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# The Levels of Vitamin D, Metalloproteinase-9 and Tissue Inhibitor Metalloproteinase-1 in COPD Patients, Healthy Smokers and Non-Smokers of Indonesian Citizens

Sri Rezeki Arbaningsih[1, 2*], Fajrinur Syarani[2], Ratna Akbari Ganie[3], Aznan Lelo[4]

*[1]Pulmonology Department, Faculty of Medicine, Muhammadiyah University of North Sumatra, Jl. Gedung Arca no.53 Medan Kota, Indonesia; [2]Pulmonology Department, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr.Mansyur no.5 Medan, Indonesia; [3]Clinical Pathology Department, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr.Mansyur no.5 Medan, Indonesia; [4]Pharmacology Department, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr.Mansyur no.5 Medan, Indonesia*

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**Keywords:** Vitamin D; MMP9; TIMP1; COPD

***Correspondence:** Sri Rezeki Arbaningsih. Pulmonology Department, Faculty of Medicine, Muhammadiyah University of North Sumatra, Jl. Gedung Arca no.53 Medan Kota, Indonesia. E-mail: arbaningsih@gmail.com

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**Abstract**

**BACKGROUND:** Exposure to cigarette smoke may stimulate the inflammatory response and activate polymorphonuclear leukocytes, thus resulting in secretion of cellular proteases. Vitamin D has the potential to modulate the inflammatory response to harmful particles in patients with Chronic Obstructive Pulmonary Disease (COPD).

**AIM:** This study aimed to determine the levels of vitamin D, MMP-9, and TIMP-1 in COPD subjects, healthy smokers and nonsmokers of Indonesian citizens

**METHODS:** Seventy-eight male subjects took part in this study. They comprised three groups, i.e. COPD (n = 26), healthy smokers (n = 25) and healthy non-smokers (n = 27). Serum 25(OHD) levels, MMP-9, and TIMP-1 concentrations measured by electrochemiluminescence binding assay (ECLIA) and enzyme-linked immunosorbent assay (ELISA).

**RESULTS:** The levels of vitamin D in COPD (21.96 ± 6.62ng/mL) and healthy smokers (27.87 ± 7.08 ng/mL) were significantly ($p < 0.001$) lower compared to that in healthy non-smokers (31.71 ± 9.24 ng/mL). On contrary, the levels of MMP-9 in COPD (11.98 ± 41.54 ng/mL) was significantly ($p = 0.003$) higher compared to that in healthy smokers (2.23 ± 3.39 ng/mL) and healthy non-smokers (0.89 ± 1.12 ng/mL). Whereas the levels of TIMP-1 in healthy smokers (24.64 ± 57.77 ng/mL) was significantly ($p < 0.001$) lower compared to that in COPD (58.40 ± 77.53 ng/mL) and healthy non-smokers (46,54 ± 71,48 ng/mL).

**CONCLUSION:** The present study showed the lowest level of vitamin D, the highest level of MMP-9 and TIMP-1 in the COPD subjects.

## Introduction

Vitamin D has reported playing a role in various body functions outside of the musculoskeletal system [1], [2] including pulmonary maturation [3]. While vitamin D deficiency can increase the risk of many diseases such as the musculoskeletal, cardiovascular and respiratory systems [3]. The Southeast Asia Nutrition Survey (SEANUTS) for children conducted during the 2010 / 2011 period in Indonesia, Malaysia, Thailand, and Vietnam [4], found that vitamin D levels did not differ between countries except Indonesia, which has a much lower level. In all countries except Vietnam, girls have vitamin D levels lower than boys after correcting age and area of residence. Urban children have lower levels of vitamin D, except in Indonesia [4]. Similarly, for adult women in Indonesia, the level of vitamin D for the rural group (20.24 ± 4.43 ng/mL) was greater than the urban group (14.9 ± 3.64 ng/mL), [5]. Various reports mentioned that patients with chronic obstructive pulmonary disease (COPD) have a low level of vitamin D [6], [7], [8], [9], [10] including the last reported by British researchers [11]. How do vitamin D levels in people with COPD in Indonesia?

