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The association between Vitamin D deficiency and tuberculosis:

Effects of different levels of vitamin D deficiency. – A meta-analysis

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Popular science summary

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Tuberculosis (TB) is one of the largest causes of death worldwide. Each hour, 120 new persons get infected and 200 dies. Despite anti-TB treatment and vaccination, the disease is still common, especially in Africa and Asia. Cost, long time of treatment, and inadequate diagnostic methods in some countries are important challenges. Vitamin D deficiency (VDD) is one of risk factors for TB. Historically, Vitamin D was used to treat TB. This also explains the association between the amount of sunlight and seasonal incidence of TB. Many experimental studies examined the immunological effects of vitamin D, and its relationship with TB infection. The result of this study confirmed the association of vitamin D deficiency and TB infection, and suggested a minimum level of 75 nmol/L of vitamin D as a safe level.

Abbreviations:

Active TB = active tuberculosis.
BCG= Bacillus Calmette-Guérin vaccine.
CI= confidence interval.
D2 ,D3 = dietary forms of vitamin D.
DM= diabetes mellitus.
HIV= human immunodeficiency virus.
I^2= heterogeneity index.
LTBI= latent tuberculosis infection.
MTB= *mycobacterium tuberculosis= M.tuberculosis*.
MD= mean difference.
ng/dl= Nanogram per milliliter.
nmol/L= Nanomoles per litre.
NOS= Newcastle- Ottawa Scale.
OR= Odd Ratio.
p =p- value (probability value).
RR= Risk Ratio.
SE= Standard Error.
TB= tuberculosis.
TLR=Toll-Like Receptors.
TST=tuberculin skin test.
VDD= vitamin D deficiency.
1.25 di-hydroxyvitamin D= active form of vitamin D.
25-hydroxyvitamin D= 25(OH)D= vitamin D.
KCDC = The Korea Centers for Disease Control and Prevention
IU = International unit = 0.025 mcg

Abstract.

Aim:

To conduct a systematic review and meta-analysis of all published studies studying the risk of vitamin D deficiency in tuberculosis patients and in healthy controls. Additionally, subgroup meta-analysis was performed based on the level of vitamin D to test the risk in TB groups and in healthy groups.

Methods:

Pubmed was searched for observational studies in human and English that discussed the association between risk of low serum vitamin D and TB. Meta-analysis was performed on all relevant studies combined and for subgroups of each vitamin D level.

Results:

22 studies were selected and pooled in the analysis. The results were consistent with previous studies examining the same risk. The overall log risk ratio (log RR) of low vitamin D was significantly higher in TB patients 1.68 times than healthy controls. In 4 subgroup meta- analyses based on vitamin D level below (20 nmol/L, 30 nmol/L, 50 nmol/L, and 75 nmol/L), the risk of having low vitamin D in TB patients was (1.82, 2.89, 1.38, 1.32) respectively. That subgroup analysis showed more clearly the higher RR were below 20 and 30 nmol/L. The smallest RR was at 75 nmol/L level.

Conclusion:

This study verified the association between risk of low vitamin D level and TB development. It also clarified that, the risk increased by decreasing vitamin D level.

Introduction:

Tuberculosis (TB), which is caused by *Mycobacterium tuberculosis* (MTB), is one of the top 10 causes of death (1.3 million deaths, WHO 2017)[1,2]. Globally, 90% of TB cases are adults (aged ≥15 years). It is estimated that, 120 new cases and 200 deaths per hour occur worldwide[55].

TB has two forms, active tuberculosis-active TB, and latent tuberculosis infection-(LTBI)[44]. *M. tuberculosis* enters the body via minute droplet nuclei carried by the air when a person with active TB coughs, talks, or sneezes[44]. Through air, the droplet nuclei are inhaled by the healthy persons into the respiratory airways and deposited on the alveolar surface[44]. In the alveolar macrophages, the microbe can remain dormant for decades causing LTBI[44]. TB becomes active when the microbes start replication causing a macrophage activation and inflammation cycle that develops active TB in patients[39,44].

Many treatment lines have been adapted to treat and prevent TB[44]. However, the cost, long period of treatment, drug-resistance, drugs side-effects and uncontrolled LTBL resist this goal[44]. Drug toxicity in liver, peripheral nerves, gastrointestinal tract and kidneys are common side-effects[44]. Also drug-resistance due to improper treatment program and long treatment time, give sufficient time for the bacteria to evolve and become resistant to the drug being used for the treatment[44].

Bacillus Calmette-Guérin (BCG) vaccine was firstly introduced in France in 1924, as TB vaccine[47]. It is used widely and is recommended by WHO in countries where the disease is endemic[47]. The vaccine provides some protection in infants, but a good estimate of the efficacy is not available[44, 47.] In adults and in immunosuppressed populations the vaccine is not effective and another customized vaccine is urgently needed[47].

Because of the drug- resistance, drug side-effects and due to non-optimal vaccination effectiveness[44,47], prevention of TB by controlling risk factors such as smoking, poverty, poor indoor air quality or ventilation, crowding and malnutrition becomes a principal part in the WHO strategy[44,48].

Malnutrition as a risk factor has been strongly associated with TB and many studies have examined the association of specific micronutrients with development of TB[6]. Generally, the relationship between nutrition, infection, and immune function is cyclical[45]. Malnourished persons more commonly get infection, and infected people are more likely to become malnourished[45,46]. Micronutrients like Zinc, Selenium, vitamin A, B and D influence the immune response to infectious diseases through different roles in the immune function[45]. In the pre-antibiotic era, high doses of vitamin D and cod liver oil- a rich source of vitamins A and D- were used to treat and prevent TB, before antimycobacterial agents discovery in the 1950s[3-5,61,63] .

Vitamin D plays an important role in infectious diseases like TB[6, 7]. A growing evidence found that 1, 25(OH) D_2D_3 stimulates antimicrobial responses leading to destruction of *M. tuberculosis*[9]. There are two sources of vitamin D. One of them is 7-dehydrocholesterol found in the skin. 7-dehydrocholesterol is converted by the ultra-violet light produced by sun to previtamin D_3[27]. Then the previtamin D_3 is metabolized by the liver and kidney to form 25-hydroxyvitamin D (25 (OH) D) and 1, 25-dihydroxyvitaminD (1, 25(OH) 2D) respectively[8]. The second source is diet. Dietary vitamin D_2 and vitamin D_3 commonly found in oily fish and egg yolk are incorporated into chylomicrons and transported by the lymphatic system into the venous circulation[8].

Previous research:

Micronutrients such as vitamins play important role in innate immunity in infectious diseases[46.]. Vitamin A deficiency may reduce natural killer cell function[46,47]. Vitamin E deficiency causes damage to both T cells and B cells[46]. Low vitamin B_{12} affects cytotoxic cell function[46].

Vitamin D deficiency (VDD) is one of the most common nutritional deficiencies known worldwide to be associated with many diseases including infections such as TB[60]. The association between vitamin D and TB was built on 2 reasons. First, it was known that vitamin D before antibiotic discovery, was used to treatment TB[3-5,61,63]. The second reason is the epidemiological phenomenon stating the seasonal variation in TB incidence during the year. This phenomenon is explained as the seasonal amount of sunlight affects vitamin D metabolism[62,64,65]. But, more explanation is needed in this interpretation, as the most TB-endemic population live in sun-rich countries[1,14,16,17,20-24,26,28,29] . The physiological role of sun on vitamin D metabolism is already known, but other factors may affect this role. Diet and ethnicity for example may affect vitamin D metabolism[30-33].

Previous studies investigated the role of vitamin D in infectious diseases, like TB in human[6-7]. Those studies revealed that *M.tuberculosis* is ingested and eliminated by macrophage cells in the innate immune system, the first arm of the immune system to destroy the pathogen[39]. Macrophages have vitamin D receptors and by these receptors, 1, 25-dihydroxyvitamin D_3, the active form of vitamin D enters the macrophage nucleus and stimulates Cathelicidin production[8]. Cathelicidin is an anti-microbial peptide capable of destroying *M. tuberculosis* as well as other infectious agents and is involved in bacterial killing[8]. Moreover, it has been revealed that 1, 25 $(OH)_2 D_3$ modulates production of cytokines in response to *M. tuberculosis* antigens[66]. It was found that vitamin D below 20 ng per milliliter (50 nmol per liter), prevents the monocyte or macrophage from initiating this innate immune response[8]. In addition, it was found that TB patients have an average lower level of vitamin D than healthy controls[10]. Two factors can explain such relation[7, 10]. The active form of vitamin D increases the ability of macrophages to prevent growth of the bacteria[10]. In addition, on triggering of *Toll-Like Receptors* TLR by molecules of the tubercle bacillus, the production of microbe-killing substances is impaired in the absence of adequate level of vitamin D[7].

