The correlation between vitamin D3 and IL-17 in rheumatoid arthritis patients

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ABSTRACT

Rheumatoid. arthritis. (RA) is define as a chronic. inflammatory disorders that have autoimmune etiologies. It is characterized. by inflamed joints. A total of 55 individual included 36 adult Rheumatoid arthritis patients from both sexes, age 21-73 yrs and 19 healthy controls (HC_S). All patients were visitors of Rheumatoid arthritis center located in Basra teaching hospital and fill a related questionnaire. The sera were tested for the existences of interlukines-17A (IL-17A) and Vitamin D (VD_3) using ELISA kit. Pearson's correlation was used to investigate various relationships. The results were statistically analyses using normality and Mann-Whiteny test in Minitab. IL-17A estimated level was higher in RA in compare to HC (121.33±71.7 and 96±57.97, respectively). In addition, the estimated level VD_3 was lower in RA in compare to HC_s (10.87±4.07 and 42.17±7.19, respectively). The correlation between the age of the study subjects and the level of IL-17A showed that there is no correlation between the two parameters in RA. The relationship between the VD_3 and the level of IL-17A was negative in RA patients.

Keywords: Rheumatoid arthritis, vitamin D3 and interleukin-17A.

Introduction

RA is one of the chronic inflammatory disorders that have autoimmune etiologies. It is characterized by inflamed joints, transition of T cell into the synovium, synovial hyperplasia and neoangiogenesis. During the course of RA, there is many catabolic cytokines as well as gradual destruction of articular cartilages and bones [1]. Numerous proinflammatory cytokines which participate in the proliferation of synovial tissue and joint destruction [2].

IL-17 is the important factor of inflammation and is contribute to the destruction of bone by increasing the migration of cells, the gene expression of chemokines and the invasiveness of synoviocytes [3;4]. T helper 1 (Th1) and Th17 play an important role as proinflammatory mediators while Th2 and T reg cells act as anti-inflammatory mediators [5]. Th1 and Th2 produce various cytokines, Th1 secret IL-2, as well as tumor necrosis Factor alpha while Th2 produce IL-4and IL-5 as well as IL-10, and IL-13.IL-17A within the IL-17 family of cytokines, which involved five interleukins such as IL-17A, IL-17 B, IL-17C, IL-17 D, IL-17E. IL-17A was the first factor in their family, and it is still the most studied [6].

Moreover, IL-17 play a role in several inflammations showing an elevated level in patients with these diseases like axial spondyloarthritis and psoriatic arthritis [7]. IL-17 is elevated in both peripheral blood and synovial fluid of RA patients [8;9]. Dhaouadi et al. have been elucidated a relationship between IL-17 in RA patients and the activity of this disease [10].

VD_3 is defined as a fat-soluble hormone is facilitates the metabolism of calcium-phosphate

in most bones. Furthermore, vitamin D contributes to other physiological functions such as the function of the immune system and pathological disorders [11;12].

VD controls the two arms of immunity, innate and adaptive, throughout various mechanisms including Toll-like receptors and T-cells differentiation as the differentiation of Th17 cells, therefore, it has a central role in the pathology of RA [13].

The purpose of the current study was to evaluate the serum levels of IL-17A and its correlation with VD_3 in patients with RA in Basra.

Materials and methods

Adult Rheumatoid arthritis patients (n=55) from both sexes, age 21-73 yrs. are eligible for our study. All patients were visitors of Rheumatoid arthritis center located in AL-Basra teacging hospital and fill a related questionnaire. In addition, samples from age matched healthy controls (HC_s) were also collected (n=19).

Five ml of blood were withdrawn and placed in a serum separation gel tube. Serum were separated at 3000 rpm for 20 min by centrifugation.

The sera were tested for the IL-17A and VD using ELISA kit from eBioscience, USA and Human Vitamin D_3(VD_3) ELISA Kit (Shanghai yehua Biological Technology Co./China), respectively, which were performed according to themanufacturer's recommendations. Pearson's correlation was used to investigate various relationships. The results were statistically analyses using normality and Mann-Whiteny test in Minitab.