Vitamin D has the potential to modulate the inflammatory response to harmful particles in patients with COPD [6]. It relates vitamin D deficiency to disease severity based on the assessment of lung function FEV1 [7], [9], [12]. In the study of Lange et

al., 2012, pulmonary function FEV1 decreased twice in smokers with vitamin D deficiency compared to smokers without vitamin D deficiency. Chronic obstructive pulmonary disease (COPD) is more common in smokers and former smokers than in non-smokers [13].

Cigarette smoking is a major risk factor for COPD. Exposure to cigarette smoke may stimulate the inflammatory response and activate polymorphonuclear leukocytes, thus resulting in secretion of cellular proteases [14]. Neutrophils produce MMP-8 and MMP-9 enzymes, while macrophages produce MMP-9 as the main proteolytic enzyme that can degrade extracellular matrix and elastin fibres [15], [16]. Many MMPs activated by smoking and oxidative stress [13]. In basal conditions, polymorphonuclear leukocytes of COPD patients released significantly more MMP-9 compared with polymorphonuclear leukocytes of healthy controls ($P = 0.016$) [14].

However, MMP-9 activity inhibited by tissue metalloproteinase (TIMP) inhibitors under normal circumstances, especially by TIMP-1, which shows it can bind to the active form and precursor form MMP-9. In smokers of COPD patients, the possibility of TIMP production does not inhibit the action of MMP-9 that occurs emphysema [15]. Therefore, COPD characterised by an imbalance between MMP-9 and TIMP-1, which may play an important role in the pathogenesis of tissue remodelling and airway obstruction [13].

MMP-9 exploded in COPD patients, and healthy smokers compared to healthy non-smokers. While TIMP-1 increases more in healthy non-smokers than COPD patients and healthy smokers [13]. The previous report showed that MMP-9 and TIMP-1 significantly increased in the serum of patients with COPD [17]. The results of the meta-analysis show that high MMP-9 and TIMP-1 protein levels can correlate with the pathogenesis of COPD, and both proteins can be important biological markers for the initial diagnosis of COPD [17].

Vitamin D can inhibit MMP-9 production, and thus, a deficiency of vitamin D can cause an increase in lung parenchymal degradation by MMP-9 [18].

Therefore, it is necessary to research how vitamin D, MMP-9, and TIMP-1 levels in COPD, healthy smokers and nonsmokers of Indonesian citizens.

## Material and Methods

We conducted this case-control study after being approved by the Ethics Committee of the University of North Sumatra, and after study, participants gave written approval.

Seventy-eight male subjects of an Indonesian citizen, aged 40-65 years, took part in this study. They comprised three groups, namely: 1, COPD group, 26 subjects were stable COPD outpatients [using (GOLD 2013) criteria], based on chest X-rays and spirometry results of at least 200 cigarettes had been smoked throughout their lives; 2, healthy smokers, 25 healthy subjects who had no abnormalities in the lungs, were known by spirometry examination, and 3, healthy non-smokers, 27 subjects were healthy people who did not have abnormalities in the lungs, known with spirometry examination, and not smoking;

We exclude subjects if they have other lung diseases, hypothyroidism, or if they have a history of taking drugs that can affect calcium and vitamin D metabolism.

We performed spirometry examination using the Minispir New spirometer (MIR-Medical International Research, Italy) MIR Spirobank II spirometer. Serum vitamin D was assessed by quantitative determination of 25-hydroxyvitamin D (25-OHD) by the electrochemiluminescence method using Elecsys Cobas® total vitamin D reagent according to the manufacturer's instructions. Serum 25-OHD concentrations of less than 20 ng/ml are considered as deficiencies, insufficiency 20-29 ng/ml and 30 ng/ml and more sufficiency [6].

