

Correlation of Vitamin D Levels With CD4+ T Cells Count in Children with HIV Infection

Vivi Sinulingga^a, Rita Evalina^b, Aridamuriany*

^a vivisinulingga@gmail.com

Resident of Departement of Child Health Medical School, University of North Sumatera/ Adam Malik Hospital, Medan

^bAllergy and Immunology Consultant of Departement of Child Health Medical School, University of North Sumatera/ Adam Malik Hospital, Medan

^cPediatric Intensive Care Consultant of Departement of Child Health Medical School, University of North Sumatera/ Adam Malik Hospital, Medan

Abstract

Background: Human immunodeficiency virus (HIV) infection has become a serious issue in the field of child health. Faster disease progression, higher mortality, higher mother-to-child transmission, lower CD4+ T cells, and slower CD4+ increases after antiretroviral use are associated with vitamin D deficiency in HIV infection.

Objective: To find out the correlation between vitamin D levels in the blood with CD4+ T cells count in children with HIV infection who received ART at Adam Malik General Hospital Medan.

Method: This study used an analytic observational design with a cross-sectional approach to see the correlation between vitamin D levels in the blood and CD4+ T cell counts in children with HIV infection who received ART more than one year. The vitamin D and CD4+ count classification is based on WHO criteria. The data collected was processed and analyzed with a 95% confidence interval and a significance of $p < 0.05$ and analysis with the Pearson and Anova correlation test.

Result: Of the 31 research subjects, 17 subjects were male (54.8%) and 14 subjects were female (45.2%). The percentage of male is 9.6% more than that of women. With a standard deviation of 3.7 years, the age of the research subjects had a mean value of 8.8 years. Anthropometric measurements showed that 18 subjects (58.1%) were subjects with good nutritional status, while 13 subjects (41.9%) were malnourished. The CD4+ T cell count had a mean value 25 % and 899 sel/mm² and mean value of vitamin D mean value 21.8 ng/dl. There was a poor correlation ($r = 0.152$), which was not significantly different ($p = 0.141$), between vitamin D levels and CD4+ cells count.

Conclusion: There was no correlation between vitamin D levels and CD4+ T cells count in children with HIV infection who received ART at Adam Malik General Hospital Medan. Further studies with more severe deficiency or severe immunodeficiency are needed, which cannot be represented in this study.

Published by IJRP.ORG.Selection and/or peer-review under responsibility of International Journal of Research Publications (IJRP.ORG)

Keyword: HIV; CD4+; Vitamin D; Children

1. Introduction

Human immunodeficiency virus (HIV) infection has become a serious issue in the field of child health (UNAIDS, 2016). Indonesia has declared HIV care as a sustainable development goal (SDGs) 2030, which has not been achieved in the Millennium Development Goals (MDGs) 2000-2015. In TCD4+ cells, vitamin D demonstrates a role. Vitamin D receptors (VDR) and vitamin D activating enzymes are found in natural or acquired immune cells (Baeke *et al*, 2016). Faster disease progression, higher mortality, higher mother-to-child transmission, lower CD4+ T cells, and slower CD4+ increases after antiretroviral use are associated with

vitamin D deficiency in HIV infection. (Bearden *et al*, 2006)

2. Method

2.1. Study Design

This study used an analytic observational design with a cross-sectional approach to find the correlation between vitamin D levels in the blood and CD4+ T cell counts in children with HIV infection who received ART at Adam Malik General Hospital Medan

2.2. Population and Sample

The population was children with HIV infection who received ART and control to an outpatient clinic at H. Adam Malik Hospital Medan between 2016-2019. Samples were children with HIV infection who received ART minimum for 1 year and meet the inclusion and exclusion criteria. The inclusion criteria were children aged 1 month to 18 years who were diagnosed with HIV by serology test or PCR. The exclusion criteria were children with severe opportunistic infection or severe malnutrition.

2.3. Assessment Procedure

Researchers evaluated children with HIV infection using the inclusion and exclusion criteria. Parents of children who met the criteria were given explanation regarding the research and were asked for consent. Interviews and questionnaires were carried out by researchers regarding basic data, such as sample identity, parent identity, medical history, type, and duration of treatment. A 2 cc asepsis technique of blood sampling was carried out through the mediana cubital vein by a skilled analyst from the Adam Malik Hospital laboratory. An examination of serum 25-hydroxyvitamin-D (25(OH)D) was carried out to the Adam Malik Hospital Medan laboratory with a mini Vidas® measuring instrument. Classification of vitamin D level is based on the WHO criteria, classified as normal, insufficiency and deficiency. A serum CD4+ T cell level was examined in the laboratory of the Adam Malik General Hospital in Medan using the Calibur Becton Dickinson® FACS measuring instrument. Performed data processing and analysis. Classification of immunodeficiency is based on the WHO criteria, classified as without immunodeficiency, mild, moreate and severe immunodeficiency.

