LITERATURE REVIEW

Combination of Vitamin D and Vitamin E Against Bacterial Infections

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ABSTRACT

Bacterial infections are a significant health problem worldwide, caused by various microorganisms that can infect different parts of the human body. Bacteria are single-celled microorganisms that can be found in various environments, including in the human body, and most of them are harmless. However, some species of bacteria can cause serious infections and inflammation that require proper medical attention. Some examples of bacteria that cause infections include *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Escherichia coli*, and *Staphylococcus aureus*. A sufficient vitamin D level in the body can enhance antimicrobial activity against various pathogens, including bacteria, viruses, and fungi. Vitamin E is known to be a powerful antioxidant and has a positive effect on the immune system. It plays a role in protecting immune cells from oxidative damage and enhancing the immune response to infection. The combination of vitamins D and E offers significant potential in the prevention and management of bacterial infections, as both contribute to improved immunological function through different and complementary mechanisms.

Keywords: bacterial infection; inflammation; *Staphlococcus aureus;* vitamin D; vitamin E

INTRODUCTION

Bacteria are single-celled or unicellular organisms, do not have a nuclear or prokaryotic membrane, do not have chlorophyll, reproduce asexually, i.e. cell division, and are of a micron size, so a microscope is needed to aid observation.[1] Bacterial infection is one of the causes of diseases commonly found in tropical areas such as Indonesia, because Indonesia has a humid climate and also warm temperatures, making it easier for microorganisms such as pathogenic bacteria to multiply well. This is also supported by inadequate existing sanitation, so that infectious diseases are more easily transmitted. One way to deal with infections caused by bacteria is to give antibiotics. Antibiotics inhibit (*bacteriostatic*) and even kill (*bactericidal*) disease-causing bacteria. When used appropriately, antibiotics provide benefits in overcoming infection problems.[2] However, if used inappropriately (irrational prescribing), it can cause losses such as the problem of resistance to the antibiotic. Resistance of chemical antibiotic drugs to bacteria continues to increase, which is caused by several influencing factors such as excessive use of antibacterial drugs, use of drugs without indications, use of drugs under recommended doses, and natural bacterial mutations. The use of chemical drugs can also cause side effects such as digestive problems, allergic reactions, fever, heart problems, and kidney failure, so the development of effective treatment strategies is needed to reduce inflammation in bacterial infections.[2] Vitamin D and vitamin E have been known to play important roles in the immune system and healing processes.[5,11] Vitamin D3, for example, has been shown to increase the activity of immune cells, such as macrophages and lymphocytes, thereby increasing the body's ability to fight infections.[3] Vitamin D inhibits proinflammatory cytokines such as IL-2, IL-6, IL-8.[4] Meanwhile, Vitamin E is a powerful antioxidant that can neutralize free radicals and reduce oxidative stress in the body.[11] In addition, vitamin E can also inhibit the production of pro-inflammatory cytokines such as TNF-α and IL-6, thus potentially reducing inflammation and protecting tissues from bacterial infections.[12] So that the combination of the two can increase effectiveness in reducing inflammation induced by bacterial infections.

Pathology of bacterial infections

Infection is the invasion of the host by microorganisms, which then multiply in close contact with the host tissue. Infection is distinguished from disease, an unnatural process that does not necessarily involve infection.[1] Bacteria can cause a wide variety of infections, ranging from invisible to severe. The capacity of bacteria can cause a wide variety of different infections, ranging from mild to severe. The capacity of bacteria to cause disease reflects their relative pathogenicity. On this basis, bacteria can be grouped into three large groups. When isolated from patients, frank pathogens or primary pathogens are considered disease-causing agents.[3] Opportunistic pathogens are pathogens isolated from patients whose host defense mechanisms have been disrupted. This pathogen is a disease agent, for example, in patients who have a tendency to urinary tract infection with *Escherichia coli* through catheterization. Finally, some bacteria, such as Lactobacillus acidophilus, are considered nonpathogenic because they rarely or never cause disease in humans.[1] However, their categorization as non-pathogenic may change, due to the adaptability of bacteria and the adverse effects of modern radiation therapy,

chemotherapy, and immunotherapy on the mechanism of resistance.[2] In fact, some bacteria that were previously thought to be non-pathogenic are now known to cause various types of diseases. *Serratia marcescens*, for example, is a common soil bacterium that causes pneumonia, urinary tract infections, and bacteriosis in disturbed hosts. Infection begins when the balance between bacterial pathogenicity and host resistance is disturbed.[1] Basically, we live in an environment that supports microbes, simply because the growth rate of bacteria far exceeds that of most eukaryotic cells. In addition, bacteria are much more flexible than eukaryotic cells in substrate utilization and biosynthesis.[2] The high rate of bacterial mutation, combined with the short generation time, results in rapid selection of the most adapted strains and species. In general, bacteria are much more resistant to toxic components in the environment than eukaryotes, especially when the main barrier of eukaryotes (skin and mucous membranes) is penetrated.[2]

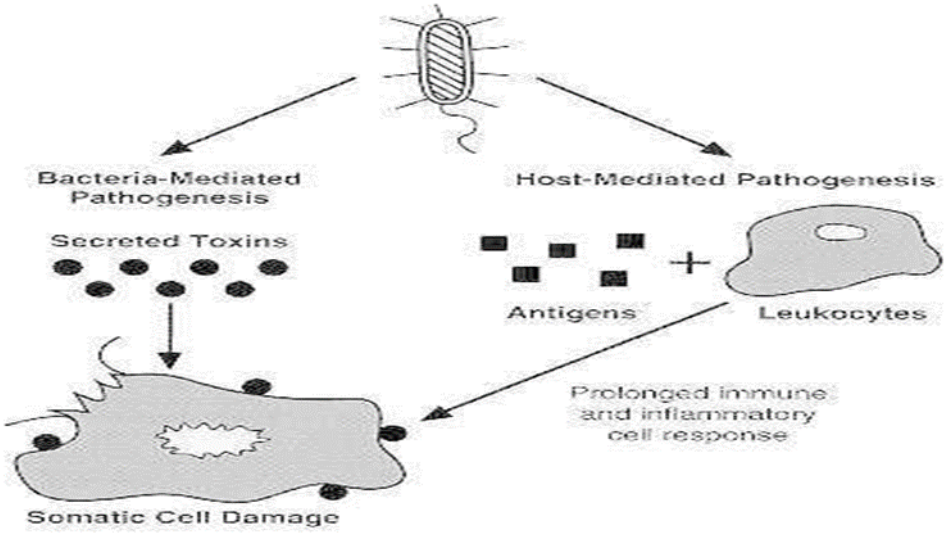

Figure 1. Pathology of bacterial infections[1]

From a practical point of view, bacteria can be said to have one purpose: to reproduce. Only a few of the large number of bacterial species in the environment consistently cause disease in a particular host. From a teleological point of view, it is not in the best interest of the pathogen to kill the host, since in many cases, the host's death means the pathogen's death. The most evolved or highly adaptable pathogens are those that acquire the nutrients necessary for growth and dispersal with the least energy expenditure and the least damage to the host. For example, *Rickettsia akari*, the etiological agent of chickenpox, causes a mild, self-healing infection consisting of headache, fever, and papulovesicular rash. Some bacteria that are poorly adapted to the host synthesize virulence factors (e.g., *tetanus* and *diphtheria toxins*) that are so potent that they can be life-threatening.[1]

