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THE IMPACT OF VITAMIN D LEVELS ON THE OXIDATIVE STRESS IN IRAQI RHEUMATOID ARTHRITIS PATIENTS

Ahmed Qasim Hwaidi and Hathama Razooki Hasan*

Department of Chemistry, College of Science, University of Baghdad, Baghdad, Iraq.
*e-mail: hathama2012@gmail.com

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ABSTRACT : This study wasundertaken tolook up the role of vitamin D in the oxidative stress present in Iraqi rheumatoid arthritis patients. The study includes 119 patients with rheumatoid arthritis, who were divided into four groups according to the level of vitamin D into : G1 (vitamin D severe deficient group ≤ 10 ng/mL, n = 31), G2 (vitamin D deficient group = 11–20 ng/mL, n = 30), G3 (vitamin D insufficient group = 21–30 ng/mL, n = 30)and G4 (vitamin D sufficient group > 30 ng/mL, n = 28). Vitamin D was measured by Roche Elecsys vitamin D total II assay, while total oxidant status (TOS) and total antioxidant status (TOS) were determined by Erel's methods. In the sera of rheumatoid arthritis patients with low levels of vitamin D (G1 & G2 groups) a highly significant increase was obvious in TOS (p = 0.000) compared with who had sufficient level of vitamin D (G4 group) and there was a significant increase (p = 0.023) in insufficient group (G3 group) when compared also with G4 group. Meanwhile a highly significant decrease in TAS in sera of each of G1, G2 and G3 groups in comparison to the G4 group (p = 0.000 in case of each of G1 & G2 and p = 0.001 in case of G3). The results of oxidative stress index (OSI) showed a highly significant increase (p=0.000) in sera of each of G1 and G2 groups and a significant increase (p = 0.002) in G3 group in comparison to that of G4 group. This study revealed a counter balance between vitamin D and oxidants and confirms the role of vitamin D as antioxidant.

Key words : Rheumatoid arthritis, vitamin D, oxidative stress index, total oxidant status, total antioxidants status.

INTRODUCTION

Rheumatoid arthritis (RA) is a long-lived inflammatory disease of joints and reported to be one of autoimmune disorder, which is characterized bychronic proliferative synovitis that results in eventually destruction of bone (Tešija, 2003). It is a most common disease of connective tissue that is reported in 0.5-1.5% of the world population, and influenced by geographic locations (Jönsson, 2008). Iraqi population of RA was determined to be 1% (Alkazzaz, 2013), all ages can beinfluenced by this disease (Gibofsky, 2012). Eventhough, its etiology and pathogenesis stayunclear, the genetic and environmental factors were suggested to cause this disease (Nielen *et al*, 2006).

Smoking cigarette is reported to be one of the environmental factors which considered the foremost frequent cause that foretells the disease susceptibility and its severity. The infectious agents and the difference in steroid hormones level like vitamin D (vit D) has been reported to make healthy persons suffered from RA (Kobayashi *et al*, 2008).

The low level of vit D is considered as a worldwide clinical problem, and it isusual in conditions that have influence on the joints (Plotnikoff and Quigley, 2003). A previous study recognized that low vit D levels were linked with the initial appearance of a number of rheumatic disorders (Cutolo, 2008). Many studies found a link between low vit D level and RA activity (Cutolo *et al*, 2006; Grazio *et al*, 2015). In contrast, discrepancies in the presence of such link has been reported by many other studies (Ediz *et al*, 2011; Tetik *et al*, 2010).

In spite of the accurate cause, the reactive oxygen species (ROS), that are a mainly important medical group created in the biological system was suggested to play acritical role in RA (Valko *et al*, 2007).

Reactive oxygen species consist of all the chemical molecules that are produced by unfinished oxygen reduction. They are quick and very reactive molecules that react with themselves and with other molecules to get stability. ROS are formed throughout the metabolism of living cell and include both useful and harmful properties (Mateen *et al*, 2016). Harmful properties of ROS are observed when a high level of free radicals present, or their removal by antioxidants become damaged. Such high

level of free radicals might lead to destruction of many physiological processes as a result of damage cellular nucleic acids, proteins, lipids as well as membrane of the cell (Hemnani and Parihar, 1998). ROS formation was measured to be five fold more in RA patients than healthy individuals (Hassan *et al*, 2011). They might be created in the joint of RA either by the chondrocytes, activated macrophages in the synovial membrane, or by the activated neutrophils in the synovial cavity (Jones, 2006). They are a pathogenic hallmark, that can deform the cartilage of joint by attacking its proteoglycan and inhibiting its synthesis (Hadjigogos, 2003; Mateen *et al*, 2016).

