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# ASSOCIATION BETWEEN SERUM 25-HYDROXYVITAMIN D LEVELS AND VITAMIN D RECEPTOR POLYMORPHISMS IN HIV-INFECTED CHILDREN

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**ABSTRACT**

**Objective:** The clinical and genetic evidence is accumulating that vitamin D may play a role in modulating human immunodeficiency virus (HIV) infection. The aim of this study was to evaluate serum 25-hydroxyvitamin D [25(OH)D] levels in HIV-infected children and its association with vitamin D receptor (VDR) gene BsmI and FokI polymorphisms.

**Methods:** Serum 25(OH)D levels were measured using 250HD Liaison XL®. The VDR genes were detected by CLART®MetaBone.

**Results:** This study included 34 HIV-infected children on highly active antiretroviral therapy (HAART) for more than a year, aged 6-14 y. The results revealed that the mean of serum 25(OH)D levels were 19.6±7.0 nmol/l. The mean of $CD4^+$T-cell counts was 724 (18–1805) cell/$mm^3$ and CD4 % was 23.72±10.77. The genotypic frequency BsmI and FokI polymorphisms in HIV-infected children were BB 29%, Bb 41%, bb 29% and FF 47%, Ff 44%, ff 9%, respectively. Serum 25(OH)D levels were associated with BsmI polymorphisms ($p<0.05$), but not with FokI polymorphisms ($p>0.05$).

**Conclusion:** The present study showed that vitamin D deficiency is common in HIV-infected children, and genetic variant could lead to altered activity of vitamin D. Therefore, it is important to consider vitamin D status routinely in preventing the development of opportunistic infections and supplementation of vitamin D is warranted among HIV-infected children on HAART.

**Keywords:** 25-hydroxyvitamin D, HIV-infected children, VDR polymorphism, BsmI, FokI


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## INTRODUCTION

There is growing evidence reported that vitamin D deficiency is a global and important health issue in all age groups, including in children. The prevalence of vitamin D deficiency varies worldwide and is estimated to range from 30% to 80% of the population [1-4]. The serum or plasma concentration of 25-hydroxyvitamin D (25(OH)D) is the best indicator of clinical vitamin D status [5, 6]. The consequences of low 25(OH)D status include increased risk of various diseases [7], including periodontitis, oral cancer, oral candidiasis [8-12].

Vitamin D is well known as a steroid hormone that plays a major role in regulating mineral metabolism and bone health [13]. More recently, there is increasing evidence that vitamin D not only has a function in bones, but is also related to cell proliferation, cell differentiation, apoptosis, and intercellular adhesion [14]. In addition, vitamin D plays a key modulator of immune function and inflammation [15-17]. The active form of the vitamin D is 1, 25-dihydroxyvitamin D (1, 25-dihydroxy cholecalciferol (DHCC)) that circulates throughout the body, exerting its effects on the tissues by binding to the vitamin D receptor (VDR) [18]. The VDR is a transcription factor regulating the expression of genes, which mediate its biologic activity. The VDR gene encodes a nuclear receptor for the active form of vitamin D, 1, 25-dihydroxy vitamin D3 (1, 25$(OH)_2D_3$). After it binds to its response element on DNA, it regulates hundreds of genes with different functions [19]. Recent studies have reported DNA sequence variations, which occur frequently in the population, commonly referred to as polymorphisms, to exist in the VDR gene. Several polymorphisms, such as BsmI (rs1544410) and FokI (rs2228570), have been described in the VDR genes that are able to alter the activity of VDR protein that may contribute to the development of the diseases [20-26].

The vitamin D receptor is widely distributed in the nucleus of a large number of cell types in almost all human tissues that are important to immune and phagocytic functions (T and B cells, macrophages, and monocytes) [18, 27, 28]. It is therefore increasingly recognized that vitamin D may also have a role in the infectious diseases, including human immunodeficiency virus type-1 (HIV-1) infection [29, 30]. Previous studies indicated that vitamin D deficiency is frequent in HIV-positive individuals. The high rates of vitamin D deficiency among HIV-infected individuals have been reported, ranging from 70.3 to 83.7%, including in children [31-33]. Clinical and genetic evidence is accumulating that vitamin D may play a role in modulating human immunodeficiency virus (HIV) infection. It has been suggested the effect of antiretroviral therapy (ART) on vitamin D deficiency [34]. The relationship between vitamin D deficiency and the degree of systemic immune, as ascertained by $CD4^+$cell counts and viral load, in HIV-infected individuals have been investigated with different results [35, 36]. Therefore an association of serum 25(OH)D levels and polymorphisms in the VDR gene and also $CD4^+$cell counts in HIV-infected individuals remains unclear.