2.4. Ethical Consideration

This study received approval from the Research Ethics Committee of the Faculty of Medicine, University of North Sumatera / Haji Adam Malik General Hospital.

2.5. Statistical Analysis

The collected data were processed and analyzed with a 95% confidence interval and a significance of $P < 0.05$. The distribution of data was tested by the Kolmogorov-Smirnov and Shapiro-Wilk normality tests. If the data is normally distributed, the data is tested with the Pearson and ANOVA correlation test. Data is processed by computerization.

3. Result

This research was conducted at the Adam Malik Haji Center General Hospital, Medan, North Sumatra, from April to November 2019, with a total sample of 31 children who met the inclusion and exclusion criteria to be included in the study. The normality test has been carried out and all data are normally distributed. Of the 31 research subjects, 17 subjects were male (54.8%) and 14 subjects were female (45.2%). The percentage of the male is 9.6% more than that of women. With a standard deviation of 3.7 years, the age of the research subjects had a mean value of 8.8 years. Anthropometric measurements showed that 18 subjects (58.1%) were subjects with good nutritional status, while 13 subjects (41.9%) were malnourished. The mean duration of ART in the study subjects had a mean of 43 months with a standard deviation of 28.23. ART was carried out with a combination of zidovudine, lamivudine, and nevirapine in 23 subjects (74.2%), while the combination of tenofovir, lamivudine, and nevirapine in 6 subjects (19.4%) and a combination of tenofovir, lamivudine, and lopinavir / ritonavir in 2 subjects (6.1%).

The physiological characteristics of the liver and kidneys in the subjects were found to be 100% within normal limits. There were no subjects with liver and kidney function disorders. Full details of the initial

subject characteristics are shown in Table 1.

Table 1. Subjects Characteristics (n=31)

| Characteristics | n = 31 |
|---|--------------|
| Age, year (mean/SD) | 8.8 ± 3.70 |
| Gender (n/%) | |
| Male (17) | 54.8% |
| Female (14) | 45.2% |
| Duration of treatment, month (mean/SD) | 43.0 ± 28.23 |
| Nutrition Status (n/%) | |
| Normal (18) | 58.1% |
| Deficit (13) | 41.9% |
| Treatment Combination (n/%) | |
| Zidovudine, lamivudine, nevirapine (23) | 74.2% |
| Tenofovir, lamivudine, and nevirapine (6) | 19.4% |
| Tenofovir, lamivudine and lopinavir/ritonavir (2) | 6.5% |
| Liver Function (n/%) | |
| Normal (31) | 100% |
| Elevated (0) | 0% |
| Kidney Function (n/%) | |
| Normal (31) | 100% |
| Elevated (0) | 0% |

All subjects were tested for the CD4+ T cell count and the mean value of the percentage of CD4+ T cell was $25\% \pm 9.24$. The CD4+ T cell absolute count had a mean of $899.9 \pm 540.62 \text{ cell/mm}^2$. Immunodeficiency status was determined based on WHO criteria and it was found that 21 subjects (67.7%) had no immunodeficiency, whereas 5 subjects (16.1%) were mild immunodeficiency, 2 subjects (6.5%) had moderate immunodeficiency and 3 subjects (9.7%) had severe immunodeficiency. The vitamin D level test was carried out on all subjects and a mean value of 21.8 ng/dl was obtained. Most vitamin D status was found in normal status (51.6%), while 13 subjects (41.9%) had insufficiency and 2 subjects (6.5%) had deficiency.

Subjects with normal vitamin D levels showed the highest status as without immunodeficiency (81.2%), while mild immunodeficiency was 6.25%, moderate immunodeficiency was 6.25%, and severe immunodeficiency was 6.25%. Subjects with insufficient vitamin D showed the highest status as without immunodeficiency (61.5%), while mild immunodeficiency was 23.1%, moderate immunodeficiency was 7.6%, and severe immunodeficiency was 7.6%. Subjects with vitamin D deficiency had mild immunodeficiency (50%) and severe immunodeficiency (50%).

