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Role of calcitriol in e.coli induced intra abdominal sepsis model of wistar rats

Dr Ashok Ku Panigrahi — Associate professor 1Dept of Pharmacology ,VSS Institute of medical science and research ,Burla-768017

Dr Sangeeta Mishra — Senior resident Dept of Obstetrics & gynecology, NRI institute of medical sciences ,Visakhapatnam, Andhrapradesh ,India – 531162

Dr Prasanna Pradhan — Assistant professor Central Research laboratory, NRI institute of medical sciences ,Visakhapatnam, Andhrapradesh ,India – 531162

ABSTRACT

OBJECTIVE: To asses the role of calcitriol in E.coli induced abdominal sepsis in wistar rats.Here we will examine the evidence behind the claims that vitamin D status could affect the course of infection.

MATERIAL AND METHOD: Wistar rats weighing 100-150 gm were taken for the study and divided into four groups. All groups were treated intraperitoneally , Group 1 recieved 0.9%Saline Group 2 recieved Calcitriol i.p Group 3 received only E.Coli bacterial suspension intraperitoneally whereas Group 4 recieved Calcitriol with E.coli bacterial suspension. Developement of symptoms were recorded after 6 hours. For liver function tests (ALP,AST) and peritoneal wash the samples were taken for PMN and Monocyte count at time intervals of 0, 24, 48 and 168 hours following infection. Subsequent changes are also observed.
RESULT: All test animals survived as compared to 25% of only E.coli treated control at 72 hours. PMN and monocyte count remain low in test group 4 as compared to E.coli treated groups. Physical signs significantly seen within observed period and blood parameters slightly increased or affected as compared to other controls.
CONCLUSION: This study is suggestive regarding role of calcitriol in prevention of E.coli . But Further studies are required to prove whether full protection is attributed to calcitriol or not.

KEYWORDS Calcitriol , Vitamin-D, abdominal sepsis ,*E.Coli*

Introduction

Calcitriol(1 ,25-dihydroxycholecalciferol) is a synthetic vitamin D analog which is active in the regulation of the absorption of calcium from the gastrointestinal tract and its utilization in the body. Several In vitro studies proved that vitamin D_3 has inhibitory activity on strains of Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli (E. coli) and other bacteria. In the presence of 50,000–90,000 IU/mL of vitamin D_3, the organisms were killed or demonstrated marked growth inhibition[1]. Gram-positive bacteria, invasive pneumococcal disease, meningococcal disease and group A streptococcal disease are more common when vitamin D levels are low, raising the possibility that pharmacological doses of vitamin D could be an effective adjuvant therapy.[2]

A number of epidemiologic studies using vitamin D status or season as the exposure have found an inverse association between vitamin D and incidence of several infections, including influenza [3], upper respiratory tract infection [4-6], HIV infection [7], and bacterial vaginosis [8]. Recent cross-sectional studies have attempted to determine whether vitamin D status was associated with serum levels of cathelicidin. Jeng et al. examined a cohort of critically ill patients with and without sepsis and healthy controls and found a weak but statistically significant positive association between serum 25(OH)D and LL-37/ hCAP18 (cathelicidin) [9]. Gombart et al. found that serum hCAP18 was associated with increased mortality from infection in patients with end-stage renal disease [10]. They did not find an association between 25(OH)D and LL-37/hCAP18(cathelicidin) likely due to the finding that 80% of the subjects had vitamin D insufficiency; however, there was a borderline (p=0.053) association between serum $1,25(OH)_2D$ and hCAP18 [10].