Increasing data indicated that vitamin D deficiency and polymorphisms in the VDR gene may be associated with various diseases in children [37-39], but few studies exist in HIV-infected children. It is well known that adequate serum concentrations of vitamin D are crucial for the developing child and play a role in immunity in pediatric patients [40]. In the present study, we investigated the serum 25-hydroxyvitamin D (25OHD) levels and its association with the frequencies of the VDR gene polymorphisms BsmI and FokI among HIV-infected children under HAART and $CD4^+$T-cell counts.

## MATERIALS AND METHODS

### Material

A cross-sectional study was conducted on 34 (thirty-four) HIV-infected children under HAART, aged between 6 and 14 y. All study participants were diagnosed HIV positive at Dr. Hasan Sadikin General Hospital, Bandung, West Java, Indonesia. Blood samples were collected from the study participants and the $CD4^+$T-cell counts were determined using the Becton Dickinson (BD) FACS Count system. The genomic DNA was extracted from whole blood using the established protocol for DNA extraction from blood cells. The VDR gene BsmI and FokI gene polymorphisms were detected by CLART®MetaBone. Serum total 25-hydroxyvitamin D (25[OH]D) levels were measured using 250HD Liaison XL®. Statistical analysis was performed by Spearman's

correlation test (P<0.01 significant). Data entry and analysis were done using software SPSS version 13. Quantitative data were presented by the mean and standard deviation. Qualitative data were presented by frequency distribution. One-way ANOVA and Kruskal-Wallis test were used to detect the mean difference across the two genotypes and the CD4+T-cell counts. A confidence interval (CI) level was set to 95% where any output p<0.05 would be interpreted as an indicator of statistical significance.

The study protocol was reviewed and approved by Ethical Committee of Faculty of Medicine, Universitas Padjadjaran number 275/UN6. C1.3.2/KEPK/PN/2015. All the parents of children provided written informed consent.

**RESULTS AND DISCUSSION**

Demographical characteristics for the participants are presented in table 1. The study involves thirty-four (34) HIV-infected children on HAART, 18 male and 16 female, aged 6 to 14 y of age (mean 9 y) who attended to Clinic Teratai, Dr. Hasan Sadikin Hospital Bandung, West Java, Indonesia. The mean duration of HAART use was 5 y. The mean of CD4+T-cell counts was 724 (18–1805) cell/mm$^3$ and CD4 % was 23.72±10.77. The mean of serum 25(OH)D levels in HIV-infected children (19.8±6.38 nmol/l).

**Table 1: Characteristics of the study participants**

| Characteristics | N (34) |
|---|---|
| Gender, n (%) | |
| • Male | 16 (47.1) |
| • Female | 18 (52.9) |
| Age (y), mean±SD | 9±3 |
| Duration of ARV exposure (years), mean±SD | 5±2 |
| Vitamin D (nmol/l), mean±SD | 19.6±7 |
| CD4 Absolut (cells/□L) | 724 (18–1805) |
| CD4 % | 23.72±10.77 |

Table 2 describes the distribution of CD4+T-cell counts in relation to the severity of immunosuppression of the study participants. The mean of the majority (70.6%) of the study participants had CD4+T-cell counts 946.08±386.94 cell/mm$^3$, and 8 (23.5%) participants reported to have CD4+T-cell counts<200/mm$^3$ (104.63±45.78/mm$^3$). The study demonstrated a positive correlation between serum 25(OH)D levels and CD4+T-cell count (p<0.05), and also correlated positively with CD4 % (p<0.05) as shown in table 3.

**Table 2: The CD4+ T-cell counts in relation to the severity of immunosuppression of the study participants**

| Classification of HIV-associated immunodeficiency | N | CD4+T-cell counts (cells/□L) Mean/SD |
|---|---|---|
| No significant immunosuppression (>500/µl) | 24 | 946.08±386.94 |
| Mild immunosuppression (350-499/µl) | 1 | 452 |
| Advanced immunosuppression (200-349/µl) | 1 | 313 |
| Severe immunosuppression (<200/µl) | 8 | 104.63±45.78 |

**Table 3: Correlation analysis between vitamin D and CD4+T-cell counts in HIV-infected children on ART**

| CD4+T-cell counts and percentage | Vitamin D | |
|---|---|---|
| | Coefficient correlation (r) | p-value |
| CD4 absolute (cells/µL) | 0.293 | 0.046* |
| CD4 % | 0.304 | 0.040* |

Data analysis using Spearman's rank correlation test, *correlation significant p<0.05

The VDR genotype of BsmI and FokI polymorphism in HIV-infected children on HAART presented in table 4. Genotype frequency of VDR polymorphisms Bmsl was high (70%, consist of Bb 41% and bb 29%) among HIV-infected children. The VDR Fokl Ff genotypes were also frequent in HIV-infected children (53%, consist of Ff 44%, ff 9%). We also found that CD4+T-cell counts were associated with Bsml polymorphisms in HIV-infected children (p<0.05), but not with Fokl the polymorphisms (p>0.05). However, our data showed that serum vitamin D levels were not associated with both of the VDR Bsml and Fokl polymorphisms in HIV-infected children table 5. Fig. 1 and 2 show the distribution of VDR Bsml polymorphism and Fokl in HIV-infected children on HAART, and its association with CD4+T-cell counts and Vitamin D serum levels.

