Hypovitaminosis D increases TB co-infection risk on HIV patients

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Abstract. Tuberculosis is causes of mortality and morbidity in patients with HIV. Hypovitaminosis D, a defective cell-mediated immune response to *Mycobacterium tuberculosis* infection has been extensively described in HIV patients, but studies assessing the role of vitamin D in TB-HIV co-infection are lacking. We, therefore, conducted a 1:1 pair-matched case-control study to verify hypovitaminosis D possible risk factor of TB- HIV co-infection. Consecutive HIV patients starting ARV and sex, age and CD4 cell count matched were by recruiting. Tuberculosis has confirmed by thepresence of acid-fast bacilli in sputum or mycobacterium detected in specimens culture/Gene Xpert/PCR. Vitamin D levels were by measuring direct chemiluminescent immunoassay on a LIAISON®25OH analyzer. The study comprised 25 cases and 25 controls, median (interquartile range) 25(OH)D3 serum concentration were 19.80 (12.15-27.45) ng/mL in cases and 33.30 (27.2-39.4) ng/mL in controls (P<0.001). After adjustment for potential confounders included anemia, smoking,and low BMI, with multivariate logistic regression analysis, hypovitaminosis Dindependently associated with the development of active tuberculosis in HIV patients.(OR 26.154 (90% CI: 4.371-156.541); p <0.001). The finding indicates that hypovitaminosis D was a risk factor of TB-HIV co-infection.

1. Introduction

TB-HIV co-infection remains a challenge to global public health. In 2015, the proportion of HIV positive TB patients on antiretroviral therapy (ART) was 78%. TB-HIV is a leading cause of death, and 99% occurs in developing countries.[1]According to Indonesian Health Ministry data until the end of December 2015, the cumulative number of reported AIDS was 735,256 cases, with TB co-infection of 11.835 cases.

Tuberculosis is a granulomatous disease caused by Mycobacterium tuberculosis (MTB). Although the alveolar macrophages employ different mechanisms to kill the engulfed microbes, MTB have developed different approaches to staying alive within the hostile environment of the phagocytes, by prevent phagosome fusion with thelysosome and avert the phagosomal acidification thus favor the survival of bacteria inside the macrophages. However, a localized inflammatory response promotes the recruitment of T lymphocytes, which leads to the formation of a granuloma,a highly organized cellular structure composed of macrophages to wall off the spread of the infection. It proposed that the increase TB-HIV co-infection is caused by the disruption of the local immune response within tuberculosis granulomas, decreasing their ability to contain MTB leading to increased replication, dissemination,and clinical disease. The effectiveness of macrophage recognition, processing,and killing of MTB is likely to play a role in the evolution of infection.[2]However the risk factors behind

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the breakdown in immune defense associated with TB-HIV co-infection are not fully understood. It is unclear why some HIV-positive people are co-infected with TB while others are not. Until reliable information on risk factors of TB-HIV co-infection is collected, it will remain difficult to design effective preventive intervention strategies to control TB in HIV- infected people.

Hypovitaminosis D is associated with impaired immune responses and increased susceptibility to some intracellular pathogens including MTB. Vitamin D is required for the macrophage responses to MTB, where it plays a critical role in macrophage activation following Toll-like receptor (TLR) signaling, tumor necrosis factor alpha (TNF-α) release, interferon gamma (IFN-γ)-mediated cathelicidin function, phagolysosome maturation, autophagy, and intracellular killing of Mtb.[3]

Several hypotheses are drawn to describe the mechanism by which HIV-1 increases the risk of TB infection. Some of thepotential mechanisms include; persistent HIV-1 replication in the lung causes immune dysfunction, HIV-1 induced CD4 T cell apoptosis and subsequent granuloma disruption depletion of MTB-specific CD4 T cells,and the recent studies have linked hypovitaminosis D with an increased risk for susceptibility to TB.[3,4]Adequate serum concentration of vitamin D_3 is critical for optimal innate immune response. Rapid sputum clearance of MTB and radiological improvement were in pulmonary tuberculosis, who received vitamin D supplementation. The physiological concentration of 1,25(OH)2 D3 have indirect antimicrobial activity against MTB and HIV through theautophagy-dependent mechanism.[5] However, to date, few studieson hypovitamin D and risk of TB-HIV co-infection have been conducted. Therefore, we initially assessed whether hypovitaminosis D as a risk factor for TB HIV co-infection.

