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Original Article

Specific Levels of Calcidiol, Calcitriol, Cathelicidin and Interferon Gamma in Diabetic Patients with TB Infection in Jakarta: Case-Control Study

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Abstract

Background: *Vitamin D plays a role in regulating the immune system via Vitamin D receptors, expressed by T-helper cells (Th). Cathelicidin LL-37 is an antimicrobial peptide that acts as the primary barrier against the M. tuberculosis bacterial infection, which is induced by calcitriol, the active form of Vitamin D3. Interferon gamma (*IFN-γ*) is a cytokine released by Th-1 cells, and is essential for the elimination of M. tuberculosis. This study aims to determine the state of, and correlation between, calcidiol, calcitriol, cathelicidin and IFN-γ levels, as well as other clinical factors among patients with Type 2 Diabetes Mellitus (T2DM) with active TB coinfection. Further analysis is also performed to differentiate between T2DM patients with active tuberculosis, latent TB and without TB infection.*

Methods: *This study using a case-control design, with a sample size of 102 T2DM patients, which are divided into 3 categories of TB infection status; active TB, latent TB and without TB coinfection. Screening for active and latent TB coinfections using Interferon Gamma Release Assay (IGRA) test, Quantiferon TB Gold Plus, GeneXpert MTB/Rif examination of the sputum and Chest X-ray. Serum calcidiol and calcitriol levels were measured using the Liquid Chromatography Double Mass Spectrometry (LC-MS/MS), whereas Cathelicidin LL-37 levels were measured using the Enzyme-linked Immunosorbent Assay (ELISA). TB specific IFN-γ levels were obtained through the IGRA test, which measured IFN-γ from CD-4 (TB1) and CD-8 (TB2) cells.*

Results: *Nearly all T2DM patients had abnormal serum calcidiol levels. Patients with an active TB infection exhibited the lowest serum calcidiol levels and were Vitamin D deficient, compared to patients with latent TB infection or without TB infections (P=0.004). T2DM patients with active TB also had high levels of calcitriol cathelicidin LL-37 and IFN-γ (TB2), compared to the other groups. Calcidiol was shown to have a negative correlation with HbA1C, calcitriol and specific IFN-γ (TB2-nil) levels in T2DM patients with active TB. Significant differences in serum calcidiol were found between T2DM patients with different smoking habits, however no significant difference was found in correlation to body mass index.*

Conclusion: *T2DM patients have lower levels of Vitamin D on average, hence require supplementation due to cases of active TB coinfection. Increases in calcitriol, cathelicidin LL-37 and specific IFN-γ can be used as potential diagnostic biomarkers of M. tuberculosis infection in T2DM patients.* ***(J Respirol Indones 2021; 41(4): 279–87)***

Keywords: *vitamin D, IFN-γ, tuberculosis, DM type 2*

Kadar Spesifik Calcidiol, Calcitriol, Cathelicidin dan Interferon Gamma pada Penderita Diabetes dengan Infeksi TB di Jakarta: Studi Kasus-Kontrol

Abstrak

Latar belakang: *Vitamin D berperan dalam pengaturan sistem imun melalui reseptor Vitamin D yang diekspresikan oleh sel T-helper (Th). Cathelicidin LL-37 adalah peptida antimikroba yang bertindak sebagai penghalang utama terhadap infeksi bakteri M. tuberculosis, yang diinduksi oleh calcitriol, bentuk aktif Vitamin D3. Interferon gamma (IFN-γ) adalah sitokin yang dilepaskan oleh sel Th-1, dan penting untuk eliminasi M. tuberculosis. Penelitian ini bertujuan untuk mengetahui keadaan, dan hubungan antara kadar kalsidiol, kalsitriol, cathelicidin dan IFN-γ, serta faktor klinis lain pada pasien Diabetes Mellitus Tipe 2 (DMT2) dengan koinfeksi TB aktif. Analisis lebih lanjut juga dilakukan untuk membedakan antara pasien DMT2 dengan TB aktif, TB laten dan tanpa infeksi TB.*

