Iraqi National Journal of Chemistry

Journal homepage:
http://iqnjc.com/Default.aspx

Prevalence of vitamin D deficiency among Rheumatoid Arthritis patients in Wasit Province / Iraq.

Hathama Razooki Hasan.
College of Science /University of Baghdad.
Email address: hathamahasan@scbaghdad.com, hathama2012@gmail.com

Abstract:

Background: Vitamin D is one of the fat-soluble vitamins, which, upon its chemical conversion in the liver and kidney, gives 25(OH) D, an indicator of vitamin D status in the serum. This vitamin plays multiple functions in the human body. Among these functions is that it is considered as a regulatory factor in human immunity, Rheumatoid arthritis **(**RA) is a most common disease of connective tissue and is considered as a long-lived inflammatory disease of joints. It is a common chronic systemic inflammatory autoimmune disease.

Aim of the study: The present study focused on measuring the prevalence of vitamin D deficiency in RA Iraqi patients who live in Wasit Province.

Method: The total number of patients enrolled in the study were 653 of both gender and different ages. Their fasting blood samples were collected during the period of January-September 2018. Vitamin D level as 25 hydroxy vitamin D(25-OHD) was measured in the patient's serum using Roche Cobas e 411 analyzer and Roche Elecsys vitamin D total II assay (Roche Diagnostics, Mannheim, Germany).

Results: The percentage of females with RA disease was higher than the males among the patients group enrolled in the present study. A high percentage (35.5%) of the studied RA patients were found to have vitamin D severe, deficiency (vitamin D level<10 ng/mL), while (19.8%) were vitamin D deficient, (8.7%) were with vitamin D insufficiency and 9.2% of them had a sufficient level of vitamin D. Furthermore, the female's patients appeared to have higher levels of vitamin D deficiency in comparison to the males. Moreover, vitamin D levels in the serum of RA patients aged 41-50 years were higher than those with other age ranges. While those with an age range of more than 61 years had the lowest.

Conclusion: The results indicated that vitamin D severe deficiency is high in the studied RA patients (43.5%), and its prevalence is high in women patients, as well as in patients with age of less than 41 years and those with age higher than 61 years. Meantime, the male patients have higher vitamin D than the female among all age groups.

Keywords: fat-soluble vitamin; 25 hydroxy vitamin D; hypovitaminosis D; Rheumatoid arthritis; inflammatory autoimmune disease.

Introduction

Vitamin D is one of the fat soluble vitamins, that upon its chemical conversion in the liver and kidney, gives 25(OH)D (which is considered an objective indicator of vitamin D status in the blood) as the first reaction product. It produces 1,25-dihydroxyvitamin D (1,25(OH)2D, the main bioactive form [1]. Many researchers have reported that this vitamin plays multiple functions in the human body. These functions are based on the receptors in most body's tissues and cells, and together, they regulate more than 200 human genes. This implies that vitamin D could have diverse roles in maintaining human health among them keeping homeostasis of phosphate and calcium metabolism [2, 3]. Furthermore, as a steroid hormone, vitamin D acts upon binding to its nuclear function as a modulator of many genes involved in inflammatory and immune modulation [4, 5]. Moreover, it acts as an anti-inflammatory and antioxidant in the body [6], functions that have gradually become apparent for this vitamin[7,8].
Even though there is a dual source for vitamin D in the human body: dietary intake and its synthesis in the skin upon exposure to sunlight [9], several reports and reviews performed in different developed and developing countries during the past decade have pointed out to the rapid growth of vitamin D deficiency globally among different population [10, 11, 12] and hypovitaminosis D has been reported as a global health problem [13, 14]. About 30-40% global population has a mild deficiency of this vitamin, which is associated with infectious & communicable diseases [15], with 7% of the global population having less than 20ng/mL [12].