Several randomized controlled trials have been conducted to examine whether vitamin D supplementation would reduce the risk of disease from viral, bacterial, fungal and protozoan infections [11]. However, given the heterogeneity in the dose, sample population, and duration of vitamin D therapy of the trials reviewed, there was insufficient data to conclusively state that vitamin D supplementation could result in lowered infection rate [11]. A meta-analysis comparing 25(OH)D concentrations in TB-infected subjects to healthy controls found a higher risk of vitamin D deficiency in TB-infected subjects [12]. Recent studies have been inadequate in dosing of vitamin D, including a large randomized controlled trial of TB-infected patients where both control and vitamin D treatment groups had similar 25(OH)D concentrations at the end of the study [13]. To determine whether vitamin D supplementation could raise cathelicidin levels in humans, Adams et al. gave osteoporotic women 50,000 IU of vitamin D_2 twice weekly for 5 weeks and found no change in serum cathelicidin levels; however, cathelicidin mRNA expression in peripheral blood monocytes was increased after high-dose vitamin D supplementation[14]. Larger doses and more rapid dosing of vitamin D are likely required to up-regulate cathelicidin expression in response to infection in humans [15]. Furthermore, levels of cathelicidin are likely to be induced in barrier sites as opposed to in the systemic circulation for localized infections.

Facts behind formulating and testing the hypothesis

Studies of a number of Gram-negative pathogens in mouse infection models have shown a role for TLR4(Toll like receptor-4), including Neisseria meningitides, E. coli, Haemophilus influenzae, Klebsiella pneumoniae, and Brucella abortus [16]. Mouse models have also shown that TLR4 is important for infection with other pathogens, including S. pneumoniae and Mycobacteria tuberculosis [16]. TLR4 has also been linked to several viral infections, including respiratory syncytial virus [17], the murine retroviruses, mouse mammary tumor virus and murine leukemia virus [18], as well as the picornavirus Coxsackievirus B4.[19]. T2.5 (TLR-2 blocking antibody) in combination with an anti-TLR4/MD-2 antibody, protects mice against sepsis induced by Salmonella enterica or E. coli when given with antibiotics [20].

TLR2/1 activation leads to vitamin D3-dependent antimycobacterial activities. TLR2/1/CD14 stimulation by mycobacterial lipoprotein LpqH can activate antibacterial autophagy through activating VDR signaling and inducing cathelicidin.[21] Vitamin D has been shown to regulate TLR-mediated events in multiple cell types. In neutrophils, 1,25(OH)2D3 suppressed the ability of LPS to induce IL-1 expression as well as inhibited some antimicrobial genes.[22] More recently, 1,25(OH)2D3 has been shown to induce the expression of antimicrobial peptides (cathelicidin and -defensin) in innate immune cells stimulated through TLR receptors, including monocytes, neutrophils and keratinocytes.[23] Stimulation through TLRs in the presence of vitamin D inhibits inflammatory cytokine production .

Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. (24).Vitamin D3 suppresses the expression of TLR2 and TLR4 protein and mRNA in human monocytes in a time- and dose-dependent fashion. Reduced TLR levels in 1,25(OH)(2)D3-treated phagocytes were accompanied by impaired NF-kappaB/RelA translocation to the nucleus and by reduced p38 and p42/44 (extracellular signal-regulated kinase 1/2) phosphorylation upon TLR-ligand engagement. 1,25(OH)(2)D3 primes monocytes to respond less effectively to bacterial cell wall components in a VDR-dependent mechanism, most likely due to decreased levels of TLR2 and TLR4.[24]