Results

IL-17A estimated level was higher in RA in compare to HC_s (121.33±71.7 and 96±57.97, respectively) as shown in figure 1. In addition, the estimated level vitamin D3 was lower in RA in compare to HC (10.87±4.07 and 42.17±7.19, respectively) as in figure 2

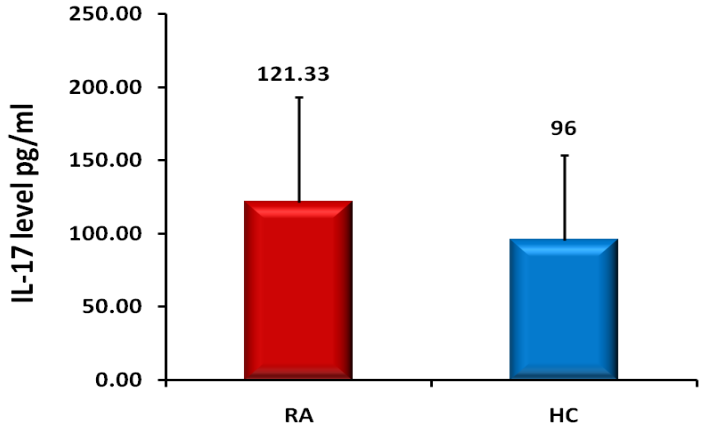

Figure 1 The level of IL-17A in RA. The level of IL-17 was higher in RA in compare to HC (121.33±71.7 and 96±57.97, respectively) measured by ELISA.

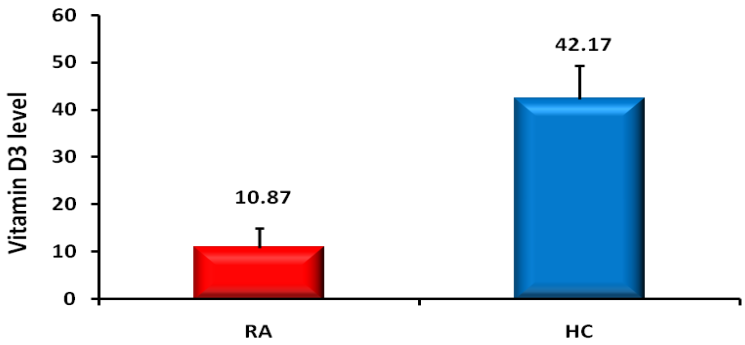

Figure 2 The level of VD3 in RA. The level of VD3 was lower in RA in compare to HC_s (10.87±4.07 and 42.17±7.19, respectively) measured by ELISA.

The correlation between the age of the study subjects and the level of IL-17A showed that there is no correlation between the two parameters in RA as the Pearson's correlation was 0.008 while the was a negative correlation between age and the level of IL-17A in HCs (Figure 3 A&B).

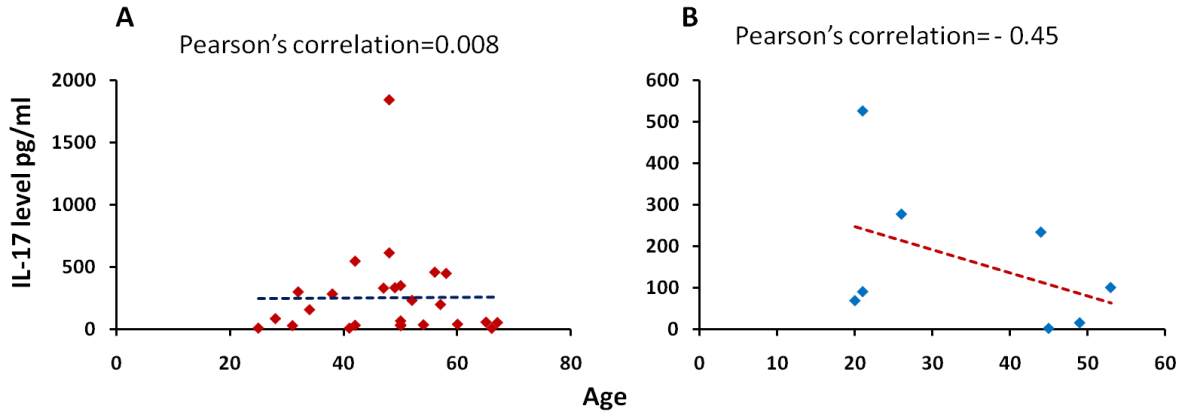

Figure 3 Pearson's correlation between Age and IL17A in the study subjects. A. No correlation between the two parameters in RA (0.008). B. negative correlation among age and the level of IL-17A in HCs (-0.45)

The correlation between the VD_3 of the study subjects and the level of IL-17A showed that there is a negative correlation between the two parameters in RA as the Pearson's correlation was – 0.2 while the was a strong positive correlation among VD_3 and the level of IL-17A in HCs, Pearson's correlation =0.6 (Figure 4 A&B).

