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Systematic Review

Signaling pathway of vitamin D, vitamin D receptor and autophagy in infection: a systematic review

Katarina Dewi Sartika[1]*, Anak Agung Ayu Yuli Gayatri[2]

[1]Krida Wacana Christian University, Jakarta, Indonesia
[2]Department of Internal Medicine, Medical Faculty of Udayana University, Sanglah Hospital, Denpasar, Bali, Indonesia

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***Correspondence:**
Dr. Katarina Dewi Sartika,
E-mail: katarinadewisartika@gmail.com

ABSTRACT

Vitamin D has an immunomodulatory effect that increases antimicrobial responses in macrophages by inducing antibacterial proteins, stimulating autophagy, and increasing autophagosome activity through various signaling pathway. This review emphasizes the several signalling pathway of vitamin D and vitamin D receptor (VDR) to induce autophagy in infection and its role in infectious disease. This study was performed based on PRISMA guidelines. Literature search conducted from Cochrane, PubMed, Science Direct and ProQuest using the terms "vitamin D" and "VDR" and "autophagy in infection". The inclusion criteria were original article, publication in English, published in 2012-2022, investigating vit D, VDR pathway and autophagy mechanism in infection. Twelves studies met our criteria. Despite numerous autophagy signalling in vitamin D and VDR, there are 2 main mechanisms vit D induces autophagy; increase expression LL-37/cathelicidin, CAMP, DEFB4 and upregulated autophagy genes (LC3B, ATG5, BECN1, MMPI, ATG16L1, PR39). While, vit D induced autophagy via expression CYP27B1 and VDR in co-infection HIV and tuberculosis. In sepsis, artesunate relates with VDR to enhance autophagy via NF-κB. Similarly, vitamin D could enhance cell resistance to *Aspergillus fumigatus* after modulating NF-κB. Vitamin D3 through PDIA3-STAT3-MCOLN3-Ca2+ axis and CAMP/LL-37 (cathelicidin antimicrobial peptide) are mediator for autophagy induction in *H. pylori*. Upregulated autophagy gene activity through vit D/VDR appears as new target therapy for infection in future.

Keywords: Autophagy, Infection, Signaling, Vitamin D, VDR

INTRODUCTION

Vitamin D is a fat-soluble vitamin that has pleiotropic effects and its deficiency can lead to various health problems. Recent studies show that vitamin D and VDR are expressed by immune cells and related with innate immune mechanisms. That mechanism is the fundamental protection against infection and needed to fight pathogens. Vitamin D has immunomodulatory effects that simultaneously stimulates antimicrobial effect through the induction of antibacterial proteins, autophagy and autophagosome activity in macrophages.[1] Vitamin D activates autophagy through various signaling pathway that the influence physiological functions of the body organs.[2]

The VDR is the main receptor that mediates most of the biological functions of 1,25(OH)2D3 or vitamin D3, the active form of vitamin D. VDR stimulates the expression of ATG16L1 at transcriptional level to induce autophagy.[3]

Autophagy is catabolic activity that consumes cytoplasmic material and dysfunctional organelles through formation of bilayered autophagosome that fuse with lysosomes for degradation and recycling to maintain cellular homeostasis. Autophagy is divided into 3 distinct types:

macro-autophagy, micro-autophagy, and chaperone-mediated autophagy. Autophagy is induced by stress response and triggered by starvation, infection, and toxin exposure to protect cells.[4] Autophagy also contributes to anti-aging, antimicrobial defense, and tumor suppression. Vit D/VDR signaling overlaps with autophagy pathway.[5]

Several studies show promising effect of vitamin D and its receptor induces autophagy as an antibacterial defense mechanism. Vitamin D-induced cathelicidin increases autophagosome-lysosome fusion leading to phagosome maturation and degradation of *M. tuberculosis* infection.[6] The possible interactions between vitamin D/ VDR and infection appear to be more complex than previously thought. Some autophagy mechanisms have been suggested to be involved with vit D or VDR.[7]

This review emphasizes the several signaling pathway of vitamin D and VDR to induce autophagy in infection and its role in infectious disease.