Pathophysiology of vitamin D

Vitamin D is a fat-soluble vitamin found in two main forms, namely vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D is also available in the form of dihydrotachysterol, alfacalcidol, and calcitriol. Vitamin D3 or cholecalciferol is one of the main isoforms of Vitamin D4. The body can produce vitamin D through exposure to UVB rays from the sun on the skin. This process converts skin cholesterol into 7-dehydrocholesterol, which is then converted into pre-vitamin D3 and finally vitamin D3 (cholecalciferol).[5] In vitamin D, absorption and metabolism begin to produce vitamin D3, which can be used in the management of vitamin D deficiency in related health problems such as *rickets*, *osteomalacia*, *hypoparathyroidism*, and chronic kidney failure. Vitamin D3 can also be used in the maintenance of immune function, as well as reducing the risk of viral infections.[6] Vitamin D also helps maintain tight junctions, gap junctions, and adherens junctions, so that it will make it harder for viruses or bacteria to enter the body.[8] Vitamin D3 also increased the expression of genes associated with antioxidants, namely *glutathione reductase* and the *glutamate-cysteine ligase* subunit.[9] Vitamin D has a bacteriostatic effect, suppresses inflammatory mediators, suppresses immune system dysregulation in activating endothelials, thus preventing damage to cell/tissue endothelials, controls mediators to prevent vasodilation, which can ultimately prevent the occurrence of MODS due to tissue hypoxia.[10]

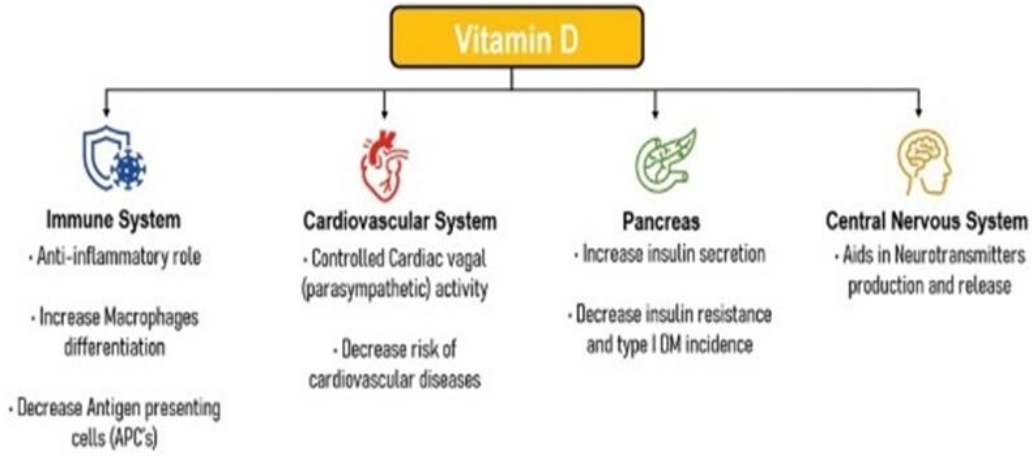

Figure 2. Pharmacology of Vitamin D[10]

Pathophysiology of vitamin E

Vitamin E is a group of fat-soluble vitamin compounds and can be divided into two groups, tocopherols and tocotrienols, with four isomers (alpha, beta, gamma, and delta).[11,12] Although vitamin E is widely known as a powerful antioxidant, some studies have also found that vitamin E has anti-inflammatory properties.[11] Vitamin E, specifically α-tocopherols, can be easily obtained and consumed by most of the world's population. Vitamin E is found in a variety of staple foods, such as vegetable oil, palm oil, bran, wheat seeds, olives, barley, soybeans, nuts, and seeds.[12] Vitamin E has attracted the attention of many researchers who can be used as a potential add-on therapy for a variety of disorders due to Vitamin E excellent antioxidant and also called as anti-inflammatory properties.[11] Inflammation is the result of an increased immune response of the body and is characterized by increased production of free radicals and pro-inflammatory mediators, which can cause excessive damage to host tissues.[12] Its occurrence can be indicated by the presence of markers or biomarkers of enzymes such as cytokines and C-reactive proteins (CRPs).[13] Vitamin E has attracted attention. Furthermore, the secretion of substances such as eicosanoids and pro-inflammatory cytokines is destructive. Regarding the anti-inflammatory properties of vitamin E, several studies have shown that vitamin E may inhibit the secretion of molecules that mediate inflammation, such as eicosanoids and the enzyme *cyclooxygenation-2* (COX-2). Vitamin E also suppresses proinflammatory signaling pathways, such as the pathway mediated by the nuclear factor kappa beta (NF-kβ) and transducer and transcription activator 3 (STAT3).[11] This process ends with excretion in the bile system and urinary tract. Levels of toxic vitamin E are not concentrated in the body in the same way as other fat-soluble vitamins such as vitamins A and D12. Although vitamin E is a fat-soluble vitamin, excess alpha-tocopherol can also be excreted through the urine and bile, reducing the accumulation of alpha-tocopherols in the circulation. As a result, toxic effects were not observed in most healthy patients who consumed less than 1000 mg of vitamin E daily. Compared to other fat-soluble vitamins, vitamin E is more evenly distributed in the body, especially in plasma, liver, and adipose tissue.[16]

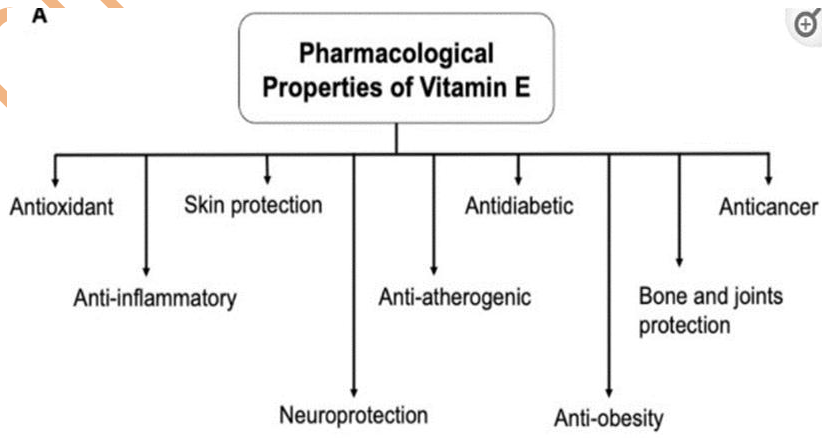

Figure 3. Pharmacology of vitamin E[11]

Doxycycline as an antibiotic

Doxycycline is a member of the tetracycline family of antibiotics used to treat various bacterial infections that have shown clinical potential. Doxycycline is a second-generation tetracycline class of antibiotics with a broad spectrum and bacteriostatic drug effects.[17] Doxycycline works by inhibiting protein synthesis to inhibit the growth of bacteria. It also exhibits pleiotropic effects, including having anti-inflammatory and antioxidant properties as well as suppressing excess nitric oxide (NO) production and matrix metalloproteinase (MMP) activity. The two molecules, NO and MMP, are strongly associated with low outcomes in NO and MMP under severe conditions, including in sepsis and septic shock. Doxycycline works by inhibiting the occurrence of translation in bacteria. Doxycycline binds to 16s units of bacterial rRNA, thus preventing the binding of tRNA to RNA-30s. As a result, protein synthesis can be prevented so that bacteria cannot multiply.[17]

