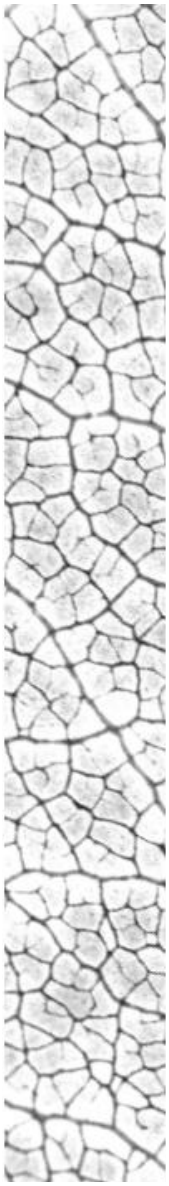

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Investigating the association of vitamin D levels with RF and HMGB1 in Rheumatoid arthritis patients in Basra, Iraq

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Abstract

Background: Rheumatoid arthritis (RA) is autoimmune disease characterized by swollen joints and the presence of autoantibodies. Many factors can affect the susceptibility of a person to this autoimmune disease, one of these factors is the level of vitamin D (VD3). The aim of the study is investigating the relationships of VD3, RF and HMGB1 in RA patients in Basra. **Materials and Methods**: A total of 154 participants were included RA patients (n=113) and healthy control (HC) (n=41) from both sexes, age range 23-75 years. The sera of RA patients and HC were tested for the level of Rheumatoid Factors (RF) included (IgG and IgM), VD3 and Human HMGB1 protein measurement by ELISA. **Results:** Our results showed that the level of RF were significantly higher and VD3 was significantly lower in RA patients in compare with HC. Furthermore, serum HMGB1 levels were higher in RA patients without treatment, but decreases upon therapy in patients while it was low in HC. A significant differences noticed in serum HMGB1 levels in RA patients depended on the types of drugs. Positive correlation between VD3, HMGB1 and the disease progress, while negative correlations were found between VD3, RF and HMGB1. **Conclusion**: The status of VD3 in RA patients living in Basra and its relation to the levels of RF and the pro inflammatory mediator HMGB1, documenting that VD3 deficiency play a role in causing the inflammation during RA.

Keywords: Rheumatoid arthritis, Rheumatoid Factor, Vitamin D3, HMGB1

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INTRODUCTION

Rheumatoid arthritis (RA) defined as a chronic inflammatory disease (Aletaha, & Smolen, 2018). Diagnosis and management of rheumatoid arthritis: a review. *Jama*, *320*(13), 1360-1372. RA is one of the autoimmune diseases that is characterized by many manifestations such as persistent synovitis and systemic inflammation. In addition, RA patients have different type of autoantibodies including Rheumatoid Factor (RF), Anti-Citrullinated Peptide antibody (ACPA) which is known as anti-Cyclic Citrullinated Peptides (anti-CCP).(Rocha, Baldo, & Andrade, 2019). RA ranged from mild and remitting to highly active and disabling, the occurring of chronic inflammation in the synovial membrane, synovial lining hyperplasia and osteoclasts over activation lead to pain and destruction of both articular cartilage and bone which is irreversible. RA in case of polyarthritis commonly affecting the small joints in hands (Meng, et al. 2018). Rheumatoid Arthritis of Knee Joints: MRI–Pathological Correlation. *Orthopaedic Surgery*, *10*(3), 247-254. Principally affecting in all synovial joints (Meng, et al. 2018). Furthermore, many organs can be affected including skin, eyes, lungs and heart (Walker, & Ranatunga, 2006). When other organs are affect it cause to decrease in life expectancy (Radner, Smolen, & Aletaha, 2011). Clinically, RA patients can be termed as seropositive RF and those patients represent 40- 80% of all RA patients while other RA patients termed as seronegative. RFs consist mostly of IgM but IgG or IgA can be also included, IgA is correlated with extra-articular manifestations while the other RF isotypes are involved in the aggressiveness of the disease (Song, & Kang, 2010). The autoantibody IgG contributes to the activation of macrophages while IgM involves in complement activation. Furthermore, autoantibodies involved in critical processes during RA such as the initiation of the inflammation and destruction of the joints (Chang, & Nigrovic, 2019). In Iraq many studies investigated the serum level of these autoantibodies (Al-Salih, & Selman, 2015; Ohajianya, et

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al, 2016). Vitamin D (VD3) is an essential hormone of endocrine functions, it involved in the regulation of cell replication and metabolic pathways related to the immune responses (Chun, et al. 2014). The role of VD3 within the immune system include the suppression of lymphocytes proliferation (Cantorna, Snyder, Lin, & Yang, 2015), the inhibition of antibodies synthesis, the inhibition of transcription factors that cause inflammation and production of various cytokines e.g. IL-6, IL-8 and IL-10[10, Liu, et al. 2018 Solidoro, et al. 2017)The mechanism in which VD3 performs its biological activity is via a receptor which acts as a transcriptional factor activated through the ligand receptor gene of VD3 (vitamin D receptor (VDR))(Mukhtar, et al. 2019). The relationship between VD3 and autoimmune diseases has been investigated by many researchers worldwide (Brown, et al. 2008. Kostoglou-Athanassiou, Iet al. 2012)..Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. *The Plant Journal*, *52*(6), 1154-1168.

