Eksakta : Berkala Ilmiah Bidang MIPA
VOLUME 24 NO 02 2023, pp 154-164
ISSN : Print 1411-3724 — Online 2549-7464
DOI : https://doi.org/10.24036/eksakta/vol24-iss02/358

Eksakta
Berkala Ilmiah Bidang MIPA
http://www.eksakta.ppj.unp.ac.id/index.php/eksakta

Article

Investigation Activities of Vitamin D, Interleukin-17A, and Alkaline Phosphatase as Biological Markers in Asthma

Article Info

Article history :

Received November 01, 2022
Revised March 09, 2023
Accepted March 28, 2023
Published June 30, 2023

Keywords :

Asthma, inflammation, vitamin D deficiency, alkaline phosphatase

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Abstract. Inflammation in asthma occurs due to the activation of various cells that produce proinflammatory cytokines. Corticosteroid treatment can affect bone formation, impacting vitamin D levels and alkaline phosphatase (ALP) enzyme activity. This study aimed to evaluate the decrease in vitamin D and increase in ALP due to inflammatory events characterized by an increase in interleukin-17A (IL-17A). The research design is a case-control design. Thirty-four adults with asthma and 34 healthy controls were monitored for blood through laboratory testing. Comparative and correlation statistics were analyzed using Medcalc ver. 19.0.7. All three laboratory parameters showed significant differences between the asthmatic and control groups ($P < 0.05$). Each vitamin D and ALP gave a good correlated wellults of the IL-17A examination ($r = -0.2950$; $P = 0.015$ and $r = 0.2590$; $P = 0.033$). There is no significant correlation between Vitamin D and ALP ($r = -0.0483$; $P = 0.696$). Moreover, ALP showed a low sensitivity (32%) in identifying vitamin D deficiency in asthmatic patients.

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1. Introduction

Asthma is a chronic inflammatory disorder of the airways that causes an increase in airway hyperresponsiveness. There are more than 350 million individuals with asthma worldwide, of which 250,000 deaths were reported from asthma attacks annually [1–3]. The prevalence of asthma is continuously increasing in developing countries, including Indonesia, which has an asthma

prevalence of 2.4% in 2018. Adults, which reached 63.9%, are known as the age group with the highest prevalence [4-5].

Chronic inflammation in asthma is associated with the body's immune response due to the activation of various body's defense cells, such as T lymphocytes, eosinophils, macrophages, masts, epithelium and fibroblasts. In mild and moderate asthma, the immune response that occurs is dominated by T helper-2 (Th-2) cells and is mediated by the cytokines interleukin-4 (IL-4), IL-5, and IL-13, eosinophil cells and immunoglobulin E (IgE).). The immune response in severe asthma is dominated by Th-17 producing IL-17A. This phenotype is characterized by a low response to therapy and an increase in morbidity and mortality. IL-17A induces neutrophil-dominated airway inflammation [6-8]. Studies on IL-17A in asthma have shown that IL-17A levels are positively correlated with asthma severity and levels are increased more than five times in asthma with exacerbations compared to healthy controls, controlled and uncontrolled asthma [9-11].

Inflammation that occurs in the lungs determines the severity of asthma. This is controlled through increased immune response by vitamin D which plays a role in enhancing immune response. High levels of vitamin D are beneficial for lung function and slow the onset of asthma exacerbations. Vitamin D deficiency is correlated with an increase in prevalence, risk for hospitalization and increase in emergency visits along with decreased lung function and increased airway hyperresponsiveness [12–13].

In these settings, corticosteroids are used to treat the inflammatory state. However, changes in bone such as density, strength, and osteoporosis in asthma occur due to the use of inhaled corticosteroids. About 55% of asthmatic patients who use corticosteroids have experienced this condition, which is characterized by decreased activity of the enzyme alkaline phosphatase (ALP) in the blood circulation. ALP is known to play a role in bone development through increased inorganic phosphate-promoting bone mineralization. Decreases in the activity of these enzymes are associated with impaired or reduced bone formation [14]. Previous studies have found a correlation indicating the involvement of ALP activity in vitamin D regulation in groups of patients with different types of disease [15-16]. Recent studies have shown that high ALP activity (>350 IU/L) can be used as a substitute for vitamin D deficiency and an important predictor of recurrent asthma [12]. However, several studies on healthy individuals also reported that there was no significant relationship between ALP activity and a decrease in Vitamin D levels.