There was a poor correlation ($r = 0.152$), which was not significantly different ($p = 0.141$), between vitamin D levels and the percentage of TCD4+ cells. The correlation between vitamin D levels and the absolute number of TCD4+ cells also showed a poor correlation ($r = 0.170$) which was not significantly different ($p = 0.361$). The correlation between vitamin D levels and immunodeficiency status of subjects also did not have a significant difference with p value 0.108. Details are described in table 2:

Table 2. Relationship between vitamin D levels and TCD4 + cells

| | CD4% (r/p) | CD4 absolut (r/p) | Immunodeficiency status (F/p) |
|-----------|---------------------------|---------------------------|-------------------------------|
| Vitamin D | 0.152(0.141) ^a | 0.170(0.361) ^b | 2.227/0.108 ^b |

^a Pearson test, ^b ANOVA test

4. Discussion

Vitamin D has a role in the immune system, both natural and acquired. Its receptors are expressed by all immune system cells, including T lymphocytes. TCD4 + cells are the target of the HIV virus, that is used as a parameter of the degree of immunodeficiency in HIV patients. Risk factors for vitamin D deficiency includes HIV infection and ARV. To this day, it is not clear how vitamin D levels are related to the number of TCD4 + cells. Various outcomes has also described from previous studies. (Hayes *et al*, 2016)

The data on initial characteristics in this study showed that the proportion of sex was almost the same between men (54.8%) and women (45.2%). It is similar to the world global data, in which it shows that in the early decades of life, there were only slight disparities based on sex. In 2018, 84,000 cases of boys below 9 years of age and 81,000 cases of girls below 9 years of age were registered (UNICEF, 2019). This data is also confirmed by Kohobondo et al, where the prevalence of male infants is almost the same as that of females, respectively 50.84% and 49.16%. (Kohobondo *et al*, 2015)

A mean level of vitamin D level of 21.8 ng/dl was found in this study. Normal vitamin D status was 51.6%, insufficiency was 41.9%, and deficiency was 6.5%. The incidence of vitamin D deficiency ranges between 12-100% in children with HIV infection varies. This disparity is due to inconsistencies in definitions of insufficiency/deficiency as well as variations in the population of the study. Inadequate intake including diet, lack of sun exposure, malabsorption, drugs that affect enzymes, and various etiologies including disease, can cause vitamin D deficiency. (Mansueto *et al*, 2015).

This study showed that the mean value of TCD4+ cells was 25% and absolute TCD4+ was 899.9 cells / mm². Based on the immunodeficiency status according to WHO, 67.7% was found with no immunodeficiency, mild immunodeficiency was 16.1%, moderate immunodeficiency was 6.5%, and severe immunodeficiency was 9.7%. The main target cells for HIV infection are TCD4+ cells, which is a good parameter to assess the degree of immunodeficiency and evaluate the response to therapy in HIV infected patients. The administration of ART has shown an effect on increasing levels of CD4+. A research in India of children under 12 years of age showed a significant increase in TCD4+ cell levels after 6 months of ART administration compared to before ART administration, which suggests immune reconstruction (Lingayat *et al*, 2015). In a study of adult patients in Ethiopia, Asfawa et al showed a two-fold increase in TCD4+ cell counts in the first 12 months of ART and three-fold in 36 months for baseline CD4+ levels <200 cells /mm (Asfaw *et al*, 2015). Weigel et al also showed an increase in the mean TCD4+ cell count after starting ART to 26.9% after two months of starting ART. Subjects for the conducted research received ART for an average of 43 months so that they showed an increase in CD4+ counts (Adedemy *et al*, 2016)

The results of this study showed that 81.2% of patients with normal vitamin D levels had no immunodeficiency, while only 6.25% had mild, moderate, and severe immunodeficiency. There was a decrease in the percentage of patients without immunodeficiency in patients with vitamin D insufficiency, namely 61.5% and an increase in the percentage of patients with mild immunodeficiency, namely 23.1%. Vitamin D deficiency patients were not seen in patients without immunodeficiency and only mild and severe immunodeficiency patients were found. However, this study did not show a statistically significant correlation between blood vitamin D levels and CD4+ T cells counts.