Metode: *Penelitian ini menggunakan desain case-control, dengan jumlah sampel 102 pasien DMT2, yang terbagi dalam 3 kategori status infeksi TB; TB aktif, TB laten dan tanpa koinfeksi TB. Skrining untuk koinfeksi TB aktif dan laten menggunakan tes Interferon Gamma Release Assay (IGRA), Quantiferon TB Gold Plus, pemeriksaan sputum GeneXpert MTB/Rif dan rontgen dada. Kadar kalsidiol dan kalsitriol serum diukur menggunakan Liquid Chromatography Double Mass Spectrometry (LC-MS/MS), sedangkan kadar Cathelicidin LL-37 diukur menggunakan Enzyme-linked Immunosorbent Assay (ELISA). Kadar IFN-γ spesifik TB diperoleh melalui uji IGRA, yang mengukur IFN- dari sel CD-4 (TB1) dan CD-8 (TB2).*

Hasil: *Hampir semua pasien DMT2 memiliki kadar serum calcidiol yang abnormal. Pasien dengan infeksi TB aktif menunjukkan kadar kalsidiol serum terendah dan kekurangan vitamin D, dibandingkan dengan pasien dengan infeksi TB laten atau tanpa infeksi TB (P=0,004). Pasien DMT2 dengan TB aktif juga memiliki kadar calcitriol cathelicidin LL-37 dan IFN-γ (TB2) yang tinggi dibandingkan dengan kelompok lain. Kalsidiol terbukti memiliki korelasi negatif dengan kadar HbA1C, kalsitriol dan IFN-γ (TB2-nil) spesifik pada pasien DMT2 dengan TB aktif. Perbedaan signifikan dalam serum kalsidiol ditemukan antara pasien DMT2 dengan kebiasaan merokok yang berbeda, namun tidak ada perbedaan signifikan yang ditemukan dalam korelasinya dengan indeks massa tubuh.*

Kesimpulan: *Pasien DMT2 rata-rata memiliki kadar Vitamin D yang lebih rendah, sehingga memerlukan suplementasi karena kasus koinfeksi TB aktif. Peningkatan calcitriol, cathelicidin LL-37 dan IFN-γ spesifik dapat digunakan sebagai biomarker diagnostik potensial infeksi M. tuberculosis pada pasien DMT2.* ***(J Respirol Indones 2021; 41(4): 272–87)***

Keywords: *vitamin D, IFN-γ, tuberkulosis, DM tipe 2*

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INTRODUCTION

Despite the consistent decline in global cases, Tuberculosis (TB) is still a major global health concern. In 2019, around 10 million new cases and 1.6 million deaths due to active TB were recorded. Diabetes mellitus (DM) has been known to increase the risk of contracting TB by at least 3 times, compared to non-diabetic individuals. Several studies have also shown the importance of DM as a risk factor for TB infection.[1,2]

Vitamin D is known to have pleiotropic effects on multiple organs, and plays an important role in innate and adaptive immunity. Calcidiol undergoes hydroxylation in the liver, catalysed by the enzyme 25-hydroxylase. Further hydroxylation by the enzyme 1-alphahydroxylase occurs in the kidneys, forming Calcitriol, the active form of vitamin D.[3] Crowle et al.[4] showed that calcitriol is an essential factor for the antimicrobial activity of human monocytes and macrophages, against *M. tuberculosis* infections. Other studies have also described the function of calcitriol as an immunomodulator in the homeostasis of the immune system, and its ability restrict the growth of *M. tuberculosis* via the induction of Cathelicidin LL-37.[4,5]

Cathelicidin LL-37, and calcitriol induce *autophagy* that acts as the first barrier against the TB infection.[6,7] Calcidiol is the primary form of the vitamin in circulation, and has a longer half-life, which makes it an effective screening marker of Vitamin D3 sufficiency. Previous studies have shown that vitamin D deficiency is correlated to the risk of active TB, as well as failure to achieve glycemic control in T2DM patients.[8,9] Despite the well-established role of vitamin D in the pathogenesis of TB, the correlation between calcidiol, calcitriol, cathelicidin and IFN-γ levels, as well as other clinical factors, among T2DM patients with active TB coinfection, latent TB and no TB infection, remains to be studied.