Recent trial of vitamin D supplementation in Indonesian pulmonary TB patients found rapid sputum clearance of acid-fast bacilli and radiological improvement[49]. Also, in a recent meta-analysis[9], it tested vitamin D association with TB in different aspects, it

found that vitamin D level was significantly lower in TB patients versus controls, and VDD was associated with an increased risk of TB infection. Moreover, it found the level of vitamin D was lower in active TB patients than vitamin D level in LTBI[9]. Additionally, it clarified the significant association between increased risks of increasing tuberculin skin test TST conversion rate and higher VDD[9].

After 2016, no meta-analysis was conducted to include recent literature studying the association between risk of low vitamin D level and TB infection. Conducting a recent meta-analysis including new literature, to verify the association based on categorized cut-off levels of vitamin D will be helpful. The previous studies used different cut off levels of vitamin D implying that the correlation between the level of vitamin D and the risk of TB could be analyzed, and that something has not been done.

Study aim:

Few meta-analyses have assessed the association between low vitamin D levels and TB. The recent study was performed in 2016 and did not include later studies[9]. No study performed a subgroup analysis based on vitamin D levels to determine explicitly the level of VDD associated with increased risk of TB[9-11]. This study aimed to include recent literature to test the association between grades of VDD and TB.

Research questions:

This study tried to answer 2 questions:

- Verify the association between VDD and TB risk?
- Does the risk increase with decreasing vitamin D levels?

Methods:

2.1. Goal:

To compare serum vitamin D levels in TB cases versus healthy controls and to examine the association between VDD level and TB. To detect at which level of VDD, TB risk is increased. To achieve this goal, a meta-analysis was conducted including studies published up to April 2019.

2.2. Retrieval of studies:

The aim of this study was to do a systematic review and meta-analysis visualizing VDD as a risk factor for TB. A systematic search was started for research papers in PubMed in April 2019. Three key search terms were used (*tuberculosis, vitamin D*, and *ergocalciferols*). The search was performed with the combination *[("tuberculosis*"[MeSH Terms] OR *"tuberculosis*"[All Fields]) AND ("*vitamin d*"[MeSH Terms] OR "*vitamin d*"[All Fields] OR "*ergocalciferols*"[MeSH Terms] OR *"ergocalciferols*"[All Fields])] AND ("*humans*"[MeSH Terms] AND *English*[lang]) .The search was restricted to human studies published in English, by adding [AND] human & English to MeSH terms.[Figure.1].

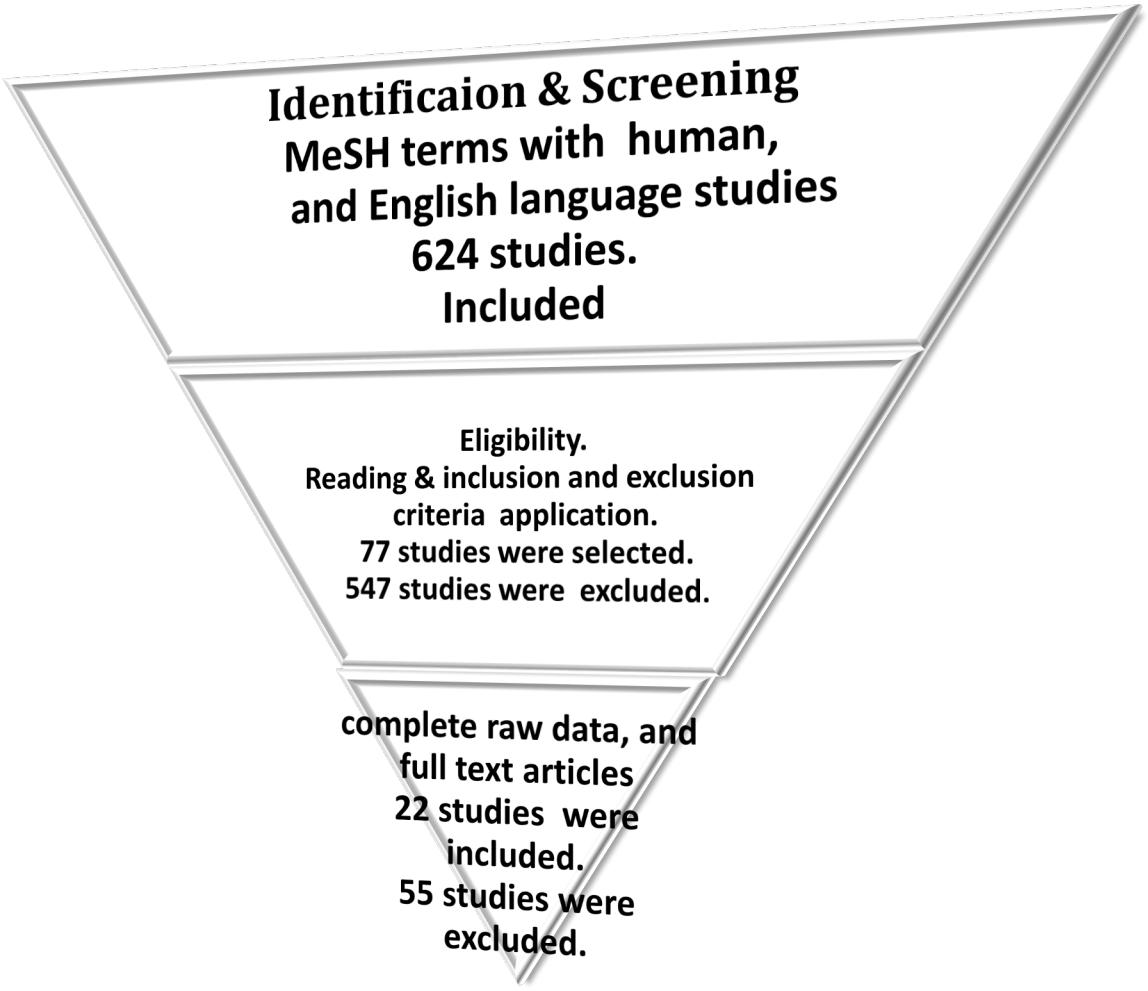

Figure.1: Flow chart for study selection. After Mesh terms use, 624 potential studies were identified. 547 of these studies were excluded after reading titles and abstracts. 55 studies were excluded by screening and applying the preselected inclusion and exclusion criteria. Finally, 22 studies were identified and included in the study.

2.3. Study selection:

The initial search identified 624 studies. By reading title and abstract to extract eligible studies, 77 studies were identified. After applying inclusion and exclusion criteria, 22 studies were identified as relevant to the study aim and were included in this study. Inclusion and exclusion criteria are summarized in [Box.1].

Box.1: Inclusion & exclusion criteria.

Inclusion criteria

1- Human studies.
2- English language.
3- Case-control, cohort studies and cross-sectional studies with Patients are clinically diagnosed as active tuberculosis (Active TB) and healthy or non-TB patients as controls.

Exclusion criteria

1- Studies conducted on patients have chronic or other diseases affecting vitamin D metabolism, such as *diabetes mellitus* (DM), human *immunodeficiency viruses* (HIV), and renal diseases.
2- Patients who had started the anti-TB treatment program.
3- Vitamin D supplementation intake.
4- Unavailable raw data and results by medians and means.
5- Meta-analyses randomized clinical trials (RCT) and case-studies.

2.4. Data handling and extraction:

The data were independently extracted from the studies and arranged including the following standardized forms: author, publication year, country, study design, sample size, and outcomes.

The examined exposure was serum vitamin D status measured as 25 (OH) D. Metabolically, vitamin D 25 (OH) D is rather stable and with 3 weeks half-time making it the most suitable indicator of vitamin D. Identifying the cut-off VDD is a matter of a much argument[6,8]. Studies included used different cut-off points for VDD. In this study, the analysis was performed in two stages. In the first stage, all studies were put in a combined meta-analysis to test the total log risk ratio (log RR). In the second stage, the

outcomes were categorized into 4 vitamin D cut-off levels (less or equal to 20 nmol/L, less or equal to 30 nmol/L, less or equal to 50 nmol/L and less or equal to 75 nmol/L). Outcome of vitamin D that was presented in ng/dl [12-17,19,20,23,26,31,33] was converted to nmol/L by multiplying by 2.496[6].

Thus, 22 studies [12-33] were included and pooled in the meta-analysis to examine log RR of low vitamin D level in TB patient compared with healthy subjects. Additionally to compare the log RR in different vitamin D cut-off points in association with TB.

2.5. Quality evaluations:

Newcastle- Ottawa Scale (NOS) [34] was used to examine all included studies for the methodological quality. The NOS is recommended for both cohort studies and case-control studies, where scores vary between 0 and 9. This scale consists of an eight-term process for assessing the selection of the study population, comparability, and the evaluation of exposure and outcome. Studies with scores of at least 5 are deemed to be high-quality studies.