Again it was found in many studies that despite a decrease in mortality in the last decade, sepsis remains the tenth-leading cause of death in western countries [25] and one of the commonest causes of death in intensive care units. Mortality in adult intensive care units may be partially linked to severe systemic inflammatory responses and sepsis [7]. Vitamin D status may determine AMP levels in patients with sepsis in the intensive care unit[7]. The epidemiology of septicemia in the United States and the variations of solar UVB, as well as the effects of vitamin D, support the hypothesis that both play important roles in reducing the risk of septicemia.[26] The risk of diseases comorbid with septicemia are generally inversely correlated with serum 25(OH)D levels. .[26] Grant also demonstrated that vitamin D supplementation of mother and infant can reduce the risk of sepsis in infants [27] and neonates.[28] Activation of TLR4, the receptor for gram-negative bacteria's outer membrane lipopolysaccharide or endotoxin, may play a potential role in determining outcomes. Vitamin D, through its modulation, may have a role as adjunctive therapy in severe sepsis and septic shock.[25] In veterans admitted to the intensive care unit, higher mortality and a longer stay were significantly linked to lower vitamin D status.[29] A total of 17% of the intensive care patients in one study had undetectable levels of vitamin D,[30] which may predispose to hypocalcemia. Zaloga et al.[31] found that 20% of critically ill patients with bacterial sepsis had hypocalcemia and that their mortality rate was significantly higher (50%) than that of normocalcemic patients with sepsis (29%). Studies in knockout mice have indicated a role for TLR4 in protection against endotoxemia also. Here we will examine the evidence behind the claims that vitamin D status could affect the course of infection.

MATERIAL AND METHOD

Animals. Adult male Wistar rats weighing 200 to 300 g each were used. All animals were housed in individual polypropylene cages under constant temperature (22°C±2°C) and humidity with a 12-h light/12-h dark cycle and had access to food and water as much as desired throughout the study. Animals were acclimatized to laboratory conditions for 7 days before experiments were carried out. Except the drug under study, no topical, systemic or oral therapy of any other drug was given to the animals. Animals were kept in aseptic condition and as much possible bacteria free environment was maintained. Sterile techniques were used to handle the animals. The study was conducted under guidline of international ethical rules and approved by the Institutional Ethical Committee, VSS Medical College,Burla,Odisha,India.

Experimental design.

A biological model of Intra-abdominal sepsis in rats [32]was adapted to test the effect of calcitriol .Intraabdominal sepsis model chosen because Microbial pathogenes within the host's intraabdominal cavity encounter three basic defense mechanisms: removal of particles through the lymphatic system; in situ elimination of the bacteria, by opsonization and phagocytosis; kidnapping of the bacteria, thus preventing its export to the bloodstream, through the products of the inflammatory response, whose fibrinogen-rich exudates retains the bacteria on its meshes[33]

Animal grouping for tests: Rats were divided into 4 groups of 4 animals in each group.

Group 1: 1.5 ml of 0.9%Saline treated control.
Group 2: Calcitriol 0.5µg once daily(qd) ×2,weekly i.p treated control
Group 3: Inoculated with only E.Coli bacterial suspension intraperitoneally.
Group 4: Calcitriol 0.5µg once daily(qd) ×2,weekly i.p treated with E.coli bacterial suspension.

Animals of group 2,4 were treated with calcitriol for three consecutive weeks.and E.coli was inoculated to group 3 and group 4 24 hour after the last dose of calcitriol.

Calcitriol Pre-treatment :

The animals of group2 and group 4 were i.p treated with calcitriol alone [8 µg/kg per day, once daily (qd) × 2, weekly].The equivalent dose was calculated for rat that is sufficient to show antitumour activity.

Inoculation with E. coli

The lethal doses (LD50) of E.coli was taken as per the model provided by damy et al.2002 . The bacteria was cultivated in heart-brain agar. For preparation of the suspensions, the bacteria were collected during the exponential growth stage and diluted in sterile 0,9% saline. Adjustment of the final concentration was achieved by Mac Farlands Scale. Control of units forming colonies (UFC/ml) was performed by the seeding of 10 µl of the bacteria suspended in Mac Conkey agar, and by reading after 18 - 24 hours of incubation, at 37°C. In parallel, and constituting the controls, groups of rats were inoculated with 1.5 ml of saline, via intra-peritoneal route. The animals were observed daily, for registrations of symptoms and deaths. All survivors were killed in ether chamber, after 7 days.