Most of the studies concentrated on the role of VD3 in the pathogenesis, activity and treatment of RA (Disanto, & Ramagopalan, 2010 Lin, et al. 2016). Two observations indicate the relationship between VD3 and RA, the first is that RA patients have low level of VD3 in their sera. The second observation is that macrophage chondrocytes and synovial cells have VDR and it has been well demonstrated (Disanto, & Ramagopalan, 2010, Lee, 2016).Interestingly, the low levels of VD3 considered to be a risk factor associated with many non-skeletal related illnesses e.g. cardiovascular diseases (Pérez-Hernández, et al. 2016)., asthma (Solidoro, et al. 2017) and cancer (Wang, et al. 2017). HMGB1 (High-mobility group box 1) was defined by Wangin in 1999 as an inflammatory cytokine during sepsis and endotoxaemia (Wang, et al. 1999). It involves in different inflammatory diseases pathogenesis including acute lung injury, ischemia, reperfusion injury and autoimmune liver damage (Wang, et al. 1999. Evankovich, et al. 2010. Tsung, et al. 2007). HMGB1 levels is increased in many autoimmune diseases e.g. RA, SLE and Behçet's disease (Nowak, et al. 2016. Magna, et al. 2014. Lu, et al. 2015). The aim of this study is to evaluate the levels of VD3, RF (IgG and IgM) and HMGB1 in RA patients then to investigate the correlation of these factors in RA patients in Basra province.

MATERIAL AND METHODS

Study Subjects

A total of 154 participants were included in this study, 113 RA patients who were visitors to the Rheumatoid arthritis center / AL-Basra general hospital from February to August of 2018. In addition, 41 healthy control samples were also collected during the same period. The subjects who are eligible to the study were adults from both sexes, age 23-75 years, categorized according to age into 6 age groups with 10 yrs. Intervals. All patients fill a questionnaire related to their medical status to confirm diagnosis. The questionnaire included information regarding sex, age, type of drugs, number of infected large joints and number of infected small joints.

Procedures

Four ml of venous blood were withdrawal from each study subject (RA = 113 and HC= 41), blood was placed in a serum separating gel tube. Serum samples were separated by centrifugation at 3000 rpm for 20 min. and 3 aliquots for each sample were made and kept at -20 °C until further analysis.

The sera of RA patients and healthy control (HC) were tested for the level of RF Factors (IgG and IgM), Human Vitamin D3 and Human HMGB1 protein and by ELISA using four kits performed according to manufacturer protocols: the Immunoenzymetric Assay by Rheumatoid Factor IgG kit from Demeditec /German, Immunoenzymetric Assay by Rheumatoid Factor IgM kit from Demeditec /German, Human Vitamin D_3(VD_3) ELISA Kit (Shanghai yehua Biological Technology Co./China) and Human High mobility group protein B1 (HMGB-1) ELISA Kit (Shanghai yehua Biological Technology Co./China). Antibody concentration was evaluated by plotting the absorbance of standards (O.D) on Y axis and antibody concentrations IU/ml on X axis. Standard curve was used to calculated the concentration of tested samples.

Statistical analysis

The data underwent statistical analysis using a normality test at beginning followed by parametric (Two sample T-Test and confidence interval) and non-parametric test (Mann-Whitney U test) in MINITAB program. In addition, the person correlation may be used to investigate the correlation between the study factors.

RESULTS

Subject demography

The results showed that the mean age of RA patients was 48.55±11.81 yrs. while the mean age of HC was 40.68±12.32 yrs. The distribution of age within RA patients showed that the high number of patients were within group 4 (48-57 yrs.) counting 43 (38.05%) patients, while the lowest number of patients were found in group 6 (68-77) counting 3 (2.65%) patients. The present study showed that during the period of sample collection the majority of RA patients were females counting 94 patients with percentage of (83.18%), while only 19 patients (16.81 %) were males.

Rheumatoid factor (IgG level) in RA

The level of IgG was significantly higher in RA patients in compare with healthy controls (p=0.0001), the mean level of IgG was 73.42 ± 121.15 ng/ml while the mean level of IgG in healthy controls was 17.78 ± 57.03 ng/ml as shown in **Fig. 1**.

Table 1. Distribution of sex among RA age groups

Age groups in yrs.	RA patients			Healthy controls		
	Total (%)	Female (%)	Male (%)	Total (%)	Female (%)	Male (%)
Group 1 (18-27)	6 (4.31)	5 (4.42)	1 (0.88)	8 (19.51)	2 (4.87)	6 (14.63)
Group 2 (28-37)	17 (15.04)	14 (12.83)	3 (2.65)	3 (7.31)	2 (4.87)	1 (2.43)
Group 3 (38-47)	23 (20.35)	21 (18.58)	2 (1.77)	15 (36.58)	8 (19.51)	7(17.07)
Group 4 (48-57)	43 (38.05)	35 (30.97)	8 (7.07)	11 (26.82)	6 (14.63)	5 (12.19)
Group 5 (58-67)	21 (18.58)	16 (14.15)	5 (4.42)	3 (7.31)	0 (0.00)	3 (7.31)
Group 6 (68-77)	3 (2.65)	3 (2.65)	0 (0)	1 (2.43)	1 (2.43)	0 (0)
Total number	113	94	19	41	19	22