2. Methods

A case-control study was conducted from September 2015 to October 2016 in Sanglah Hospital. Consecutive adult patients (age >12 years old) presenting at the participating hospital with ARV naïf-HIV infection. Cases were co-infection TB-HIV patients, new TB active diagnosed by the National TB Program. A confirmed diagnosis of TB infection was made by clinical symptoms suggestive of TB. One of the following is a positive sputum smear on Ziehl Nielsen stain for acid-fast bacilli (AFB) or MTB detected on specimen culture or Gene Xpert MTB-RIF or PCR.[1] Controls were HIV infected patients without TB, matched by gender, age (±5 years) and CD4 cell count (≤200;>200).

After obtaining informed consent, CRF was used to collect information about the socio-demographic characteristic data, smoking history(ever daily smoking), drug history (including use of steroids, vitamin D supplementation, anti TB drug, and highly active antiretroviral therapy),and laboratory tests for CD4 cell count and hemoglobin level. All participants also underwent baseline anthropometric measurements of weight and height for calculation of the body mass index (BMI). Exclusion criteria were patients with Vitamin D supplementation or immunosuppressant therapy within the last 2 weeks, diabetes mellitus, chronic kidney disease

The serum concentration of 25-hydroxyvitamin D or 25(OH)D was by determininga LIAISON® direct chemiluminescent immunoassay (CLIA), hypovitaminosis D was as serum vitamin D levels <30 ng/ml (<75 nmol/L).[6]

The study has agreement from theMedical faculty of Udayana University/ Sanglah Hospital research and ethics committee. All patients gave informed consent before enrolment into the study.

Based on the minimal case-control samples formula (Sopyudin Dahlan, 2009) 25 characteristic of TB-HIV co-infection patients were compared to 25 controls. Student's t-test was used to assess thedifference in means between 2 groups when there was a normal distribution, and Wilcoxon's rank-sum test was when thenonparametric analysis was needed. The association between hypovitaminosis D and TB-HIV co-infection was by evaluatinglogistic regression. Statistical analysis was performed using SPSS version 16. *P*-value<0.05 was considered significant.

3. Results

At enrolment, 25 cases compared with 25 controls had complete information on 25(OH)D status and relevant covariates. Demographic and characteristics of the patients are in table 1.

Table 1. Socio-demographic factors and clinical characteristics of study participants.

Characteristics	Cases (n=25)	Controls(n=25)	*p* value
Mean age, years (±SD)	34.92±9.0	35.44±8.77	0.837
Gender			
- male	22 (88%)	22(88%)	1.000
- female	3(12%)	3(12%)	
CD4 cell count mean (cell/ µL),(±SD)	79.64±110.96	80.0±132.82	0.567
Smoking			
- yes	12 (48%)	3 (12%)	**0.012**
- no	13 (52%)	22 (88%)	
Anemia (Hb level<10g/dL)			
- yes	15 (60%)	6 (24%)	**0.010**
- no	10 (40%)	19 (76%)	
BMI			
- low (BMI<18.5Kg/m^2)	10 (40%)	5 (20%)	0.123
- normal (BMI≥18.5Kg/m^2)	15 (60%)	20 (80%)	
HIV risk factors			
- multipartner sexual	22(88%)	23(92%)	0.837
- homosexual	1(4.0%)	1(4.0%)	
- ivdu	2(8.0%)	1(4.0%)	
Stage of HIV (WHO)			
- stage 2	0 (0%)	2(8.0%)	0.112
- stage 3	1(4.0%)	4(16%)	
- stage 4	24(96%)	19(76%)	
Confirmation for active TB	25 (100%)		
- Sputum AFB (+)	19 (76%)		
- Sputum *Gene Expert* MTB (+)	19 (76%)		
- PCR MTB (+)	4 (16%)		