Vit D has been recently reported to have a non-calcemic function, it acts as antioxidant (Mokhtari *et al*, 2017). Eventhough a lot of researches have been done on the vit D action. A little information is available about its actual role in RA disease. Therefore, the aim of the current project is to study the role of vit D level on the oxidative stress (OS) present in RA patients.

MATERIALS AND METHODS

Chemicals

All chemicals used throughout this work were of highly purified grads.

Studied groups

The study was carried out on a total number of 119 patients. They were Iraqi RA patients with age ranged from (40 - 60 years). All patients were medically diagnosed and had a positive C-Reactive Protein (CRP). The study participants were divided into four groups according to their vit D levels based on references (Holick, 2007; Spiro and Buttriss, 2014) into: G1 (vit D severe deficient group ≤ 10 ng/mL, n = 31), G2 (vit D deficient group = 11–20 ng/mL, n = 30), G3 (vit D insufficient group = 21–30 ng/mL, n = 30) and G4 (vit D sufficient group > 30 ng/mL, n = 28). In order to investigate the role of vit D on OS present in these patients, G4 was used as a control in the current study. All patients were attending to different hospitals and medical centers in Iraq. Patients of RA having chronic renal failure, systemic lupus erythematosus, diabetes mellitus, any systemic illness and patients on enzyme inducer drugs or on calcium and vit D supplements were excluded from the study. Blood samples were collected from RA patients, then the sera were separated and stored at -20ºC until been used.

The study protocol was proved by the Ethics Committee of College of Science, University of Baghdad.

Determination of vitamin D level

The assay was performed using Roche Cobas e 411 analyzer and Roche Elecsys vit D total II assay (Roche Diagnostics, Mannheim, Germany). Elecsys Vit D assay employs VDBP to capture both 25-hydroxyvitamin D3 and D2. This assay is intended for the quantitative determination of total vit D (25-OH) in human serum.

Determination of total oxidant status (TOS)

Total oxidant status value was determined using Erel's method (Erel, 2005). The assay was calibrated with hydrogen peroxide using different concentrations (0, 25, 50, 100, 150, 200 ìmol/L) of (10 mM) standard hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (ìmol H2O2 Equiv./L). Then the TOS level of all RA samples were calculated by the equation that derived from the standard curve, that was constructed from by plotting the absorbances of the standard solutions measured at a wave length λ = 560 nm against their concentrations.

Determination of total antioxidant status (TAS)

The total antioxidant status value was determined using Erel's method (Erel, 2004). The assay was calibrated using different concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0 mmol/L) of (1.0 mM) standard vit C. The results were expressed as a term of millimolar vit C equivalent per liter. Then the TAS level of all RA samples were calculated from the equation that derived from the standard curve, that was constructed by plottingthe absorbances of the standard solutions measured at a wave length λ = 444 nm against their concentrations.

Calculation of oxidative stress index (OSI)

Oxidative stress index value was calculated from the below equation (Karahan *et al*, 2013).

OSI (arbitrary unit) = TOS (ìmol H_2O_2 Eq/L)/TAS (ìmol Vit C Eq/L)

Statistical analysis

The data throughout this work was reported in the form of (mean value ± the standard deviation). The data were compared by SPSS version 20 (One-Way ANOVA and Pearson correlation), where the difference is considered as highly significant when ($P < 0.001$), significant when ($P < 0.05$) and non-significant when ($P > 0.05$).

RESULTS

The mean value and standard deviation (mean ± SD) of the level of vit D, ages and gender of the patients are shown in Table 1.

Total Oxidant Status (TOS) {which can be defined

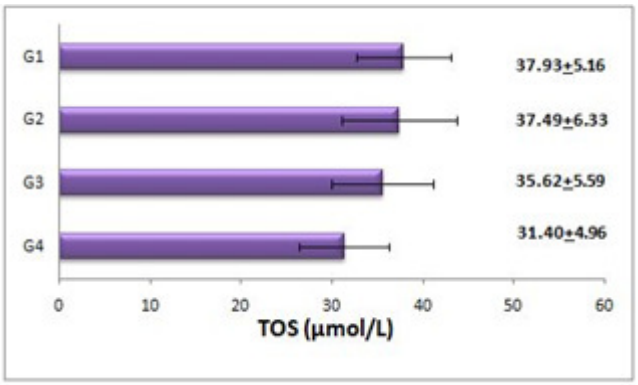

Fig. 1 : Comparison between level of TOS among the different studied groups.