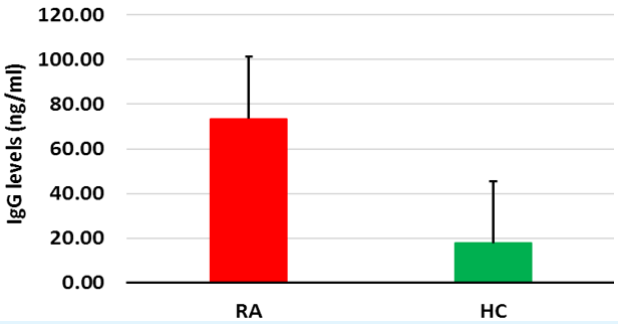

Fig. 1. IgG levels in RA patients and HC. The IgG levels were measured by ELISA

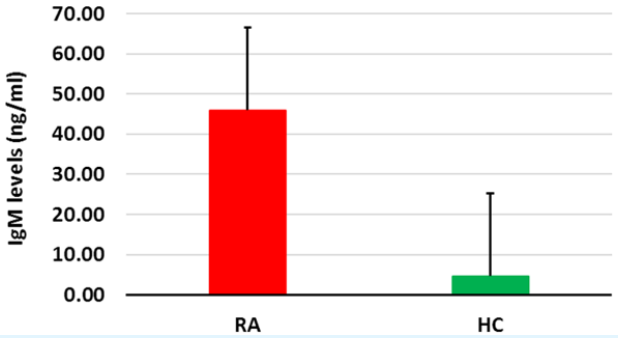

Fig. 2. IgM levels in RA patients and HC. The IgM levels were measured by ELISA

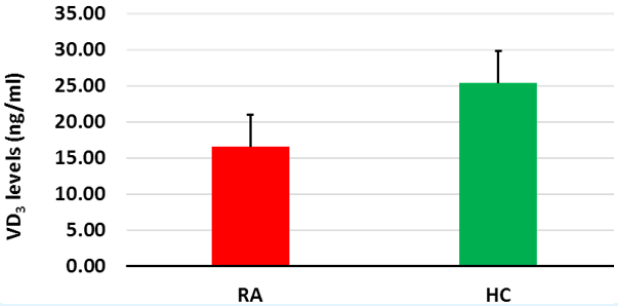

Fig. 3. The mean of VD3 level in RA patients and HC. The serum VD3 levels were measured by ELISA

Regarding the seropositive and seronegative cases of RA patients in terms of IgG levels, the results showed that 55% of RA patients were seropositive for RF IgG leaving 45% of RA patients as seronegative cases.

Rheumatoid factor (IgM level) in RA

The current study showed that the level of IgM was significantly higher in RA patients in compare to healthy controls (p = 0.0002) and the mean levels were 45.99 ± 69.54 ng/ml and 4.65 ±1 ng/ml, respectively (**Fig. 2**).

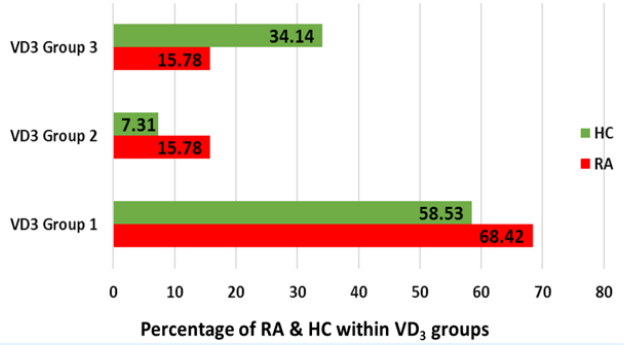

Fig. 4. Percentage of RA patients and HC within VD_3 groups. VD_3 Group 1= VD_3 deficiency (<20 ng/ml), VD_3 group 2 = VD_3 insufficient (20-30 ng/ml) and VD_3 group 3 = VD_3 optimal (≥ 30 ng/ml)

Regarding the seropositive and seronegative cases of RA patients in terms of IgM levels. The results showed that 45.61% of RA patients were seropositive for IgM leaving 54.31% of RA patients as seronegative cases.

Serum Human Vitamin D3 (VD3) Levels in RA

The serum level of VD3 was non-significantly lower in RA and the mean levels in RA and healthy controls were 16.8 2± 22.47 ng/ml and 25.91 ±27.25 ng/ml, respectively as shown in **Fig. 3**.

Additionally, we categorized the level of VD3 into 3 groups (VD3 Group 1= VD3 deficiency (<20 ng/ml), VD3 Group 2 = VD3 insufficient (20-30 ng/ml) and VD3 Group 3 = VD3 optimal (≥ 30 ng/ml)). Investigation on the distribution of RA patient's within VD3 groups showed that the percentages of RA patients were the highest (68.42%, mean of VD3 = 6.12±5.97 ng/ml) in VD3 Groups 1, while the lowest percentages of RA patients found in VD3 Group 2 and 3 (15.78%, mean of VD3 = 24.21±2.59 ng/ml; 15.77%, mean of VD3 = 55.62± 31.35 ng/ml, respectively). In heathy control, the percentages of subjects' distribution were 58.53% (mean of VD3 = 6.80 ±4.77 ng/ml) in VD3 Groups 1, 7.31% (mean of VD3 = 25.68 ±1.32 ng/ml) in VD3 Group 2 and 34.14% (mean of VD3 = 55.17 ±22.80 ng/ml) in group 3 (**Fig. 4**).

The mean of serum VD3 levels varied according to the number of effected joints (small and large) in RA patients. Firstly, the results of the association of the number of affected large joints with VD3 levels showed that the lowest serum VD3 level were association with the highest number of large joint affected with RA (14.15± 14.72 ng/ml). Secondly, the results of the association of the number of affected small joints with

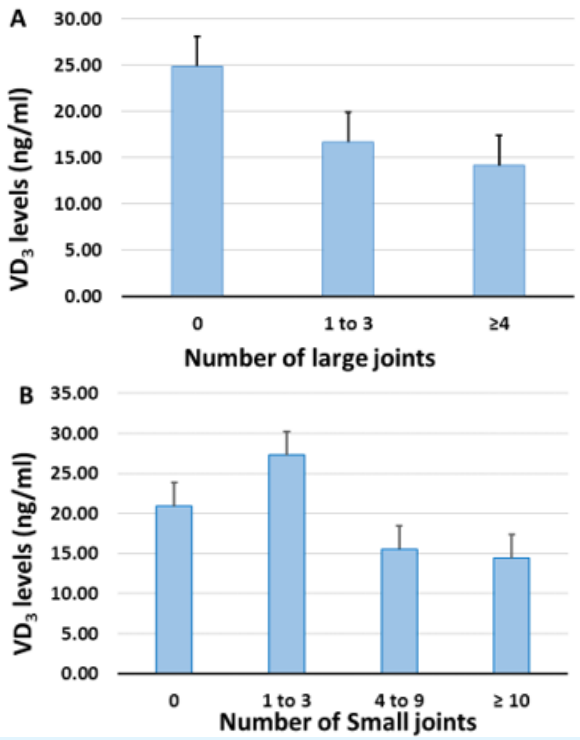

Fig. 5. Serum VD3 levels in RA patients according to the number of large and small joints affected. A. the association with affected large joints. B. the association with affected small joints, the serum VD3 level were measured by ELISA