2.6. Statistical analysis:

All studies included are measuring the levels of vitamin D in TB cases and healthy controls. The raw data was presented as below different levels of vitamin D. The tested 4 levels were (≤20 nmol/L, ≤30 nmol/L, ≤50 nmol/L, and ≤75 nmol/L). The risk for individual patients to be below the different levels of vitamin D in the two groups, TB positive and TB negative was calculated. Using the package metafor[36]in R[38] the effect size was calculated of each study as the log RR showing the difference in risk for being vitamin D deficient in the TB positive group compared with the TB negative group. A random effect model was used to test effect size, corresponding sampling variance and heterogeneity between the studies (I^2)[36]. Restricted Maximum –Likelihood estimation ''REML'' was used to test random effect model as this model deals with the variability in the results caused by heterogeneity between the studies[36]. Forest plot was used for graphical overview of the results[37]. Funnel plot of standard errors (SEs), the square root of the sampling variances on the vertical axis versus log RRs was on the horizontal axis

used to assess publication bias[37]. Finally the same calculation was performed for combined studies and for each vitamin D level separately to examine log RR and effect size with 95% CI and *p-value* for each level, p-value is considered significant < 0.05. In addition, subgroup meta-analysis was performed for the 17 free HIV or DM to address if the inclusion of HIV or DM studies affected log RR in each vitamin D level.

Results:

3.1. Literature search:

624 potentially applicable studies were identified in PubMed. Of which 77 studies were selected after careful reading titles, abstracts and conclusions. By applying preselected inclusion and exclusion criteria, 22 studies[12-33] were selected and included. Those studies testing the risk of low vitamin D levels or status in TB patients and healthy controls were included in this meta-analysis. [Figure.1].The characteristics of study population, publication year, and study design, size of cases & controls and status of HIV/DM in the studies are summarized in [Table.1].

Table.1: Studies characteristics: name of trial. Published year, country of trial, study design, sample size, no of cases & controls, and HIV/DM status. ▲Studies measured vitamin D level
■ studies recruited controls from another studies.

Study	Year	Country	Study population	Study design number of Cases / Controls
Zhang[12]	2018	China	Patients in hospital & healthy volunteers	Case-control 152 / 59
Maceda[13]	2018	Brazil	Cases & controls were incarcerated in same prison.	Case-control 24 / 48
Musarurwa[14]	2017	Zimbabwe	Cases from Harare Clinics Controls were volunteers	Cross-section 134 / 133
Arnedo-Pena[15]	2015	Spain	TB Cases & healthy controls were pulmonary TB contacts in public health center in Spain.	Cohort 3 / 520
Jubulis[17]	2014	India	< 5 TB hospitalized children as cases .controls were healthy ones attended immunization clinic in same hospital (Children)	Case-control 60 / 118
Joshi[1]	2014	India	Cases were patients in clinic. Control were household contacts & free subjects	Case-control 25 / 50
Hong[16]	2014	Korea	Cases were patients in hospital. Controls were recruited from national Korean health survey▪	Case-control 94 / 282
Kim[19]	2013	Korea	Cases were patients in hospital, controls were healthy subjects	Case-control 165 / 197
Iftikhar[20]	2013	Pakistan	Cases were outdoor TB patients. Controls were community randomly selected.	Case-control 105 / 255
Mastala[21]	2013	Malawi	Cases were hospitalized patients in Malawi. Control were non- TB patients taken from previous cross section study in same hospital▪	Case-control 55 / 105
Conesa Botella[22]	2012	Uganda	Cases were patients in hospital. Controls were healthy subjects.	Case-control 27 / 23
Koo[23]	2012	Korea	Cases were TB patients in hospital. Controls were healthy subjects and non-TB patients.	Case-control 116 / 86
Gray[24]	2012	Australia	Refugee children in hospital with TB cases, and non-TB controls (Children)	Cross-section 11 / 236
Nielsen[25]	2010	Greenland	Cases were hospital patients, Control were randomly community selected.	Case-control 72 / 72
Ho-Pham[26]	2010	Vietnam	Cases were hospital patients. Controls were randomly community selected. ▪	Case-control 166 / 219
Martineau[27]	2010	UK	Cases were outdoor patients. Controls were healthy adult contacts. Both were Gujarati Asians lived in London	Case-control 84 / 55
Gibney[28]	2008	Australia	Cases were TB patients and controls were non-TB patients in same hospital.	Cohort 40 / 34
Wejse[29]	2007	Guinea-Bissau	TB cases and healthy controls selected from same area. ▪	Case-control 362 /494

Sita-Lumsden[30]	**2007**	**UK**	**Cases were hospitalized patients controls were healthy and same ethnic contacts.**	**Case-control 178 / 128**
Sasidharan[31]	**2002**	**India**	**Cases were hospital patients. Controls were randomly selected.**	**Case-control 35 / 16**
Wilkinson[32]	**2000**	**UK**	**hospital-based case-control analysis of Asians of Gujarati origin.**	**Case-control 103 / 42**
Grange[33]	**1985**	**Indonesia**	**Cases were outdoor patients . controls were randomly selected in same age.**	**Case-control 40 / 38**

3.2. Quality evaluation

All studies were tested by Newcastle-Ottawa Scale (NOS) [34] to examine their methodological quality.

Table.2: (NOS) is a grading system of articles consists of 3categories (selection, comparability, and exposure) maximum grade is 9. Study graded 5 is deemed to be high-quality study. All selected studies were above 5 and no study was excluded.

Author	**Selection**				**Comparability**		**Exposure**			**Score**
Zhang[12]	★	★		★	★	★	★	★	★	**8**
Maceda[13]	★			★	★	★	★	★	★	**7**
Musarurwa[14]	★	★	★		★	★	★	★		**7**
Arnedo-Pena[15]	★	★	★	★	★		★	★		**7**
Hong[16]	★	★	★	★	★	★	★		★	**8**
Jubulis[17]	★	★	★	★	★	★	★	★	★	**9**
Joshi[18]	★	★	★	★	★	★	★	★	★	**9**
Kim[19]	★	★		★	★	★	★	★		**7**
Iftikhar[20]	★	★	★	★	★		★			**6**
Mastala[21]	★	★	★	★	★	★	★			**7**
Conesa-Botella[22]	★	★	★	★	★	★	★	★	★	**9**
Koo[23]	★	★	★	★	★	★	★	★		**8**
Gray[24]	★	★		★	★		★	★		**6**
Nielsen[25]	★	★	★	★	★		★	★	★	**8**
Ho-Pham[26]	★	★	★	★	★		★	★		**7**
Martineau[27]	★	★		★	★		★	★		**6**
Gibney[28]	★	★		★	★		★	★		**6**
Wejse[29]	★	★	★	★	★	★	★	★	★	**9**
Sita-Lumsden[30]	★	★	★	★	★	★	★	★		**9**
Sasidharan[31]	★	★		★	★		★			**5**
Wilkinson[32]	★	★	★	★	★	★	★			**7**
Grange[33]	★	★	★	★	★			★		**6**

By applying NOS testing the studies quality. All studies selected had more than 5 and were thus deemed as high quality studies. No study was excluded at this stage. 5 studies scored 9, 4 studies scored 8, 7 studies scored7, 5 studies scored 6, and 1 study scored 5. Score 5 is a minimum score to identify the study as a high-quality study. All selected studies had scores above 5. [Table.2]

3.3. Characteristics and quality of the included studies:

The included studies were published between 1985 and April 2019. 18/22 studies studies[12,13.16-23,25-27,29-33]were case-control studies (81.8%), two studies[15,28] were cohort studies (9.1%) and two studies[14,24] were cross-sectional studies (9.1%). [Table.1].

3 studies[18, 24, 28] (13.6%) examined vitamin D levels, in both active TB and LTBI. The data was extracted for active TB only. Two studies[17, 24 were] conducted on children (9.1%).

4 studies (18.2%) [16,24,26,29] had recruited their controls from other studies:

1-In Hong[16] study, the control group was randomly recruited from subjects who participated in the KNHANES IV–V study between 2008 and 2011[40]. KNHANES is a nationwide survey that measures the health status of the Korean people and produces basic statistics for establishing health policy[40].

2-The Mastala[24] study compared 2 cross-sectional studies, the first study investigated VDD in non-TB patients (2013) in Queen Elizabeth Central Hospital (QECH) in Blantyre, Malawi. The second study was performed by the same author in 2011 investigated vitamin D status in TB patients in same hospital[41].

3- The Ho-Pham[26] recruited the control group by random selection from another study on vitamin D status in Vietnamese urban population which was conducted by same author[42].

4-The Wejse[29] enrolled the control group by a random population sample from another study testing genetic risk factors for TB, conducted by the same author in the same area between April 2005 and February 2006[43].