LITERATURE SEARCH

This study was performed based on PRISMA guidelines.[8] A literature search was conducted in Cochrane, PubMed, Science Direct, ProQuest to obtain the experimental study, animal study, and clinical trials. Following keywords used: "vitamin D" and “VDR” and "autophagy in infection". Inclusion criteria were original article, publication in English, published in 2012-2022, and use vitamin D, VDR. Articles were excluded if only an abstract was available, literature review, case report, systematic review, meta-analysis, no use vitamin D/ VDR pathway and mechanism aside from autophagy in infection (Figure 1).

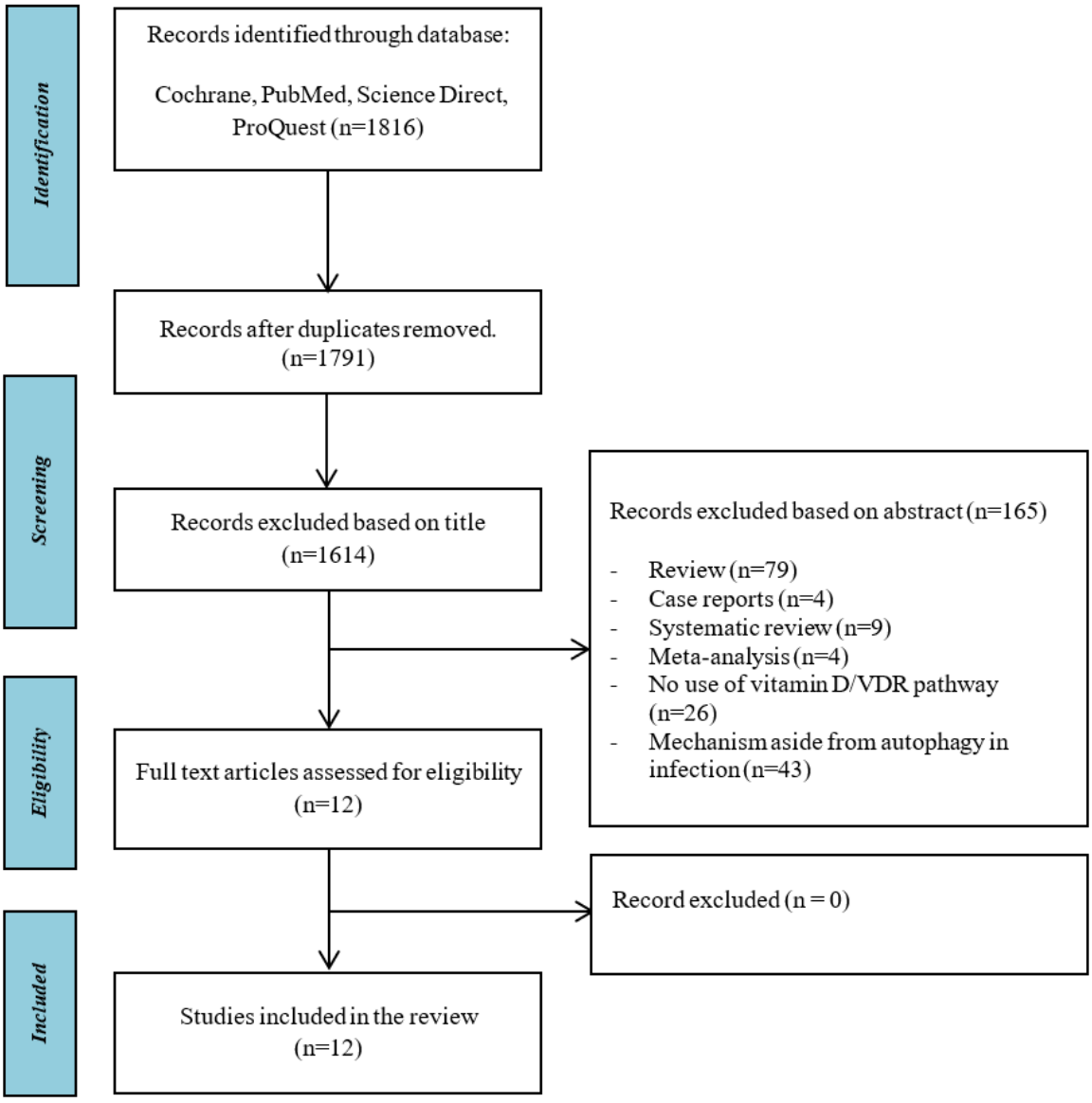

Figure 1: The systematic review process.

RESULTS

Our final search, conducted on 9 April 2022, retrieved 1861 articles after filtering. Retrieved articles were imported and independently screened for duplicity, eligibility by author KDS and checked by author AAYG. Twelve relevant studies were included in the final review. A data extraction tool is designed to guide information from each study. We extracted data including: year, study design, detailed mechanism, genes involved and type of infection. Study characteristics and data extraction represented in Table 1. Our review consists of 12 studies of several infections; *M. tuberculosis* (n=6 articles), HIV type 1 and *M. tuberculosis* (n=1 article), *M. marinum* (n=1 article), sepsis (n=1), *Helicobacter pylori* (n=1 article), rotavirus (n=1 article) and *Aspergillus fumigatus* (n=1 article). This review involves 4 experimental studies, 2 animal studies and one prospective study of tuberculosis infection. In addition, there was one experimental study of *M, marinum* infection, one animal study of sepsis-induced immunosuppression, *in vivo* and *in vitro* study of *H. pylori*, animal study of rotavirus infection and *in vitro* and *in vivo* study of *Aspergillus fumigatus* infection.

Table 1: Signaling pathways of vitamin D, VDR and autophagy in infection.

References	Study design	Infection	Autophagy mechanisms	Involved genes