METHODS

This research was an observational study with a case-control design. The sample size was obtained based on a significance level of 0.05 and power of 0.8. Research subjects included T2DM patients who had visited the endocrinology and pulmonology clinic at Dr. Cipto Mangunkusumo Hospital, Persahabatan Hospital, Harapan Jaya Hospital, and Islam Pondok Kopi Jakarta Hospital. The subjects were selected via consecutive sampling. The subjects included T2DM patients who had been diagnosed with DM for at least 1 year, were not pregnant, did not suffer from liver or kidney disorders, had not been diagnosed with cancer, and were not consuming immunosuppressant drugs.

A total of 102 patients were collected based on the criteria. The research subjects were screened using the IGRA test (Quantiferon TB Gold Plus/QFT Plus), chest x-ray, and GeneXpert MTB/Rif sputum examination. Subjects with a positive IGRA result, but a normal chest x-ray and sputum examination were classified as DM with latent TB, whereas subjects with positive IGRA and sputum examinations were grouped as DM patients with active TB, regardless of chest x-ray results indicative of TB infection. Subjects who tested negative in every screening method were grouped as DM without TB coinfection. Baseline characteristics of the research subjects, such as sex and age, from every group were matched. Serum calcidiol and calcitriol levels were measured using the LC-MS/MS method, Cathelicidin levels were measured using the competitive ELISA method and TB specific IFN-γ levels were obtained from the QFT Plus (TB1 and TB2) results.

In this study, cathelicidin LL-37 levels were measured via the ELISA method from MyBioSource, with a detection threshold of 1.56-100 ng/mL. Calcitriol and calcidiol levels were measured using the LC-MS method, with a limit of detection (LOD) of 1 ppb, via the Agilent LC system 1290, with the Agilent Triple Quad 6460 (LC-MS). Vitamin D status was classified based on the Hollick criteria; normal (>30 ng/mL), insufficiency (20-29.9 ng/mL), deficiency (10-19.9 ng/mL) and severe deficiency (<10 ng/mL), however in this study, the criteria categories are simplified to normal, insufficient and deficiency.

Variables such as T2DM and TB status are analysed by using the Chi Square or Fisher test.

Results from the hypothesis testing wil include p-values, which are considered significant if P<0.05, as well as the corresponding confidence intervals. To study the correlation between calcidiol, calcitriol, cathelicidin and IFN-γ levels, and other clinical factors, and the TB status among T2DM patients, appropriate hypothesis testing will be conducted. Statistical analysis of the data are performed using the SPSS for Windows 20 program. This study had been approved by the Ethical Clearance Committee, Faculty of Medicine, Universitas Indonesia (610/UN2.F1/ETIK/2017), and the Health Reseach Ethics Commission Persahabatan Hospital (41/KEPK-RSUPP/09/20) on the 19 September 2017.

RESULTS

This study was performed on 102 diabetic patients, who were divided into 3 groups, based on their TB status; active TB, latent TB and without TB. Based on the sex, there were more female subjects (52.9%) than male subjects (47.1%). Every group had the same number of female (n=18) and male subjects (n=16). There were no significant differences in the age of the subjects between the groups, since the subjects were matched based on their age. On average, the age of the T2DM subjects without TB was 48.4 years, compared to 49.8 years for the latent TB group and 47.3 years for the active TB group.

Majority of the patients from each group did not have a history of smoking, however there were 8 subjects (23.5%) who were smokers in the group without TB infection, compared to 3 subjects (8.8%) in the latent TB group and 11 subjects (32.4%) in the active TB group. Duration of diabetes was varying among the subject groups. On average, T2DM patients with latent TB had a longer history of diabetes (median = 8.5 years), compared to without TB (median = 5.5 years) and active TB (median = 3 years).