Measuring leukocytes in the peritoneal wash

PMN and MN study from abdominal cavity, male and female rats were inoculated with 1 LD 50 of E. coli suspension. For liver function tests, at time intervals of 0, 24, 48 and 168 hours following infection, rats were anaesthetized with an association of Ketamine 25mg/kg, intramuscularly, and blood samples collected, by cardiac puncture. The same animals were then killed in carbon dioxide chamber and had 10 ml of saline injected into the peritoneal cavity. This dilution factor was included on the final calculation. After massaging the abdominal wall for one minute, a Pasteur pipette was introduced in the midline for the collecting of peritoneal wash. Total leukocytes count was performed on cells counter. Samples were centrifuged for five minutes at 2000 rpm, supernatant was despised, and the sediment re-suspended in 0.5 ml of saline was homogenized and distributed in two slides. Cells were stained by the Leishmann method after drying, 100 cells were counted, and neutrophils (PMN) and monocytes (MN) were differentiated. Final calculation of number of cells at each collecting point was achieved by multiplying the number of PMN or MN by the total number of cells registered on the counter, followed by division of the result by 100. In parallel, control groups of males and females were inoculated with 1.5 ml of saline and submitted to the same procedures described for the infected groups of animals.

Laboratory Tests

Serum calcium, phosphorus, magnesium and alkaline phosphatase and 24-hour urinary calcium and phosphorus were determined periodically. During the initial phase of the medication,

serum calcium and phosphorus determined more frequently (twice weekly).

Liver function tests

From the described, same groups of animals, serum levels of AP, AST and ALT were determined by analyses of enzyme activities using the Cobas-Mira/Plus* (Roche, Germany) automated system. The collected blood was centrifuged at 2000 rpm for 10 minutes, serum was separated in tubes and stored at -200 C.

RESULT

Wistar rats Group-3(only E.coli), six hours after E. coli inoculation, showed the following symptoms: Piloerection, Hyperpnea Decreased motor activity but no such symptoms found in Group-4(calcitriol +E.coli) at 6,8,10,12,16,20,24 hours.

Regarding neutrophil count ,In the group-3 of E. coli infected rats the PMN cells count was higher than the count of PMN cells in the GR-4

Table-1 Polymorphonuclear leucocyte cell count.

	0 hours	**24 hours**	**48 hours**	**≥48 and ≤168 hours**
Group-1(n=8)	$1.67x10^5$ ±0.41	$1.8x10^5$ ±0.32	$1.47x10^5$ ±0.23	$1.4x10^5$ ±0.10
Group-2(n=8)	$1.82x10^5$ ±0.3	$1.6x10^5$ ±0.35	$1.33x10^5$ ±0.15	$1.3x10^5$ ±0.1
Group-3(n=8)	$1.75x10^5$ ±0.38	$3.3x10^5$±0.59	$4.87x10^5$±0.42	$2.77x10^5$±0.15
Group-4(n=8)	$1.87x10^5$ ±0.31	$1.9x10^5$±0.4	$1.33x10^5$± 0.13	$1.78x10^5$±0.31
	P=0.7	P=0.0001	P=0.0046	P=0.0007

Table- 2 is showing the monocyte count is also reduced in th vita D treated group as compared to e.coli alone. Values of neutrophil and monocyte count of both e.coli and vita D treated group are significantly reduced as compared to e.coli treated group alone.

Table-2 Monocyte count

	0 hours	24 hours	48 hours	≥ 48 and ≤168 hours
Group-1(n=8)	$0.55X10^4$±0.11	$0.67X10^4$±0.11	$0.51X10^4$±0.08	$0.46X10^4$±0.07
Group-2(n=8)	$0.65X10^4$±0.10	$0.45X10^4$±0.09	$0.47X10^4$±0.1	$0.41X10^4$±0.08
Group-3(n=8)	$0.45X10^4$±0.2	$1.35X10^4$±0.18	$1.52X10^4$±0.19	$0.92X10^4$±0.12
Group-4(n=8)	$0.33X10^4$ ±0.1	$0.61X10^4$±0.08	$0.62X10^4$±0.08	$0.4X10^4$±0.07
	P=0.0001	P=0.0005	P=0.001	P=0.0003