Therefore, it is important to observe an increase in ALP activity in adults with asthma as a result of decreased levels of vitamin D and inflammation. This condition was determined based on the level of IL-17A which has been proven as a biomarker of inflammation in asthma in several previous studies [13][17]. This study aimed to demonstrate the potential of ALP enzyme activity assays in prognostic considerations regarding the biological changes of bone formation due to inflammation in asthma.

2. Methods

This observational study with a case-control approach was approved by the Health Research Ethics Committee of the Health Polytechnic of Jambi with approval number: IB.02.06/2/136/2022 dated 27/05/2022. The study population was adult asthmatic patients aged 15-64 years who had signed informed consent before. The selection of research subjects was carried out by accidental sampling until it reached a certain number. Calculation with OR 4.8 (95% CI) obtained a total sample of 68 participants divided into two groups with the same proportion (1:1). Thirty-four people in the asthmatic group and 34 healthy people in the control group participated voluntarily in this study.

Participants from the asthma group were included in the study after they had previously received confirmation of their disease from a clinician or pulmonologist. Individuals with asthma are categorized as intermittent, or persistent (mild, moderate to severe) according to the Global Initiative for Asthma (GINA) classification. This category is limited to the frequency characteristics of daily

symptoms, exacerbations, and nocturnal symptoms based on the results of in-depth interviews with each patient. The control group consisted of individuals who had no history of asthma and were not currently suffering from acute respiratory infections, allergies, chronic diseases, or other systemic infections.

The study was conducted from March to July 2022. Interviews were conducted to assess the characteristics of all participants and continued with venous blood sampling which was carried out using a closed system technique with a vacuum tube, five milliliters per participant. Determination of IL-17A in serum was carried out at Eureka Research Laboratory Palembang, South Sumatra using the Enzyme-Linked Immunosorbent Assay (ELISA) method using a recommended value of <20pg/mL. Determination of vitamin D levels using Enzyme-Linked Fluorescent Assay (ELFA) and ALP enzyme activity (photometry) at the Immunology and Clinical Chemistry Laboratory, Department of Medical Laboratory Technology at Health Polytechnic of Jambi. The reference value for ALP is <115 IU/L [14], while vitamin D is categorized based on the classification of vitamin D status, namely "deficiency" (<20 ng/mL), "deficiency" (21 - 29 ng/mL) and "normal" / "sufficient" (>30 ng/mL) [15]. The flow charts used in this study include:

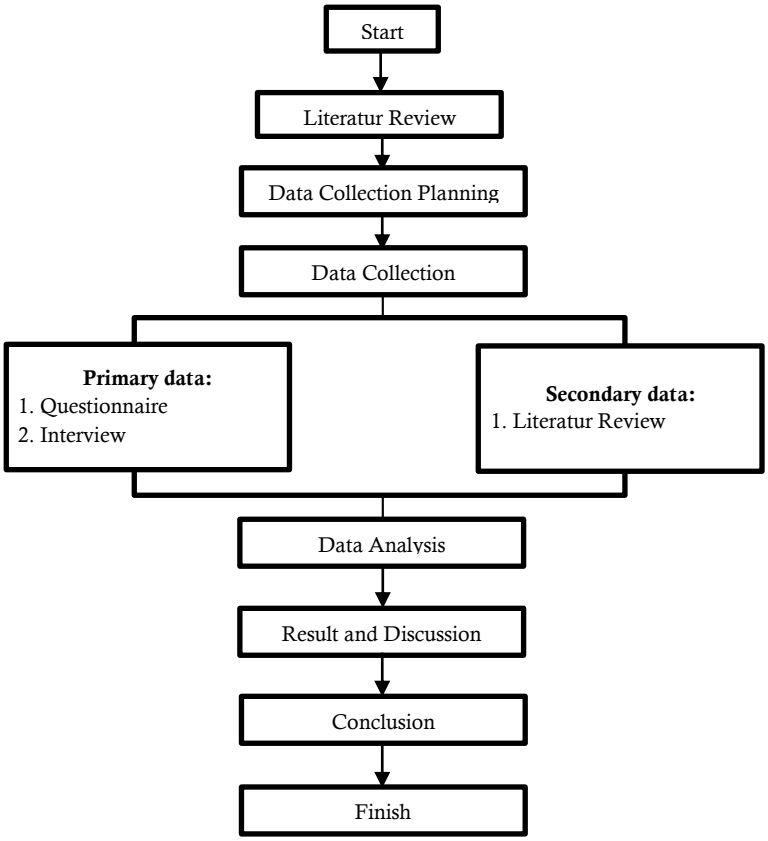

Figure 1. Research flowchart

Data were analyzed using Medcalc version 19.0.7 software for Windows and presented in the form of tables and graphs. Percentages were used to describe qualitative data such as gender and asthma severity. From the Shapiro-Wilk test analysis, if the data is normally distributed, it will be described as the mean and standard deviation values, followed by statistical tests using independent t-tests to see the differences between the asthmatic group and the control group and the Pearson correlation coefficient to see the relationship between laboratory test variables. If the data distribution

is not normal, then a medium and interquartile range are used, followed by Mann-Whitney's test and Spearman's coefficient of rank correlation. Statistical tests for frequency data were analyzed by Chi-square (sex, and vitamin D status) or Fisher exact test (ALP). All statistical analyzes were carried out at a significance level of <0.05. The ROC (Received Operating Curve) test is used to show the observed diagnostic power based on the sensitivity, specificity, and AUC (Area Under Curve) values of each laboratory test parameter [18].

3. Result and Discussions

Sixty-eight participants were involved in this study, 34 asthmatics and 34 healthy controls. There was no significant difference in the age of the participants between the asthmatic and control groups. Significant differences were found in gender, where in the asthma group there were more women than men which corresponded to global prevalence [3]. On the other hand, in the control group, there were more men because of this gender, they were more willing to participate voluntarily. Asthma duration and severity were only carried out in the asthmatic group because the controls in this study were healthy individuals (Table 1). Asthma patients in this group generally have had asthma since their teens, some even started at an early age. The severity level obtained shows a higher percentage than the global survey results (5%) that have been previously reported [19-21]. However, similar to the report, the severe persistent category is the lowest proportion.

This study is the first study aimed at looking at the description of IL-17A levels, vitamin D levels, and serum ALP activity among a population of adults with asthma who are not undergoing treatment (outpatients) in Indonesia. In addition, this study also examines the correlation between the three laboratory test parameters, especially to see the potential of serum ALP which is assumed to replace the role of determining vitamin D levels which have also never been observed before in this population. This is to show phenomena related to bone formation due to inflammation and asthma treatment.

Table 1. Demographic and laboratory characteristics

Characteristics	Asthmatic group (n=34)	Control group (n=34)	*P*
Age (years)	31.2 + 15.9	29.8 + 10.4	0.3088
Sex - Male	10 (29.41%)	24 (70.59%)	0.0016
- Female	23 (67.65%)	11 (32.35%)	
Asthma duration (years)	9 [4 - 15]*	N/A	
Severity			
Intermitten	18 (52.94%)	N/A	
Mild persistent	6 (17.65%)	N/A	
Moderate persistent	6 (17.65%)	N/A	
Severe persistent	4 (11.76%)	N/A	
IL-17A (pg/mL)	13.3 [12.4 - 14.3]*	11.4 [24.5 - 31.4]*	<0.0001
Vitamin D (ng/mL)	21.4 $\pm$ 8.2	27.1 $\pm$ 5.5	0.0012
Sufficiency	5 (14.71%)	14 (41.18%)	
Insufficiency	11 (32.35%)	15 (44.12%)	0.0022
Defficiency	18 (52.94%)	5 (14.70%)	
ALP (IU/L)	73.3 $\pm$ 20.0	60.7 $\pm$ 16.7	0.0063
Normal	33 (97.04%)	34 (100%)	>0.05
Increased	1 (2.94%)	0 (0%)	

The value is in mean + SD or n (%); *medium [interquartile range]; N/A=not available

The test results on the three observed laboratory parameters showed significant differences between the asthmatic group and the control group. IL-17A levels and ALP enzyme activity showed an increase in the asthmatic group, whereas serum vitamin D levels were significantly lower [22-23]. Vitamin D status categorized according to the GINA guidelines showed a significant difference, where most of the asthma group tended to have more deficiency status, while the control group tended to normal status (Figure 2). However, the results of all measurements of ALP enzyme activity in both groups were mostly still in the normal range (< 115 IU/L). An increase that exceeded the reference value of the enzyme activity was only found in one participant from the asthma group [24].