Markers of glycemic control, such as Fasting Blood Glucose (FBG), 2-hour Post Prandial Blood Glucose (PPG) and HbA1c were poor on average. T2DM subjects without TB had a median FBG of 140.5 mg/dL, whereas patients with latent TB and active TB had median values of 140 mg/dL and 229 mg/dL, respectively. Post prandial glucose levels were higher on average for DM patients with active TB coinfections (310.71 mg/dL), followed by latent TB coinfections (229.65 mg/dL) and without TB coinfection (195.76 mg/dL).

Table 1. Characteristics of Research Subjects

Characteristics	DM without TB infection (n=34)	DM with Latent TB (n = 34)	DM with Active TB (n=34)	P
Smoking History				0,19
Smokers	8 (23.5%)	3 (8.8%)	11 (32.4%)	
Ex-smokers	7 (20.6%)	6 (17.6%)	5 (14.7%)	
Non-smokers	19 (55.9%)	25 (73.5%)	18 (52.9%)	
Duration of DM (years)	5.5 (1–18)	8.5 (1-26)	3 (1–21)	0,01
≤5	17 (50.0%)	9 (26.5%)	24 (70.6%)	
6–15	12 (38.7%)	19 (55.9%)	8 (23.5%)	
>15	5 (14.7%)	6 (17.6%)	2 (5.9%)	
FBG (mg/dL)	140.5(84–273)	140 (82–343)	229 (94–403)	<0,001
2hPP(mg/dL)	195.76 ± 58.59	229.65 ± 77.16	310.71 ± 82.59	<0,001
HbA1c (%)	6.9 (5.7–12.9)	8.45 (5.5–12.5)	10.35 (6.4–15.7)	<0,001
< 7	18 (52.9%)	7 (20.6%)	2 (5.9%)	
7–9.9	10 (29.4%)	16 (47.1%)	14 (46.7%)	
≥ 10	6 (17.6%)	11 (32.4%)	18 (52.9%)	
BMI (kg/m^2)	25.66 (18.31–37.78)	25.18 (16.22–36)	23 (15.63–28.44)	0,01
< 18.5	1 (2.9%)	4 (11.8%)	4 (11.8%)	
18.5–22.9	11 (32.4%)	10 (29.4%)	14 (41.2%)	
23.0–24.9	5 (14.7%)	10 (29.4%)	14 (41.2%)	
≥ 25.0	17 (50.0%)	10 (29.4%)	2 (5.9%)	

Similarly, HbA1c levels were lowest in patients without TB (6.9%), followed by latent TB (8.45%) and active TB coinfections (10.35%). Markers of glycemic control were significantly different among the subject groups, with active TB coinfection group displaying the worst glycemic control on average.

On average, calcitriol levels in the active TB group were the highest (53.88 ng/mL), followed by latent TB (51.38 ng/mL) and no TB infection (43.5 ng/mL). The difference between the calcitriol levels between the three groups was not statistically significant (P=0.99).

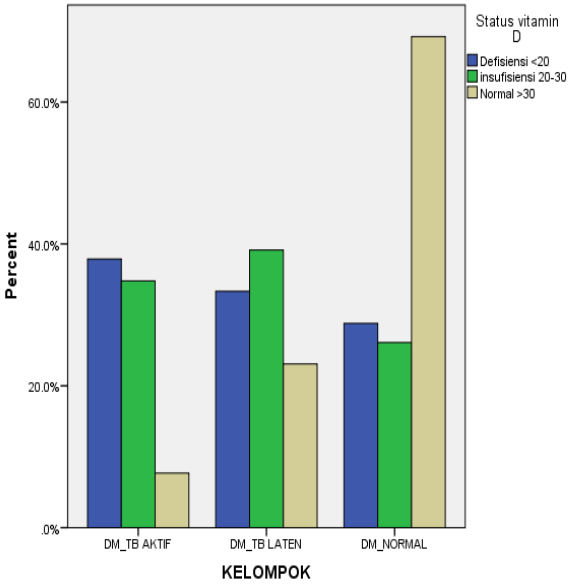

Figure 1. Vitamin D3 (Calcidiol) status in DM patients with active TB, latent TB and without TB infection

Cathelicidin levels in T2DM subjects with active pulmonary TB (49.6 ng/mL) were higher than that of subjects with latent TB (23.49 ng/mL) and without TB infection (10.46 ng/mL) on average, and this difference was statistically significant (P<0.0001).