Wan et al, 2018 (Sweden)[18]	Experimental study (human macrophages)	*M. tuberculosis*	PGE2 inhibits LL-37 expression in human macrophages and reduces host defense against Mtb. PGE2 binds to EP2 and EP4 which activates cAMP/PKA signaling to elevate repressor CREM/ICER expression and reduce VDR expression. Increased binding of CREM/ICER to CRE and reduced binding of VDR to VDREs in promoter of human cathelicidin expression and autophagy, resulting in increased Mtb survival.	LL-37 EP2 EP4 cAMP/PKA CREM/ICER
Klug-Micu et al, 2013 (Germany)[13]	Experimental study (peripheral blood mononuclear cell)	*M. tuberculosis*	Activation of human monocytes via CD40 ligand and IFN-y induces CYP27b1-hydroxylase, responsible for conversion vit D and VDR that stimulated an antimicrobial activity against intracellular *M. tuberculosis* through upregulated expression of cathelicidin and DEFB4. 2nd key event in vit D antimicrobial pathway is induction of autophagy. Increase in LC3-II is required to overcome phagosome maturation block in infected monocytes /macrophages.	LC3-II Cathelicidin DEFB4
Afsal et al, 2016 (India)[15]	Experimental study (peripheral blood mononuclear cells)	*Pulmonary tuberculosis*	Supplementation vitamin D during anti-TB treatment induces the expression of autophagy genes (ATG5 and BECN1) that help remove the pathogen-induced phagosome maturation block and restrict the growth of intracellular pathogen such as *Mycobacterium tuberculosis*	ATG5 BECN1 CAMP
Vermay et al, 2013 (Canada)[16]	Animal study (mice)	*M. tuberculosis*	Vitamin D stimulates autophagy in macrophages but dependent upon epithelial IL1R1 signaling and IL-1B driven epithelial production of the antimicrobial peptide DEFB4/HBD2	IL1R1 signaling IL-1B DEFB4/HBD2
Wahyunitisari et al, 2022 (Indonesia)[12]	Animal study (mice)	Tuberculosis	Supplementation vit D3 on 2nd line anti-TB therapy increases vit D3 receptor, CRAMP, LC3B, caspase-3, presses MMP1 and number of bacteria. Vit D increase the conversion of LC3B-1 to LC3B-II. Structural equation modeling shows that increasing autophagy pathways reduces necrosis by lowering MMPI	Cathelin-related antimicrobial peptide CRAMP, LC3B, caspase-3, MMP1
Meca et al, 2021 (Romania)[11]	Prospective study (30 newly diagnosed pulmonary tuberculosis	*Tuberculosis*	After two months of 1st line anti TB treatment in negative sputum-culture patients both vitamin D and VDR status were increased. VDR promotes cathelicidin (LL-37) synthesis and activity leading to bacterial membrane	Cathelicidin (LL-37)