In this study, it was shown that IL-17A, vitamin D, and serum ALP were significantly different between asthmatic groups and healthy controls [25-26]. This is similar to many previous studies of inflammatory conditions in various diseases, including asthma. In the asthma group, IL-7A and ALP were obtained which gave higher examination results, whereas vitamin D levels were known to be lower than in the control group.

Vitamin D showed a significant reduction in levels in adults with asthma (21.4 ng/mL) compared to the healthy control group (27.1 ng/mL). In a previous study, it was found that subjects with asthma had lower vitamin D measurements than healthy controls (12.31 ± 3.6 versus 15.50 ± 6.37 ng/mL; P = 0.04). Categorization of vitamin D status most of the respondents in both groups were included in the deficiency category, where the asthma group was: deficiency = 90.5%; and insufficiency = 9.5%, while the control group: deficiency = 65.3%; insufficiency = 30.7%; and sufficiency = 4.0% [27-28]. This study showed similar results, but the mean value of vitamin levels in both groups was in the insufficiency category (21.4 + 8.2 versus 27.1 + 5.5 ng/mL; P = 0.0012) with vitamin D status, asthma group: deficiency = 52.9%; insufficiency = 32.4%, and normal = 14.7%, while the control group: deficiency = 14.7%; insufficiency = 44.1%, and normal = 41.2%.

Meanwhile, there is no case-control study for ALP enzyme activity in adult asthma had been carried out before, including in outpatients with a risk of vitamin D deficiency due to inflammation [29-30]. Most of the existing study reports were conducted on children with asthma who were undergoing treatment in a hospital. In these studies, it was reported that ALP in children with asthma tended to increase beyond the normal range and was significantly different from the control group. This study also found a significant difference between the two groups, but the average value of both was still in the normal range (73.3 versus 60.7 IU/L; P = 0.0063).

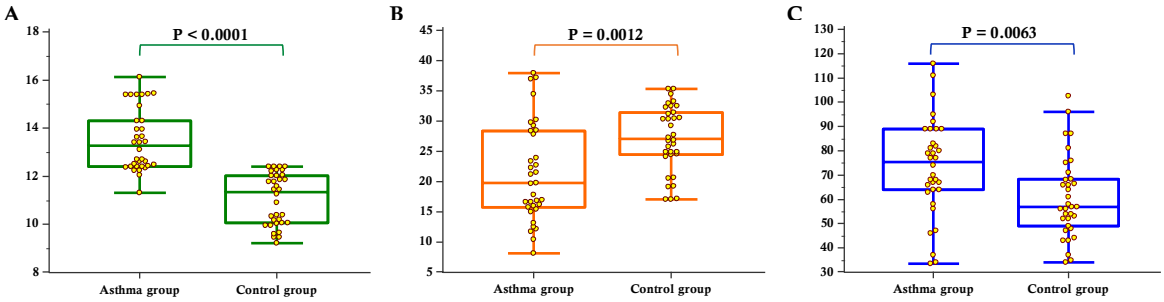

Figure 2. The boxplot graph showing the comparison between asthma and control group (A= IL-17A; B= Vitamin D; C= ALP)

The significant difference of each laboratory test variable is generally similar to several previous studies conducted on asthmatic patients with an exacerbation status and undergoing hospitalization. The same results were also obtained, but this study successfully demonstrated the phenomenon in adult asthmatic outpatients who were used as the primary research subjects. Furthermore, correlation

analysis was carried out to show a significant relationship between variables. The level of correlation was shown in the table and graph below.