The twenty two studies had a total sample size of 5236, of which 2051 were cases (39.2%) and 3185 were controls (60.8%). 6 studies (27.3%) tested cut- off vitamin D ≤ 20 nmol/L for deficiency with 1424 sample size, 658 (46.2%) cases and 766 (53.8%) control [23,24,26,27,30,32] . 11 studies (50%) tested cut-off vitamin D ≤ 30 nmol/L with 3554 sample size, 1278 (36%) as cases and 2276 (64%) as control[12,14-16,19,23,24,26,28,29,31] . 15 studies (60.2%) tested cut-off vitamin D ≤ 50 nmol/L with 4227 sample size 1528 (36.2%) cases and 2699 (63.8%) controls[12-19,21,22,24-26,29,30] . 10 studies (45.5%) tested cut-off vitamin D ≤75 nmol/L with 2560 sample size, 847 (33%) cases and 1713 (67%) control [13-15,18,20-22,25,29,33] . [Table.3]

Table.3: the studies' authors and number of studies included in each cut-off and sample size of each cut-off (Number of cases / Number of controls).

Vitamin D cut-off	Trials names	No. studies	Sample size (Cases /control)
≤ 20 nmol/L	Koo[23], Gray[24], Ho-Pham[26], Martineau[27], Sita-Lumsden[30], Wilkinson[32]	6	1424 (658/766)
≤ 30 nmol/l	Zhang[12], Musarurwa[14], Arnedo-Pena[15], Hong[16], Kim[19], Koo[23], Gray[24], Ho-Pham[26], Gibney[28], Wejse[29], Sasidharan[31]	11	3554 (1728/2276)
≤ 50 nmol/L	Zhang[12],Maceda[13],Musarurwa[14],Arnedo-Pena[15], Jubulis[17], Joshi[12], Hong[16], Kim[19], Mastala[21], Conesa-Botella[22], Gray[24], Nielsen, Ho-Pham[26], Wejse[29], Sita-Lumsden[30]	15	4187 (1528/2659)
≤ 75 nmol/l	Maceda[13], Musarurwa[14], Arnedo-Pena[15], Joshi[18], Iftikhar[20], Mastala[21], Conesa-Botella[22], Nielsen, Wejse[29], Grange[33]	10	2560 (847/1713)
Total	Total studies[12-33]	22	5236 (2051 / 3185)

17 studies (72.7%) [14,15,18-21,22,,24-33] were HIV and DM free. Five[13,15-17,23] of the studies had a small number of HIV/DM patients, which are known as a risk factor in TB development. That small number couldn't be separately identified and thus had to be included in the study population.

The number of HIV/DM was 59 persons in the five study population (59/1351). The percentage of HIV/DM subjects were 3.9% in these 5 studies. By including these HIV/DM subjects they corresponded to 1.1% in the total material (59 /5236). Also the percentage of HIV/DM was calculated in each level separately to demonstrate its

contribution of each level. The percentage ranged from 0.8% to 1.5%. Due to this small portion of HIV or DM in these studies, they were not excluded from the analysis. [Figure.2]

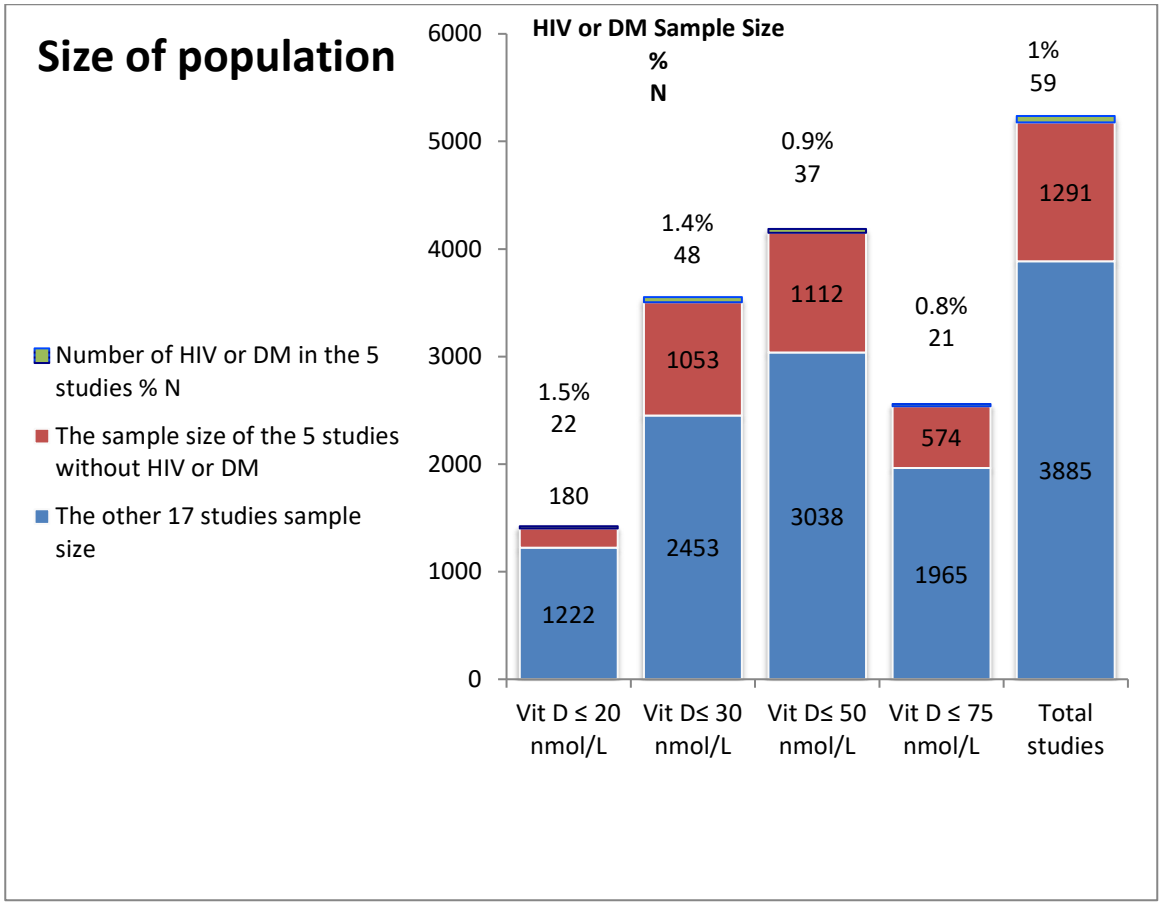

Figure.2: Sample size of population in the different cut-off point of vitamin D and percentage of HIV/DM in each level per total study population and the 5 HIV/DM including studies.

3.4. Quantitative results (meta-analysis):

The meta-analysis was performed using a random effects model. Log RR was calculated in 2 stages (combined studies and subgroup meta-analysis for each level).

First stage: combined 22 studies (overall log risk ratio)

The risk of having lower vitamin D level in TB patients was 1.68 times higher than in healthy controls [Log RR 1.68, 95% CI= 1.37-2.06]; $p< 0.0001$; $I^2=96.14\%$. Total 22 Studies with a population size 5236, were published between 1985 and 2018. [Figure.3].