In this study, there were no difference in mean age(SD) (p=0.837) and gender (*p*=1.000), between cases (mean age 34.92±9.0; male 88%) and controls (mean age 35.44±8.77; male 80%) and mean CD4 cell count (79.64±110.96 for cases, 80.0±132.82 for controls, *p*=0.567) was found.A higher proportion of cases had a history of smoking compared to controls (p=0.012). Individuals who had anemia (hemoglobin<10.0g/dl) were more likely to have active TB (p=0.10). A higher proportion of cases (40%) had a BMI less than 18.5kg/m2 compared to controls (20%), p value=0.123.There was no significant difference in risk factor for HIV between cases and controls, including multipartner sexual (cases 88%: controls 92%), homosexual (cases 4%: controls 4%), and intravenous drug users (ivdu) (cases 8%: control 4%;p=0.837). In addition there was no significance difference concerning HIV stage (WHO) between cases (stage 4 (96%), stage 3 (4%), stage 2 (4%)), and controls (stage 4 (76%), stage 3 (16%), stage 2 (8%); p value=0.1120). Among the 25 cases of TB-HIV co-infection, 19(76%) cases were diagnosed by AFB sputum smear, 19(76%) cases were Gene-XpertMtb positive Rif susceptible while the remaining 4(16%) cases were diagnosed by PCR.

Table 2. Association of hypovitaminosis D and TB –HIV co-infection.

Vitamin D	Case	control	OR(95%CI)	*p* value
Hypovitaminosis D (vit D<30 ng/dl)	22(88%)	5(20%)	29.333	**<0.001**
Normal vitamin D (vitD≥30ng/dl)	3(12%)	20(80%)	(6.200-138.781)	

We observed in cases have lower 25(OH)D3 serum concentration with median (interquartile range) 19.80 (12.15-27.45) ng/mL compared to controls 33.30 (27.2-39.4) ng/mL (*p*<0.001). Further significant interaction of hypovitaminosis D and TB-HIV infection were seen (OR: 29.333 (90% CI: 6.200-138.781); *p* <0.001) (table 2). Adjustment for potential confounders included anemia, smoking and low BMI (p<0,25, in univariate analysis), with multivariate logistic regression analysis,

hypovitaminosis D were independently associated with the development of active tuberculosis in HIV patients.(OR 26.154 (90% CI: 4.371-156.541); p <0.001) (table 3).

Table 3. Multivariate analysis logistic regression of hypovitaminosis D and another potential confounder variables.

Variable	B	SEB	*P value*	*Adjusted OR*	*95% CI OR*	
					lower	*upper*
Hypovitaminosis D, yes/no	3.264	0.913	**<0.001**	**26.154**	4.371	156.458
- Ref. no						
Smoking, yes/no	1.238	0.993	0.187	3.448	0.548	21.702
- Ref. no						
Anemia, yes/no	1.030	0.856	0.229	2.802	0.523	15.005
- Ref. no						
Low BMI, yes/no	1.233	0.940	0.190	3.430	0.543	21.656
- Ref. no						

4. Discussion

The evaluation of risk factors for TB –HIV co-infection and determinant factors, it is often challenging, however, to differentiate the direct impact of TB-HIV infection from the effect of traditional risk factors which may be over-expressed. At the same time, hypovitaminosis is more frequent in HIV-infected patients. Several studies have shown an association between hypovitaminosis D and TB[3,7] or hypovitaminosis D and HIV infection.[8] Hypovitaminosis D is believed to increase the risk of progression and long-term complications of HIV infection, while increased levels have associated with inhibition of HIV replication and lower risk of all-cause mortality.[9]

This study shows that TB-HIV patients have lower 25(OH)D3 serum concentrationmedian (interquartile range) 19.80 (12.15-27.45) ng/mL compared to controls 33.30 (27.2-39.4) ng/mL (p<0.001). It is similar to the reported for HIV patients in Cape Town; 28.7nmol in TB patients and 54.7 nmol/L in non-TB patients (p<0.001)[10] and previous study in Tanzania which reported 25(OH)D3 level as 7.4 nmol lower in cases (p=0.02)[7]