MMP-9 and TIMP-1 levels were examined by human Qayee-Bio® MMP-9 ELISA (enzyme-related immunosorbent test).

### *Statistical analysis*

We express all data as mean ± standard deviation. We analysed comparisons between groups with Kruskal Wallis (one-way); $p < 0.05$ was considered significant.

## Results

We can see the normality of data for each variable level of vitamin D, in Table 1. Based on the Shapiro-Wilk test for normality test, we found that only vitamin D levels in the COPD group, they normally distributed only vitamin D levels in the COPD group.

Table 1 above also shows a significant difference ($p < 0.05$) for the mean level of vitamin D, MMP-9, and TIMP-1 among subjects with COPD, healthy people who smoke and healthy people who are not smokers.

Healthy smokers followed low levels of vitamin D in COPD subjects and the highest in healthy non-smokers (Table 1). We showed the opposite in

MMP-9 levels, where the highest MMP-9 levels in COPD subjects followed by healthy smokers and the lowest in healthy non-smokers (Table 1) whereas a group of healthy non-smokers followed the highest TIMP-1 level in the COPD group and the lowest in healthy smokers (Table 1).

**Table 1: Normality of vitamin D, MMP-9 and TIMP-1 levels in COPD subjects, healthy smokers and healthy non-smokers**

| | COPD (n = 26) | Healthy Smoker (n = 25) | Healthy Non-Smoker n = 27) | p-value |
|---|---|---|---|---|
| Vitamin D 25(OH)D (ng/mL) | | | | |
| Mean | 21.96 | 27.87 | 31.71 | < 0.001 |
| Median | 21.35 | 27.44 | 30.21 | |
| SD | 6.62 | 7.08 | 9.24 | |
| Minimum | 3.38 | 17.19 | 19.97 | |
| Maximum | 32.72 | 53.80 | 60.60 | |
| 95% CI | 19.28 – 24.63 | 24.95-30.79 | 28.06-35.37 | |
| p (Shapiro-Wilk) | 0.35 | 0.001 | 0.02 | |
| MMP-9(ng/mL) | | | | |
| Mean | 11.98 | 2.23 | 0.89 | 0.003 |
| Median | 1.74 | 0.99 | 0.50 | |
| SD | 41.54 | 3.94 | 1.12 | |
| Minimum | 0.25 | 0.21 | 0.08 | |
| Maximum | 214 | 19.7 | 4.5 | |
| 95% CI | -4.80-28.76 | 0.61-3.86 | 0.44-1.33 | |
| p (Shapiro-Wilk) | < 0.001 | < 0.001 | < 0.001 | |
| TIMP-1(ng/mL) | | | | |
| Mean | 58.40 | 24.64 | 46.54 | < 0.001 |
| Median | 17.43 | 5.66 | 12.6 | |
| SD | 77.53 | 57.77 | 71.48 | |
| Minimum | 10.66 | 1.66 | 0.28 | |
| Maximum | 297.2 | 290 | 327 | |
| 95% CI | 27.09-89.72 | 0.80-48.49 | 18.27-74.82 | |
| p (Shapiro Wilk) | < 0.001 | < 0.001 | < 0.001 | |

## Discussion

Although previous studies have suggested that vitamin D levels in Indonesian society are insufficient in children and adult women, in the group of healthy non-smoker Indonesian men, the level of vitamin D is sufficient. Sari et al., (2017) found that the level of vitamin D in adult Indonesian women from rural area (20.24 ± 4.43 ng/mL) is higher than those from urban area (14.9 ± 3.64 ng/mL), it turns out this does not apply to however all urban male subjects took part in the present study, ie: healthy nonsmokers (31.71 ± 9.24 ng/mL) and COPD patients (21.96 ± 6.62 ng/mL) are not deficient. The difference in vitamin D levels in adult Indonesians is like that reported in Indonesian children [4].