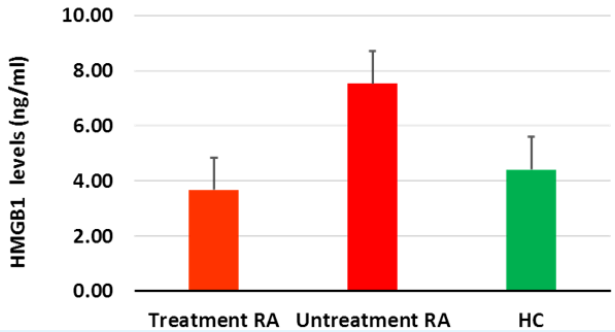

Fig. 6. HMGB1 levels in RA patients and HC. The HMGB1 levels were measured by ELISA

VD3 levels showed that the lowest serum VD3 level were association with the highest number of smalls joint affected with RA (14.4± 17.58 ng/ml) as shown in **Fig. 5A** & **B**.

Serum Human High mobility group B1 protein (HMGB-1) levels in RA levels of HMGB1 were higher in RA patients without treatment (7.52± 11.22 ng/ml) but decreases upon therapy in patients (3.67 ± 3.01 ng/ml) nearly to its levels in healthy controls (3.33±1.21 ng/ml). The differences were significant (p =0.007) between treated and untreated RA patients, no significant differences (p = 0.72) between treated RA patients and healthy controls as shown in **Fig. 6**.

Regarding the treatment choice that the RA patients received and their effect on HMGB1 levels, the current

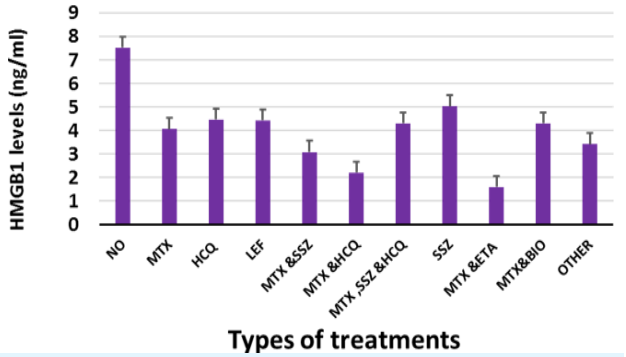

Fig. 7. Serum Human HMGB-1 levels of RA patients with and without treatment. Abbreviations: NO, no treatment; MTX, methotrexate; HCQ, hydroxychloroquine; LEF, leflunomide; SSZ, sulfasalazine; ETA, Etanercept and OTHER, another drugs

study revealed that DMARD monotherapy such as methotrexate (MTX), sulfasalazine (SSZ), hydroxylchloroquine (HCQ), leflunomide (LEF) and Etanercept (ETA) were the common used for RA patients. The results showed that 63.06% of the RA patients received DMARD monotherapy while Double therapy was used in 21.62% of RA patients followed by 4.50% who received triple-DMARD therapy. But, the use of biologicals and immunosuppressive drugs were less common. Among different drugs, methotrexate was the major frequently used of the patients 41%. The patients were treated with sulfasalazine and methotrexate as second treatment in 14.41%. Moreover,10.81 % of the patient did not received any treatment. Early stage of RA patients (without therapy) had the highest levels of Serum Human HMGB1 protein in compare with other patients who received treatments. This high value was significant at p=0.03, no differences in HMGB1 levels among patients who received various therapies (**Fig. 7**).

Our results reveled that there is a relationship between levels of serum Human HMGB1 protein and number of large joints as the highest level of HMGB1 reported in RA patients with more than 4 large joints being affected, while the result of the protein level was variable in association with affected small joints ($p < 0.05$) as shown in **Fig. 8 A** & **B**.

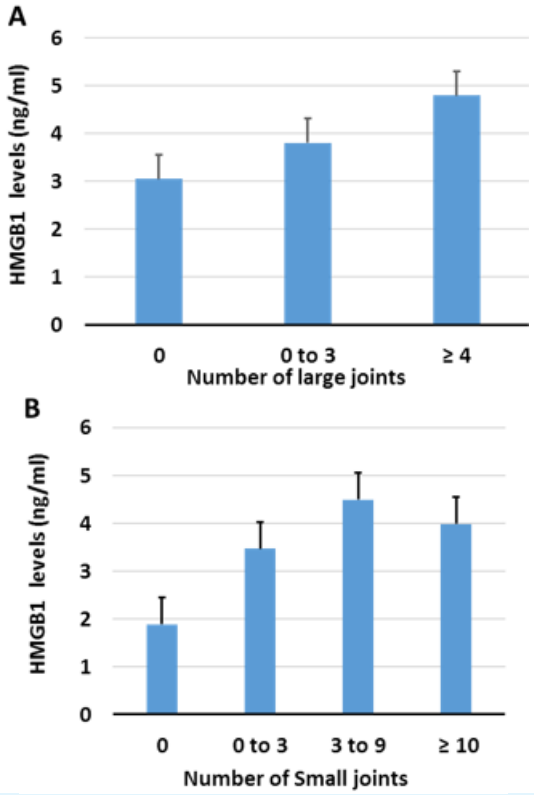

Fig. 8. Serum Human HMGB-1 levels of RA patients according to the number of large and small joints affected. A. the association with affected large joints. B. the association with affected small joints. the serum VD3 level were measured by ELISA