With a cut of point 30 ng/dl further significant interaction of hypovitaminosis D and TB-HIV infection were seen (OR: 29.333 (90% CI: 6.200-138.781); p <0.001), hypovitaminosis D was more common in the cases (88%)compared to controls (20%). Our results are similar with previous studies which assessing 25(OH)D levels in those with active pulmonary TB (both in HIV positive and healthy subjects), the prevalence of hypovitaminosis D was 86%.[7]A study in South Koreademonstrates through significantly lower vitamin D levels in TB patients compared to control subjects.[16]After adjustment for potential confounders included anemia, smoking,and low BMI, with multivariate logistic regression analysis, hypovitaminosis D independently associated with the development of active tuberculosis in HIV patients.(OR 26.154 (90% CI: 4.371-156.541); p <0.001) These results are similar with two previous studies, first; In Tanzanian, adult HIV patients initiating antiretroviral therapy showing that low vitamin D (<20ng/dl) had asignificantly greater association with incident pulmonary tuberculosis.(OR 2.89;95%CI, 1.31-7.41; p=0.027),[8]seconds, Jarvis 2012, found vitamin D deficiency (plasma 25[OH]D≤50nmol/L) is not associated with cryptococcal meningitis. But itisassociated with increased odds of active TB, with worsening deficiency (OR 1.47 [95% CI, 0.5-4.7] for vitamin D insufficiency, OR 1.51[95% CI0.5-4.5] for vitamin D deficiency and OR 2.52[95%CI, 0.8-7.9] for severe vitamin D deficiency, all compared to a baseline of normal vitamin D status (for p trend=0.69)[4]

Of note, the same or near the same age, gender and CD4 Cell count in both groups is due to matched controls. Patients were majority males (p=1.000), in mean (SD) age (cases 34.92±9.0 :controls 35.44±8.77; p= 0.837) and low CD4 cell count (p=0.567). Similarly, a systematic review to determine the burden and risk factors for TB-HIV co-infection in Europe, reflecting the higher

proportion of HIV and TB-HIV co-infection in males with the mean of age was 38±10.45 years.[11] Both TB and HIV are more common among males and young adult population.[1] There can be several explanations for this finding, first; the majority of the HIV infected people fall in this group and second; males of this age group are relatively higher exposure to the outside environment. There has been a long-standing interest in the effect of sex hormones on immune function. Sex different which is affected by the immunosuppressive effect of testosterone in males and an immune-enhancing effect of estrogen seems given that females tend to have better immune function.[12]

This study also showed the majority patients in stage 3 or 4 with low CD4 Cell count (<200 cell/ml) and multipartner sexual was the strongest risk factor for HIV infection compared with homosexual and IVDU, and there is no significant difference between cases and controls. This finding was likely explained by the fact that ARV was more frequent initiated for the HIV patient when they developed stage 3 or 4 characteristics or in severe opportunistic infections with very low CD4 cell count. There were similar to the report from previous studies inSouth Africa[13] and Europe.[11]

Of the variables in table 1, we examine smoking as the potential determinants of theTB-HIV risk factor, but in multivariate analysis reviled no association between these variables and TB-HIV co-infection. Several studies have linked tobacco smoking with increased risk active TB[14,15] but there were not in this study, that could be probably due to the low prevalence of smoking in our study population. There could also be a social desirability bias whereby smokers denied their smoking status.

According to our results, anemia and low BMI do not increase the risk of TB-HIVco-infection. There were consistent with the results of study in Seoul which found there was no significant correlation between anemia and low BMI with TB-HIV co-infection and vitamin D status.[16]

Hypovitaminosis D has long been accepted to be anassociation with an increased risk for active TB and HIV disease progression.[8]Vitamin D sufficiency is a critical factor to activate autophagy pathways. The previous studies have shown that vitamin D improves phagocytosis and induces monocytes maturation, induced higher expression of cathelicidin antimicrobial peptide (CAMP), which can act against intracellular MTB. The promoter regions of CAMP and DEFb2 genes are reported to have vitamin D response elements that mediate vitamin D3-dependent gene expression, and these defensins also play an important role in theinduction of autophagy to control mycobacterial growth.[7,16]In addition, vitamin D also suppresses the macrophage inflammatory response by downregulating the production of pro-inflammatory cytokines.[5]

This study was limited by; first, the lack of information on the other chronic diseases and malignancies which could be the cause of hypovitaminosis D, second, vitamin D level was measured at a single time point.We are unable to determine whether hypovitaminosis D at a single time point or long-term deficiency is biologically relevant.

5. Conclusion

The study shows that hypovitaminosis D was a risk factor of TB-HIV co-infection. The results provide an importance of vitamin D sufficiency in HIV infected persons and provide new insight into approaches to prevent and treat TB-HIV co-infection.

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