Rheumatoid arthritis (RA) is a systemic and progressive chronic inflammatory disease that has been reported to be an autoimmune disorder that primarily affects the joints. It is the most common disease of connective tissue and is considered a long-lived inflammatory disease of joints that primarily affects the lining of the synovial joints [16]. RA is characterized by chronic proliferative synovitis that eventually leads to many complications, such as progressive joint destruction, deformation, and progressive disability. This joint disease is associated with autoantibodies that target various molecules and eventually lead to the destruction of bone, several systemic complications, and premature death [17].
It has been reported that about 2-3% of the world population suffers from this disease [18]. A recent research mentioned that RA occurs in about 5 per 1000 people [16]. In Iraq, 1% of the Iraqi population was reported to suffer from this disease [19], (a percentage which we think is much higher nowadays due to Iraq's present pollution situation). This disease occurs in all age ranges, even though its onset occurs in middle age and is more common in adults of older age, with the possibility of its development in younger ages and children [20]. It also can occur in all ethnic as well as in all races [2]. Furthermore, it is influenced by the geographic locations [21. The exact etiology of this disease is still unknown to a certain extent, and many genetic and environmental factors have been reported to have a role in its development, including hypovitaminosis D [6].
In Iraq, few studies have been carried out to look up the association of vitamin D deficiency with different diseases such as Parkinson's Disease, primary fibromyalgia syndrome, and asthma [22-24]. No report in the literature indicates vitamin D status in Iraqi RA patients. Therefore, the present study explored the prevalence of vitamin D deficiency among Iraqi Rheumatoid Arthritis patients, taking RA patients living in Waist Province / Iraq as the study participants.

Materials and Methods

Study population:
The present study's participants were RA patients (n=653), who live in Waist Province in Iraq. They were attending Alzhraa Hospital in Waist during the period January- September 2018 and were diagnosed by the hospital specialist to have RA. Those who were excluded from the study

were: those with diabetes mellitus, or chronic renal failure, or any systemic illness, and those who were on calcium and vitamin D supplements or enzyme inducer drugs. Fasting blood samples were collected from the patients, and the serum was obtained by centrifugation of the collected blood samples. then obtained serum samples were used for vitamin D measurements as explained below.

Determination of vitamin D level

Roche Cobas e 411 analyzer (Roche Company, Germany) and Roche Elecsys vitamin D total II assay (Roche Diagnostics, Mannheim, Germany) were used to measure this vitamin's level in the collected serum samples. The assay was performed using Elecsys Vitamin D assay employs Vitamin D Binding Protein (VDBP). This assay is intended for quantitatively determining total vitamin D (25-OH) in human serum. It is considered the best method to measure vitamin D in serum due to its prolonged half-life [(1)].

Statistical analysis

The data throughout this work was revised and tabulated using SPPS, version 25 (IBM SPSS, Inc.). Data were expressed as median, and the value of categorical data was presented as a number (percentage).

Results

The grouping of the participants of the present study according to their serum vitamin D level was based on [(25, 26)]. Therefore the participants were divided into four groups: severe deficient group (vitamin D level < 10 ng/mL, deficient group (vitamin D level = 11-20 ng/mL), insufficient group (vitamin D level= 20-30 ng/mL) and sufficient group (vitamin D> 30 ng/mL). The serum vitamin D measurement results show that our RA patients (n=653) can be divided into these four groups, as illustrated in Figure 1.