The correlation of serum levels of VD_3 with RF and serum HMGB1 in RA

The correlation between the serum levels of VD_3 and RF (IgG and IgM) and HMGB1 protein can be illustrated as follow: Firstly, from plotting the VD3 values with the IgG levels in RA patients, the results showed a weak negative correlation between the two factors. The R value was -0.022 which is nearly no correlation (**Fig. 9 A**). Secondly, plotting the VD3 values with the IgM levels in RA patients, the results showed a weak negative correlation between the two factors. The R value was -0.1 (**Fig. 9 B**). Thirdly, from plotting the VD3 values with the serum HMGB1 levels in RA patients, the results showed a weak negative correlation between the two factors. The R value was -0.1 (**Fig. 9 C**).

DISCUSSION

This article investigated the levels of VD3 along with other factors in RA patients living in Basra. First of all, our study reported the age that most of RA patients in Basra placed in, this group was age group 4 (48-57yrs.) followed by the age group 3 (38-47 yrs.). Another study conducted in Iraq reported a mean of 46.5±10.4 yrs. (Ihsan, et al. 2015). In different study similar age mean were also reported (Mayyadah & Rashid, 2017) The tendency of RA to infect older people is well defined in Iraq as the majority of the patients were placed within the age range of 40-59 yrs.(Hussein, MezherAl-Rayahi, Taha, 2018). The high variability of factors during the aging process are involved in the explanation of why RA occur more frequently in elderly than in young people,

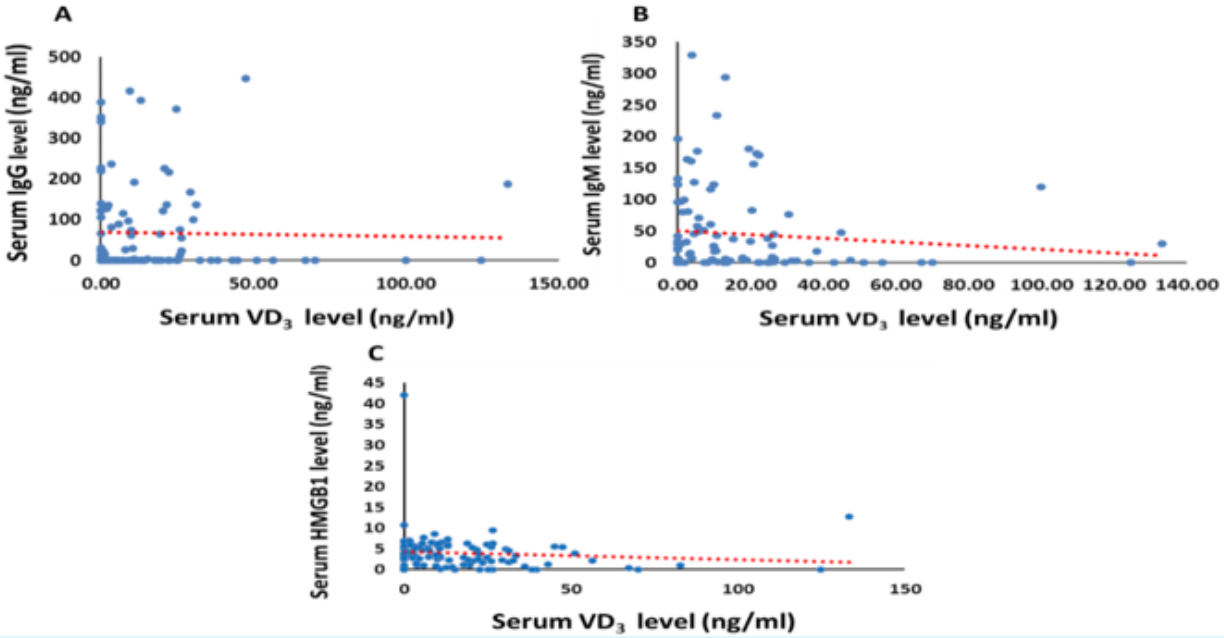

Fig. 9. The correlation between VD_3 and other study parameters. A. The correlation between VD_3 and RF-IgG levels in RA. The results showed a weak negative correlation. B. The correlation between VD_3 and RF-IgM levels in RA. The results showed a weak negative correlation. C. The correlation between VD_3 and serum HMGB1 levels in RA. The results showed a weak negative correlation