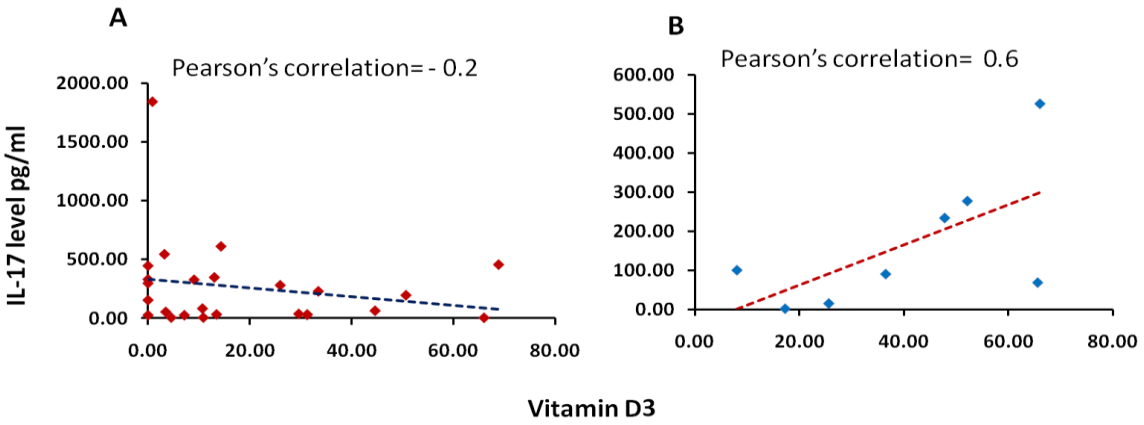

Figure 4: Pearson's correlation among VD_3 and IL17A. A. Negative correlation between the two parameters in RA (-0.2). B. A positive correlation among VD_3 and the level of IL-17A in HCs (0.6)

Discussion

RA is defining chronic systemic polyarthritis. Which is described as a systemic inflammatory disease due to the production of pro-inflammatory cytokines in high levels like IL-17. Our study revealed that IL-17A level in patients with RA was high compared to HCs, and possible IL-17A was associated with RA. IL17A is a proinflammatory cytokine, it has a role in the pathogenesis of autoimmune diseases [14], it affects several cell types by its role in the early and late stages of several diseases. IL-17A has a role on keratinocytes to stimulate many chemokines which cause to the induce of immune cells that activation in rheumatoid arthritis [15]. As well as IL-17A effect on synoviocytes and osteoblasts that participate in synovitis and destruction of joints [16;17].

In the current investigation, the VD_3 level in RA patients was low compared to HCs. Several studies were suggested that VD_3 deficiency causes many autoimmune diseases including RA [18;19]. VD_3 deficiency was endemic in several countries. This is probably by decreased exposure to the sun and VD_3 is

naturally found in a few types of foods so a person cannot get the optimal amount of VD_3. The mechanism of VD_3 during RA disease is that it decreases monocytes differentiation to Dendritic cells that acts to inhibition of antigen -presenting cells that induce T cells [20;21]. As well as, VD_3 decrease the proliferation of B cell and differentiated these cells into plasma cells, therefore, occurrence the inhibition of immunoglobulin production [22].

The current study results demonstrate no correlation between age and the level of IL-17A in RA patients. Rea et al were showed that cytokine dysregulation play important role in the immune response at an older age, as well as inhibition control inflammation, which is a characteristic of unsuccessful aging [23], on other hand, the inflammatory response can occur by the interaction between our genes, environments and lifestyles [24-26].Therefore IL-17 induces inflammation as well as it is overexpressed in several autoimmune and infection diseases[27].

Our study showed a negative relation between age and IL-17A in HCs. Aging is a factor associated with dysregulated immune response and inflammation. Many extrinsic factors that affect the age-associated decline in T cell function [28]. In other words, Elderly people have a decrease in IL-17-producing cells in comparison to healthy younger people [29].

In the current study, a negative correlation between VD_3 and IL-17A in RA patients was observed. VD_3 is a hormone-like substance, which support the synthesis of cytokines like IL-17 by dendritic cells and lymphocytes. Dankers et al were investigated the treatment with VD_3 that inhibited pro-inflammatory cytokines like IL-17A, IL-22 and IFNγ in memory CCR6+ T helper cells from HCs and RA patients [30], in another study, VD_3 had a potential role in modulating CD4+T cells by the response to allergens and down-regulated the allergen-induced expression of IL-17[31]. Our results are supported by studies observing that induce of CD4 T cells with VD_3 declines that affect the expression of IL17A, and IL17F [32:33] may be by VDR binding to the IL17A promotor, in result occurred reduction in production of IL17A [33].