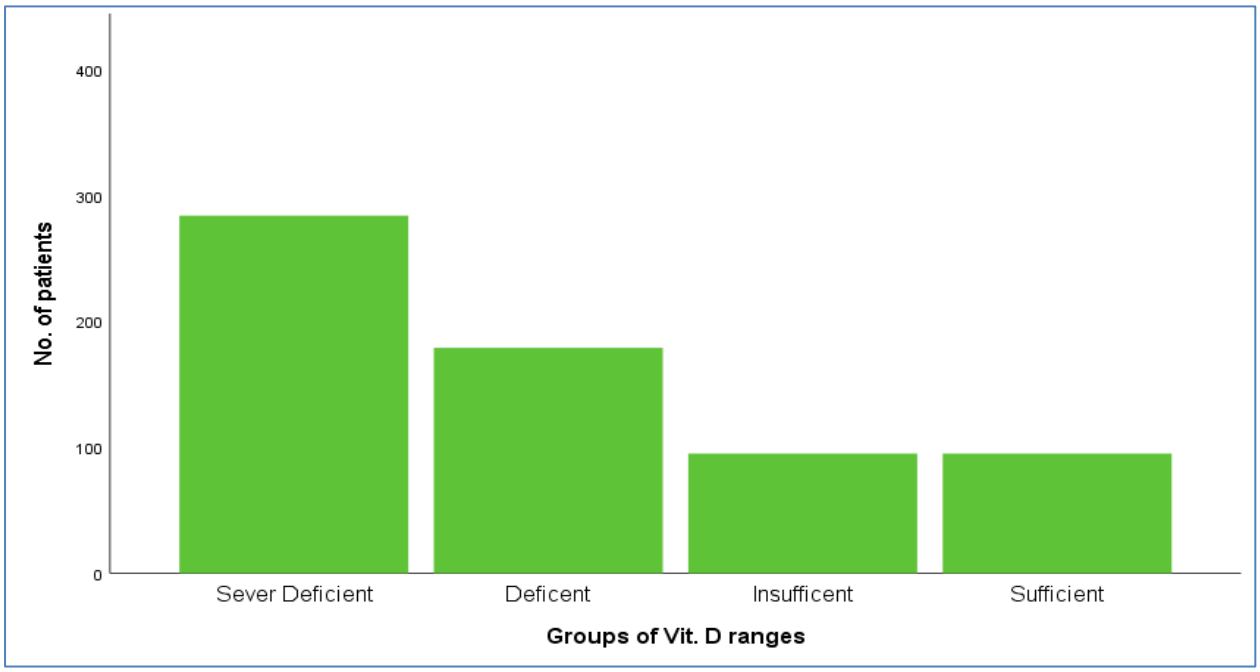

Figure 1. The vitamin D status among the studied RA patients.

It is clear from the above results that the number of RA patients with serum vitamin D levels less than 20ng/mL (those with severe D deficient level and deficient level) is much higher than the number in the other two groups. The percentage of RA patients within each group was calculated, and the results are presented in Table 1.

Table 1: Vitamin D status among the total studied Iraqi RA patients.

Group			Total
Vitamin D levels (ng/ml)	Severe Deficiency	N	284
		%	43.5%
	Deficient	N	179
		%	27.4%
	Insufficient	N	95
		%	14.5%
	Sufficient	N	95
		%	14.5%
Number and percentage of total patients		N	653
		%	100%

These results show that (43.5%) of the total studied RA patients had severely deficient vitamin D levels, followed by those with deficient levels (27.4%), and then by both the insufficient and the sufficient groups.

The RA patients enrolled in the present study were of both genders. Therefore, the serum 25(OH) D level was analyzed considering the gender distribution, and the results are presented in Figure 2 and Table 2.

