to degranulation of neutrophil, production various cytokines, and endothelial death. The presence of auto-antigens can activate auto-reactive B cells and T helper cells, which can then induce small vessel vasculitis.[8,29,30]

In this experiment, we also found a moderate correlation among histone externalization and apoptosis. This result showed that increase in histones externalization can also increase the number of apoptotic cells. Our result has similarities with the study conducted by Saffardzeh et al. (2012), who found that MPO and histones were dominant substances that induced endothelial death.[17]

The optimal vitamin D level in serum was >30 ng/ml (>75 nM).[29] In this study, 10 nM was better than 100 nM 1.25 $(OH)_2D3$ in decreasing histone externalization. This result revealed that vitamin D [1.25 $(OH)_2D3$] has attained the optimum dose (1 nM dose equals to 0.4 ng/ml; therefore 100 nM equals to 40 ng/ml).[31] If 100 nM is added to the culture, it would result in an inverse effect.[32,33] Similarly, in another study conducted using dendritic cells, $1x10^{-7}$ M of 1.25 $(OH)_2D3$ treatment had inverse effect, indicated by an increase in cytokine IL-17A and Th17.[2]

Conclusion

Vitamin D, especially at $1x10^{-8}$ M dose, significantly inhibited histone and defensin externalization during NETosis and endothelial apoptosis. Increasing doses however, may give inverse results due to opposite effects.

Peer-review
Externally peer-reviewed.

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
This study was financed by the Directorate General of Higher Education research grant of 2013–2014.

ACKNOWLEDGEMENTS
This study was supported by the Directorate General of Higher Education research grant of 2013-2014

References

1. Handono K, Sari TL, Adam AA. Homocysteine, Folic Acid, Vitamin B6, Vitamin B12, and Biochemical Parameters of Bone Metabolism in Female Patients with Systemic Lupus Erythematosus. J Clin Cell Immunol 2014;5:217. doi: 10.4172/2155-9899.1000217
2. Wahono CS, Rusmini H, Soelistyoningsih D, Hakim R, Handono K, Endharti AT, et al. Effects of 1,25(OH)2 D3 in immune response regulation of systemic lupus erithematosus (SLE) patient with hypovitamin D. Int J Clin Exp Med 2014 2014;7:22–31.
3. Migita K, Miyashita T, Maeda Y, Nakamura M, Yatsuhashi H, Kimura H, et al. Toll-like receptor expression in lupus peripheral blood mononuclear cells. J Rheumatol 2007;34:493–500.
4. Yu Y, Su K. Neutrophil Extracellular Traps and Systemic Lupus Erythematosus. J Clin Cell Immunol 2013;4. doi: 10.4172/2155-9899.1000139
5. Villanueva E, Yalavarthi S, Berthier CC, Hodgin JB, Khandpur R, Lin AM, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol 2011;187:538–52. doi: 10.4049/jimmunol.1100450
6. Leffler J, Martin M, Gullstrand B, Tydén H, Lood C, Truedsson L, et al. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. J Immunol 2012;188:3522–31. doi: 10.4049/jimmunol.1102404
7. Charles Jennette J, Xiao H, Hu P. Complement in ANCA-associated vasculitis. Semin Nephrol 2013;33:557–64. doi: 10.1016/j.semnephrol.2013.08.006
8. Kaplan MJ, Radic M. Neutrophil extracellular traps: double-edged swords of innate immunity. J Immunol 2012;189:2689–95. doi: 10.4049/jimmunol.1201719
9. Zawrotniak M, Rapala-Kozik M. Neutrophil extracellular traps (NETs) - formation and implications. Acta Biochim Pol 2013;60(3):277–84.
10. DiMartino SJ, Trujillo G, McVoy LA, Zhang J, Kew RR. Upregulation of Vitamin D Binding Protein (Gc-Globulin) Binding Sites During Neutrophil Activation from a Latent Reservoir in Azurophil Granules. Mol Immunol 2007;44:2370–7. doi: 10.1016/j.molimm.2006.10.011