as with aging the influences of the environment make the people more susceptible to the disease, in addition, the strange features of ageing and the sociocultural factors may increase the degree of complication that effect people in this age, furthermore, in this age the alteration of the immune responses may play a role as well, especially when aging is associated with the decrease of T cell proliferation (Makinodan, & Kay, 1980) It also been demonstrated that the variability in the immune regulatory mechanisms that occur along with aging may participate in the pathogenesis of RA (Gamerith, et al. 1993). Nonetheless, RA can occur in all ages (Duboiset al. 2017) Regarding the sex of the patients, the majority of RA patients were females this can be explained by the gender differences in regard of biology and hormones. In addition, the differences in the behaviors between the two genders such as smoking and others that may affect RA susceptibility of RA (Alpízar-Rodríguez, et al. 2017).The female to male ratio of RA is 1.5-2 in females to 1 male, but this ratio increased with age and reaches 4-4.5 females to 1 male, other studies reported that RA was predominant in women more than men with a ratio of 2.6:1 (Muss, et al. 2018). In other studies, the female to male ratio vary but it is consistence with the fact that RA prevalence in higher in females than males (Hajjaj-Hassouni, et al. 2017). This variation can be explained by the specific inclusion criteria of each study, additionally, several factors such as gonadal hormones, genetic variation, life style many also cause this variation (Deane, et al. 2017. Malm, et al. 2016)..The elevation of (RF) IgG and IgM is expected as previously reported in many studies (Hussein, MezherAl-Rayahi, Taha, 2018). The RF seronegative cases of RA patients were also reported as well as the seropositive RF cases in healthy controls (Al-Herz, et al. 2016. Børretzen, et al. 1997. Ingegnoli, Castelli, & Gualtierotti, 2013). Our study showed that RA patients had decreased levels of VD3 which is a reasonable result as VD3 has been associated with many autoimmune diseases pathogenicity (Mukhtar, et al. 2019. Arnson, Amital, & Shoenfeld, 2007) The mechanism in which VD3 is involved in during RA is that it inhibits monocytes differentiation into Dendritic cells (DCs) leading to the reduction of antigen presenting cells (APCs) that stimulate T cells (Solidoro, P., et al. 2017. Di Rosa, Malaguarnera, Nicoletti & Malaguarnera, 2011) Moreover, VD3 inhibits the proliferation of B cell before it differentiated to plasma cells resulting in the reduction of immunoglobulin production (Di Rosa, Malaguarnera, Nicoletti & Malaguarnera, 2011). Globally the reasons of VD3 deficiency include lack of awareness that people can obtain VD3 in an inexpensive and safe way by exposing themselves to the sun, VD3 naturally found in few foods therefore a person cannot get the proper amount of VD3 through a balanced diet (Holick, & Chen, 2008) The association of low levels of VD3 and RA also reported in many studies and underwent meta analyzed (Lin, Liu, Davies, & Chen, 2016). which make our result that the majority of RA patients falls into the group of VD3 deficiency more reliable. The association of low VD3 levels and the highest number of affected joints is expected .(Garfinkel, Dilisio, & Agrawal, 2017) In contrast, other studies reported no correlation between low levels of VD3 and the disease activity score 28(DAS 28) (Azzeh, 2012). It is important to mention the low VD3 level in RA patients may associated with cardio metabolic risk factors (Haque, Bathon, & Giles, 2012) It also disturbs the immune tolerance which induce autoimmune disease in the first place in addition to its immunomodulatory properties (Altieri, et al. 2017. Meena, et al. 2018). The serum level of HMGB1 is high in RA patients who did not received treatment suggesting that RA therapy has an effect of HMGB1 levels. As it is known HMGB1 is a proinflammatory mediator that can induce inflammation via binding to cell surface receptors (Pilzweger, & Holdenrieder, 2015). Many cells can produce this protein include neutrophils, macrophages and endothelial cells, it serves as a stimulator of the activation of macrophages (Yuen, et al. 2011). A common drug used in RA treatment is methotrexate, it has been reported that this drug decreases the levels of HMGB1 in the synovial tissue (Li, et al. 2016). Moreover, other studies stated that the levels of HMGB1 reaches the highest level in the early disease onset (before therapy) which confirm our results (Schierbeck, et al. 2013). The correlation between the level of HMGB1 and the number of affected joints showed no clear results as the majority of our study subjects had received treatment making it difficult to interpret the results in this case but it is reported that the serum levels of HMGB1 correlation significantly with DAS 28 in RA (Chiang, 2017). The negative correlation of VD3 levels HMGB1 and RF (IgG and IgM) support the role of VD3 deficiency in the progression of autoimmune diseases in general, specially RA as it suppresses the differentiation and proliferation of effector cell such as B cell (Bugatti, Vitolo, Caporali, Montecucco, & Manzo, 2014).

CONCLUSION

The status of VD3 in RA patients living in Basra and its relation to the levels of RF and the pro inflammatory mediator HMGB1, documenting that VD3 deficiency play a role in causing the inflammation during RA.

REFERENCES

Aletaha, D., & Smolen, J. S. (2018). Diagnosis and management of rheumatoid arthritis: a review. Jama, 320(13), 1360-1372.

Al-Herz, A., Al-Awadhi, A., Saleh, K., Al-Kandari, W., Hasan, E., Ghanem, A., ... & Khadrawy, A. (2016). A comparison of rheumatoid arthritis patients in Kuwait with other populations: results from the KRRD registry. Journal of Advances in Medicine and Medical Research, 1-11.

Alpízar-Rodríguez, D., Pluchino, N., Canny, G., Gabay, C., & Finckh, A. (2017). The role of female hormonal factors in the development of rheumatoid arthritis. Rheumatology, 56(8), 1254-1263.

Al-Salih, M. W., & Selman, A. N. (2015). Evaluation of heat shock proteins 70 (HSP70) and some risk factors in sera of rheumatoid arthritis patients in Thi-Qar province. Al-Nahrain Journal of Science, 18(1), 123-129.

Altieri, B., Muscogiuri, G., Barrea, L., Mathieu, C., Vallone, C. V., Mascitelli, L., ... & Savastano, S. (2017). Does vitamin D play a role in autoimmune endocrine disorders? A proof of concept. Reviews in Endocrine and Metabolic Disorders, 18(3), 335-346.

Arnson, Y., Amital, H., & Shoenfeld, Y. (2007). Vitamin D and autoimmunity: new aetiological and therapeutic considerations. Annals of the rheumatic diseases, 66(9), 1137-1142.

Azzeh, F. S. (2012). Relationship between vitamin D and rheumatoid arthritis disease. Pakistan Journal of Nutrition, 11(3), 293.

Børretzen, M., Chapman, C., Natvig, J. B., & Thompson, K. M. (1997). Differences in mutational patterns between rheumatoid factors in health and disease are related to variable heavy chain family and germ-line gene usage. European journal of immunology, 27(3), 735-741.