Levels of specific IFN-γ TB2-Nil were found to be greater than IFN-γ TB1-Nil levels across all subject groups. On average, specific IFN-γ levels were greater in subjects with active TB, compared to the other groups (P<0.0001).

Among the T2DM subjects with active TB, vitamin D levels were found to be negatively correlated to HbA1c (P=0.004; r=0.5), calcitriol (P=0.023; r=0.39) and specific IFN-γ (TB2-Nil) (P=0.04; r=0.36) levels, as illustrated in Figure 2.

Figure 3 shows that latent TB DMT2 subjects had Calcidiol levels only moderately correlated with Calcitriol levels (P=0.006; r=-0.46), and not correlated with specific HbA1c and IFN-γ levels.

No significant correlation was found between Calcidiol levels and the variables assessed in the group of T2DM patients without TB coinfection. Smoking history was significantly associated with Calcidiol levels, with smokers having lower levels (15.09±7.57) on average, compared to former smokers (16.04±9.01) and non-smokers (22.10±12.18).

Median vitamin D-25OH levels among subjects who were obese, overweight, normoweight and underweight were 17.23 ng/mL, 18.65 ng/mL, 15.9 ng/mL and 11.98 ng/mL respectively. No significant differences in vitamin D-25OH levels were found between T2DM subjects across all nutritional statuses (P=0.32).

Vitamin D is an essential nutritional component, which has unique metabolic and physiologic functions, when compared to other vitamins. Vitamin D has shown to have a role in diabetes mellitus and has an impact on the risk of TB infection.

Table 2. Calcidiol, Calcitriol, Catheliciidn LL-37 and IFN-γ levels

Indeks	DM without TB infection	DM TB Latent	DM with Active Tb	P
Calcidiol (ng/mL)	18,61 ± 10,92	17,77 ± 8,53	15 ± 10,14	0,04
Normal (>30 ng/mL)	9 (26,5%)	3 (8,92%)	1	
Insufficiency (20 – 30 ng/mL)	16 (47,05%)	9(26,47%)	8	
Deficiency (< 20 ng/mL)	19 (55,89)	22 (64,7%)	25	
Calcitriol (ng/mL)	43,5 ± 43,5	51,38 ± 49,31	53,88 ± 59	0,99
Cathelicidin LL-37 (ng/mL)				<0,001
IFN-γ (IU/L)	10,46 (0,26–78,01)	23,49 (2,57–53,13)	49,6 (9,3–174,11)	<0,001
IFN-γ (TB1-Nil)	0,03 (0–0,29)	1,4 (0,18–7,89)	1,79 (0,01–10)	
IFN-γ (TB2-Nil)	0,04 (0–0,30)	1,4 (0,22–8,02)	3,7 (0,06–10)	