**Table 4: Genotypes distribution of the VDR gene polymorphisms in HIV-infected children on HAART**

| Genotype | Frequency N (%) |
|---|---|
| VDR Bsml | |
| - BB | 10 (29.4) |
| - Bb | 14 (41.2) |
| - bb | 10 (29.4) |
| VDR Fokl | |
| - FF | 16 (47.1) |
| - Ff | 15 (44.1) |
| - ff | 3 (8.8) |

**Table 5: Correlation analysis between VDR Bsml/Folk polymorphism and CD4/Vitamin D in HIV-infected children on HAART**

| Variable | VDR Bsml polymorphism | | VDR Folk polymorphism | |
|---|---|---|---|---|
| | Coefficient correlation (r) | p-value | Coefficient correlation (r) | p-value |
| CD4 Absolute | 0.344 | 0.023* | 0.140 | 0.215 |
| Vitamin D | -0.055 | 0.379 | -0.212 | 0.114 |

Data analysis using the point-biserial correlation, *correlation significant p<0.05

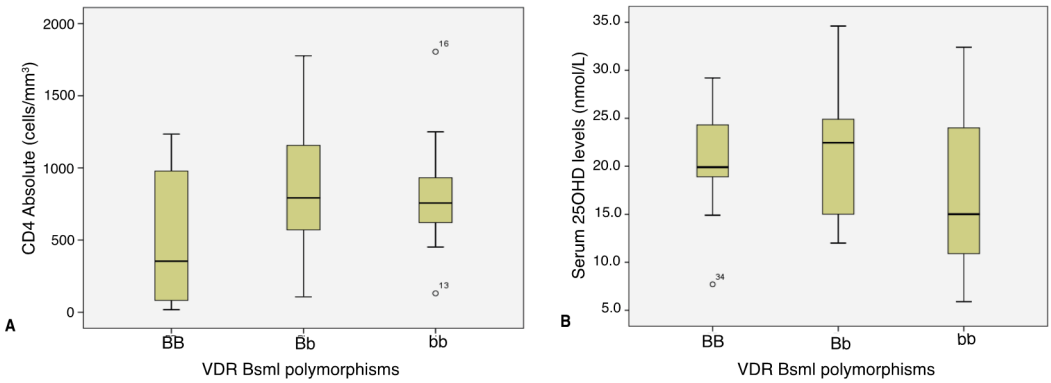


**Fig. 1: Distribution of VDR Bsml polymorphism in HIV-infected children on HAART. A. VDR Bsml polymorphism and CD4⁺T-cell counts. B. Vitamin D serum levels and CD4⁺T-cell counts**

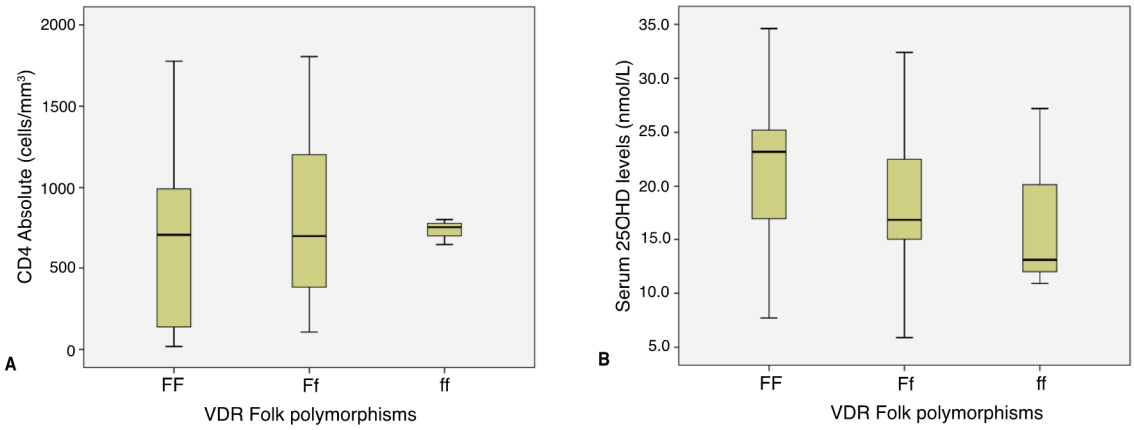


**Fig. 2: Distribution of VDR Folk polymorphism in HIV-infected children on HAART. A. VDR Bsml polymorphism and CD4⁺T-cell counts. B. Vitamin D serum levels and CD4⁺T-cell counts distribution**