Brown, D. M., Goubet, F., Wong, V. W., Goodacre, R., Stephens, E., Dupree, P., & Turner, S. R. (2008). Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. The Plant Journal, 52(6), 1154-1168.

Bugatti, S., Vitolo, B., Caporali, R., Montecucco, C., & Manzo, A. (2014). B cells in rheumatoid arthritis: from pathogenic players to disease biomarkers. BioMed research international, 2014.

Cantorna, M. T., Snyder, L., Lin, Y. D., & Yang, L. (2015). Vitamin D and 1, 25 (OH) 2D regulation of T cells. Nutrients, 7(4), 3011-3021.

Chang, M. H., & Nigrovic, P. A. (2019). Antibody-dependent and-independent mechanisms of inflammatory arthritis. JCI insight, 4(5).

Chiang, H. C. (2017). Comparison of Hybridization in DNA Brushes and in Solution (Doctoral dissertation, Polytechnic Institute of New York University).

Chun, R. F., Liu, P. T., Modlin, R. L., Adams, J. S., & Hewison, M. (2014). Impact of vitamin D on immune function: lessons learned from genome-wide analysis. Frontiers in physiology, 5, 151.

Deane, K. D., Demoruelle, M. K., Kelmenson, L. B., Kuhn, K. A., Norris, J. M., & Holers, V. M. (2017). Genetic and environmental risk factors for rheumatoid arthritis. Best practice & research Clinical rheumatology, 31(1), 3-18.

Di Rosa, M., Malaguarnera, M., Nicoletti, F., & Malaguarnera, L. (2011). Vitamin D3: a helpful immuno-modulator. Immunology, 134(2), 123-139.

Disanto, G., & Ramagopalan, S. V. (2010). Vitamin D: shining a light on autoimmune disease. International Journal of Clinical Rheumatology, 5(6), 605.

Dubois, B., Kobelt, G., Berg, J., Capsa, D., Gannedahl, M., & European Multiple Sclerosis Platform. (2017). New insights into the burden and costs of multiple sclerosis in Europe: Results for Belgium. Multiple Sclerosis Journal, 23(2_suppl), 29-40.

Evankovich, J., Cho, S. W., Zhang, R., Cardinal, J., Dhupar, R., Zhang, L., ... & Tsung, A. (2010). High mobility group box 1 release from hepatocytes during ischemia and reperfusion injury is mediated by decreased histone deacetylase activity. Journal of Biological Chemistry, 285(51), 39888-39897.

Gamerith, F., Zlabinger, G. J., Scherak, O., Kolarz, G., Schemper, M., Heinzl, H., & Menzel, J. E. (1993). Differences in anti-Fab antibodies in adult and late onset rheumatoid arthritis. Rheumatology international, 13(3), 107-112.

Garfinkel, R. J., Dilisio, M. F., & Agrawal, D. K. (2017). Vitamin D and its effects on articular cartilage and osteoarthritis. Orthopaedic Journal of Sports Medicine, 5(6), 2325967117711376.

Hajjaj-Hassouni, N., Mawani, N., Allali, F., Rkain, H., Hassouni, K., Hmamouchi, I., & Dougados, M. (2017). Evaluation of vitamin D status in rheumatoid arthritis and its association with disease activity across 15 countries:"The COMORA Study". International journal of rheumatology, 2017.

Haque, U. J., Bathon, J. M., & Giles, J. T. (2012). Association of vitamin D with cardiometabolic risk factors in rheumatoid arthritis. Arthritis care & research, 64(10), 1497-1504.

Holick, M. F., & Chen, T. C. (2008). Vitamin D deficiency: a worldwide problem with health consequences. The American journal of clinical nutrition, 87(4), 1080S-1086S.

Hussein, R.H., MezherAl-Rayahi, I.A., Taha,K.(2018). Rheumatoid factor isotypes in a sample of Iraqi rheumatoid arthritis patients, J. Glob. Pharma Technol. 10 141–145.

Ihsan, M.A.A. Chiad1*, A. Hanaa S.Yossef, Muntadher A. Al-Saidi2,(2015). Evaluation of Some Immunological Markers in the Rheumatoid Arthritis Patients, 56 7 2488–2493.

Ingegnoli, F., Castelli, R., & Gualtierotti, R. (2013). Rheumatoid factors: clinical applications. Disease markers, 35.

Kostoglou-Athanassiou, I., Athanassiou, P., Lyraki, A., Raftakis, I., & Antoniadis, C. (2012). Vitamin D and rheumatoid arthritis. Therapeutic advances in endocrinology and metabolism, 3(6), 181-187.

Lee, Y. H., & Bae, S. C. (2016). Vitamin D level in rheumatoid arthritis and its correlation with the disease activity: a meta-analysis. Clin Exp Rheumatol, 34(5), 827-833.

Li, Y. B., Xu, P., Xu, K., Cai, Y. S., Sun, M. Y., Yang, L., ... & Lu, S. M. (2016). Methotrexate affects HMGB1 expression in rheumatoid arthritis, and the downregulation of HMGB1 prevents rheumatoid arthritis progression. Molecular and cellular biochemistry, 420(1-2), 161-170.

Lin, J., Liu, J., Davies, M. L., & Chen, W. (2016). Serum vitamin D level and rheumatoid arthritis disease activity: review and meta-analysis. PloS one, 11(1), e0146351.

Liu, W., Zhang, L., Xu, H. J., Li, Y., Hu, C. M., Yang, J. Y., & Sun, M. Y. (2018). The anti-inflammatory effects of vitamin D in tumorigenesis. International journal of molecular sciences, 19(9), 2736.

Lu, M., Yu, S., Xu, W., Gao, B., & Xiong, S. (2015). HMGB1 promotes systemic lupus erythematosus by enhancing macrophage inflammatory response. Journal of immunology research, 2015.