Table-3 Alkaline Phosphotase(ALP) level (Values are in U/dL)

	0 hours	24 hours	48 hours	≥48 and ≤168 hours
Group-1(n=8)	6.5±0.93	6.12±0.63	6.01±0.59	5.93±0.30
Group-2(n=8)	5.9±0.68	6.8±0.72	5.87±0.38	6.76± 0.67
Group-3(n=8)	7.2±0.87	7.5±0.75	7.11±0.54	10.6± 0.56
Group-4(n=8)	6.9±0.55	6.2±0.81	6.15± 0.6	7.36 ± 0.45
	P=0.0151	P= 0.0023	P=0.0003	P= 0.0009

Table-4 Aspartate transaminase (AST) level (values are U/dL)

	0 hours	24 hours	48 hours	≥48 and ≤168 hours
Group-1(n=8)	21.8±1.21	24.6± 1.4	25.31± 1.37	23.8± 1.03
Group-2(n=8)	34.9±1.76	30.07±1.9	30.75±2.57	31.8± 1.76
Group-3(n=8)	23.7±1.25	32.33±2.13	40.46± 2.3	47.22±2.55
Group-4(n=8)	31.9± 2.18	33.7±1.9	32.26± 2.51	30.82± 1.76
	P=0. 0008	P=0.0001	P=0.0003	P=0.0023

P<0.05 is considered as significant

In table-3 and table-4 the AST level values significantly reduced in group 4 as compared to group 3.

There is observable increase in leucocyte migration in terms of neutrophil and monocyte in e.coli treated group-3 animals as compared to group-4(calcitriol + e.coli) AST and ALP levels also significantly changed in group-3 and group 4.

DISCUSSION AND CONCLUSION

In our present study the leucocyte count was significantly reduced in Group-4 as compared to e.coli treated groups. Many In vitro studies proved vitamin D3 has inhibitory activity on strains of Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Escherichia coli (E. coli) and other bacteria. In the presence of 50,000–90,000 IU/mL of vitamin D3, the organisms were killed or demonstrated marked growth inhibition.1 Gram-positive bacteria, invasive pneumococcal disease, meningococcal disease and group A streptococcal disease are more common when vitamin D levels are low, raising the possibility that pharmacological doses of vitamin D could be an effective adjuvant therapy.2

Despite a decrease in mortality in the last decade, sepsis remains the tenth-leading cause of death in western countries25 and one of the commonest causes of death in intensive care units. Mortality in adult intensive care units may be partially linked to severe systemic inflammatory responses and sepsis.9 Vitamin D status may determine AMP levels in patients with sepsis inthe intensive care unit.9 The epidemiology of septicemia in the United States and the variations of solar UVB, as well as the effects of vitamin D, support the hypothesis that both play important roles in reducing the risk of septicemia.26 The risk of diseases comorbid with septicemia are generally inversely correlated with serum 25(OH)D levels.26 Grant also demonstrated that vitamin D supplementation of mother and infant can reduce the risk of sepsis in infants27 and neonates.28 Activation of TLR4, the receptor for gram-negative bacteria's outer membrane lipopolysaccharide or endotoxin, may play a potential role in determining outcomes. Vitamin D, through its modulation, may have a role as adjunctive therapy in severe sepsis and septic shock.25 In veterans admitted to the intensive care unit, higher mortality and a longer stay were significantly linked to lower vitamin D status.29 A total of 17% of the intensive care patients in one study had undetectable levels of vitamin D,30 which may predispose to hypocalcemia.31 found that 20% of critically ill patients with bacterial sepsis had hypocalcemia and that their mortality rate was significantly higher (50%) than that of normocalcemic patients with sepsis (29%).