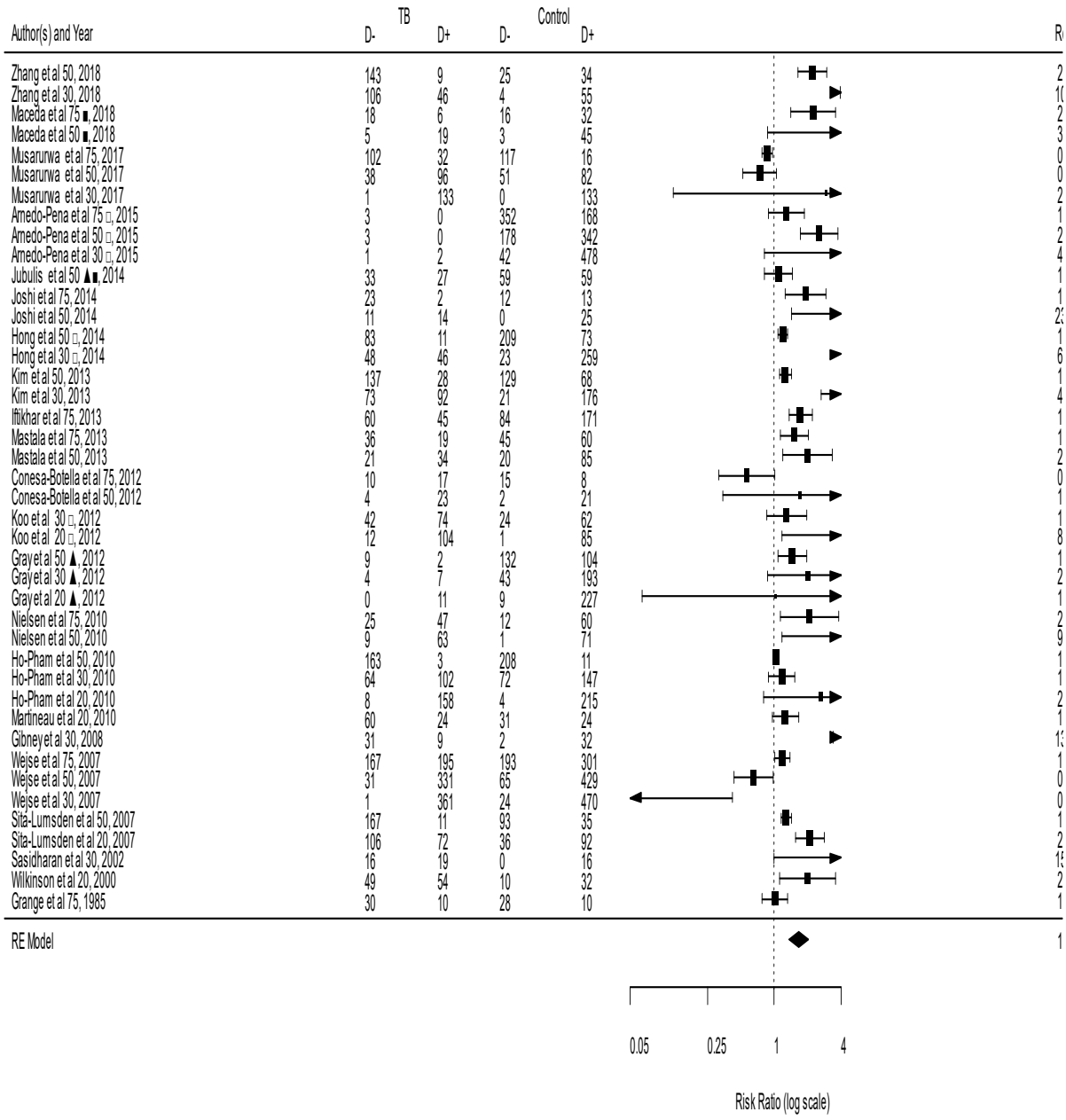

Figure.3: Forest plot of the 22 combined pooled log RR for lower vitamin D in the TB patients

TB = tuberculosis, control = healthy patients, □ = DM, ■ = HIV, ▲ = conducted on children.

Second stage:

A subgroup meta-analysis was performed for each pre-designed cut off point of vitamin D level, to examine Log RR in each group separately.

Cut-off ≤ 20 nmol/L:

The cut-off below20 nmol/L showed that the risk of having vitamin D levels ≤ 20 nmol/L in TB patients was 1.82 times higher than in healthy controls [Log RR 1.82, 95% CI =1.29-2.57]; p=0.0006; I^2=48.11%. Six studies were pooled in this level with a population size 1424. All studies were published between 2000 and 2012. [Figure.4].

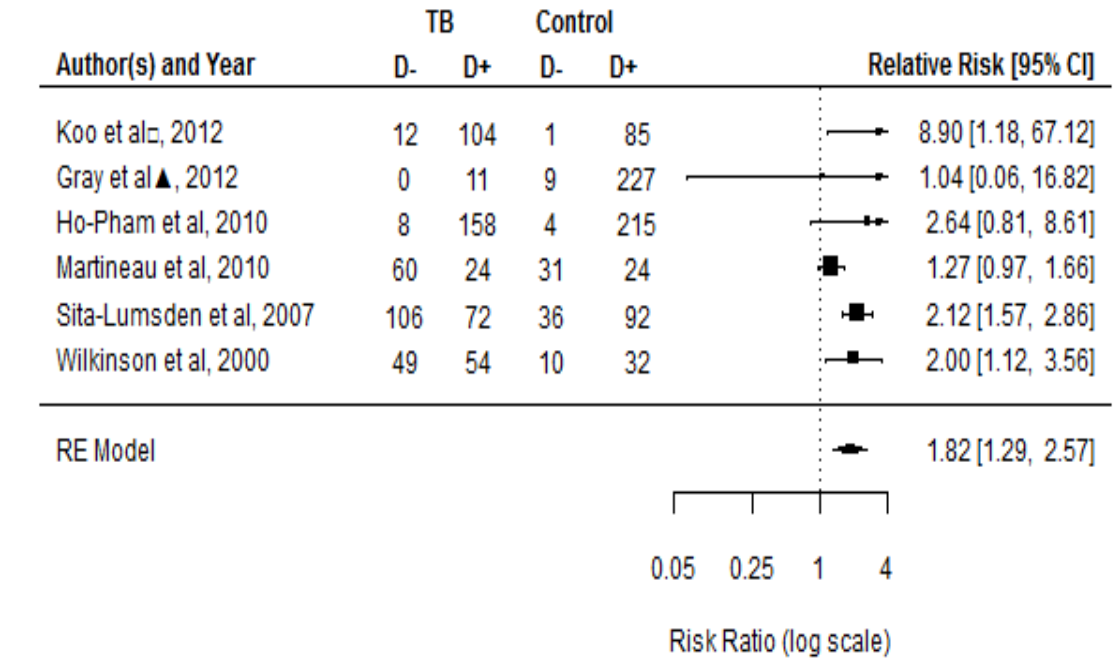

Figure.4: Forest plot of pooled log RR for vitamin D cut-off ≤ 20 nmol/L in the TB patients. TB = tuberculosis, control = healthy patients, □ = DM, ▲ = conducted on children.

Cut-off ≤ 30 nmol/L:

The cut-off below30 nmol/L shows that the risk of having vitamin D levels ≤ 30 nmol/L in TB patients was 2.89 times higher than in healthy controls [Log RR 2.89, 95% CI =1.32-6.35]; p=0.008; I^2=92.92%. Eleven studies were pooled with a population size 3554. Studies were published between 2002 and 2018. [Figure.5].

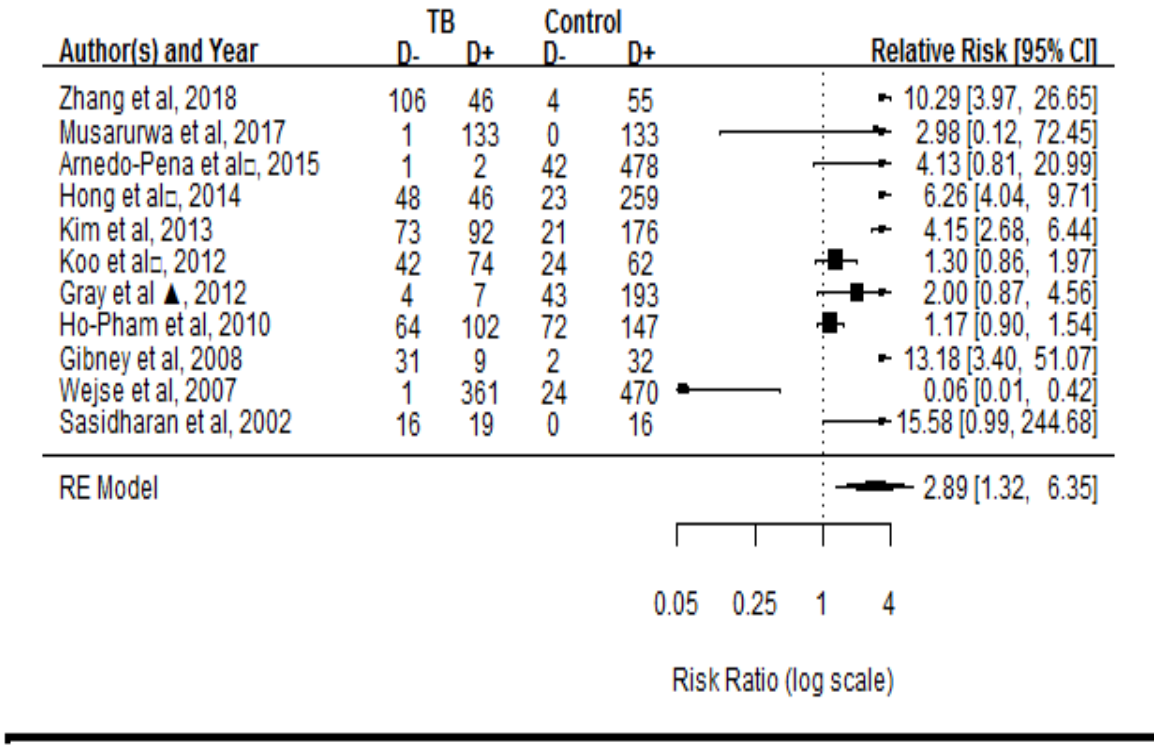

Figure.5: Forest plot of pooled log RR for vitamin D cut-off ≤ 30 nmol/L in the TB patients. TB= tuberculosis, control = healthy patients, ,□ = DM, ▲ = conducted on children.