Application of the rat model to *Staphylococcus aureus* infection

Approximately 90% of mouse genes have strict direct homologs in the genomes of mice and humans. The larger size of mice and larger vascular, cardiac, blood, and urine volumes allow for more complex physiological measurements and surgical maneuvers. As a promising preclinical model, the mouse model has provided many insights into understanding the pathogenic mechanisms of *Staphylococcus aureus* and exploring therapeutic approaches, which also promotes vaccine research. *Staphylococcus aureus* is a major causative bacterium of bacteremia and can cause high morbidity and mortality. Channabasappa et al. investigated the efficacy of P128, a chimeric ectolysin derived from bacteriophage, in a mouse model of bacteremia caused by *Staphylococcus aureus*. Female Wistar albino mice were injected intravenously with CFU of MRSA USA300. The CFU count of MRSA USA300 in blood was found to be between 2 × 108 ± 8 × 107 CFU/mL, in the first minute after injection of MRSA USA300. The number dropped by one to three times within 4 hours. The amount of CFU in the blood remained in the range of 2 × 104 CFU/mL to 2 × 106 CFU/mL for the next four days. At 96 hours post-infection, the CFU count was 1.89 × 107 ± 9.84 × 106 in the kidneys, 2.37 × 105 ± 2.19 × 105 in the liver, 2.16 × 104 ± 1.26 × 104 in the spleen, and 2.02 × 104 ± 1.93 × 104 in the lungs. In the kidneys, a diffuse abscess is visible after a rough necropsy. Treatment with P128 contributed to a dose-dependent increase in survival, and treatment with P128 (2.5 mg/kg) led to few or no abscesses compared to mice treated with saline solution. The average body weight of mice treated with P128 (2.5 mg/kg) increased significantly on day 7 compared to the control group. Thus, P128 can be used as a new option for the treatment of *Staphylococcus aureus* bacteriosis. This model of bacteremia in mice can be reproduced and shows a gradual progression of the disease, which can lead to death for several days, and therefore more closely resembles an infection in humans.[7] Sepsis is a systemic inflammatory reaction with high mortality. The burden of pathogens in the blood correlates with the severity and mortality of the disease in patients with sepsis. Kang et al. developed an external blood purification device, based on the microstructure of mouse biospleen, that is capable of removing pathogens such as *Staphylococcus aureus* bacteria from the blood flowing from patients indicated for sepsis. Rats

were injected intraperitoneally with 1 mL of PBS containing *Staphylococcus aureus* (ATCC 12598, 5 × 108 CFU/mL). The level of the pathogen in the blood of the mice increased 3-4 hours after the injection and peaked at about 10 hours. At the 10th hour, the mice were anesthetized, the mice's jugular venous catheter was connected to the tube of the biosplain device, and a saline solution containing heparin, magnetic opsonin, and magnetic beads was injected using a syringe pump at a flow rate of 7.1 μL min-1. The findings showed that biospleen could clear >90% of *Staphylococcus aureus* from the blood, reduce the burden of *Staphylococcus aureus* bacterial infection, and reduce immune cell infiltration in many organs, as well as reduce several levels of cytokine inflammation (such as interleukin-1α (IL-1α), interleukin-4/IL-4, interleukin-6 (IL-6), interferon-γ (IFN-γ).[7]

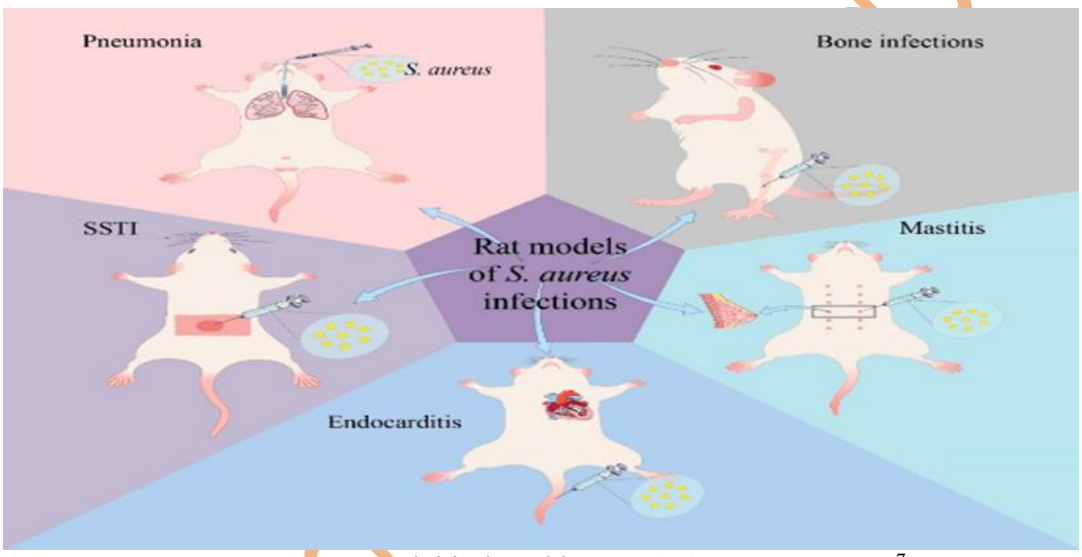

Figure 4. Rat model induced by *Staphylococcus aureus*[7]

Table 1. Rat infects by *Staphylococcus aureus* results[7]

Ref	*Staphylococcus aureus* strain	Species of rat	Main Results
Huang	AH1252	SD	Iclarprim significantly reduced the colony counts of MRSA compared with the control group, and the survival rate of the rats treated with Iclarprim was 100%
Prazak	MRSA-AW7	Wistar	Treatment with aerophages enhanced the survival rate of the rats compared with the control group significantly, and aerophages combined with intravenous phages rescued 91% of rats compared to either therapy alone
Valente	MRSA-AW7	Wistar	Intravenous daptomycin combined with aerosolized phage treatment, improving survival and reducing bacterial load in the lungs or spleen of rats, was not superior to aeroophage treatment alone
Duan	8325-4	SD	Treatment with diphenyl pyrimidine could prevent increased mortality induced by *Staphylococcus aureus* effectively and prevent lung injury by inhibiting the expression of NLRP3 and inflammatory factors in rats
Wu	8325-4	-	Isoxanthanol could mitigate chronic obstructive pulmonary disease induced by *Staphylococcus aureus* in the rat model through suppressing the production of inflammatory cytokines and upregulating the expression of miR-145-5p
Mcelroy	8325-4 DU5883	-	Compared with rats vaccinated with 8325-4 and DU5883 (p fnbpA 4), *Staphylococcus aureus* growth and the degree of lung damage were increased in rats vaccinated with the deletion mutant DU5883, suggesting that fibronectin-binding protein-mediated internalization of alveolar epithelial cells could reduce the virulence of *Staphylococcus aureus* in pneumonia
Niu	MRSA B3180	SD	All the rats in the infected group developed pneumonia with a marked cellular inflammatory response compared with the negative control group. In contrast to the untreated controls, rats in the colistins and SB203580 + Colistin group show a reduction in alveolar septal thickening and cellular inflammatory infiltration
Montgomery	USA400 USA300	SD	In a rat necrotizing pneumonia model, USA300 isolates were more virulent than USA400
Mcelroy	8325-4 DU1090	SD	α-toxing is an important cause of air-blood barrier damage in vivo, but it may not act directly on alveolar epithelial type I cells

Inflammatory response and histopathological changes in a Rat model infected with *Staphylococcus aureus*