A major component of the antimicrobial action of Vitamin D is through the production of peptides which have antimicrobial as well as anti-endotoxin activity. Vitamin D stimulates the expression of potent antimicrobial peptides, such as cathelicidin and defensin34,35 which exist in neutrophils, monocytes, natural killer (NK) cells and epithelial cells lining the respiratory tract.36 Macrophages, lymphocytes and monocytes have VDRs that, with 25(OH)D stimulation, increase the expression of these antimicrobial peptides.37,38 .In our study also found the migration of neutrophils and monocytes decreased significantly in

vita D and E.coli treated group as compared to e.coli alone. In sepsis liver enzymes also altered but by giving vitamin D the liver enzyme elevation also reduced.

Vitamin D is emerging as an important and cost-effective option in the therapeutic options in reducing many infections either as a sole agent or as an adjunct to current antimicrobial agents. Further research is needed to prove the exact mechanism.

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REFERENCES

1 Feindt E, Stroder J. Studies on the antimicrobial effect of vitamin D. Klin Wochenschr 1977; 55:507-8.

2. Cannell JJ, Hollis BW. Use of vitamin D in clinical practice. Altern Med Rev 2008; 13:6-20.

3. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, et al. Epidemic influenza and vitamin D. Epidemiol Infect. 2006;134(6):1129–1140.

4. Laaksi I, Ruohola JP, Tuohimaa P, Auvinen A, Haataja R, Pihlajamaki H, et al. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr. 2007;86(3):714–717.

5. Cannell JJ, Vieth R, Willett W, Zasloff M, Hathcock JN, White JH, et al. Cod liver oil, vitamin A toxicity, frequent respiratory infections, and the vitamin D deficiency epidemic. Ann Otol Rhinol Laryngol. 2008;117(11):864–870.

6. Ginde AA, Mansbach JM, Camargo CA., Jr Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med. 2009;169(4):384–390.

7. Villamor E. A potential role for vitamin D on HIV infection? Nutr Rev. 2006;64(5 Pt 1):226–233.

8 Bodnar LM, Krohn MA, Simhan HN. Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. J Nutr. 2009;139(6):1157–1161.

9. Jeng L, Yamshchikov AV, Judd SE, Blumberg HM, Martin GS, Ziegler TR, et al. Alterations in vitamin D status and antimicrobial peptide levels in patients in the intensive care unit with sepsis. J Transl Med. 2009;7:28.

10. Gombart AF, Bhan I, Borregaard N, Tamez H, Camargo CA, Jr, Koeffler HP, et al. Low plasma level of cathelicidin antimicrobial peptide (hCAP18) predicts increased infectious disease mortality in patients undergoing hemodialysis. Clin Infect Dis. 2009;48(4):418–424.

11. 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;15(5):438–449.

12. Nnoaham KE, Clarke A. Low serum vitamin D levels and tuberculosis: a systematic review and meta-analysis. Int J Epidemiol. 2008;37(1):113–119.

13. Wejse C, Gomes VF, Rabna P, Gustafson P, Aaby P, Lisse IM, et al. Vitamin D as supplementary treatment for tuberculosis: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med. 2009;179(9):843–850.

14. Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, et al. Vitamin d-directed rheostatic regulation of monocyte antibacterial responses. J Immunol.2009;182(7):4289–4295.

15. Yamshchikov AV, Oladele A, Leonard MK, Jr, Blumberg HM, Ziegler TR, Tangpricha V. Vitamin D as Adjunctive Therapy in Refractory Pulmonary Tuberculosis: A Case Report. South Med J. 2010 in press

16. Schnare M, Rollinghoff M, and Qureshi S (2006) Toll-like receptors: sentinels of host defence against bacterial infection. Int Arch Allergy Immunol 139:75–85.

17. Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, Walsh EE, Freeman MW, Golenbock DT, Anderson LJ, et al. (2000) Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol 1:398–401.

18. Rassa JC, Meyers JL, Zhang Y, Kudaravalli R, and Ross SR (2002) Murine retroviruses activate B cells via interaction with toll-like receptor 4. Proc Natl Acad Sci U S A 99:2281–2286.

19. Triantafilou K and Triantafilou M (2004) Coxsackievirus B4-induced cytokine production in pancreatic cells is mediated through toll-like receptor 4. J Virol 78: 11313–11320.