Cut-off ≤ 50 nmol/L:

The cut-off below 50 nmol/L shows that the risk of having vitamin D levels ≤ 50 nmol/L in TB patients was 1.38 times higher than in healthy controls [Log RR 1.38, 95% CI =1.09-1.74]; p=0.0072; I^2=95.18%. Fifteen studies were pooled, with a population size 4178. Studies were published between 2007 and 2018. [Figure.6].

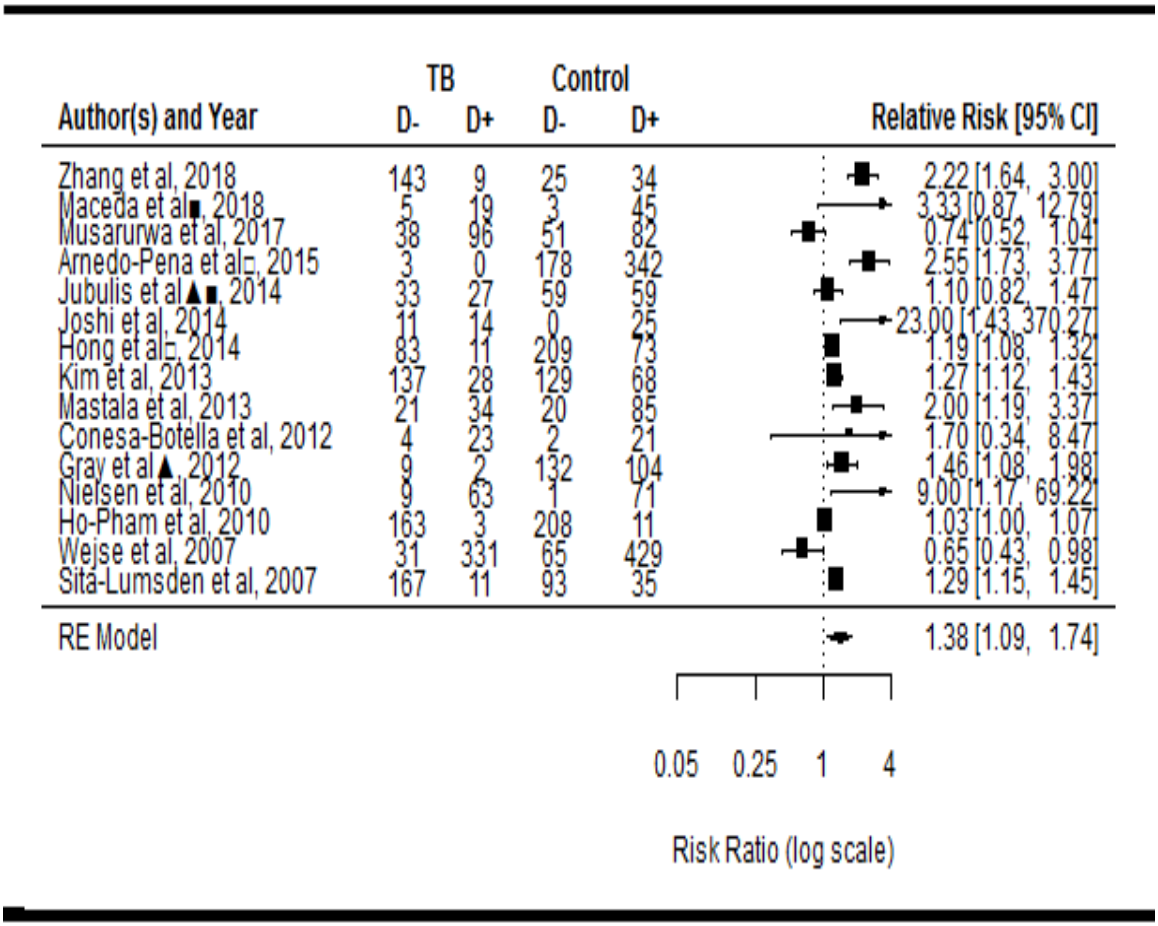

Figure.6: Forest plot of pooled log RR for vitamin D cut-off ≤ 50 nmol/L in the TB patients.

TB = tuberculosis, control = healthy patients, ■ = HIV,□ = DM, ▲ = conducted on children.

Cut-off ≤ 75 nmol/L:

The cut-off below 75 nmol/L shows that the risk of having vitamin D levels ≤ 75 nmol/L in TB patients was 1.32 times higher than in healthy controls [Log RR 1.32, 95% CI =1.04-1.67]; p=0.0223; I^2=87.17%. The population size was 4178. Ten studies were pooled. Studies were published between 1985 and 2018. [Figure.7]

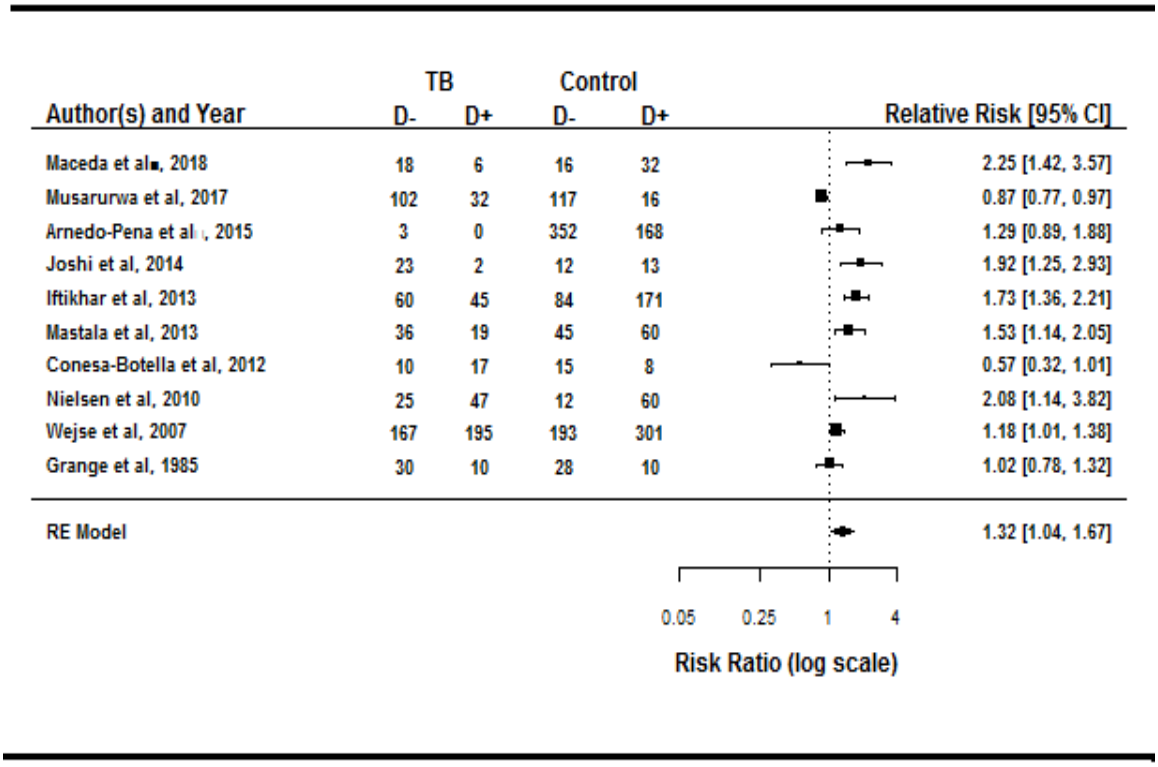

Figure.7: Forest plot of pooled log risk RR for vitamin D cut-off ≤ 75 nmol/L in the TB patients.

TB = tuberculosis, control = healthy patients, ■ = HIV.

All log risk ratios for the overall studies and the 4 subgroup meta-analyses were put in one chart for easier interpretation and comparison. The charts show that the level ≤30 nmol/l had the highest log RR and 2 levels had log RR above the overall log RR. [Figure.8].