(G4 group). Meanwhile there is a significant increase in TOS level (p = 0.023) of the group that hadinsufficient vit D level (G3 group) when compared also withthat of G4 group.

When Pearson correlation was done between vit D level and TOS level in each of G1, G2, G3 and G4 groups, the results of such correlation are presented in Fig. 2. It is obvious that vit D has a negative correlation with TOS in all groups.

Total Antioxidant Status (TAS) {which is a biomarker for measuring the antioxidant potential of body fluids can be defined as the moles of oxidants neutralized by one

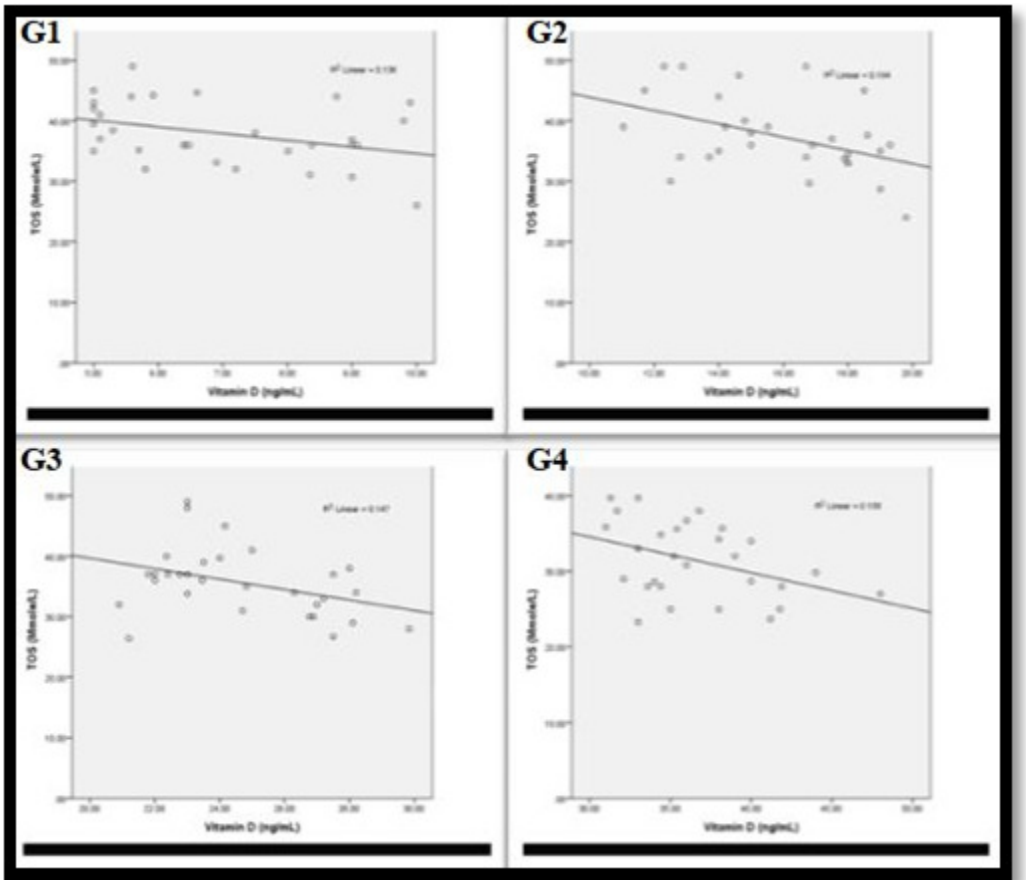

Fig. 2 : Association of TOS level with vitamin D level in: G1: The correlation coefficient -0.370 and the correlation is significant (p=0.05). G2: The correlation coefficient -0.441 and the correlation is significant (p=0.05). G3: The correlation coefficient -0.384 and the correlation is significant (p=0.05). G4: The correlation coefficient -0.397 and the correlation is significant (p=0.05).

as the *in vivo* marker of a shift developing in an oxidative/ anti oxidative ratio in favor of the oxidative side (GÜNEY *et al*, 2015)} was measured in serum of the different studied patients groups as aforementioned in materials & methods sectionand the results are presented in Fig. 1.

It clear from the above results that in the sera of patients with low levels of vit D (G1 & G2 groups) a highly significant increase in TOS is obvious (p=0.001) compared with the one who had sufficient level of vit D

liter of solution (Peluso and Raguzzini, 2016)} was measured in the studied groups as mentioned in methods & materials section and the results are shown in Fig. 3.