The incidence of bloodstream infections caused by *Staphylococcus aureus* has increased significantly in critically ill patients and accounts for 20%–30% of deaths worldwide.[29] Staphylococcus aureus bacteria can survive in environments that have high salt levels.[22] In the human body, many normal flora can be found that have the potential to become pathogens, including the bacterium *Staphylococcus aureus.*[21] Bloodstream infections caused by *Staphylococcus aureus* bacteria can cause sepsis or septic shock, and the incidence of sepsis and sepsis-related deaths continues to increase. To date, several models of sepsis in experimental animals have been developed, including exogenous administration of toxins or live bacteria, ligation and puncture of the sepsis administration of toxins or live bacteria, ligation and puncture of the sepsis (CLP), and abdominal sepsis, which mimics the pathophysiological changes typically seen in sepsis patients.[3] Levels of cytokines, IL-1β, IL-6, and TNF-α, in serum and tissue homogenates were measured with an *enzyme-linked immunosorbent assay* (ELISA) kit (Neo Bioscience, Shenzhen, China) according to the manufacturer's instructions. The blood is coagulated for 1 hour at room temperature and centrifuged (4000 × g, 4°C, 15 min) in a refrigerated centrifuge, and then the supernatant is carefully aspirated to clean the tube and stored at -80°C until further analysis. A total of 60 mice (n = 10/group) were infected (iv) with 4.5 × 109, 4.5 × 108, 4.5 × 107, 4.5 × 106, 4.5 × 105, or 4.5 × 104 CFU/mL *Staphylococcus aureus* (0.1 mL/10 g body weight). The survival rate was monitored for 7 consecutive days after infection. In BSI mice, the survival rate was 0% in the 4.5 × 109 CFU/mL group, 70% in the 4.5 × 108 CFU/mL group 90% in the 4.5 × 107 CFU/mL group, 100% in the 4.5 × 106 CFU/mL group, 4.5 × 105 CFU/mL, and the 4.5 × 104 CFU/mL group. In the PBS control group (n = 10), the survival rate was 100% throughout the trial. Therefore, 4.5 × 108 CFU/mL was chosen as the best concentration, based on survival rate and median lethal dose. Serum and homogenate levels of IL-1β, IL-6, and TNF-α (liver, lungs, and kidneys) were evaluated during the 7-day post-infection period. Cytokine levels in serum and lung, and liver homogenates peak 6-24 hours post-infection. IL-1β levels in renal homogenates peaked at 48 hours post-infection, while IL-6 levels in liver and kidney homogenates and TNF-α in renal homogenates continued to increase during the 7-day post-infection period. In contrast, serum cytokine levels and tissue homogenates in the PBS control group were very low. IL-1β, IL-6, and TNF-α are inflammatory markers typical of sepsis, which are significantly increased in mice. Van den Berg et al. assessed the levels of proinflammatory cytokines in a murine model with *Staphylococcus aureus* bacteremia and showed that serum levels of IL-1, IL-6, and TNF-α were significantly increased. The results showed that *Staphylococcus aureus* increased the production of these three cytokines in serum and tissue homogenates (lung, liver, and kidney) in BSI mice over a 7-day observation period.[3] These results are similar to other studies, where most of the cytokines (IL-1β, IL-6, and TNF-α) in serum and tissues homogenates begin to increase at 1-3 hours post-infection and peak at 12-24 hours post-infection. However, we also found that IL-6 in liver and kidney homogenates, and TNF-α in renal homogenates increased continuously

during the 7-day observation period, The results showed that IL-1β, IL-6 and TNF-α levels were indicative of the presence of *Staphylococcus aureus* bacteremia by showing a significant positive correlation between TNF-α and IL-6 levels in serum, liver, lungs, and kidneys (p <0.001).[31]

Table 2. Association IL-1β, IL-6, and TNF-α on liver, kidney, and lungs in rats induced by *Staphylococcus aureus*[31]

		IL-6		TNF-α	
		r	p	r	p
IL-1β	Serum	0.928	<0.001**	0.983	<0.001**
	Liver	0.989	<0.001**	0.643	<0.001**
	Lungs	0.603	<0.001**	0.623	0.006*
	Kidneys	0.237	0.098	0.180	0.210
IL-6	Serum	-	-	0.932	<0.001**
	Liver	-	-	0.642	<0.001**
	Lungs	-	-	0.942	<0.001**
	Kidneys	-	-	0.857	<0.001**

Vitamin D promotes wound healing and reduces the production of interleukin-6 in those infected with *Staphylococcus aureus*

In particular, *Staphylococcus aureus* is the most common agent in chronic wounds, oftenappearing as biofilm-forming bacteria resistant to antimicrobial therapy, and it has been shown that vitamin D can reduce the risk of infection through several mechanisms appearing as biofilm-forming bacteria resistant to antimicrobial therapy.[28] It has been shown that vitamin D can reduce the risk of infection through several mechanisms, such as enhancing innate immunity through anti-microbial peptide (AMP) modulation and pro-inflammatory cytokine production. Preclinical and clinical studies strongly suggest that vitamin D exerts an influence on host immunity, infectious diseases, and autoimmune conditions.[14,15] Clinical data have shown that vitamin D deficiency is involved in many pathological processes, including some viral, bacterial, and fungal infections, and also wounds. The inflammatory phase causes neutrophil migration, then continues to the wound area, which is replaced by monocytes.[25] Specifically, vitamin D decreases the production of pro-inflammatory cytokines such as IL-6, interferon-γ (IFN-γ), IL-2, and TNF-α, which indicate its immunomodulatory effects. Some evidence from studies has shown that *Staphylococcus aureus* is associated with increased production of IL-6 by HaCaT cells. According to Ngo et al., the secretion of IL-6 by HaCaT cells is proportional to the rate of internalization of *Staphylococcus aureus*. In addition, IL-6 can prevent *Staphylococcus aureus* from spreading to healthy host cells that are still unaffected by inducing *keratinocyte* differentiation, thus accelerating the removal of already infected tissue from the surrounding area. Interestingly, treatment with vitamin D attenuates the release of IL-6 by *Staphylococcus aureus-infected* cells, demonstrates the inflammatory immunomodulatory activity of vitamin D, and is a novel strategy to attenuate pathogenesis caused by *Staphylococcus aureus*, promote wound healing, and reduce bacteria-mediated inflammation.[15]

Protective effects of vitamin D

Vitamin D3 is an antioxidant agent, and activation of the vitamin D3-VDR complex is associated with increased antioxidant activity.[21] Several animal studies have shown a close association between vitamin D deficiency and increased oxidative stress.[21] The molecular mechanism behind the action of vitamin D3 in nephrotoxicity induced by VCM may be based on reduced oxidative stress. In pathophysiological conditions, excessive production of ROS, such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, will lower anti-oxidative defenses and cause oxidative stress, which is involved in the development of endothelial dysfunction. Vitamin D exerts antioxidant effects through increased expression of anti-oxidative enzymes, including SOD, which can scavenge free radicals. In addition, the genetic action of vitamin D causes the expression of erythroid 2-related factor-2 nuclear factor (Nrf2), which is a key transcription factor that suppresses the production of ROS from various sources and increases the expression of antioxidants. Vitamin D3 showed anti-inflammatory activity by inhibiting the expression of RANTES and TNF-α in a mouse model.[22] Vitamin D3 mediates the pro-inflammatory response of the NF-κB pathway, specifically binding to the vitamin D3-VDR complex to the p65 subunit, thereby preventing NF-κB from interacting with DNA elements. Vitamin D3-VDR also likely attenuates the expression of P53-Upregulated Modulator of Apoptosis (PUMA) and miR-155 in tubule epithelial cells by interfering with NF-Kb activation. Vitamin D3 has a pronounced positive effect on VCM-induced nephrotoxicity and improves oxidative status, biochemical damage, and histopathological changes. As a result, vitamin D3 may have a useful role as a novel retrospective agent.[22]

Protective effects of vitamin E

Vitamin E, in particular, has a significant antioxidant function for cell membrane lipoproteins.[11] The role of vitamin E in supporting the immune system is enhanced by selenium.[20] Vitamin E administration to newborns with pancreatic cystic fibrosis has been found to help alleviate respiratory distress and act as an antioxidant. The effects of vitamin E are more pronounced at high oxygen concentrations. It serves as a natural antioxidant that reduces oxidized cellular components, decomposes the reactive oxygen species, and detoxifies toxic oxidation products. Vitamin E also protects erythrocytes against hemolysis in premature infants and reduces DNA damage due to its antioxidant effects.[12] During sepsis, oxidative stress increases while antioxidant levels such as selenium and vitamin E decrease.[18] Because the immune response is extraordinarily complex and complex in the sepsis process, antimicrobial treatment alone is not enough to improve patient survival. It is now thought that the use of antioxidants may play a role in the treatment of sepsis.[23] In oxidative cases such as sepsis, the release of free radicals causes tissue damage due to lipid peroxidation. Antioxidants such as vitamin E, selenium, and glutathione peroxidase inhibit lipid peroxidation.[18] In septic rats, short-term high-dose enteral vitamin E supplementation modulated monocyte and macrophage responses to endotoxins. In patients with severe burns, vitamin E levels were seen to decrease, while serum lipid peroxide increased. In patients treated with vitamin E, serum levels increased, and lipid peroxide decreased to healthy control group levels. Kono et al. showed that a novel synthetic vitamin E

derivative, E-Ant-S-GS, suppresses the effects of CLP sepsis-induced inflammatory changes in the lungs without any change in systemic side effects.[20] E-Ant-S-GS is highly effective and can be applied to future cases of severe sepsis, as monotherapy or in combination with other antioxidants. This study supports this as the above findings suggest that the application of selenium and vitamin E, alone or together as a combination of therapies, may be beneficial for sepsis model animals.[18]