In conclusion, the levels of IL-17A were observed to be elevated in patients compared with HCs, the data suggest that the serum levels of IL-17A can potentially be a biomarker for RA. In addition, the levels of VD_3 were decreased in patients and IL-17A levels were not affected with the age of the patients.

References

1. Tateiwa D, Yoshikawa H, Kaito T. Cartilage and Bone Destruction in Arthritis: Pathogenesis and Treatment Strategy: A Literature Review. *Cells*. (2019);8(8):818. Published 2019 Aug 2. doi:10.3390/cells8080818
2. 2. Takeuchi Y, Hirota K and Sakaguch S. Synovial Tissue Inflammation Mediated by Autoimmune T Cells. Front. Immunol., 21 August(2019) https://doi.org/10.3389/fimmu.2019.01989
3. Kurebayashi Y, Nagai S, Ikejiri A, Koyasu S. Recent advances in understanding the molecular mechanisms of the development and function of Th17 cells. Genes Cells (2013) 18: 247–265.
4. Miossec, P. Local and systemic effects of IL-17 in joint inflammation: a historical perspective from discovery to targeting. *Cell Mol Immunol* (2021).**18,** 860–865 https://doi.org/10.1038/s41423-021-00644-
5. Raphael I, Nalawade S, Eagar TN, Forsthuber TG. T cell subsets and their signature cytokines in autoimmune and inflammatory diseases. *Cytokine*. (2015);74(1):5-17. doi:10.1016/j.cyto.2014.09.
6. 6.McGeachy, M.J., D.J. Cua, and S.L. Gaffen.. The IL-17 Family of Cytokines in Health and Disease. Immunity. (2019) 50:892–906. https://doi.org/10.1016/j
7. McGonagle DG, McInnes IB, Kirkham BW, Sherlock J, Moots R. The role of IL-17A in axial spondyloarthritis and psoriatic arthritis: recent advances and controversies [published correction appears in Ann Rheum Dis. 2020 Jan;79(1):e12].

8. Robert M, Miossec P. IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels. *Front Med (Lausanne)*.(2019);5:364. Published 2019 Jan 14. doi:10.3389/fmed.2018.00364
9. 9 . Li, X., Lei, Y., Gao, Z. *et al.* Effect of IL-34 on T helper 17 cell proliferation and IL-17 secretion by peripheral blood mononuclear cells from rheumatoid arthritis patients. *Sci Rep* (2020). **10,** 22239 https://doi.org/10.1038/s41598-020-79312-z
10. 10- Dhaouadi T, Chahbi M, Haouami Y, Sfar I, Abdelmoula L, Ben Abdallah T et al. IL-17A, IL-17RC polymorphisms, IL17 plasma levels in Tunisian cases with rheumatoid arthritis. Plos One(2018); 13 (3): 194-201. 15.
11. Bivona G, Agnello L, Ciaccio M. The immunological implication of the new vitamin D metabolism. *Central Euro J Immunol.* (2018) 43:331–4. doi: 10.5114/ceji.2018.80053
12. 12.Medrano M, Carrillo-Cruz E, Montero I, Perez-Simon JA. Vitamin D: effect on haematopoiesis and immune system and clinical applications. *Int J Mol Sci.* (2018) 19:2663. doi: 10.3390/ijms19092663
13. Higgins MJ, Mackie SL, Thalayasingam N, Bingham SJ, Hamilton J, Kelly CA. The effect of vitamin D levels on the assessment of disease activity in rheumatoid arthritis. *ClinRheumatol.* (2013) 32:863–7. doi: 10.1007/s10067-013-2174-x
14. Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic inflammation. *Nat Rev Drug Discov.* (2012) 11:763–76. 10.1038/nrd3794
15. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet* (2016) 388:2023–38. 10.1016/S0140-6736(16)30173-8
16. Hot A, Miossec P. Effects of interleukin (IL)-17A and IL-17F in human rheumatoid arthritis synoviocytes. *Ann Rheum Dis.* (2011) 70:727–32. 10.1136/ard.2010.143768
17. Ndongo-Thiam N, Miossec P. A cell-based bioassay for circulating bioactive IL-17: application to destruction in rheumatoid arthritis. *Ann Rheum Dis.* (2015) 74:1629–31. 10.1136/annrheumdis-2014-207110
18. 18 . Mukhtar M, Sheikh N, Suqaina SK, Batool A, Fatima N, Mehmood R, et al. Vitamin D Receptor Gene Polymorphism: An Important Predictor of Arthritis Development. Biomed Res Int 2019;2019. https://doi.org/10.1155/2019/8326246.
19. Dupuis, M.L., Pagano, M.T., Pierdominici, M. *et al.* The role of vitamin D in autoimmune diseases: could sex make the difference?. *Biol Sex Differ* (2021) **12,** 12 https://doi.org/10.1186/s13293-021-00358-3
20. Solidoro P, Bellocchia M, Aredano I, Mattei A, Pivetta E, Patrucco F, et al. Asthmatic patients with vitamin D deficiency have decreased exacerbations after vitamin replacement. Nutrients 2017;9. https://doi.org/10.3390/nu9111234.
21. Van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: Basic concepts. J Steroid Biochem Mol Biol 2005;97:93–101. https://doi.org/10.1016/j.jsbmb.2005.06.002.
22. Di Rosa M, Malaguarnera M, Nicoletti F, Malaguarnera L. Vitamin D3: A helpful immuno-modulator. Immunology 2011;134:123–39. https://doi.org/10.1111/j.1365-2567.2011.03482.x.
23. 23.Rea I M, Gibson D S., McGilligan V, McNerlan S E., Alexander H.D and A. Ross O A. .Age and Age-Related Diseases: Role of inflammation Triggers and Cytokines. Frontiers in Immunology . 2018; 9 PP. 586.