From the above results, it is clear that there is a highly significant decrease (p = 0.000 in G1 & G2 and p = 0.001 in G3) in this parameterin serum of all G1, G2 and G3 groups in comparison to the G4 group.

Upon performing Pearson correlation between the level of vit D in each groups (G1, G2, G3 and G4) with

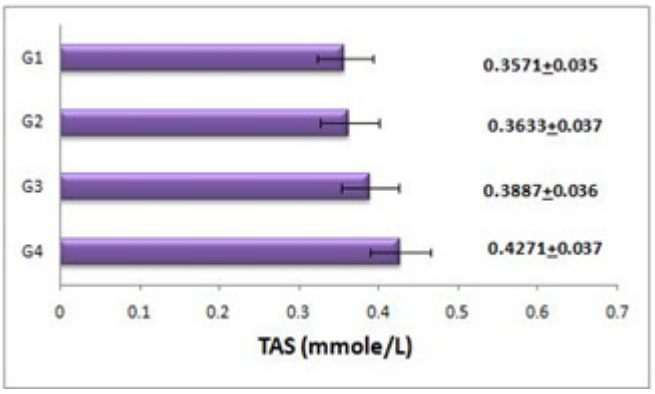

Fig. 3 : Comparison between level of TAS among the different studied groups.

groups, and there is a significant increase (p = 0.002) in G3 group in comparison to the G4 group.

Pearson correlations between vit D level and OSI in each of the studied groups (G1, G2, G3 and G4)are presented in Fig. 6. It can conclude from Fig. 6 that vit D level a negative correlation with OSI in all of these studied groups.

DISCUSSION

Oxidative stress (OS) is a term applied to the cell as a result of excess presence of oxidants accompanied with antioxidants deficiency (Kashmiri and Mankar, 2014). In this expression, the fact that OS reveals the final