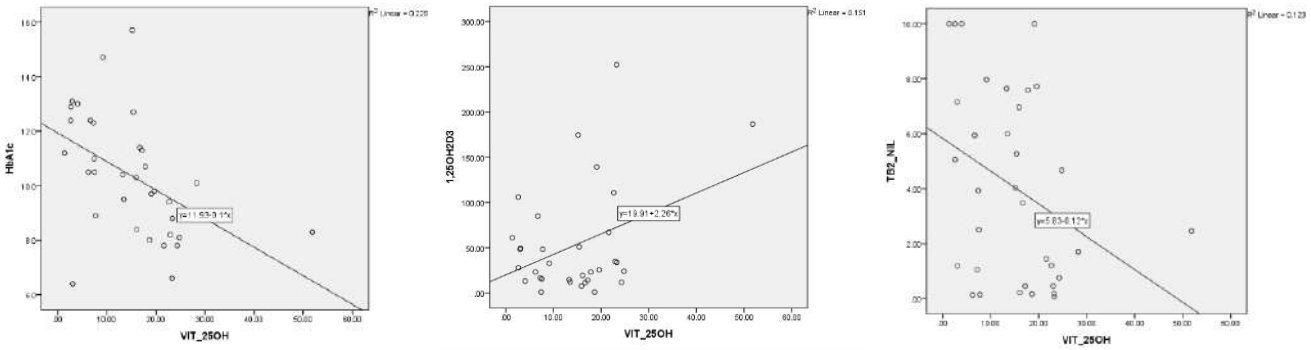

Figure 2. Correlation of vitamin 25OHD3 levels with HbA1c, calcitriol and specific IFN-γ

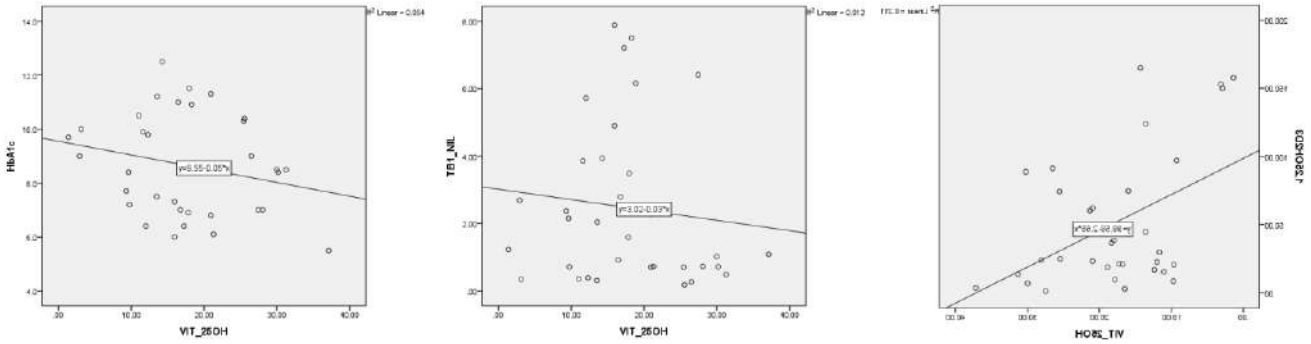

Figure 3. Correlation of vitamin 25OHD3 levels with HbA1c, calcitriol and specific IFN-γ

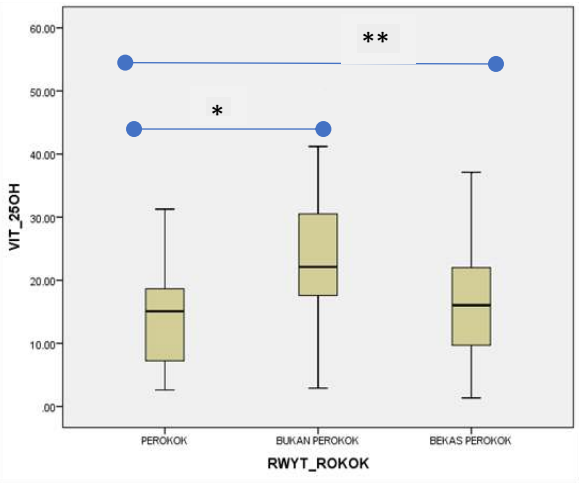

Figure 4. Calcidiol levels in DMT2 subjects based on smoking history

DISCUSSION

Median calcidiol levels across all subject groups are classified as deficient levels of Vitamin D (<20 ng/mL). The median vitamin D levels among T2DM patients without TB coinfection is 18.6 ng/mL, which is similar to levels described by Chaudhary et al.[10] which is 19.42 ng/mL. Average calcidiol levels in T2DM patients with active TB in this study were found to be 115 ng/mL. These results are confirmed by Chaudary et al. who have found average calcidiol levels among similar subjects to be as high as 15.96 ng/mL. However, studies by Zhao et al.[11] and Zhan et al.[12] have recorded average calcidiol levels among T2DM subjects with active TB to be 12.1 ng/mL and 11.36 ng/mL respectively. These results are much lower when compared to healthy subjects with similar baseline characteristics to the subjects in this study.