Vitamin D has a role in immune system regulation. Its receptors are expressed by all immune system cells, including T lymphocytes. The association between vitamin D levels and TCD4+ cell count is still unclear to date, although a positive correlation has been shown by most studies (Mansueto *et al*, 2015). Beukel et al conducted study with large sample and showed the same outcome as our study. This study showed no significant difference of CD4+ count between normal and deficiency vitamin D. In addition, there was no significant difference in the rate CD4+ count recovery when patients were stratified by baseline CD4+ count. Kakalia et al studied the vitamin D supplementation effect in children with HIV infection and showed no significant correlation between vitamin D and CD4+ count. Another research by Ezeamama et al shows the correlation between vitamin D and CD4+ count and a slower increased CD4+ T cell in patient with deficiency. These result differed with our study could be due to the various vitamin D status, degree of immunodeficiency, local dietary patterns and confounding factors.

5. Conclusion

There was no correlation between blood vitamin D levels and CD4+ T cells count in children with HIV infection who received ART at Adam Malik General Hospital Medan. Further research is needed to find out the effect of vitamin D to CD4+ T cell count in a more severe disease condition with lower CD+ cell count.

Acknowledgements

The researcher would like to thank all staff of the allergy and immunology division at the Faculty of Medicine, University of North Sumatra and Tambor Kambaren, Rosmayanti Siregar, and Olga R Siregar for their time and guidance.

References

- Adedemy JD, Zohoun L, Alihonou F, d'Almeida M, Couringa Y, Agossou J, et al. Screening for Malnutrition and Nutritional Care in HIV-Infected Children Followed up in the Pediatric Unit of CNHU-KHM in Cotonou. *Matern Pediatr Nutr* 2016;109:2-7.
- Asfaw A, Ali D, Eticha T, Alemayehu A, Alemayehu M, Kindeya F. CD4 cell count trends after commencement of antiretroviral therapy among HIV infected patients in Trigay, Northern Ethiopia: a retrospective cross sectional study. *Plos one* 2015;10: 1-9.
- Badan Pusat Statistik Indonesia. Potret awal tujuan pembangunan berkelanjutan (*sustainable development goals*) di Indonesia. Jakarta: BPS;2016.
- Baeke F, Takiishi, Korf H, Gysemans C, Mathieu C. Vitamin D: modular of the immune system. *Curr Opin Pharmacol* 2010; 10: 482-86.
- Bearden A, Abad C, Gangnon R, Sosman JM, Binkley N, Safdar N. Cross sectional study of vitamin D levels, immunologic and virologic outcomes in HIV infected adults. *J Clin Endocrinol Metab* 2013; 98: 1726-33.
- Beukel C, Fievez L, Michels M, Sweep F, Hermus AR, Bosch ME, dkk. Vitamin D deficiency among HIV type 1 infected individuals in Netherlands: effects of antiretroviral Therapy. *AIDS Res Hum Retroviruses* 2008; 24: 1375-82.
- Dinas Kesehatan Provinsi Sumatera Utara. Profil Kesehatan Provinsi Sumatera Utara. Medan: Dinas Kesehatan Sumatera Utara;2013.
- Hayes CE, Hubler SL, Moore JR, Barta LE, Praska CE, Nashold FE. Vitamin D actions on CD4+ T cells in autoimmune disease. *Front Immunol* 2015;6: 1-22.
- Kakalia S, Sochetti EB, Stephens D, Assor E, Read SE, Bitnun A. Vitamin D supplementation and CD4 count in children infected with human immunodeficiency Virus. *J pediatr* 2011;159: 951-7.
- Khobondo JO, Gicheru MM, Khamadi SA. Pediatric HIV-AIDS in Nairobi: prevalence, gender and implication for prevention of mother to child transmission. *European journal of Research in Medical Sciences* 2015: 52-9.
- Lingayat AM, Kamble P. Study of clinical profile of CD4 count and outcome in children with HIV/AIDS below 12 years. *Int J Cur Res Rev* 2015;7: 8-11.
- Mansueto P, Seidita A, Vitale G, Gangemi S, Laria C, Cascio A. Vitamin D in HIV-Infected Patients: not only bone disorder. *Biomed Res Int* 2015; 1-19.
- UNAIDS. Children and HIV. Switzerland: UNAIDS; 2016.
- UNAIDS. Start free stayfree AIDS free. Switzerland: UNAIDS; 2017.
- UNICEF. Gender and HIV/AIDS. Switzerland: UNICEF; 2019.
- Weigel R, Phiri S, Chiputula F, Gumulira J, Brinkhof M, Gsponer T *et al.* Growth response to antiretroviral treatment in HIV-infected children: a cohort study from Lilongwe, Malawi. *Trop Med Infect Dis* 2010; 15: 934-44.