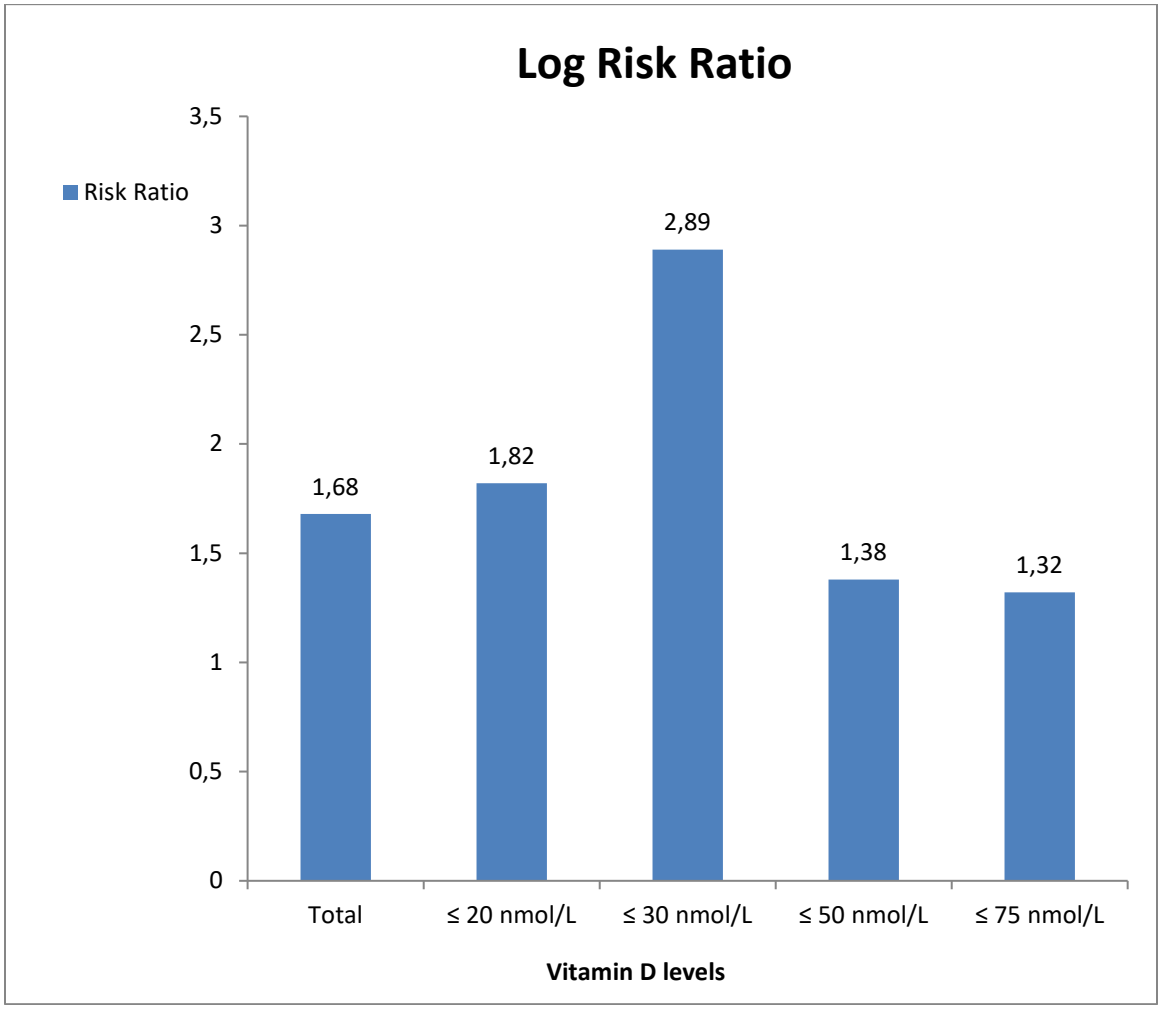

Figure .8: Log RR of total studies included and in each vitamin D cut-off. The results showed the inverse relation between the vitamin D level and risk ratio.

Subgroup meta-analysis was also performed for the 17 free HIV/ DM to address if the inclusion of HIV/DM studies affected the risk ratios of the different vitamin D level. The results were compared with those of the total 22 studies. The results of both were similar showing that the small percentage of HIV/DM did not affect the results. [Table.4].

Table.4: Log RR of vitamin D cut-off points for the 17 free HIV/DM studies & total 22 studies were similar, and the percentage of persons having HIV/DM in each level in both cases or control groups were ranged from(0.1% to 1.5%).

Vitamin D Level	Free HIV/DM studies		Combined free and HIV/DM studies		
	Log RR	**No. of studies 17**	**Log RR**	**No. of studies 22**	**HIV- DM Cases / HIV - DM control (% - %)**
≤ 20 nmol/L	**1.74**	**5/17**	**1.82**	**6/22**	**1 1 /658 – 11 / 766 (1.7% - 1.4%)**
≤ 30 nmol/L	**2.27**	**8/17**	**2.89**	**11/22**	**19 / 1278 -29 / 2276 (1.5% - 1.3%)**
≤ 50 nmol/L	**1.31**	**11/17**	**1.38**	**15/22**	**17 / 1528 – 20 /2659 (1.1% - 0.8%)**
≤ 75 nmol/L	**1.25**	**8/17**	**1.32**	**10/22**	**1 /847 – 20 / 1713 (0.1% - 1.2%)**
All studies	**1.57**	**17/17**	**1.68**	**22/22**	**28 / 2051 -3 1 /3185 (1.4% - 1%)**

3.5.Between –studies heterogeneity:

The amount of heterogeneity was measured by I^2 index. I^2 index statistically estimated in percent how much of the total variability in the effect size estimates. In this analysis, the heterogeneity varied from 48.11% to 95.18% in the total and subgroup meta-analyses with significant p-values for all levels, except the cut-off 20 nmol/L.

3.6.Publication bias:

Symmetry and publication bias were evaluated by a funnel plot. The pooled results of all included studies showed very little publication bias. Larger studies were located at narrowing top in both sides. Smaller studies were scattered at the bottom. [Figure.9]

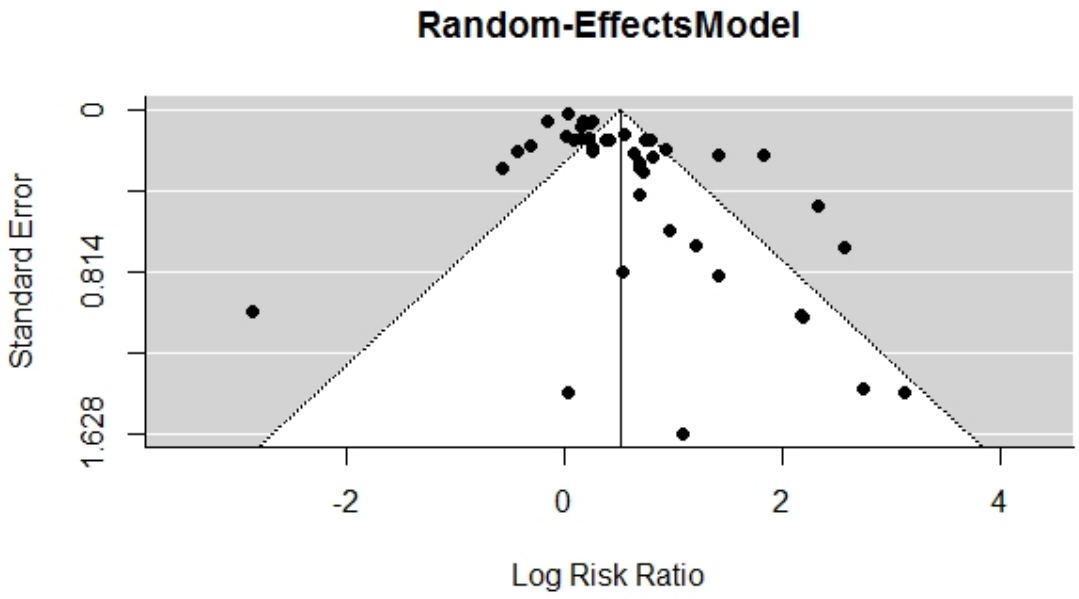

Figure.9: Funnel plot for all included studies.

Discussion:

This meta-analysis of the association between vitamin DDand TB patients showed a significant overall risk of lower level of vitamin D in TB patients versus control (first stage). The overall log risk ratio was consistent with previous meta-analysis studies comparing lower vitamin D in TB and healthy controls. Nnooahm et al[10] stated that TB patients had 0.68 times lower vitamin D level compared with controls. Also the results were agreed with Haung et al[9] who demonstrated a significant mean difference of serum vitamin D level between TB and healthy controls. Additionally, another meta-analysis was performed to test odds ratio (OR) of lower vitamin D levels in patients and controls in children, the results was OR 2.09[11].

Despite that study result is consistent with previous studies in the association and its direction; the values are slightly different as some studies used mean difference MD or OR to compare the risk. Also, some previous meta-analyses mixed active TB and LTBI which may have affected results, since their immune response is not quite the same.

Some studies used medians or means instead of raw data, and pooled studies which recruited HIV- infected patients or diabetic patients which may have affected the results. No previous study tested the risk of lower vitamin D in multiple different levels to illustrate clearly which level is suggested to be risky or safe.

The association was also confirmed by subgroup meta-analyses conducted on the multiple vitamin D levels in this study (second stage). All four vitamin D levels showed significant lower vitamin D levels in TB patients compared with healthy controls.