20. Spiller S, Elson G, Ferstl R, Dreher S, Mueller T, Freudenberg M, Daubeuf B, Wagner H, and Kirschning CJ (2008) TLR4-induced IFN-gamma production increases TLR2 sensitivity and drives Gram-negative sepsis in mice. J Exp Med 205:1747–1754.

21. Shin DM, Yuk JM, Lee HM, Lee SH, Son JW, Harding CV, et al. Mycobacterial Lipoprotein Activates Autophagy via TLR2/1/CD14 and a Functional Vitamin D Receptor Signaling. Cell Microbiol 2010; 12:1648-65.

22. Takahashi K, Nakayama Y, Horiuchi H, Ohta T, Komoriya K, Ohmori H, Kamimura T. Human neutrophils express messenger RNA of vitamin D receptor and respond to 1alpha,25-dihydroxyvitamin D3. Immuno pharmacol Immunotoxicol 2002;24 :335–47

23. Liu PT, Modlin RL. Human macrophage host defense against Mycobacterium tuberculosis. Curr Opin Immunol 2008;20:371–6

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24. Wessner B, Laggner U, Ploder M, Tamandl D, Friedl J, Zügel U, Steinmeyer A, Pollak A, Roth E, Boltz-Nitulescu G, Spittler A.Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns:European Journal of Immunology:Volume 36, Issue 2, pages 361–370, 2006

25. Wittebole X, Castanares-Zapatero D, Laterre PF. Tolllike receptor 4 modulation as a strategy to treat sepsis. Mediators Inflamm 2010; 2010:568396.

26. Grant WB. Solar ultraviolet-B irradiance and vitamin D may reduce the risk of septicemia. Dermatoendocrinol 2009; 1:37-42.

27. Grant WB.(A) Vitamin D supplementation could reduce risk of sepsis in infants. World J Pediatr 2010; 6:185.

28. Grant WB.(B) Vitamin D supplementation of mother and infant could reduce risk of sepsis in premature infants. Early Hum Dev 2010; 86:133

29. McKinney JD, Bailey BA, Garrett LH, Peiris P, Manning T, Peiris AN. Relationship between vitamin D status and ICU outcomes in veterans. J Am Med Dir Assoc 2011; 12:208-11

30. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med 2009; 360:1912-4.

31. Zaloga GP, Chernow B, Cook D, Snyder R, Clapper M, O'Brian JT. Assessment of calcium homeostasis in the critically ill surgical patient. The diagnostic pitfalls of the McLean-Hastings nomogram. Ann Surg 1985; 202:587-94.

32. DAMY, S.B.; EBISUI, L.; SPINELLI, M.O.; OSAKA, J.T.; TOLOSA, E.M.C.; ORTIZ, S.C.B.C. Inbred F344 rats as a biologic model of intraabdominal sepsis. Braz. J. vet. Res. anim. Sci., São Paulo, v.39, n.1, p. 21-26, 2002.

33. DUNN, D. L.; BARKE, R. A.; KNIGHT, N. B.; HUMPHREY, E. W.; SIMMONS, R. L. Role of resident macrophages, peripheral neutrophils, and translymphatic absorption in bacterial clearance from peritoneal cavity. Infection and Immunity, v. 49, n. 3, p.257-264, 1985

34. Thomas DR. Vitamins in aging, health and longevity. Clin Interv Aging 2006; 1:81-91.

35. Bartley J. Vitamin D: emerging roles in infection and immunity. Expert Rev Anti Infect Ther 2010; 8:1359-69.

36. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009; 169:384-90.

37. Schwalfenberg GK. A review of the critical role of vitamin D in the functioning of the immune system and the clinical implications of vitamin D deficiency. Mol Nutr Food Res 2011; 55:96-108.

38. Alitalo A. Human anti-infectious defence may be enhanced by vitamin D. Duodecim 2010; 126:1127-34