The low level of vitamin D in the COPD group aged between 40-65 years in this study is under other previous research reports, apart from the influence of age, as stated by Heidari et al. (2015). Many studies suggest that vitamin D deficiency is common in all COPD patients [6], [7], [8], [9], [10] including the last report from British researchers [11]. Also, there is a dose-response relationship between vitamin D levels and pulmonary function FEV1 [6], [9].

People with COPD have a high risk of vitamin D deficiency for low intake of vitamin D from food, decreased synthesis of vitamin D along with aging of the skin, lack of outside activity and lack of sun exposure, increased glucocorticoid catabolism, disorders activation because of renal dysfunction, and decreased muscle mass [8] while Indonesian researchers found that predictive factors independently related to the risk of low levels of vitamin D were sun exposure, work, and intake of vitamin D [5].

Matrix metalloproteinases (MMPs) are a large group of calcium-dependent zinc-containing endopeptidases it mainly concerns which with the remodelling of tissue along with degradation of the extracellular matrix. In the clinical trials sectors, we examine various MMPs along with to import the properties of being a high biomarker in various disorders such as COPD [16].

MMP-9 is the main elastolytic enzyme produced by stromal cells such as alveolar and neutrophil macrophages, which play a major role in lung diseases such as COPD. Increased serum MMP-9 concentrations in COPD subjects illustrate increased proteolytic activity associated with disease severity [19]. In the present study, they find the highest MMP-9 levels in the COPD subjects (11.98 ± 41.54 ng/ml) and the lowest in the healthy subjects who did not smoke (0.89 ± 1.12 ng/ml). The findings in the present study are like those reported by Esa et al., (2014). Constantly raised the amount of it may involve MMP-9 in the degradation of extracellular matrix in the lungs as seen in COPD patients [14].

Although the results of our study are in line with those reported by Esa et al., (2014) but the MMP-9 levels in this study were lower than those reported, both in the COPD subjects (11.98 ± 41.54 ng/ml vs 194.4 ± 100.6 ng/ml), healthy smoker (2.23 ± 3.94 ng/ml vs 104.5 ± 42.1 ng/ml) or healthy non-smokers (0.89 ± 1.12 ng/ml vs 34.5 ± 36.1 ng/ml). It is probable that differences in nutritional status, race, genetics, gender, location of the area between the subjects may cause this studied. This finding is in line with the previous report [3], which found that sputum MMP-9 concentrations increased in COPD subjects compared to those who had never smoked but were similar to healthy smokers.

MMP activity is regulated by proteolytic activation of the inactive proenzyme and through inhibition of active enzymes by TIMP. TIMP-1 binds both the active and precursor form of MMP-9 in a ratio of 1: 1 and selectively inhibits enzyme activity [20]. In the study of Esa et al., (2014), it shows that MMP-9 levels may relate to the severity of increased according to COPD severity, while TIMP-1 levels did not change, this may cause MMP-9 / TIMP-1 ratio to be greater than one. Mild COPD has an MMP-9 / TIMP-1 ratio of less than one (Esa et al., 2014). The COPD subjects who took part in our study seemed to classified as mild COPD with a small MMP-9/TIMP-1 ratio. However, the MMP-9 / TIMP-1 ratio value in healthy smoking and non-smoking subjects cannot attribute to the lung function. This study showed that TIMP-1 levels were highest in the COPD subjects

(58.40 ± 77.83 ng/ml) compared with the group of healthy non-smokers (46.54, 71.84 ng/ml) and the lowest in the healthy smoker group (24.64 ± 57.77 ng/ml).

Regarding TIMP-1, we found that the COPD group showed the highest average TIMP-1 compared to the healthy group of non-smokers and the healthy smoker group (table 3). This result is not inherent with the previous report. It shows increased tIMP-1 more in healthy non-smokers (192.7 ± 37.7 ng/ml) than healthy smokers (145.3 ± 35.1 ng/ml) and COPD patients (115 ± 55.5 ng/ml) (Esa et al., 2014). The lowest value of TIMP-1 in the study of Esa et al. (2014) found in COPD subjects may relate to the severity of the disease. While the present study showed the low level of vitamin D in COPD followed by the high level of MMP-9 but it does not reduce the TIMP-1, this might be the severity of COPD subjects took part in the present study is mild.