This study clearly shows that TB patients were more likely having vitamin D levels 20-75 nmol/L, while healthy group was more likely having vitamin D more than 75 nmol/L. The highest log RR was at ≤ 30 nmol/L vitamin D level 2.89. It could have been expected that log RR at ≤ 20 nmol/L would have been the highest. Except for the cut-off point of ≤ 20 nmol/L, the relative risk gradually decreased as the vitamin D cut-off level increased. That may be due to few studies and consequently, low size of population in that group, which could have affected the results. The lower log RR in vitamin D ≤ 20 nmol/L was also probably due to the low cut-off level, with only the patients with extreme vitamin DD was included in the exposed group. That leads to inclusion of many TB cases in the non-exposed group, which decrease the log RR.

The log RR of vitamin D ≤ 50 nmol/L and ≤ 75 nmol/L were nearly similar and were below the total log RR. The results of those two categories were consistent with Liu et al, who stated that when serum levels of 25-hydroxyvitamin D fallen below 20 ng per milliliter (50 nmol per liter), the monocyte or macrophage was prevented from initiating this innate immune response[7]. Also those results were consistent with some experts' definition of VDD below 50 nmol/L.[8]. Identification of a common acceptable definition of vitamin DD will help in diagnosis and treatment protocols for infectious diseases and TB[8].

For better illustration, the subgroup meta-analysis based on the multiple vitamin D levels reflected more clear and specific results than the overall did. This categorization

should help in deciding when supplementation should be included in treatment plans and in preventive procedures.

The debate of cause or consequence has been lively discussed whether the vitamin D deficiency as an indicator of malnutrition, would be a cause of TB or that TB is causing VDD[9]. The present results of this study supported the opinion, that VDD is a cause and not a consequence of TB infection. The results of this analysis showed an inverse relationship between the level of vitamin D and log RR. In addition, it has been shown that the levels of 1,25 $(OH)_2$ D_3, active form of vitamin D did not decrease after TB infection[8]. Furthermore, in a study tested vitamin D levels in TB- patients receiving their anti-TB treatment and TB-patients did not receive their anti-TB treatment, the vitamin D levels did not increase after anti-TB treatment[16, 19,23,58,59]. In another study[19], after one year of TB treatment, serum 25 (OH) D_3 levels in TB patients did not increase and there was no significant improvement compared to baseline.

Use of Vitamin D supplementation in TB patient's treatment was discussed and performed in the pre-antibiotic era[3-5]. Recently, after the role of vitamin D in the immune system has been discovered, many questions about its efficacy and dose in the TB patient treatment program were raised. Positive effect of vitamin D supplementation was confirmed by Nursyam et al[49], TB patients were administered vitamin D or placebo following the sixth week of standard TB treatment, with dose 25 mg/day vitamin D for six months, the vitamin D group had higher sputum conversion and radiological improvement 100% compared to the placebo group 76%. On the other hand, another randomized trial did not find a significant effect on TB patients after vitamin D supplementation[*50*]. They based their choice of dose on what has been shown effective toward osteomalacia (demineralization of bone). 25 mcg of cholecalciferol was used 3 times in 1-5 and 8 month of study. The optimal dose for an anti-TB effect is not known and they thought that dose for TB patients may be higher. Additionally, the interval between the 3 doses was long and infrequent, and more 30% of population was HIV-infected. All these factors could affect that study result.

Range and colleagues[51] studied the effect of vitamin D supplementation on the mortality rate. They reported a 50% reduction in mortality among HIV infected patients with TB treated with multivitamin supplementation including vitamin D in a randomized clinical trial in Tanzania. The role of Vitamin D supplementation in children with TB was studied by Amaresh et al[52]. They found a significant improvement in TB score and weight in the supplementation group compared with the placebo group[52]. Additionally a recent meta-analysis[53] clarified the efficacy and safety of vitamin D supplementation in TB patients' treatment, found that vitamin D supplementation increased the proportion of sputum smear and culture conversions (OR 1.21, 95% CI 1.05~ 1.39, P = 0.007) and(OR 1.22, 95%CI 1.04~ 1.43, P = 0.02) respectively. Also, a systematic review[54] conducted on case-control studies and randomized controlled trials published between 2002 and 2014, concluded that Individuals with TB had lower vitamin D status than healthy individuals, and vitamin D Supplementation improved their clinical outcomes.

LTBI is the main factor of active TB[55]. 2.5 billion people are estimated to have LTBI[5]. Its progressive risk for activation increases in specific populations including active TB household contacts and HIV-infected persons[5, 9, 24, 28, 55]. 5-10% of individuals with LTBI develop active TB[9]. WHO has targeted for the complete eradication of TB by 2050 and the reduction of TB occurrence to no more than one case per one million of population[55].

Treating LTBI is a primary to eradicate TB globally[55]. The association of VDD with an increased risk of developing active TB in LTBI subjects was confirmed by a recent meta-analysis[9] that stated VDD was positively and significantly associated with an increased risk of developing active TB in LTBI subjects [OR 4.26, 95% CI = 2.48,7.30]. In a cross-sectional study of refugee children in Australia, they found a significant association between low vitamin D levels and active TB / LTBI[24].

A cohort study[28] showed lower vitamin D levels in patients with LTBI than in those with no TB, and the levels were more decreased in active TB patients than in those with LBTI, that study examined vitamin D deficiency association with TB and LTBI in immigrants from Sub-Saharan Africa attending Royal Melbourne Hospital, Australia.

concluding that higher vitamin D levels were associated with lower probability of any TB infection and lower probability of tuberculosis compared with LTBI.

Martineau et al[56] determined the significant effect of vitamin D supplementation on antimycobacterial immunity and vitamin D status in LTBI patients. Those results were supported by a conducted trial in Mongolia[57]. The lower conversion rate of the tuberculin skin test TST among Mongolian school-age children taking VD capsule of 20 mcg vitamin D3 daily for 6 months[57] was significantly observed. In Korea[35], 73.3% of Korean adolescents had VDD < 20 ng/mL, and about 90% of Korean adolescents had a VD level < 10 ng/mL. The Korea Centers for Disease Control and Prevention (KCDC) started a project in 2017 to administer prophylactic anti-TB drugs to persons who had tested positive for LTBI[55]. The KCDC announced that results were effective and promising in some persons received the treatment and recommended vitamin D supplementation for LTBI persons who refuse anti-TB treatment[55]. But they did not state whether the results were significant[55]. Further clinical trials are needed to confirm those results.

Based on the knowledge of vitamin D action in the immune system[7,8,60-62-65i] , the studies that associated its deficiency with TB development[12-33], its affordable cost, the studies illustrated the effect of vitamin D supplementation[55] and the results of this study, vitamin D should be a part of the TB treatment program and might also be a prophylactic factor in TB prevention[55]. Vitamin D supplementation could play a protective role against active TB infection and LTBI activation[5,8,9,24,28,55]. Further studies are needed to detect the actual effect and dose.

Heterogeneity was found in this analysis. The high percentage corresponds to high heterogeneity (more 80-90 %) was found in all sub-groups of analysis with significant p-value. Except a p-value of >20 nmol/L was insignificant. The high range of heterogeneity in this study can have many causes such as different methods and characteristics of studies included, the long time interval of the included studies between 1985 and 2018, the inconsistent definition of VDD used in the studies, small size of population in some studies and some confounders that affect the study results such as diet, sunlight exposure,

ethnicity, diseases influencing vitamin D metabolism and poverty. No publication bias has been observed, the studies were equally distributed.

This study had some limitations; it included studies published in English which may affect the results. The limited numbers of subjects and studies in the lowest level of vitamin D ≤ 20 nmol/L and below may have affected the results. This meta-analysis included observational studies with many uncontrolled confounders that could have affected the results. The studies included were conducted with different research design. Finally, inconsistent vitamin D scaling, that some studies considered many cut-off levels as optimum.

Conclusion:

This study included all relevant studies with reasonable quality. It confirmed the association between low vitamin D and TB. It calculated the log risk ratio in different cut-off points of vitamin D. It assumed that TB infection is more susceptible to be found in persons with lower 75 nmol/L of vitamin D. Higher than 75 nmol/L seems a safe level. But, further studies needed to examine the association of vitamin D level above and TB infection.

By including recent literature, this study verified an overall significant risk of lower vitamin D levels in TB patients. Also, it was a first trial to clarify the association between decreased levels of vitamin D and increased probability of TB infection. It implied that lower than 30 nmol/L of vitamin D had high log RR. The risk at 50 and 75 nmol/L was too close. This average 50 -75 nmol/L was previously assumed as inadequate level of vitamin D.

It showed also, that higher than the 75 nmol/L is needed to abolish the increased probability of TB infection. Further studies are needed to verify such association and the use of vitamin D supplementation in a preventive or prophylactic treatment for TB patient, in both active and latent forms.

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