Continued.

References	Study design	Infection	Autophagy mechanisms	Involved genes
	patients and none of them received vit D supplement undergoing evaluation of vit D status and culture before and after 2 months of 1st line anti TB treatment)		disintegration by increased autophagy upregulated though phagosomal maturation	
Sato et al, 2013 (Japan)[17]	Experimental study (human monocytes)	*M. marinum*	Cathelicidin antimicrobacterial peptide (CAMP) is up-regulated by vitamin D/VDR pathway and exogenous added LL-37 accelerate antimicrobial function of autophagolysosome and decreased proliferation of *Mycobacterium marinum*	CAMP Cathelicidin (LL-37)
Campbell et al, 2012 (USA)[18]	Experimental study (human macrophages)	HIV type 1 and *M. tuberculosis*	Vitamin D induced autophagy in human macrophages during co infection HIV and tuberculosis via downregulation of Beclin-1 and light chain 3B (LC3B)-II. During autophagy, cytosolic LC3B-I is converted to LC3B-II by a ubiquitin-like system that involves autophagy related protein-7 (ATG7), ATG3 and the ATG5-ATG12 complex.	LC3B-II BECN1 ATG3 ATG7 ATG5-ATG12 complex
Shang et al, 2019 (China)[19]	Animal study (mice)	Sepsis-induced immunosuppression	Artesunate interacts with VDR to reverse sepsis-induced immunosuppression in an autophagy and NF-kB-dependent manner. Artesunate enhanced autophagy in LPS-tolerant cells to eliminate bacteria.	ATG16L1 siRNA
Hu et al, 2019 (China)[20]	Experimental (mouse gastric tissue) and animal study (mice)	*Helicobacter pylori*	Vitamin D3 reactivate the lysosomal acidification and degradation function of autolysosomes. which is the key signal pathway for the antibacterial both in cells and in animals	PDIA3-STAT3-MCLN3-Ca^{2+}
Gang et al, 2016 (China)[21]	Animal study (pigs)	Rotavirus	Vitamin D3 could regulate the expression of the autophagy related genes (LC3-II/I) and PR39 mRNA induce autophagy in porcine intestine and IPEC-J2 cells. Vitamin D3 alleviate RV infection via the regulation of autophagic maturation and the expression of porcine cathelicidin genes	LC3-II/LC3-I PR 39
Suxia et al, 2020 (China)[22]	*In vitro* (macrophage mice) aAnimal study (mice)	*Aspergillus fumigatus*	Cells stimulated by vitamin D is resistance to Aspergillus fumigatus via modulating NF-κB. Vitamin D reduces LC3 expression, maintain low level autophagy and prevent damage excessive autophagy. Vitamin D can inhibit excess autophagy and helps to reduce programmed cell death that continued to increase antifungal ability. Vitamin D deficiency can aggravate inflammatory damage in the lungs and the survival rate was lower than the mice with sufficient vitamin D.	TNF-α, IL-1β, IL-6 and LC3BII, NF-κB