Immunomodulatory effects of vitamin D on innate and adaptive immune response to *Streptococcus pneumoniae*

Invasive infections caused by the human-specific *Streptococcus pneumoniae* bacteria are still a serious problem worldwide. *Streptococcus pneumoniae* is part of the normal nasopharyngeal flora but can also cause a broad spectrum of inflammatory diseases.[20] Monocyte-derived dendritic cells (DCs) were stimulated with live *pneumococcus* (MOI 1), *pneumococcal* PGN (1 µg/mL), and MDP (5 µg/mL) in a 96-well flat base plate. Each treatment for each donor is done in triplicate. For stimulation of live *pneumococcus*, *gentamicin* 100 µg/mL (Invitrogen) is added after 30 minutes to kill extracellular bacteria. In some trials, DC was incubated first with cytocalcin D (1 µg/mL), vitamin D (1,25-(OH)2 D3; 100 nmol/L), vitamin D proform (25(OH)D3; 500 nmol/L), or itraconazole (10 µmol/L). The stimulated cells were incubated at 37°C in a humid atmosphere with 5% CO2. At 24 hours after infection, cells are analyzed for gene and protein expression or co-cultured with CD4 T cells. Vitamin D greatly increases the expression of genes and IL-1β proteins in PGN-stimulated DC. A compilation of results from several donors showed that after 24 hours of stimulation, gene expression increased 100-fold and protein expression increased 7-fold. Exogenous vitamin D eliminates the production of IFN-γ and IL-17, while the production of IL-10 is enhanced. The compilation of results from 5 donors shows that IFN-γ and IL-17 are muted by 63% and 75%, respectively, while IL-10 production increased 2.6-fold. Thus, vitamin D can modulate the host's response to pneumococci from the inflammatory Th1/Th17 response to the IL-10-regulated anti-inflammatory response. Exogenous vitamin D causes increased DC maturation, as well as increased regulation of CCR7, a surface marker for DC migration to lymph nodes. In particular, increased regulation in CD86, CD80, and CCR7 expression was offset by reduced cellular uptake from intact *pneumococcus*. Overall, these results suggest that vitamin D promotes a mature, migratory, and non-phagocytosis DC phenotype.[27]

Vitamin E against *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* bacteria

In humans, the main biologically active form of vitamin E is *α-tocopherol*.[26] *Burkholderia cenocepacia* increases antibiotic resistance by isolating bactericidal antibiotics into extracellular spaces. Computational guided analysis of X-ray crystallography and experimental structures, and ligand docking modeling, tested the binding mode of BcnA.[26] While antibiotics tested, such as norfloxacin, rifampicin, seftazidine, and gentamicin, interact with the lipokalin edges, more lipophilic structures such as *α-tocopherols* bind to the inside of the lipokalin tunnel.[26] In the Nile-Red displacement test, *α-tocopherol* showed an insufficient mean binding inhibition constant, while the average binding inhibition constant of

the antibiotics tested was two to four times higher, indicating that α-tocopherol had a superior binding affinity compared to the applied antibiotics.[26] In the group treated with vitamin E (α-tocopherol), survival rates were significantly higher compared to the untreated control group.[26] Another study investigated the significant effects of α-tocopherol on the severity of *Pseudomonas aeruginosa*-induced pneumonia. Initial treatment with α-tocopherol significantly decreased the increase in endothelial paracellular permeability mediated by *Pseudomonas aeruginosa*, a model of pneumonia induced by *Pseudomonas aeruginosa* in rats. Mice were treated with *α-tocopherol* by intraperitoneal injection before and after induction using *Pseudomonas aeruginosa* bacteria, and in the results, the application of *α-tocopherol* supplementation decreased the permeability of pulmonary histopathology, reducing the forming units colonization of *Pseudomonas aeruginosa* bacteria and the release of *myeloperoxidase*, resulting in a significant reduction in mortality in mice infected with *Pseudomonas aeruginosa* bacteria.[26]

Vitamin E and K1 as anti-quorum-sensing agents against *Pseudomonas aeruginosa* bacteria

Currently, antivirulence compounds that weaken bacterial pathogenicity and do not interfere with bacterial viability or growth are being introduced as next-generation antibacterial agents. Vitamins E and K1, as anti-quorum-sensing agents against *Pseudomonas aeruginosa*, are pathogens that are harmful to human life and are responsible for several diseases. Both vitamins showed significant anti-biofilm activity (62% and 40.3% reduction by vitamins E and K1, respectively), and the expression of virulence factors, including pyocyanin, pyoverdine, and proteases, could be significantly inhibited, especially in the presence of vitamin E.[24] Cotreatment of biofilms built with the supplementation of this vitamin plus the antimicrobial tobramycin can significantly reduce the number of protected bacterial cells within the impermeable matrix (71.6% and 69% by the combination of tobramycin and vitamin E or K1, respectively). Vitamins E and K1 can significantly suppress biofilm formation and virulence factor production by *Pseudomonas aeruginosa*. This antipathogenic effect is important to know, especially because of the important role of *Pseudomonas aeruginosa* in many opportunistic infections.[24]

Vitamin D3 increases autophagy in THP-1 cells infected with *Mycobacterium tuberculosis*

Vitamin D has been widely used to treat tuberculosis, where its active metabolite, 1,25-dihydroxy vitamin D, can increase the immune response to the invasion of *Mycobacterium tuberculosis*. In addition, macrophages infected with *Mycobacterium tuberculosis* have a high need for Ca2+. Experiment on mice at the indicated time point (7 days after infection with *Mycobacterium tuberculosis* and treatment with vitamin D3). Next, pulmonary histopathology is seen. The lungs were extracted from the mice, washed once using PBS, and fixed in a 4% solution of paraformaldehyde for 24 hours at room temperature. Lung cell tissue is grown in paraffin and sliced into 4-µm-thick sections using microtomes. The samples were then stained with H&E for 10 minutes at room temperature, and the images were taken with a microscope light (magnification x 100). MTT tests showed that vitamin D3 lowered the viability of THP-1 cells in a

dose- and time-dependent manner. Factors associated with autophagy in THP-1 cells infected with *Mycobacterium tuberculosis* were analyzed by western blotting and RT-qPCR, and the results showed that vitamin D3 significantly increased the expression levels of p62, LC3II/LC3I, Beclin-1, ATG-5, and AMPK in THP-1 cells after infection with *Mycobacterium tuberculosis* bacteria. Ca2+ concentration tests show that vitamin D3 can promote cellular autophagy by inhibiting Ca2+ concentrations. After the Balb/c mice were treated intranatally with vitamin D3, the pathological parts of the lungs infected with *Mycobacterium tuberculosis* were removed and stained using H&E. The lung tissue portion of the control group showed a clear alveolar structure with no edema, no inflammation, or no fluid infiltrating inflammatory cells in the lung tissue. However, the lung tissue of the group infected with *Mycobacterium tuberculosis* bacteria was damaged and full of inflammatory cell infiltrates and pathological changes in the lung tissue. In the *Mycobacterium tuberculosis* infection group and the vitamin D3 + *Mycobacterium tuberculosis* infection group, supplementation was administered through the nose and repeated for 7 days. Compared to the *Mycobacterium tuberculosis* bacterial infection group, vitamin D3 group + *Mycobacterium tuberculosis* infection, the level of alveolar wall damage and inflammatory infiltration in the vitamin D3 supplementation group + *Mycobacterium tuberculosis* infection decreased significantly and significantly.[31] These results suggest that vitamin D3 can reduce lung damage after *Mycobacterium tuberculosis* infection by increasing cell autophagy and suggest that the molecular mechanism of vitamin D3 increases autophagy in THP-1 cells infected by *Mycobacterium tuberculosis.*[30]