Vitamin D plays a role in regulating human lung fibroblast functions in wound repair, and tissue remodelling through not only inhibiting IL-1β stimulated MMP-9 production and conversion to its active form but also inhibiting IL-1β inhibition on TIMP-1 production [18]. Vitamin D deficiency can not reduce MMP-9 activity, which results in increased lung parenchymal degradation [18].

In conclusion, the present study showed that the levels of vitamin D were lowest in COPD subjects compared to that in healthy smokers and healthy non-smokers. The levels of MMP-9 and TIMP-1 were highest in COPD subjects of an Indonesian citizen.

## References


1. Kadhim KA, NafeaLT, FawziHA, Hameed EA, Gasim GA. Assessment Of Vitamin D Therapy Effect On Inflammatory Markers In Pediatric Patients With Type I Diabetic. Asian J Pharm Clin Res. 2018; 11(10):552-4. https://doi.org/10.22159/ajpcr.2018.v11i10.28936

2. Mohammed AK, Alqani VHA. Association between maternal serum vitamin D and early pregnancy spontaneous abortion in Iraqi women. Asian J Pharm Clin Res. 2018; 11(2):432-4. https://doi.org/10.22159/ajpcr.2018.v11i2.24588

3. Gatera VA, Abdulah R, Musfiroh I, Judistiani RT, Setiabudiawan B. Updates on the status of vitamin D as a risk factor for respiratory distress syndrome. Adv Pharmacol Sci. 2018; 2018. https://doi.org/10.1155/2018/8494816 PMid:30364026 PMCid:PMC6186338

4. Poh BK, Rojroongwasinkul N, Nguyen BK, Sandjaja, Ruzita AT, Yamborisut U, Hong TN, Ernawati F, Deurenberg P, Parikh P; SEANUTS Study Group.25-Hydroxy-Vitamin D Demography and The Risk of Vitamin D Insufficiency In The South East Asian Nutrition Surveys (SEANUTS). Asia Pac J ClinNutr. 2016; 25(3):538-48.

5. Sari DK, Tala ZZ, Lestari S, Hutagalung S, Ganie RA. Lifestyle differences in rural and urban areas affected the level of vitamin D in women with single nucleotide polymorphism in North Sumatera. Asian J ClinNutr. 2017; 9(2):57-63. https://doi.org/10.3923/ajcn.2017.57.63

6. Heidari B, Javadian Y, Monadi M, Dankob Y, Firouzjahi A. Vitamin D status and distribution in patients with chronic obstructive pulmonary disease versus healthy controls. Caspian J Intern Med. 2015; 6(2):93-7.

7. Janssens W, Bouillon R, Claes B, Carremans C, Lehouck A, Buysschaert I, et al. Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene. Thorax. 2010; 65:215-20. https://doi.org/10.1136/thx.2009.120659 PMid:19996341

8. Janssens W, Mathieu C, Boonen S, Decramer M. Vitamin D deficiency and chronic obstructive pulmonary disease: a vicious circle. VitamHorm. 2011; 86:379-99. https://doi.org/10.1016/B978-0-12-386960-9.00017-4 PMid:21419281

9. Lange NE, Sparrow D, Vokonas P, Litonjua AA. Vitamin D deficiency, smoking, and lung function in the normative aging study. Am J RespirCrit Care Med. 2012; 186:616-21. https://doi.org/10.1164/rccm.201110-1868OC PMid:22822023 PMCid:PMC3480523