Table 2. Correlation between variables

Variable	*r*	*P*
Asthma Duration		
x Interleukin-17A	-0.1440	0.4153
x Vitamin D	-0.1212	0.4949
x Alkaline Phosphatase	0.0747	0.6744
Severity		
x Interleukin-17A	0.6284	0.0001*
x Vitamin D	-0.0488	0.7840
x Alkaline Phosphatase	0.0549	0.7580
Interleukin-17A x Vitamin D	-0.2950	0.0146**
Interleukin-17A x Alkaline Phosphatase	0.2590	0.0327**
Vitamin D x Alkaline Phosphatase	-0.0483	0.6955**

r= correlation coefficient; **P*<0.05 for asthma group; ** *P* <0.05 for both group

All laboratory test parameters did not show a good relationship in terms of asthma duration. It is assumed that the signs of inflammation cannot be judged by the duration of asthma in the individual. From the r value obtained, it can be seen that IL-17A and Vitamin D have a tendency to experience a slight decrease in terms of asthma duration, while ALP does not have that tendency [31-32]. Likewise, there was no significant correlation between the severity of asthma and vitamin D and ALP levels. In contrast, IL-17A showed a significant correlation with severity. In fact, the r value given from the Pearson correlation test analysis shows a relationship that is included in the "moderate to good" category, so that IL-17A is proven to be able to show ability as a marker of asthma severity. Although this phenomenon is similar to several previous studies, the investigation in this study was carried out on outpatients who were not experiencing exacerbations at all, where blood sampling was carried out when asthma patients were not undergoing treatment.

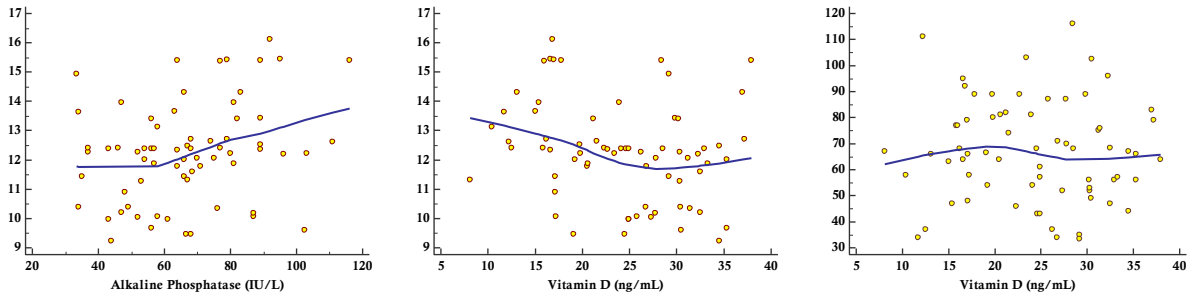

Figure 3. The scattered graph showing the corellation among laboratory test results.

Analysis of the correlation between laboratory parameters found a significant relationship between IL-17A and the other two test parameters ($p<0.05$). However, both show a correlation value that is still included in the "reasonable" category. IL-17A which was shown to increase in severity conditions tended to show a decrease in vitamin D levels. In contrast to ALP, which tended to experience an increase in enzyme activity along with the increase in IL-17A examination results. No

relationship has shown between vitamin D levels and the measuring ALP activity obtained in this study (p>0.05).

Table 3. AUC, sensitivity and specificity of IL-17A, vitamin D and ALP

Parameter	P	AUC	Sensitivity in % [95% CI]	Specificity in % [95% CI]	Associated criterion
IL-17A	<0.0001	0.960	79.4 [62.1 - 91.3]	100.0 [89.7 - 100.0]	>12.39 pg/mL
Vit. D	0.0002	0.736	70.59 [52.5 - 84.9]	79.41 [62.1 - 91.3]	≤23.9 ng/mL
ALP	0.0022	0.701	79.41 [62.1 - 91.3]	58.83 [40.7 - 75.4]	>61 IU/L