Comparison of anti-inflammatory effects of vitamin E and vitamin D in a mouse model of dextran sulfate sodium (DSS)-induced ulcerative colitis

This study aims to compare the clinical effects of vitamin E and vitamin D in a mouse model of ulcerative colitis (UC) induced with dextran sulfate sodium (DSS), and to elucidate the underlying mechanisms associated with changes in cytokine proinflammatory levels. After successful formation of DSS-induced UC mouse models, prednisolone (1 mg/kg), vitamin D (50 ng), and vitamin E (6, 30, and 150 IU/kg) were administered orally for 1 week. The mice were divided into eight groups [(G1, G3, G3, G4, G5A, G5B, G5C and G6], and the doses were summarized. Rats in G2 had more severe macroscopic inflammation than in G1. Consistent with macroscopic observations, the length of the intestine in G2 at day 8 was shorter than that of G1. On day 15, the length of the colon in G3 and G6 is longer than that in G2. Compared to G2, there is no difference in intestinal length in G4 and G5, but there is a noticeable difference in intestinal length between G6 and G2. Although the length of the colon in G6 is not as long as it is in G3, the experimental results provide new evidence for the treatment of UC. These findings suggest that combined vitamin D and vitamin E supplementation may be better than individual use or as monotherapy. The weight/length ratio of the colon (%) at G1 and G2 at day 8 was calculated. Compared to G1, the weight/length ratio of the bowel at day 8 in G2 was significantly improved than that of G1 ($F = -5.38$, $p = 0.01$), which suggests that the DSS-induced UC mouse model was successfully applied.[19]

Compared to G2, the weight/length ratio of the colon at day 15 in G3, G4, G5B and G6 was significantly lower (F 3 = 5.67, p = 0.03; F4 = 3,049; F5B = 4.08, p = 0.049; 0,048; F6 = 4.97, p = 0.04), this suggests that vitamin D and vitamin E inhibit DSS-induced colon inflammation. To determine the anti-inflammatory effects of vitamin D and vitamin E in a DSS-induced UC mouse model, five inflammatory markers (IL-6, IL-12, IL-18, TNF-α, and IFN-γ) were assessed. Compared to G1, the levels of five cytokines in G2 were significantly increased on day 8 (ftnf-α = -2.99, p = 0.03; fifn-γ = -172.53, p = 0.04; fil-18 = 16.39, p = 0.01; fil-12 = 33.76, p = 0.03; fil-6 = 2.88, p = 0.04;). On day 15, the remaining mice were retested, and their colon tissue was collected for cytokine analysis. The results showed that levels four of inflammatory cytokines (TNF-α, IL-12, IL-12, and IFN-γ) in G3 and G4 were significantly decreased compared to the G2 (TNF-α: G3 VS. G2 group: F = -3.82, p = 0.01; G4 vs G2 Group, F = -8.81, p = 0.01. IFN-γ: G3 vs. G2 Group: F = 138.62, P = 0.01; G4 vs. G2 Group, F = 122.99, p = 0.02. IL-18: G3 vs. G2 Group: F = 51.04, p = 0.01; G4 vs. G2 Group, P = 22.17, P = 0.01. IL-12: G3 vs. G2 Group: F = 42.09, p = 0.01; G4 vs. G2 Group, F = 13.78, p = 0.04.), while there was no obvious change in the IL-6 level (G3 vs. G2 Group: F = 0.66, p = 0.65; G4 vs G2 Group, F = 0.49, p = 0.81). In addition, in colon tissue, levels of four inflammatory cytokines (TNF-α, IL-18, IL-12 and IL-6) decreased in the G5B group compared to the G2 group (fil-12 = -34.97, p = 0.03; FIFN-γ = 2.86, p = 0.049; f TNF-α = -3.91, p = 0.03; f IL-6 = -8.54, p = 0.04; f il-18 = 46.19, p = 0.01), while there was no obvious change in the IFN-γ level (F = 1.75, p = 0.14). Notably, levels of five inflammatory cytokines (IL-6, IL-12, IL-18, TNF-α and IFN-γ) were significantly reduced in the G6 group compared to the rat group in the G2.[19] Group (F TNF-α = -4.77, p = 0.03; f IFN-γ = 138.66, p = 0.04; fil-12 = 42.18, p = 0.03; f IL-6 = 8.04, p = 0.01; fil-18 = 17.39, p = 0.01;). Collectively, these data show that both vitamins, either vitamin D or vitamin E, given as monotherapy, can partially reduce inflammatory cytokine levels in mice with DSS-UC, while the application of a combination using vitamin D and vitamin E supplementation shows a more pronounced inflammatory inhibitory effect[21]. These data strongly indicate that using a combination of vitamin D and vitamin E supplementation as adjuvant therapy has a clear anti-inflammatory effect on UC and may have a positive therapeutic effect in patients with inflammatory bowel disease (IBD). In conclusion, the combination of vitamin D and vitamin E is feasible and effective in current research, and vitamins D and E are relatively safe supplements that have been used clinically for a long time.[19]

Table 3. Dosage of the combination of vitamin D and E on wistar[19] rats

Group Name	Treatment	Number of Rats	Route of adminstration	Dosage of administration
G1	Control (Vehicle)	24	Drinking water	
G2	DSS (Day 1-7)	24	Dissolved in drinking water	5%
G3	DSS (Day 1-7) + prednisolone (Day 8-14)	12	Orally administrated (Day 8-14)	1.0 mg/kg
G4	DSS (Day 1-7) + Vitamin D (low dose) (Day 8-14)	12	Orally administrated (Day 8-14)	50 mg
G5a	DSS (Day 1-7) + Vitamin E (low dose) (Day 8-14)	12	Orally administrated (Day 8-14)	6 IU/kg
G5b	DSS (Day 1-7) + Vitamin E (medium dose) (Day 8-14)	12	Orally administrated (Day 8-14)	30 IU/Kg
G5c	DSS (Day 1-7) + Vitamin E (high dose) (Day 8-14)	12	Orally administrated (Day 8-14)	150 IU/Kg
G6	DSS (Day 1-7) + Vitamin D +Vitamin E (Day 8-14)	12	Orally administrated (Day 8-14)	50 mg + 30 IU/Kg

The clinical trial in humans demonstrating the effectiveness of the combination of vitamin D and E in addressing bacterial infections

A systematic review identified 13 controlled trials assessing vitamin D for infectious diseases, with five focusing specifically on bacterial infections, primarily tuberculosis (TB). These studies indicated that vitamin D supplementation could serve as an adjunctive therapy, enhancing treatment outcomes for patients with TB and other bacterial infections. In a randomized controlled trial, high-dose vitamin D3 supplementation (4000 IU daily) significantly reduced symptoms and antibiotic consumption among patients with recurrent respiratory tract infections (RTIs), suggesting its utility as a strategy to mitigate antibiotic use in susceptible populations, the currently available data from studies in humans regarding the potential value of vitamin D as adjunctive therapy in bacterial infection remain conflicting. Three of the 4 TB trials and the 1 trial of vitamin D therapy to prevent *H pylori*-related gastrointestinal disease demonstrated positive outcomes, although these studies were hampered by major limitations, such as poor sample size and limited information regarding the effectiveness of the repletion strategy.[32] The most recent and the most rigorously designed trial of the series, reported by Wejse, demonstrated no clear benefit of adjunctive vitamin D therapy in TB treatment. As discussed in the foregoing material, vitamin D administered at doses higher than the total of 300,000 IU given to the intervention group in this study, along with careful attention to potential confounding factors affecting vitamin D levels in the placebo group, may be necessary to improve the statistical power of future studies. More prospectively designed, intervention-based trials are needed for further evaluation of the relationship between adequate vitamin D repletion and treatment or prevention (or both) of bacterial infections such as TB.[33] While there is substantial evidence supporting the role of vitamin D in managing bacterial infections, research specifically examining vitamin E's effectiveness or its combination with vitamin D is scarce. Vitamin E is known for its antioxidant properties and potential immune-

modulatory effects, but direct clinical evidence linking it to improved outcomes in bacterial infections remains limited because while current clinical trials predominantly focus on vitamin D's role in addressing bacterial infections, there is a clear need for further research to explore the combined effects of vitamins D and E. Such studies could enhance our understanding of their potential as adjunctive therapies in combating bacterial infections and reducing antibiotic resistance.