Over the last 20 y several definitions of vitamin D deficiency for children have been published. The Institute of Medicine (IOM) and the American Academy of Pediatrics (AAP) have defined vitamin D deficiency among infants and young children as a serum 25 (OH)D level of<27.5 nmol/l (11 ng/ml) [41,42]. The Canadian Paediatric Society (2007) defined vitamin D deficiency as a serum 25(OH)D level of<25 nmol/l [43]. The most recent version, a review written on behalf of the Lawson Wilkins Pediatric Endocrine Society (2008) reported that vitamin D deficiency was defined as a serum 25(OH)D level of<37.5 nmol/l [44]. This study also revealed that HIV-infected children on HAART had vitamin D deficiency, and had a positive correlation with CD4+T-cell count and CD4%. Our research also supports past findings that vitamin D insufficiency is associated with impaired late CD4 recovery in HIV-infected patients on highly active antiretroviral therapy (HAART) [35]. Whereas, another study showed that serum 25(OH)D deficiency was not associated with CD4⁺T-cell counts, HIV viral load, or clinical stage [36].

A number of risk factors for low 25(OH)D levels in HIV-infected individuals have been suggested. First, factors related to the HIV infection itself may have contributed for the low vitamin D levels seen in the HIV-1-infected patients. The HIV-infected individuals are more exposed to chronic complications caused by the disease itself, and the increase of pro-inflammatory cytokines. TNF-alpha release may be responsible for renal 1a-hydroxylase impairment, reducing the PTH (parathyroid hormone) stimulatory effect on the production of the hormonally active 1, 25(OH)2D (1,25-dihydroxy vitamin D). It is then suggested that chronic HIV-associated inflammation and immune activation may also contribute to low vitamin D [31]. Second, to the interference of antiretroviral drugs may also have contributed to vitamin D deficiency in HIV-1-infected patients. The mean duration of HAART use of the study participant was 5 y. It is well known that vitamin D is metabolized in the body through cytochrome P450 enzymes [18]. It has been suggested that certain drugs can induce CYP3A4 expression in the liver and small intestine, accelerate vitamin D catabolism, and may contribute to vitamin D deficiency [45]. Prior studies have investigated the potential impact of HAART regimens on vitamin D levels in HIV patients, such as protease inhibitions and non-nucleoside reverse transcriptase inhibitors [46, 47]. Third, another possible reasons is due to traditional risk factors such as female sex, increasing age, lack of sunlight exposure, low vitamin D intake, poor absorption, greater body mass index (BMI), kidney or liver impairment, and multiple cardiovascular disease risk factors, and darker skin pigmentation [31, 48, 49].

Recently, many studies indicated that VDR gene BsmI and FokI polymorphisms have been associated with various diseases [20-24], including those in the oral cavity [25, 26]. Those studies indicated that VDR gene polymorphisms will help elucidate the pathogenesis of the various diseases and in the design of new approaches for prevention and treatment. It has also been reported the prevalence of vitamin D receptor gene polymorphisms in HIV-infected and its association with susceptibility and progression of HIV infection [50-53]. We herein also investigated the VDR genotype of BsmI and FokI polymorphism in HIV-infected children on HAART, and the results showed that genotype frequency of VDR polymorphisms Bmsl was high and associated with $CD4^+$T-cell counts, different with VDR Fokl polymorphisms. Our data also showed that serum vitamin D levels were not associated with both of the VDR Bsml and Fokl polymorphisms in HIV-infected children. Further studies with a larger patient population are needed to prove a relation between VDR gene polymorphism and vitamin D. In addition, further investigations may also be needed to evaluate an impact of vitamin D supplementation and other genetic variations in vitamin D receptors on the progression of HIV infection.

## CONCLUSION

The results of this study showed that vitamin D deficiency is common among HIV-infected children on HAART and the VDR gene Bmsl polymorphisms were significantly associated with low $CD4^+$T-cell count. It is important to consider vitamin D status routinely in preventing the development of opportunistic infections and supplementation of vitamin D is warranted.

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## AUTHORS CONTRIBUTIONS

Irna Sufiawati: Conception or design of the study, data collection, data analysis and interpretation, drafting the article, critical revision of the article, final approval of the version to be published. Risti Sapatarini: Conception or design of the study, data collection, drafting the article, final approval of the study to be published. Eriska Riyanti: Conception or design of the study, data collection, drafting the article, final approval of the study to be published. Intan Maulani: Data collection, data analysis and interpretation, drafting the article, final approval of the study to be published.

## CONFLICT OF INTERESTS

The authors declared no conflict of interest

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