DISCUSSION

Vitamin D induces autophagy through different signaling pathway. Vitamin D signalling express a crucial role in the innate defence against intracellular microorganisms through autophagy, cathelicidin directly bind and kill many pathogens. It also turns as a second messenger, driving vitamin D-mediated inflammation during infection.[9] This review showed that signaling pathway of vitamin D and VDR could affect autophagy in bacterial, viral and fungal infection. *Mycobacterium tuberculosis* is an intracellular pathogen. It damages macrophages and eludes eradication by interfering with host defence mechanisms. Recent studies have shown that autophagy is a very preserved process that sheds and degrades proteins and entire organelles through lysosomes, and limit mycobacterial proliferation. VD-mediated innate immunity and autophagy have been shown to offer protection against tuberculosis infection.[10] Vitamin D stimulates autophagy as an antimicrobial defense mechanism. Vitamin D increases the expression of human cathelicidin/LL-37 peptide to promote autophagy. Meca et al revealed after 1st line anti-TB treatment, vitamin D, VDR and cathelicidin (LL-37) expression is increased leading to increased autophagy.[11] Similarly with Sato et al revealed vitamin D also has benefit in *Mycobacterium marinum* infection, a nontuberculous photochromogenic mycobacterium species that caused cutaneous skin infection.[12] Vitamin D/VDR accelerate antimicrobial activity both intracellular/extracellular via cathelicidin antimycobacterial peptide (CAMP)/LL-37 to contradictory infection via auto-phagolysosome in human monocytes. In another study, vitamin D3 through PDIA3-STAT3-MCOLN3-Ca2+ axis to reactivate the lysosomal acidification and degradation function of autolysosomes. CAMP/LL-37 is induced by VD3, which is a mediator for autophagy induction. This mechanism of action would offer new therapeutic approaches for H. pylori treatment.[13] Vitamin D and VDR manage autophagy through distinct signaling pathways. LC3 acts directly as a VDR ligand, promoting VDR and activating VDR signaling. While, Wahyunitisari et al demonstrated co-treatment of vitamin D3 and 2nd line anti-tuberculosis drugs might increase cathelin-related antimicrobial peptide and LC3B expression.[14] LC3 is an autophagic membrane precursor divided as three variants/post-translational isoforms of LC3A, LC3B and LC3C. Vitamin D upregulates the conversion of LC3B-1 to LC3B-II. Following lipidation, the translated LC3B converts to the LC3-I cytoplasmic form and moves to the LC3-II membrane form. Co-localization of LC3 with phagosomes results in delivery of immature phagosomes to lysosomes via the mitogen activated protein kinase (MAPK) pathway. Though LC3 has some homologues, cytoplasmic LC3B is frequently used as an autophagy marker. LC3-II is present on the autophagosome membrane, and the former is degraded. This finding is support by Klug-Micu et al the activation of vitamin D pathway by CD40L and IFN-y results in upregulated expression of the antimicrobial peptides, cathelicidin and DEFB4 also induction of autophagy and reduction of VDR expression inhibits LL-37 expression in human macrophages will decrease host defense against *Mycobacterium tuberculosis*.[15,16] A second key event in the vitamin D antimicrobial pathway is the induction of autophagy, which is required to overcome the phagosome maturation block in *M. tuberculosis* infected monocytes/macrophages. Increase in LC3-II is associated with the induction of autophagy. Monocyte LC3-II was significantly improved in *Mycobacterium tuberculosis* stimulated cultures compared with untreated cultures.

Other pathway has shown that vitamin D could enhance innate immune functions through the expression of autophagy genes (ATG5 and BECN1) and help regulate intracellular growth of mycobacteria in macrophages.[17] However, Vermay et al revealed that using vitamin D increase IL1B signaling leads to epithelial antimicrobial peptide production (DEFB4/HBD2).[18]

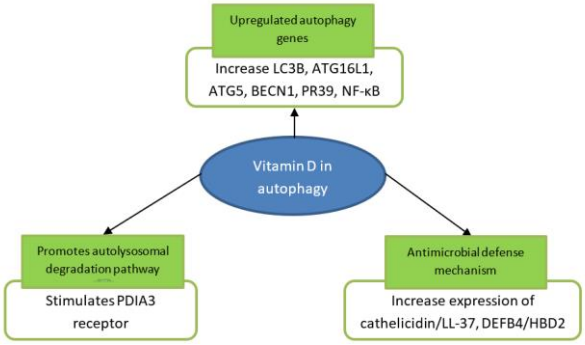

Figure 2: Potential mechanism of vitamin D induce autophagy.