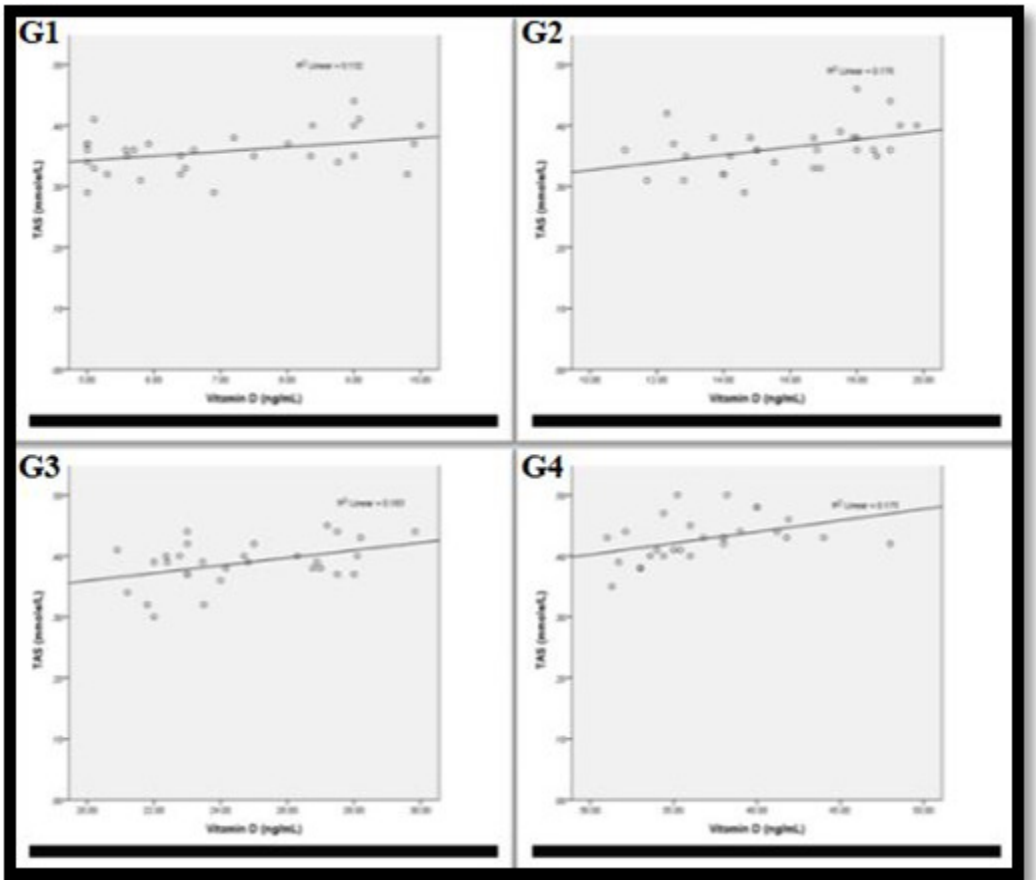

Fig. 4 : Association of TAS level with vitamin D level in : G1: The correlation coefficient 0.363 and the correlation is significant (p=0.05).G2: The correlation coefficient 0.420 and the correlation is significant (p=0.05). G3: The correlation coefficient 0.427 and the correlation is significant (p = 0.05). G4: The correlation coefficient 0.419 and the correlation is significant (p=0.05).

their TAS level. Fig. 4 shows that vit D level has a positive correlation with TAS in all of G1, G2, G3 and G4.

The Oxidative Stress Index (OSI), which is the most accurate method to express OS (Karahan *et al*, 2013) was calculated in serum samples of all patients groups, asmentionedin the materials and methods section. The results are presented in Fig. 5.

It is obvious from Fig. 5 that there is a highly significant increase (p = 0.000) in this index in serum of G1 and G2

properties of the pooled action of oxidants and antioxidants is not considered (Quiñonez-Flores *et al*, 2016). The OS being one of the important cause of various rampant diseases of modern age such as RA, cancer, osteoarthritis, osteoporosis and atherosclerosis, they occur due to the disruption in the balance between body's oxidants load and antioxidants reservoir (Demirbag *et al*, 2005).

RA is a relapsing, inflammatory autoimmune disorder with synovial proliferation, destruction of bone and

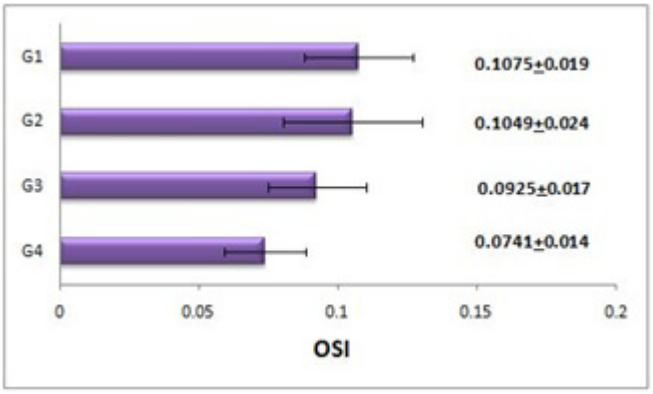

Fig. 5 : Comparison between OSI among the different studied groups.

activated polymorphonuclear cells (PMNs) and by ischemia–reperfusion in the inflamed joints (Taysi *et al*, 2002).

In the current study, the effect of different level of vit D on measured OS in RA patients was investigated. The obtained results are expressed as [TOS], [TAS] and OSI because they are the mainly suitable and reliable method to measure OS as whole (Bozkus *et al*, 2013). The results revealed that the level of oxidants and OSI decline (Figs. 1 & 5) with an increase in the level of antioxidants (Fig. 3) as vit D level increase. Meanwhile