The difference in average vitamin D levels across various studies may be attributed to differences in testing protocol, geographical variation of the study sites, seasonal changes and genetic or ethnic variation in the study subjects.

The study by Zhao et al.[11] in China, illustrates the impact of seasonal variation and time of data collection on the vitamin D measurements, which led to deficiency in vitamin D among TB patients and T2DM subjects with active TB coinfection. Subjects who were tested in the colder months were found to be more deficient on average, compared to T2DM subjects who were tested in the warmer months. The low Calcidiol levels in T2DM patients with active TB coinfection can also be caused by lower mRNA expression of *Vitamin D Binding Protein* (VDBP), which is in concordance to overall lower albumin levels among active TB patients compared to normal healthy controls.[9,11] The lack of protein formation may

be associated with the general nutritional deficiency seen in cases of TB. Roughly 70-90% of TB patients have shown to have calcidiol levels lower than 20 ng/mL. These results can be attributed to the tendency of patients with chronic lung disease to limit physical activity and exposure to sunlight, due to the overall limited capacity to perform activities. In addition, advanced age leads to greater catabolism of calcidiol, with the simultaneous reduction in synthesis, which leads to lower serum calcidiol levels.[13]

The variation in calcidiol levels may be accredited to polymorphism of the VDR gene. Polymorphism of the *Fok*I on the VDR gene has shown to increase the risk of TB among Asian individuals, as is the case with the polymorphism of the *Tag*I alleles of the VDR gene.

Low vitamin D levels have been long regarded as a significant risk factor for glucose intolerance. Type 2 Diabetes Mellitus has consistently been shown to affect individuals with vitamin D deficiency more often. Available evidence has described the role of vitamin D in insulin secretion, via the presence of VDR on the Beta cells, and vitamin D dependent calcium binding protein on the pancreatic tissue.[14–16]

Low calcidiol levels are found in South and South East Asian regions, where TB and DM cases are common. Other factors that play a role include old age and obesity.[17] Old age is associated with the lack of *7-dehydrocholesterol* within the skin, leading to a reduction in the production of vitamin D3 by 4 times, within individuals aged 60 years compared to 20 year old individuals. This can further be attributed to lower outdoor activities and poor absorption from food.

This study has demonstrated that among individuals with T2DM and active TB coinfection, serum vitamin D levels are negatively correlated to HbA1c, calcitriol and specific IFN-γ levels, which is in concordance with results obtained by Zhao et al.(11)

Vitamin D has an impact on the secretion and sensitivity of insulin, and the chronic inflammation that is the primary mechanism of pathogenesis of T2DM. Results of a meta-analysis have shown that supplementation of Vitamin D, can increase serum Vitamin D 25-OH levels and improve glycemic control in T2DM patients.[18]

Patients with T2DM and active TB coinfection have the highest serum calcitriol levels, followed by latent TB coinfection and patients without TB infection. Currently, limited data is available on the expression of calcitriol in patients with T2DM and TB coinfection. Davies et al.[19] have conducted *ex vivo* studies, which show that the median calcitriol levels in pulmonary TB patients was 35.7 pg/mL prior to treatment, compared to healthy controls which was 28.7 pg/mL. Similar results were obtained by Selvaraj et al.[20] where higher calcitriol levels were found in pulmonary TB patients compared to healthy controls, albeit still within normal limits. Conflicting results were reported by Gao et al.[21] with lower calcitriol levels being reported, compared to healthy controls (365,9 µg/L vs. 464,3 µg/L), which predisposed the patients towards being infected with TB and developing disease.