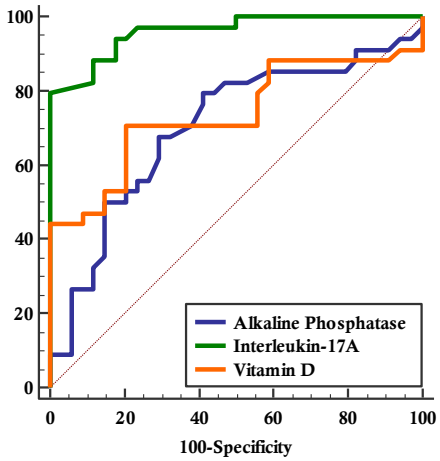

Figure 4. The ROC graph of IL-17A, vitamin D and ALP in identifying asthma

The ability to diagnose asthma from the three laboratory parameters was carried out by analysis of the ROC curve. This is done to see the potential of each parameter in differentiating individuals with asthma and without asthma. The area under curve (AUC) value shown by IL-17A looks the widest and almost fills the entire area. IL-17A was shown to be highly capable of demonstrating the inflammatory state of asthma in the subjects studied. Even based on the interpretation of the AUC value, it shows that the IL-17A chart is included in the "outstanding" category [33-34]. For 100% specificity, the maximum sensitivity was about 79.4% at the cut-off value of 12.39 pg/mL. While the AUC for ALP enzyme activity and Vitamin D levels, although most of the curve area is still above the diagonal line, the picture shown is not comparable to the AUC value of IL-17A, where the maximum specificity of both is <80%. Pairwise comparison of ROC curves analysis showed that the AUC of IL-17A was significantly different from the other two tests (P<0.05).

Report elevated IL-17 levels in severe asthma and find a value greater than 20 pg/mL to distinguish it from other severity categories [35-36]. In this study, it was found that this value can also be used as a comparison between outpatients with asthma and healthy controls, where the value is much lower, namely 12.4 pg/mL. Previously Hynes & Hinks (2019) stated that elevated levels of IL-17A in the airways have been repeatedly observed in severe asthma and contribute to airway hyperresponsiveness [19]. However, the direction of its relationship with disease pathology is still not clearly understood.

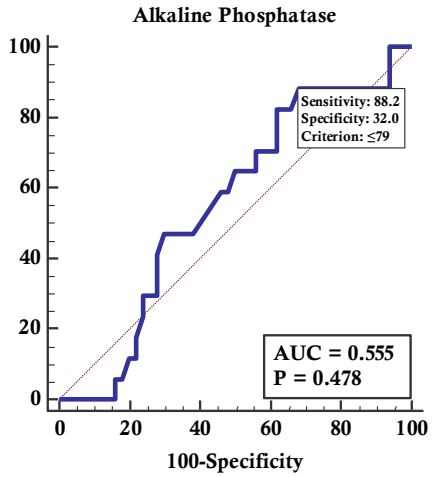

Figure 5. The ROC graph of ALP in identifying vitamin D deficiency

ROC analysis of alkaline phosphatase did not show good potential in indicating vitamin D deficiency. This is following the statistical results which stated that there was no correlation between the two (table 3). The low ability is also indicated by the low specificity value (32%) from this analysis. Thus, it can be assumed that ALP cannot be used as an alternative in considering vitamin D deficiency conditions in corticosteroid users in the treatment of asthma.

From the correlation analysis, it is known that IL-17A correlates with vitamin D and ALP values although only in the fair category. This is similar to many previous studies looking at this correlation. However, one of the aims of this study is to see the correlation between ALP and vitamin D. There was no such correlation, so ALP, as a laboratory test method that is much cheaper and easier, was not recommended as an alternative substitute for vitamin D determination. Several previous studies have shown varying results on the relationship between these laboratory tests, both in asthma and other inflammatory diseases [37-39]. The ROC analysis carried out also concluded that the two parameters were not comparable to the potency of IL-17A in indicating inflammation or its absence in asthma.

4. Conclusion

The IL-17A, vitamin D, and ALP showed significant differences in the laboratory results between the asthma group and the healthy control group. Vitamin D deficiency in the asthma group (85.29%) was higher than in the control group (58.82%), while increased ALP activity was only found in asthma (2.94%). The level of IL-17A had a fair relationship with vitamin D levels and ALP activities. There was no relation between vitamin D levels and ALP activity. ALP is not recommended to be used as an alternative substitute for identifying vitamin D deficiency in asthma.

5. Acknowledgement

This study was supported by Hibah Penelitian Kerjasama Antar Perguruan Tinggi (PKAPT) PPSDM-Kes 2021 Number : DP.02.01/4.4/06/2022 Politeknik Kesehatan Kemenkes Jambi.

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