The risks, side effects of vitamin D and vitamin E in contraindicated conditions

Vitamin D deficiency is therefore associated with reduced calcium absorption and hence secondary hyperparathyroidism, resulting in the recruitment of calcium from the bone to maintain adequate plasma calcium concentrations. Similarly, vitamin D toxicity is associated with increased absorption of calcium from the gut and hypercalcaemia. Furthermore, vitamin D excess may increase bone resorption, further increasing calcium levels, Koutkia *et al.*[34] reported severe hypercalcaemia and renal failure due to vitamin D toxicity in a subject taking vitamin D3 at a stated dose of 2000 IU day^{-1}, yet analysis of the medication revealed actual doses of up to 2.6 million IU day^{-1}. Another US study reported on a woman with vitamin D toxicity associated with the use of a vitamin D supplement containing 188 000 IU of cholecalciferol rather than the stated dose of 400 IU.[35] Benemei *et al.* reported three cases of vitamin D intoxication with severe hypercalcaemia, where the patients had been treated with a vitamin D formulation with a stated dose of 600 IU, whereas the actual content was 52 800 IU. The causes of vitamin D toxicity appear to be multiple and include the use of unlicensed and poorly manufactured products. In addition, there is widespread availability and inappropriate use of high-dose over-the-counter supplements, and prescribing errors arising from the injudicious use of high-dose supplements. Prescribers and dispensers need to appreciate the potential dangers to their patients and, wherever possible, mitigate against causing them harm. While on Vitamin E, it may lead to vitamin E toxicity, most notably manifested in an increased risk of bleeding. Vitamin E deficiency is rare and is characterized by the development of progressively worsening peripheral neuropathy, ataxia, and hyporeflexia. It may develop in patients with difficulty absorbing fat, such as cystic fibrosis, Crohn's disease, and chronic pancreatitis. It has been explored in osteoporosis as an antioxidant and anti-inflammatory with mixed benefits. In diabetes, alpha-tocopherol was found to help with wound healing. In inflammatory diseases, such as asthma, Crohn's, and rheumatoid arthritis, it has been shown that tocotrienols are more effective compared to alpha-tocopherols at decreasing inflammation. In patients exposed to large amounts of radiation, vitamin E is effective in helping hematopoiesis after radiation. “Toxic” vitamin E levels do not concentrate in the body in the same fashion as other fat-soluble vitamins like vitamins A and D. Although vitamin E is a lipid-soluble vitamin, excess alpha-tocopherols are excreted in the urine and bile, thereby decreasing the accumulation of circulating alpha-tocopherols. As a result, toxic effects are not observed in most healthy patients taking less than 1000 mg of vitamin E daily.[36]

The role of vitamin D in modifying outcomes after surgery

Vitamin D supplementation could potentially improve postoperative outcomes. However, studies correlating vitamin D levels with outcomes are often subject to considerable confounding and selection bias, given that several risk factors for hypovitaminosis D are also associated with patient outcomes (eg, obesity and frailty). As such, observational associations between vitamin D levels and postoperative outcomes do not necessarily imply that vitamin D supplementation will result in improved outcomes. Consequently, there has recently been interest in the potential benefits of vitamin D supplementation for critically ill patients. A recent meta-analysis assessed the impact of vitamin D supplementation in critically ill patients and found no evidence of a significant survival benefit, but did find a significant reduction in the length of hospital stay.[37] Additionally, a large-scale clinical trial in clinically ill, vitamin D-deficient patients found no significant benefit of vitamin D supplementation for any clinical outcomes assessed. A similar investigation into the effect of vitamin D supplementation on the population of patients undergoing surgery was warranted. the postoperative mortality rate in these studies was generally low, with a total of only 12 deaths in 631 patients. As such, all four studies were considerably underpowered to be able to detect a clinically meaningful difference in this outcome, leading to an inflated false-negative rate. Consequently, there were insufficient data to draw any reliable conclusions on the association between vitamin D supplementation and postoperative mortality. A meta-analysis of this outcome was not performed on account of the variability in the time at which postoperative mortality was assessed. Surgical mortality has traditionally been assessed at arbitrary intervals out to 1 year without an agreed-upon optimum time point, as evidenced by the fact that all four studies reporting this outcome measured it at different time points. It has been suggested that surgery has the greatest impact on the risk of death when assessed closest to the time of the operation, It has been suggested that vitamin D status at the time of surgery is the most relevant predictor of long-term outcomes, compared with vitamin D status in the days to weeks after surgery and that minimal benefits can be gained by supplementation at the time of surgery.[38]

SUMMARY

The synergistic effects of vitamin D and vitamin E in preventing and managing bacterial infections. Vitamin D enhances immune responses by promoting antimicrobial peptides, regulating cytokines, and supporting epithelial barriers, while vitamin E acts as a powerful antioxidant that reduces oxidative stress and inflammation. Includes numerous animal studies especially involving *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Mycobacterium tuberculosis.* demonstrating that the combined supplementation of these vitamins reduces bacterial load, lowers pro-inflammatory cytokines, promotes healing, and improves survival outcomes. The findings suggest that vitamins D and E, through their complementary immune-modulating and anti-inflammatory mechanisms, hold promise as safe and effective adjunct therapies against bacterial infections, especially in the face of growing antibiotic resistance.