Abnormal metabolism of vitamin D is a common finding during infections, when the levels of active form of calcitriol increase due to the increase in the extrarenal production of calcitriol. As a result, the increase in calcitriol leads to a decrease in calcidiol levels, due to the increase in vitamin D3 breakdown. Deficiency of calcidiol has been associated with the risk of T2DM onset. Low levels of calcidiol, with an associated increase in calcitriol, can cause insulin resistance and inhibit glucose absorption by adipocytes. Low serum calcidiol levels are also associated with an increase in proinflammatory cytokines among diabetics.[22] Selvaraj et al.[20] have shown that among subjects with pulmonary TB, a decrease in VDR protein levels were found when compared to control groups, due to the downregulation of the VRD gene expression and increase in the synthesis of calcitriol. This decrease is associated with increased risk of TB infection via cathelicidin LL-37 and inflammatory cytokines.

Levels of cathelicidin LL-37 in T2DM patients with active TB coinfection have been found to be different, when compared to T2DM groups with latent or without TB. The difference in cathelicidin LL-37 levels are statistically significant, and is in

concordance with studies by Yamshchikov et al.[23] and Kumar et al.[24]

Cathelicidin LL-37 is considered as an essential component for the control of TB. *In vitro* studies have shown that cathelicidin LL-37 play an important role in the antimicrobial activity of macrophages. Expression of cathelicidin increases to its peak values post infection; the first day post infection, 21 days post infection during the development of protective immunity and 60 days post infection, when the disease becomes progressive, which is evident in animal models by the total number of pathogens and degree of organ damage. High expression of cathelicidin in the macrophage vacuole indicates the greater immunomodulatory effect of cathelicidin, compared to the antimicrobial effect in progressive disease. As a result, an increase in cathelicidin is observed in active TB, compared to latent or without TB, in T2DM patients.[25]

Relatively higher levels of cathelicidin LL-37 in T2DM without TB indicates the multiplication process of *M. tuberculosis* or ongoing inflammatory process.

Multiple studies that have been conducted previously, have shown that specific IFN-γ response by TB2 is associated with an active TB infection and a more severe disease profile. The research conducted by Lee et al.[26] showed that greater CD8+ cell response to QFT Plus tests were found in subjects with active TB, confirmed by culture, compared to latent or subjects without TB. The results of the study are concordant with our findings, which show that specific IFN-γ from TB2 is greater in the T2DM with active pulmonary TB group, compared to the latent TB or without TB groups. Other studies have shown that T2DM subjects with latent TB have a minimal CD8+ response.[27,28]

Latent TB in patients with T2DM express fewer proinflammatory cytokines specific to *M. tuberculosis*, lesser anti-inflammatory activity and weaker Th2 cellular response compared to normoglycemic patients with latent TB. Activation of TB infection increases cytokine levels from CD4+, CD8+ lymphocytes and NK cells. The increase in cytokine levels is indicative of the high *bacterial load* in patients with TB DM coinfection, as a consequence of delay in initial control of *M. tuberculosis* replication. In addition, greater organ damage is also observed as a consequence of weaker cytokine response in latent TB patients.[29]

This study has a few limitations, as the measurements of vitamin D were not taken at the same time period. Furthermore, VDR and CYP27B1 testing was not conducted, which would describe the complete status of vitamin D and its metabolites, as well as polymorphism factors were not studied, which could influence the vitamin D status. The study did not also assess nutritional adequacy and sunlight exposure of the research subjects.

CONCLUSIONS

Based on the results of this study, it can be concluded that lower levels of Vitamin D 25(OH) are in T2DM patients with concurrent TB infection, both active and latent, when compared to without TB infection. Levels of active vitamin D3 and cathelicidin are higher in T2DM patients with active TB, compared to latent or without TB infection. Vitamin D has the potential to be used as adjunctive therapy for TB, as well as prevention of TB infection and reactivation of latent TB. Vitamin D supplementation can improve glycemic control in T2DM patients, and hence reduce the risk of TB infection. Increases in the levels of cathelicidin and IFN-γ can act as potential biomarkers of active TB and latent in T2DM, and in cases without TB.

Limitations of this study include lack of vitamin D measurements during the same time period, as well as lack of assessment of VDR and CYP27B1 status, in order to have a complete assessment of vitamin D status, and its metabolites. Polymorphic factors that affect vitamin D status has also not been studied in this research. Finally, measurements of nutritional adequacy and sunlight exposure on the subjects have not been addressed.

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