24. Ter Horst R, Jaeger M, Smeekens SP, Oosting M, Swertz MA, Li Y, et al. Host and environmental factors influencing individual human cytokine responses. *Cell* (2016) 167(4):1111e–24e. doi:10.1016/j.cell.2016.10.018
25. Govindaraju D, Atzmon G, Barzilai N. Genetics, lifestyle and longevity: lessons from centenarians. *Appl Transl Genom* (2015) 4:23–32. doi:10.1016/j. atg.2015.01.001
26. Rea JNM, Carvalho A, McNerlan SE, Alexander HD, Rea IM. Genes and life-style factors in BELFAST nonagenarians: nature, nurture and narrative. *Biogerontology* (2015) 16(5):587–97. doi:10.1007/s10522-015-9567-y
27. Zambrano-Zaragoza JF, Romo-Martínez EJ, Durán-Avelar Mde J, García-Magallanes N, Vibanco-Pérez N. Th17 cells in autoimmune and infectious diseases. *Int J Inflam* (2014) :651503. doi:10.1155/2014/651503
28. 28 Wu D, Meydani SN. Age-associated changes in immune and inflammatory responses: impact of vitamin E intervention. J Leukoc Biol. (2008) Oct;84(4):900-14.
29. Lee JS, Lee WW, Kim SH, Kang Y, Lee N, Shin MS, et al. Age-associated alteration in naive and memory Th17 cell response in humans. *Clin Immunol* (2011) 140(1):84–91.
30. 30 Dankers W, Davelaar N, van Hamburg JP, van de Peppel J, Colin EM, Lubberts E. Human Memory Th17 Cell Populations Change Into Anti-inflammatory Cells With Regulatory Capacity Upon Exposure to Active Vitamin D. Front Immunol. 2019 Jul 17;10:1504. doi: 10.3389/fimmu.2019.01504. PMID: 31379807; PMCID: PMC6651215.
31. 31.Zhong H, Zhou XJ, Hong JG. The effects of vitamin D on allergen-induced expression of interleukin-13 and interleukin-17 in cord blood $CD4^{+}$T cells. Iran J Allergy Asthma Immunol. (2014) Apr;13(2):93-7.
32. Colin, E.M.;Asmawidjaja, P.S.; vanHamburg, J.P.;Mus,A.M.C.; vanDriel,M.;Hazes, J.M.W.;Van Leeuwen, J.P.T.M.; Lubberts, E. 1,25-dihydroxyvitaminD3modulates Th17 polarization and interleukin-22 expression by memory T cells from patients with early rheumatoid arthritis. Arthritis Rheum.(2010), 62, 132–142.
33. Joshi, S.; Pantalena, L.C.; Liu, X.K.; Ga_en, S.L.; Liu, H.; Rohowsky-Kochan, C.; Ichiyama, K.; Yoshimura, A.; Steinman, L.; Christakos, S.; et al. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. Mol. Cell Biol. (2011**)**, 31, 3653–3669.