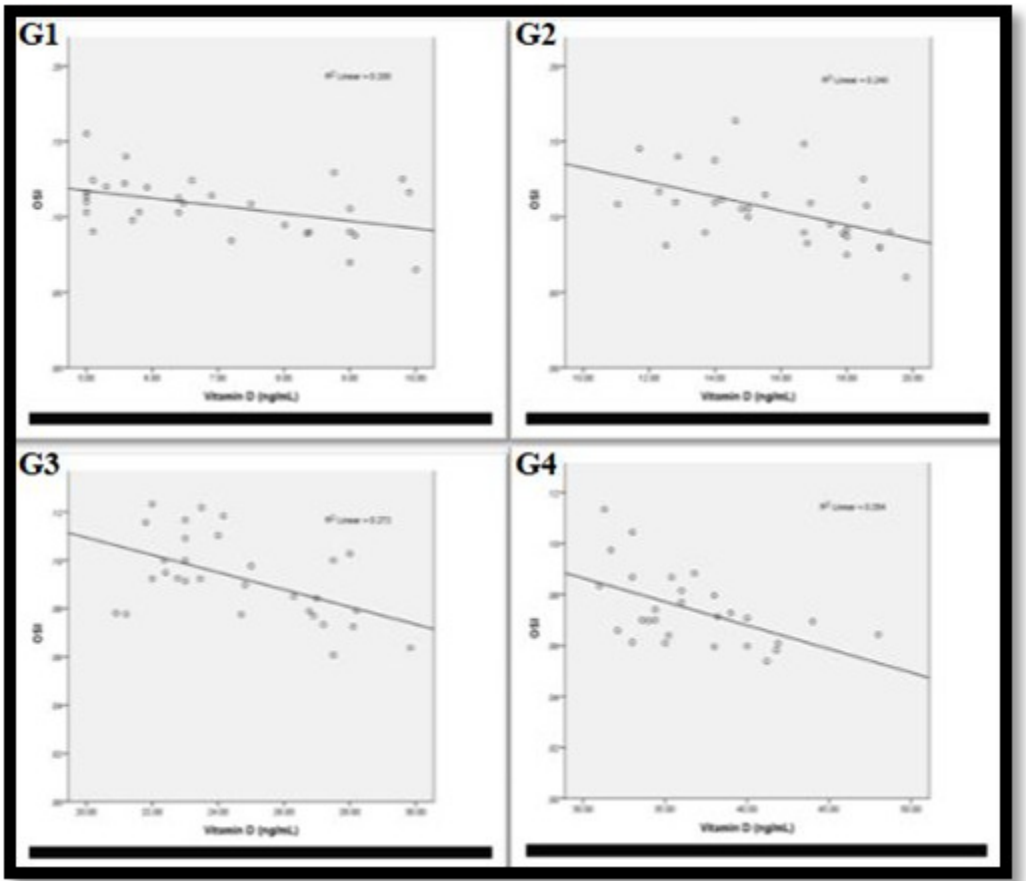

Fig. 6 : Association of OSI with vitamin D level in:G1: The correlation coefficient -0.447 and the correlation is significant (p= 0.05). G2: The correlation coefficient -0.499 and the correlation is significant (p= 0.01). G3: The correlation coefficient -0.522 and the correlation is significant (p= 0.01). G4: The correlation coefficient -0.533 and the correlation is significant (p=0.01).

cartilage degradation (Guo *et al*, 2018). Although, the etiology of RA is still not very much clear, several studies have established a role of oxidant in the pathogenesis of inflammatory chronic disease (Bauerova and Bezek, 2000; Filippin *et al*, 2008; Hitchon and El-Gabalawy, 2004). Macrophages and polymorphonuclear cells present at the site of synovitis promotes the formation of reactive oxygen species that cause activation of inflammatory molecules, which are involved in the progression of RA (Mateen *et al*, 2016). In this disease two common critical mechanisms of oxidant formation was reported by

the level of vit D has a negative correlation with [TOS] and OSI as shown if (Figs. 2 and 6), while a positive one with [TAS] as shown in Fig. 4.

The presence of the oxidative stress which is clear from (Figs. 1 and 3) in RA patients, agrees with the results of Jazayeri *et al* (2010), who measured glutathione (GSH) and vit C levels (as antioxidants) as well as Malondialdehyde (MDA, as index of lipid peroxidation) and found that [MDA] decrease as [GSH] and [vit C] increase in their RA patients. As well as agree with results

Table 1: The mean value and standard deviation (mean ± SD) of level of vit D in the different studied groups with distribution of their ages and gender.

Groups	n	Ages (year)	Male	Female	Vit D (ng/mL)
G1	31	49.80±5.85	10	21	6.96±1.72
G2	30	51.30±4.55	8	22	15.82±2.54
G3	30	50.40±5.49	11	19	24.68±2.49
G4	28	48.46±6.19	10	18	36.64±4.18

of Chandankhede *et al* (2013), who reported that there is a disruption in the balance of glutathione peroxidase, superoxide dismuatase (as antioxidants) with [MDA] (as index of lipid peroxidation). They suggested that the disruption in the balance might play a role in the tissue injure and inflammation action of RA.

The positive correlation between vit D level and [TAS] can be explained as that vitamin D regulates OS through induction of several molecules involved in antioxidants defence system like glutathione peroxidase, superoxide dismuatase and GSH (Mokhtari *et al*, 2017). Also, vit D could reduce the oxidants production by induction of the cellular pool of reduced thiols (Jain *et al*, 2014). Furthermore, it reduces the oxidants production by suppression of NADPH oxidase gene (which its activation is considered as a positive marker of OS) (Kono *et al*, 2013; Labudzynskyi *et al*, 2015). Another suggested mechanism of vit D role is through the reduction of lipid peroxidation and induction of superoxide dismuatase activity as Hamden et al suggested from their study of rat with induced diabetes (Hamden *et al*, 2008).