Magna, M., & Pisetsky, D. S. (2014). The role of HMGB1 in the pathogenesis of inflammatory and autoimmune diseases. Molecular medicine, 20(1), 138-146.

Makinodan, T., & Kay, M. M. (1980). Age influence on the immune system. In Advances in immunology (Vol. 29, pp. 287-330). Academic Press.

Malm, K. Bremander, A. Arvidsson,B. Andersson, M.L.E. Bergman,S. Larsson,(2016). I. The influence of lifestyle habits on quality of life in patients with established rheumatoid arthritis - A constant balancing between ideality and reality, Int. J. Qual. Stud. Health Well-Being. 11 https://doi.org/10.3402/qhw.v11.30534.

Mayyadah R.H.A.-J. & Rashid, M.H.A. H.(2017). Plasma microRNA-16 expression as a potential biomarker for disease assessment in newly diagnosed theumatoid arthritis patients, IJABR, VOL.7 2017 257-262.

Meena, N., Chawla, S. P. S., Garg, R., Batta, A., & Kaur, S. (2018). Assessment of vitamin D in rheumatoid arthritis and its correlation with disease activity. Journal of natural science, biology, and medicine, 9(1), 54.

Meng, X. H., Wang, Z., Zhang, X. N., Xu, J., & Hu, Y. C. (2018). Rheumatoid Arthritis of Knee Joints: MRI–Pathological Correlation. Orthopaedic Surgery, 10(3), 247-254.

Mukhtar, M., Sheikh, N., Suqaina, S. K., Batool, A., Fatima, N., Mehmood, R., & Nazir, S. (2019). Vitamin D receptor gene polymorphism: an important predictor of arthritis development. BioMed Research International, 2019.

Muss, T. Q., AL-Faham, M., & Gorial, F. I.(2018). Assessment of Serum Galectin-8 in Iraqi Patients with Rheumatoid arthritis.

Nowak, A., Boesch, L., Andres, E., Battegay, E., Hornemann, T., Schmid, C., ... & Krayenbuehl, P. A. (2016). Effect of vitamin D3 on self-perceived fatigue: A double-blind randomized placebo-controlled trial. Medicine, 95(52).

Ohajianya, D. O., Okwara, M. O., Ugwu, J. N., Tim Ashama, A., Mbah, R. O., & Dike, N. F. (2016). Productivity of Farmland Values in Food Crop Production in the Natural Disasters Prone Areas of Imo State, Nigeria. International Journal of Sustainable Agricultural Research, 3(2), 42-48

Pérez-Hernández, N., Aptilon-Duque, G., Nostroza-Hernández, M. C., Vargas-Alarcón, G., Rodríguez-Pérez, J. M., & Blachman-Braun, R. (2016). Vitamin D and its effects on cardiovascular diseases: a comprehensive review. The Korean journal of internal medicine, 31(6), 1018.

Pilzweger, C., & Holdenrieder, S. (2015). Circulating HMGB1 and RAGE as clinical biomarkers in malignant and autoimmune diseases. Diagnostics, 5(2), 219-253.

Radner, H., Smolen, J. S., & Aletaha, D. (2011). Comorbidity affects all domains of physical function and quality of life in patients with rheumatoid arthritis. Rheumatology, 50(2), 381-388.

Rocha, S. D. B., Baldo, D. C., & Andrade, L. E. C. (2019). Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Advances in Rheumatology, 59.

Schierbeck, H. Pullerits, R. Pruunsild, C. Fischer,M. D. Holzinger, Å. Laestadius, E. Sundberg, Harris, H.E. (2013).HMGB1 levels are increased in patients with juvenile idiopathic arthritis, correlate with early onset of disease,,andare independent of disease duration, J. Rheumatol. 40 1604–1613. https://doi.org/10.3899/jrheum.120987.

Solidoro, P., Bellocchia, M., Aredano, I., Mattei, A., Pivetta, E., Patrucco, F., ... & Bucca, C. (2017). Asthmatic patients with vitamin D deficiency have decreased exacerbations after vitamin replacement. Nutrients, 9(11), 1234.

Song, Y. W., & Kang, E. H. (2010). The pathogenic role of rheumatoid factor in rheumatoid arthritis. International Journal of Clinical Rheumatology, 5(6), 651.

Tsung, A., Klune, J. R., Zhang, X., Jeyabalan, G., Cao, Z., Peng, X., ... & Billiar, T. R. (2007). HMGB1 release induced by liver ischemia involves Toll-like receptor 4–dependent reactive oxygen species production and calcium-mediated signaling. The Journal of experimental medicine, 204(12), 2913-2923.

van Etten, E., & Mathieu, C. (2005). Immunoregulation by 1, 25-dihydroxyvitamin D3: basic concepts. The Journal of steroid biochemistry and molecular biology, 97(1-2), 93-101.

Walker, S. E., & Ranatunga, S. K. (2006). Rheumatoid arthritis: A review. Missouri medicine, 103(5), 539-544.

Wang, H., Bloom, O., Zhang, M., Vishnubhakat, J. M., Ombrellino, M., Che, J., ... & Manogue, K. R. (1999). HMG-1 as a late mediator of endotoxin lethality in mice. Science, 285(5425), 248-251.

Wang, W., Wu, A., Zhou, Y., Wang, Y., & Cao, K. (2017). Association between Vitamin D Receptor polymorphisms and rheumatoid arthritis risk: a meta-analysis. International Journal of Clinical & Experimental Medicine, 10(2).

Yuen, E. Y., Liu, W., Karatsoreos, I. N., Ren, Y., Feng, J., McEwen, B. S., & Yan, Z. (2011). Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory. Molecular psychiatry, 16(2), 156-170.