11. Kaynar AM, Houghton AM, Lum EH, Pitt BR, Shapiro SD. Neutrophil elastase is needed for neutrophil emigration into lungs in ventilator-induced lung injury. Am J Respir Cell Mol Biol 2008;39:53–60. doi: 10.1165/rcmb.2007-0315OC
12. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 2008;4:80–90. doi: 10.1038/ncpendmet0716
13. Kuhn A, Beissert S, Krammer PH. CD4(+)CD25(+) regulatory T cells in human lupus erythematosus. Arch Dermatol Res 2009;301:71–81. doi: 10.1007/s00403-008-0891-9
14. Handono K, Marisa D, Kalim H. Association between the low levels of vitamin D and Treg function in systemic lupus erythematosus patients. Acta Med Indones 2013;45:26–31.
15. Hirsch D, Archer FE, Joshi-Kale M, Vetrano AM, Weinberger B. Decreased anti-inflammatory responses to vitamin D in neonatal neutrophils. Mediators Inflamm 2011;2011:598345. doi: 10.1155/2011/598345
16. Cloke T, Munder M, Taylor G, Müller I, Kropf P. Characterization of a Novel Population of Low-Density Granulocytes Associated with Disease Severity in HIV-1 Infection. PLoS One 2012;7:1–7. doi: 10.1371/journal.pone.0048939
17. Saffarzadeh M, Juenemann C, Queisser MA, Lochnit G, Barreto G, Galuska SP, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: A predominant role of histones. PLoS One 2012;7. doi: 10.1371/journal.pone.0032366
18. Denny MF, Yalavarthi S, Zhao W, Thacker SG, Anderson M, Sandy AR, et al. A distinct subset of proinflammatory neutrophils isolated from patients with systemic lupus erythematosus induces vascular damage and synthesizes type I IFNs. J Immunol 2010;184:3284–97. doi: 10.4049/jimmunol.0902199
19. Kessenbrock K, Krumbholz M, Schönermarck U, Back W, Gross WL, Werb Z, et al. Netting neutrophils in autoimmune small-vessel vasculitis. Nature Medicine 2009;15:623–5. doi:10.1038/nm.1959
20. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. J Cell Biol 2010;191:677–91. doi: 10.1083/jcb.201006052
21. Whitcomb JP, Deagostino M, Ballentine M, Fu J, Tenniswood M, Welsh J, et al. The role of vitamin D and vitamin D receptor in immunity to Leishmania major infection. J Parasitol Res 2012;2012:134645. doi: 10.1155/2012/134645
22. Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: is immunity the second function of chromatin? J Cell Biol 2012;198:773–83. doi: 10.1083/jcb.201203170
23. Kim TH, Choi SJ, Lee YH, Song GG, Ji JD. Combined therapeutic application of mTOR inhibitor and vitamin D(3) for inflammatory bone destruction of rheumatoid arthritis. Med Hypotheses 2016;79:757–60. doi: 10.1016/j.mehy.2012.08.022
24. McInturff AM, Cody MJ, Elliott EA, Glenn JW, Rowley JW, Rondina MT, et al. Mammalian target of rapamycin regulates neutrophil extracellular trap formation via induction of hypoxia-inducible factor 1α. Blood 2012;120:3118——25. doi: 10.1182/blood-2012-01-405993
25. Kim BJ, Rho YK, Lee HI, Jeong MS, Li K, Seo SJ, et al. The effect of calcipotriol on the expression of human β defensin-2 and LL-37 in cultured human keratinocytes. Clin Dev Immunol 2009;2009:645898. doi: 10.1155/2009/645898
26. White JH. Vitamin D signaling, infectious diseases, and regulation of innate immunity. Infect Immun 2008;76:3837–43. doi: 10.1128/IAI.00353-08
27. Abrams ST, Zhang N, Dart C, Wang SS, Thachil J, Guan Y, et al. Human CRP defends against the toxicity of circulating histones. J Immunol 2013;191:2495–502. doi: 10.4049/jimmunol.1203181
28. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007;35:495–516. doi: 10.1080/01926230701320337
29. Zhang X, Liu X, Shang H, Xu Y, Qian M. Monocyte chemoattractant protein-1 induces endothelial cell apoptosis in vitro through a p53-dependent mitochondrial pathway. Acta Biochim Biophys Sin (Shanghai) 2011;43:787–95. doi: 10.1093/abbs/gmr072
30. Kaplan MJ. Role of neutrophils in systemic autoimmune diseases. Arthritis Res Ther 2013;15:219. doi: 10.1186/ar4325
31. Hewison M. An update on vitamin D and human immunity. Clin Endocrinol (Oxf) 2012;76:315–25. doi: 10.1111/j.1365-2265.2011.04261.x
32. Blumberg JM, Tzameli I, Astapova I, Lam FS, Flier JS, Hollenberg AN. Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. J Biol Chem 2006;281:11205–13. doi: 10.1074/jbc.M510343200
33. Garcia LA, King KK, Ferrini MG, Norris KC, Artaza JN. 1,25(OH)2vitamin D3 stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. Endocrinology 2011;152:2976–86. doi: 10.1210/en.2011-0159