Additional investigation is necessary to reveal the precise mechanism behind the antioxidant property of vit D. This would be very useful for identification of possible new therapeutic plans.

CONCLUSION

During recent years, vit D was suggested to prevent chronic disease such as diabetes disease, cardiovascular and chronic kidney disease by regulation of OS (Wang *et al*, 2017).

Based on these reported literatures and on the present study results which reveal the presence of counterbalance between vit D level and the [TOS] and OSI.One can conclude that vit D has an antioxidant action in RA patients and by this confirm the new suggested non-calcemic of this vitamin in other chronic diseases. Therefore, taking this vit as a supplement maybe suggested to aid in relief the symptoms of this disease.

REFERENCES

Alkazzaz A M H (2013) Incdence of Rheumatoid Arthritis [2001 to 2011]. *Iraqi Academic Scientific Journal* **12**(4), 568-572.

Bauerova K and Bezek S (2000) Role of reactive oxygen and nitrogen species in etiopathogenesis of rheumatoid arthritis. *General Physiology and Biophysics* **18**, 15-20.

Bozkus F, San I, Ulas T, Iynen I, Yesilova Y, Guler Y and Aksoy N (2013) Evaluation of total oxidative stress parameters in patients with nasal polyps. *Acta Otorhinolaryngologica Italica* **33**(4), 248.

Chandankhede M S and Gupta M M (2013) Oxidative stress and antioxidant status in patients with rheumatoid arthritis. *Int J Biol Med Res.* **4**(2), 3088-3090.

Cutolo M (2008) Vitamin D and autoimmune rheumatic diseases: Oxford University Press.

Cutolo M, Otsa K, Laas K, Yprus M, Lehtme R, Secchi M and Seriolo B (2006) Circannual vitamin D serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. *Clin Exp Rheumatol.* **24**(6), 702-704.

Demirbag R, Yilmaz R, Erel O, Gultekin U, Asci D and Elbasan Z (2005) The relationship between potency of oxidative stress and severity of dilated cardiomyopathy. *The Canadian Journal of Cardiology* **21**(10), 851-855.

Ediz L, Hiz O, Ozkol H, Gulcu E, Toprak M and Ceylan M F (2011) Relationship between anti-CCP antibodies and oxidant and anti-oxidant activity in patients with rheumatoid arthritis. *International Journal of Medical Sciences* **8**(2), 139.

Erel O (2004) A novel automated method to measure total antioxidant response against potent free radical reactions. *Clinical Biochemistry* **37**(2), 112-119.

Erel O (2005) A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry* **38**(12), 1103-1111.

Filippin L I, Vercelino R, Marroni N and Xavier R M (2008) Redox signalling and the inflammatory response in rheumatoid arthritis. *Clinical & Experimental Immunology* **152**(3), 415-422.

Gibofsky A (2012) Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. *The American Journal of Managed Care* **18**(13 Suppl), S295-302.

Grazio S, Grubišiæ F, Kavanagh H S, Nagliæ Đ B, Aniæ B, Bakula M and Cvijetiæ S (2015) Vitamin D serum level, disease activity and functional ability in different rheumatic patients. *The American Journal of the Medical Sciences* **349**(1), 46-49.

GÜNEY T, ALIÞIK M, Akinci S, NEÞELÝOĐLU S, Dilek I and EREL Ö (2015) Evaluation of oxidant and antioxidant status in patients with vitamin B12 deficiency. *Turkish Journal of Medical Sciences* **45**(6), 1280-1284.

Guo Q, Wang Y, Xu D, Nossent J, Pavlos N J and Xu J (2018) Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. *Bone Research* **6**.

Hadjigogos K (2003) The role of free radicals in the pathogenesis of rheumatoid arthritis. *Panminerva Medica* **45**(1), 7-13.

Hamden K, Carreau S, Jamoussi K, Ayadi F, Garmazi F, Mezgenni N and Elfeki A (2008) Inhibitory effects of 1α, 25dihydroxyvitamin D3 and Ajuga iva extract on oxidative stress, toxicity and hypo-fertility in diabetic rat testes. *Journal of Physiology and Biochemistry* **64**(3), 231-239.

Hassan S Z, Gheita T A, Kenawy S A, Fahim A T, EL SOROUGY I M and Abdou M S (2011) Oxidative stress in systemic lupus erythematosus and rheumatoid arthritis patients: relationship to disease manifestations and activity. *International Journal of Rheumatic Diseases* **14**(4), 325-331.