17. Li Y, Lu Y, Zhao Z, Wang J, Li J, Wang W, et al. Relationships of MMP-9 and TIMP-1 proteins with chronic obstructive pulmonary disease risk: A Systematic Review And Meta-Analysis. J Res Med Sci. 2016; 21:12. https://doi.org/10.4103/1735-1995.178737 PMid:27904558 PMCid:PMC5122186

10. Persson LJP, Aanerud M, Hiemstra PS, Hardie JA, Bakke PS, Eagan TML. Chronic obstructive pulmonary disease is associated with low levels of vitamin D. PloS ONE. 2012; 7:e38934. https://doi.org/10.1371/journal.pone.0038934 PMid:22737223 PMCid:PMC3380863

11. Jolliffe DA, James WY, Hooper RL, Barnes NC, Greiller CL, Islam K, et al. Prevalence, Determinants and clinical correlates of vitamin D deficiency in patients with chronic obstructive pulmonary disease in London, UK. J Steroid BiochemMol Biol. 2018; 175:138-145. https://doi.org/10.1016/j.jsbmb.2017.01.019 PMid:28161533

12. Færk G, Çolak Y, Afzal S, Nordestgaard BG. Low concentrations of 25-hydroxyvitamin D and long-term prognosis of PPOK: a prospective cohort study. Eur J Epidemiol. 2018; 33(6):567-577. https://doi.org/10.1007/s10654-018-0393-9 PMid:29691706

13. Esa SA, .Rawy AM, El-Behissy MM,Kamel MH, Mustafael-Hwaitty HMM. Study of the level of sputum Matrix Metalloproteinase-9 (MMP-9) and Tissue Inhibitor Metalloproteinase-1 (TIMP-1) in COPD patients Egyptian Journal Of Chest Diseases and Tuberculosis. 2014; 63(4):861-7. https://doi.org/10.1016/j.ejcdt.2014.06.014

14. Somborac-Bačura A, Popović-Grle S, Zovko V, Žanić-Grubišić T. Cigarette Smoke Induces Activation of Polymorphonuclear Leukocytes. Lung. 2018; 196(1):27-31. https://doi.org/10.1007/s00408-017-0077-3 PMid:29222599

15. Barnes PJ. Chronic obstructive pulmonary disease. N Engl J Med. 2000; 343:269-80. https://doi.org/10.1056/NEJM200007273430407 PMid:10911010

16. Robertson S, Rajan AK, Maheshwaran A, Senthilnathan B, et al. Matrix Metalloproteinases 2 and 9 in Avocation of Multitudinal Complications in Explicitly to Carcinoma: Review. Asian J Pharm Clin Res. 2017; 10(1):47-54. https://doi.org/10.22159/ajpcr.2017.v10i1.14214

17. Li Y, Lu Y, Zhao Z, Wang J, Li J, Wang W, et al. Relationships of MMP-9 and TIMP-1 proteins with chronic obstructive pulmonary disease risk: A Systematic Review And Meta-Analysis. J Res Med Sci. 2016; 21:12. https://doi.org/10.4103/1735-1995.178737 PMid:27904558 PMCid:PMC5122186

18. Kim SH, Baek MS, Yoon DS, Park JS, Yoon BW, Oh BS, et al. Vitamin D inhibits expression and activity of matrix metalloproteinase in human lung fibroblasts (HFL-1) Cells. TubercRespir Dis (Seoul). 2014; 77(2):73-80. https://doi.org/10.4046/trd.2014.77.2.73 PMid:25237378 PMCid:PMC4165663

19. Louhelainen N, Stark H, Mazur W, Rytilä P, Djukanovic R, Kinnula VL. Elevation of sputum matrix metalloproteinase-9 persists up to 6 months after smoking cessation: a research study. BMC Pulm Med. 2010; 10:13. https://doi.org/10.1186/1471-2466-10-13 PMid:20226090 PMCid:PMC2841651

20. Glowinska-Olszewska B, Urban M. Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. Metabolism. 2007; 56:799-805. https://doi.org/10.1016/j.metabol.2007.01.011 PMid:17512313