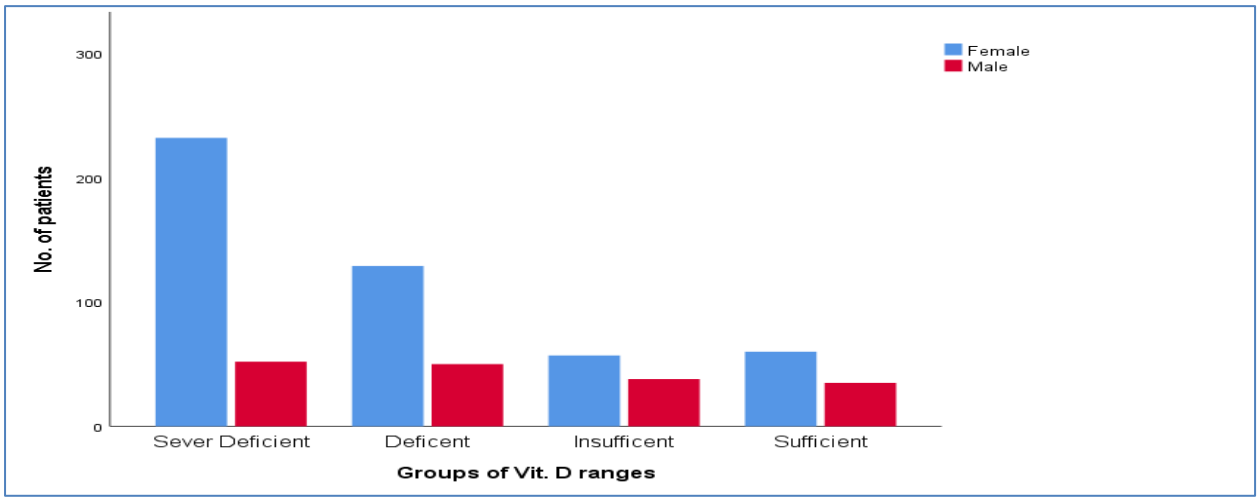

Figure 2. Vitamin D status in female & male RA patients versus their number.

Table 2. The number and percentages of RA incidences and vitamin D status according to gender.

		Gender		Total
		Female	Male	
Severe Deficient	N	232	52	284
	%	35.5%	8.0%	43.5%
Deficient	N	129	50	179
	%	19.8%	7.7%	27.4%
In sufficient	N	57	38	95
	%	8.7%	5.8%	14.5%
Sufficient	N	60	35	95
	%	9.2%	5.4%	14.5%
Number & percentage of total patients	N	478	175	653
	%	73.2%	26.8%	100.0%

As it is clear from the above results, among the total number of studied RA patients, the incidence of RA in females is higher than in males, and the percentage of female patients was 73.2% of the total patients, while only 26.8% were male. On the other hand, the female registered a higher percentage among all vitamin D-level groups. (Figure 2 &Table 2).

The patients included in this study were at different ages. Therefore, we analyzed the obtained results of vitamin levels measurements among the different age's ranges and the results are presented in Figure 3.