Hemnani T and Parihar M (1998) Reactive oxygen species and oxidative DNA damage. *Indian Journal of Physiology and Pharmacology* **42**, 440-452.

Hitchon C A and El-Gabalawy H S (2004) Oxidation in rheumatoid arthritis. *Arthritis Res Ther.* **6**(6), 265.

Holick M F (2007) Vitamin D deficiency. *New England Journal of Medicine* **357**(3), 266-281.

Jain S K, Micinski D, Huning L, Kahlon G, Bass P and Levine S N (2014) Vitamin D and L-cysteine levels correlate positively with GSH and negatively with insulin resistance levels in the blood of type 2 diabetic patients. *European Journal of Clinical Nutrition* **68**(10), 1148.

Jazayeri S, Hoshyarrad A, Hoseini F and Fasihi-Radmandi M (2010) Effects of antioxidant supplementations on oxidative stress in rheumatoid arthritis patients. *Journal of Biological Sciences* **10**(1), 63-66.

Jones D P (2006) Disruption of mitochondrial redox circuitry in oxidative stress. *Chemico-Biological Interactions* **163**(1-2), 38-53.

Jönsson B (2008) Patient access to rheumatoid arthritis treatments. *The European Journal of Health Economics* **8**(2), 35-38.

Karahan O, Manduz S, Bektasoglu G, Zorlu A, Turkdogan K and Bozok S (2013) A high oxidative stress index predicts endothelial dysfunction in young male smokers. *Bratislavske lekarske listy* **114**(12), 721-725.

Kashmiri Z and Mankar S (2014) Free radicals and oxidative stress in bacteria. *Int J Curr Microbiol App Sci*. **3**(9), 34-40.

Kobayashi S, Momohara S, Kamatani N and Okamoto H (2008) Molecular aspects of rheumatoid arthritis: role of environmental factors. *The FEBS Journal* **275**(18), 4456-4462.

Kono K, Fujii H, Nakai K, Goto S, Kitazawa R, Kitazawa S and Nishi S (2013) Anti-oxidative effect of vitamin D analog on incipient vascular lesion in non-obese type 2 diabetic rats. *American Journal of Nephrology* **37**(2), 167-174.

Labudzynskyi D, Zaitseva O, Latyshko N, Gudkova O and Veliky M (2015) Vitamin D3 contribution to the regulation of oxidative metabolism in the liver of diabetic mice. *The Ukrainian Biochemical Journal* **87**(3), 75-90.

Mateen S, Moin S, Khan A Q, Zafar A and Fatima N (2016) Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. *PloS One* **11**(4), e0152925.

Mateen S, Moin S, Zafar A and Khan A Q (2016) Redox signaling in rheumatoid arthritis and the preventive role of polyphenols. *Clinica Chimica Acta* **463**, 4-10.

Mokhtari Z, Hekmatdoost A and Nourian M (2017) Antioxidant efficacy of vitamin D. *J Parathyr Dis*. **5**(1), 11-16.

Nielen M M, van Schaardenburg D, Lems W F, van de Stadt R J, de Koning M H, Reesink H W and Dijkmans B A (2006) Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino *et al*. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology* **54**(11), 3719-3720.

Peluso I and Raguzzini A (2016) Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. *Pathology Research International*.

Plotnikoff G A and Quigley J M (2003) *Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain*. Paper presented at the Mayo clinic proceedings.

Quiñonez-Flores C M, González-Chávez S A, Del Río Nájera D and Pacheco-Tena C (2016) Oxidative stress relevance in the pathogenesis of the rheumatoid arthritis: a systematic review. *BioMed research International*.

Spiro A and Buttriss J (2014) Vitamin D: An overview of vitamin D status and intake in E urope. *Nutrition Bulletin* **39**(4), 322-350.

Taysi S, Polat F, Gul M, Sari R and Bakan E (2002) Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. *Rheumatology International* **21**(5), 200-204.

Tešija A (2003) Novi biomarkeri u dijagnostici i praæenju bolesnika s reumatoidnim artritisom. *Biochem Med.* **13**(1-2), 141-142.

Tetik S, Ahmad S, Alturfan A A, Fresko I, Disbudak M, Sahin Y and Yardimci K T (2010) Determination of oxidant stress in plasma of rheumatoid arthritis and primary osteoarthritis patients.

Valko M, Leibfritz D, Moncol J, Cronin M T, Mazur M and Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology* **39**(1), 44-84.

Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N and Zheng S G (2017) Vitamin D and chronic diseases. *Aging and Disease* **8**(3), 346.