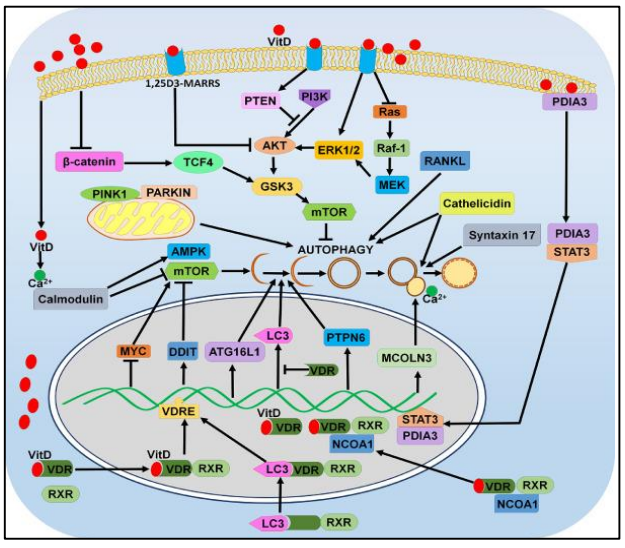

Figure 3: Vitamin D and its receptors in autophagy signaling. Vitamin D induces autophagy through cathelicidin, stimulates the PD1A3 membrane receptor, interaction of protein LC3 and VDR increases its nuclear translocation, subsequently promoting VDR retinoid X receptor heterodimerization and activating VDR signaling.[2]

Moreover, individual with HIV is at high risk of being infected with *Mycobacterium tuberculosis*. Campbell et al exposed that 1,25 D3 inhibit both co-infection HIV and tuberculosis replication in human macrophages through an autophagy and CAMP dependent mechanism.[19] Vitamin D induced autophagy in human macrophages through downregulation Beclin-1 and light chain 3B (LC3B)-II. In autophagy, cytosolic LC3B-I is converted to LC3B-II by a ubiquitin-like system that involves autophagy related protein-7 (ATG7), ATG3 and the ATG5-ATG12 complex. The ATG5-ATG12 complex ligates LC3B-II to the nascent autophagosome membrane through phosphatidylethanolamine with the LC3B-II associated with the inner membrane degraded after fusion of the autophagosome with lysosomes. Consequently, the conversion of LC3B-I to LC3B-II and its turnover is an indicator of autophagy induction and flux. This finding indicated that vitamin D can act as a potent stimulator of autophagy in co-infection HIV and tuberculosis patients.

Furthermore, vitamin D3 improves rotavirus infection via the regulation of autophagic maturation and the expression of porcine cathelicidin genes. Vitamin D increases the autophagy related genes (LC3-II/I) and PR 39 mRNA expression in porcine jejunum and ileum.[20] Likewise, vitamin D could reduce LC3 expression and modulate NF-κB to maintain low level autophagy and prevent damage excessive autophagy in *Aspergillus fumigatus* infection.[21] A promising finding has been reported that artesunate interacts with VDR decreases its adverse regulation of autophagy-related target genes such as ATG16L1 and increases autophagy. Moreover, artesunate interaction with VDR promoting pro-inflammatory cytokine release from macrophages via NF-kB-dependent manner in the sepsis model.[22]

CONCLUSION

Stimulation of vitamin D and VDR are associated with better outcomes in infection population. While, the mechanism between vitamin D, VDR, and autophagy in infections is more complex than previously thought. Vitamin D and VDR induced autophagy are associated with upregulation of cathelicidin and autophagy related genes, which appears to be new therapeutic strategies. Further study in different model is needed to discover autophagy related gene activity as new target therapy for infection in the future.

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