REFERENCES

1. Doron S, Gorbach SL. Bacterial Infections: Overview [Internet]. Heggenhougen HK (Kris), editor. ScienceDirect. Oxford: Academic Press; 2008. p. 273–82.
2. Fadrian. ANTIBIOTIK, INFEKSI DAN RESISTENSI. Pertama. Hidayat S, editor. Padang: Andalas University Press ; 2023.
3. Taylor TA, Unakal CG. *Staphylococcus Aureus* Infection [Internet]. National Library of Medicine. StatPearls Publishing; 2023.
4. Audran M, Kumar R. The Physiology and Pathophysiology of Vitamin D. Mayo Clinic Proceedings [Internet]. 1985 Dec 1;60(12):851–66.
5. PubChem. Cholecalciferol. Nih.gov. PubChem; 2019.
6. Ardiaria M. Peran Vitamin D Dalam Pencegahan Influenza Dan Covid-19. Actanutrica. 2020 May 22;8(2):79–85.
7. Liang H, Wang Y, Liu F, Duan G, Long J, Jin Y, et al. The Application of Rat Models in *Staphylococcus aureus* Infections. Pathogens [Internet]. 2024 May 21 [cited 2024 Sep 12];13(6):434–4.
8. Yisak H, Ewunetei A, Kefale B, Mamuye M, et al. Effect of Vitamin D on COVID-19 infection and Prognosis : A systematic review. Risk Management and Healthcare Policy. 2021 Jan; Volume 14:31-8
9. Bikle D. Vitamin D: Production, Metabolism, and Mechanisms of Action [Internet]. Nih.gov. MDText.com, Inc.; 2017.
10. Amrein K, Scherkl M, Hoffmann M, Neuwersch-Sommeregger S, Köstenberger M, Tmava Berisha A, et al. Vitamin D deficiency 2.0: an update on the current status worldwide. European Journal of Clinical Nutrition. 2020 Jan 20;74(11):1–16.
11. Vitamin E [Internet]. Mims.com. 2022 [cited 2024 Sep 11]. Available from:https://mims.com/indonesia/drug/info/vitamin%20e?mtype=generic
12. Mohd Zaffarin AS, Ng SF, Ng MH, Hassan H, Alias E. Pharmacology and Pharmacokinetics of Vitamin E: Nanoformulations to Enhance Bioavailability. International Journal of Nanomedicine. 2020 Dec;Volume 15:9961–74.
13. PubChem. Vitamin E [Internet]. Nih.gov. PubChem; 2019. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Vitamin-E
14. Irawan, Alvian Oscar. Pengaruh Vitamin D3 terhadap Kadar IL-6, HS-CRP, dan Tekanan Darah pada Tikus Putih Jantan Model Penyakit Ginjal Kronis Unilateral Obstruction (UUO). DISERTASI UNIVERSITAS NEGERI SEBELAS NOVEMBER [Internet]. 2024
15. Alsufiani HM, AlGhamdi SA, AlShaibi HF, Khoja SO, Saif SF, Carlberg C. A Single Vitamin D3 Bolus Supplementation Improves Vitamin D Status and Reduces Proinflammatory Cytokines in Healthy Females. Nutrients [Internet]. 2022 Sep 24 [cited 2024 Sep 13];14(19):3963–3
16. M Sakarya, Fo Eris, Ab Derbent, Koca U, S Tüzün, T Onat, et al. The antioxidant effects of vitamin C and vitamin E on oxidative stress. Clinical intensive care. 1999 Dec 1;10(6):245–50.
17. Holmes NE, Charles PGP. Safety and Efficacy Review of Doxycycline. Clinical Medicine Therapeutics [Internet]. 2009 Jan;1:CMT.S2035

18. Mustafa Atli, Mehmet Erikoglu, Adnan Kaynak, Esen HH, Kurban S. *The effects of selenium and vitamin E on lung tissue in rats with sepsis. Clinical and investigative medicine*. 2012 Apr 1;35(2):48–8.
19. Fan X, Yin J, Yin J, Weng X, Ding R. Comparison of the anti-inflammatory effects of vitamin E and vitamin D on a rat model of dextran sulfate sodium-induced ulcerative colitis. Experimental and Therapeutic Medicine. 2023 Jan 13;25(2).
20. Olliver M, Spelmink L, Hiew J, Meyer-Hoffert U, Henriques-Normark B, Bergman P. Immunomodulatory Effects of Vitamin D on Innate and Adaptive Immune Responses to *Streptococcus pneumoniae*. The Journal of Infectious Diseases. 2013 Aug 6;208(9):1474–81.
21. Charoenngam N, Holick MF. Immunologic Effects of Vitamin D on Human Health and Disease. Nutrients [Internet]. 2020 Jul 15;12(7):2097.
22. Sîrbe C, Rednic S, Grama A, Pop TL. An Update on the Effects of Vitamin D on the Immune System and Autoimmune Diseases. International Journal of Molecular Sciences [Internet]. 2022 Aug 29;23(17):9784.
23. Hartmann Ms, Mousavi S, Bereswill S, Heimesaat Mm. Vitamin E as promising adjunct treatment option in the combat of infectious diseases caused by bacterial including multi-drug resistant pathogens – Results from a comprehensive literature survey. European Journal of Microbiology & Immunology [Internet]. 2020 Nov 5 10(4):193–201.
24. Soltani S, Sedigheh B, Farzin Hadizadeh, Fatemeh Roodbari, Vahid Soheili. New Insight into Vitamins E and K1 as Anti-Quorum-Sensing Agents against *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy. 2021 May 18;65(6).
25. Camargo CA, Sluyter J, Stewart AW, Khaw KT, Lawes CMM, Toop L, et al. Effect of Monthly High-Dose Vitamin D Supplementation on Acute Respiratory Infections in Older Adults: A Randomized Controlled Trial. Clinical Infectious Diseases. 2019 Aug 17;
26. Olszowiec-Chlebna M, Koniarek-Maniecka A, Brzozowska A, Błauż A, Rychlik B, Stelmach I. Vitamin D inhibits pro-inflammatory cytokines in the airways of cystic fibrosis patients infected by *Pseudomonas aeruginosa*- pilot study. Italian Journal of Pediatrics. 2019 Mar 29;45(1).
27. Bou Ghanem EN, Lee JN, Joma BH, Meydani SN, Leong JM, Panda A. The Alpha-Tocopherol Form of Vitamin E Boosts Elastase Activity of Human PMNs and Their Ability to Kill *Streptococcus pneumoniae*. Frontiers in Cellular and Infection Microbiology. 2017 May 3;7.
28. Muharraran F, Gusbakti Rusip, Rosa Paradita Dalimunthe. Potential of Hydrogel Acemannan Aloe Vera (Aloe vera) on Wound Healing After Tooth Extraction In vivo Via Regulation of Inflammatory Response. Bioscmed [Internet]. 2022Apr.25;6(6):1908-13
29. Siregar, Angraini, et al. "Uji Aktivitas Antibakteri Ekstrak Etanol Daun Pegagan (Centella Asiatica (L.) Urb) pada Bakteri S. Aureus." *Pharmasipha*, vol. 6, no. 1, 31 Mar. 2022, pp. 21-28

30. Wu, Y., Lin, X., Song, F., Xue, D., Wang, Y."Vitamin D3 promotes autophagy in THP-1 cells infected with *Mycobacterium tuberculosis* Experimental and Therapeutic Medicine 23, no. 3 (2022): 240. https://doi.org/10.3892/etm.2022.11
31. Wu, D., Zhou, S., Hu, S., & Liu, B. (2017). Inflammatory responses and histopathological changes in a mouse model of *Staphylococcus aureus*-induced bloodstream infections. *Journal of infection in developing countries*, *11*(4), 294–305. https://doi.org/10.3855/jidc.7800
32. Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocr Pract. 2009 Jul-Aug;15(5):438-49. doi: 10.4158/EP09101.ORR. PMID: 19491064; PMCID: PMC2855046.
33. Wejse C. Vitamin D as supplementary treatment for tuberculosis—a double-blind randomized, placebo-controlled trial. Am J Respir Crit Care Med. 2009;179:843–850. doi: 10.1164/rccm.200804-567OC
34. Koutkia P, Chen TC, Holick MF. Vitamin D intoxication associated with an over-the-counter supplement. N Engl J Med 2001; 345: 66–67.
35. Benemei S, Gallo E, Giocaliere E, Bartolucci G, Menniti-Ippolito F, Firenzuoli F, *et al* It's time for new rules on vitamin D food supplements. Br J Clin Pharmacol 2013; 76: 825–826.
36. Traber MG. Vitamin E inadequacy in humans: causes and consequences. Adv Nutr. 2014 Sep;5(5):503-14
37. Patel A, Caruana EJ, Hodson J, Morrison R, Khor B, Gysling S, Trevis J, Mangel T, Benson R, Zakeri R, Manders J, Vaja R, Rogers L, Baker P, Pournaras DJ, Thickett D, Hewison M, Naidu B, Lim E. Role of vitamin D supplementation in modifying outcomes after surgery: a systematic review of randomised controlled trials. BMJ Open. 2024 Jan 17;14(1):e073431. doi: 10.1136/bmjopen-2023-073431. PMID: 38233048; PMCID: PMC10806719.
38. Iglar PJ, Hogan KJ. Vitamin D status and surgical outcomes: a systematic review. Patient Saf Surg 2015;9:14. 10.1186/s13037-015-0060-y