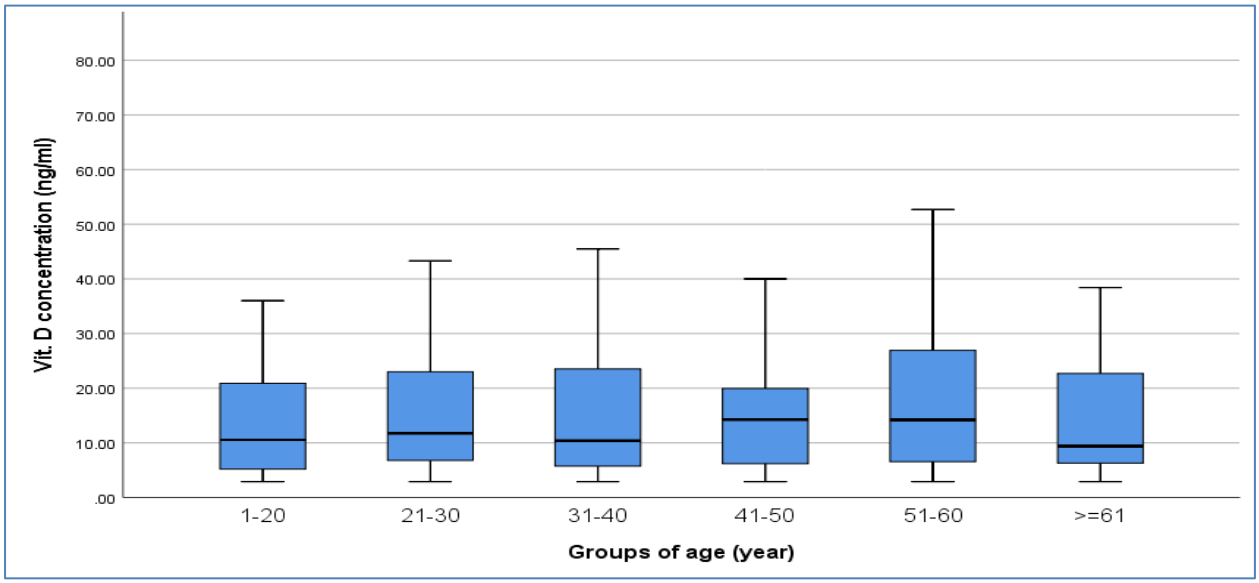

Figure 3: Median of vitamin D concentrations according to age category.

These results show that the patients with an age range between 41-50 years old have a higher median value of vitamin D level than the other age groups, while those with an age higher than 61 years and those age< 20 years have the lowest median value. Meanwhile, the results in Figure 4

and Figure 5 show vitamin D concentrations versus the age of RA females and male patients. As these Figures show, the male patients have a higher vitamin D concentration than the females in all age categories, and the highest value is in those with the age category of 31-40 years old and the lowest in those aged> 61 years.

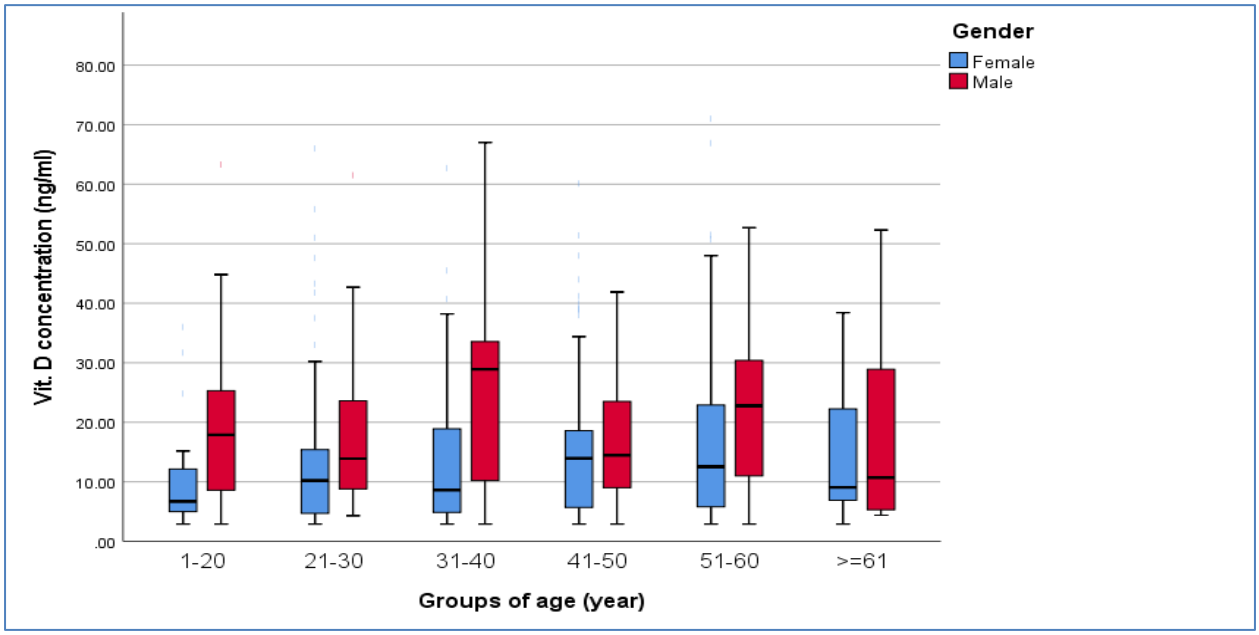

Figure 4: The median of vitamin D concentration in Iraqi females and male RA patients according their age category.

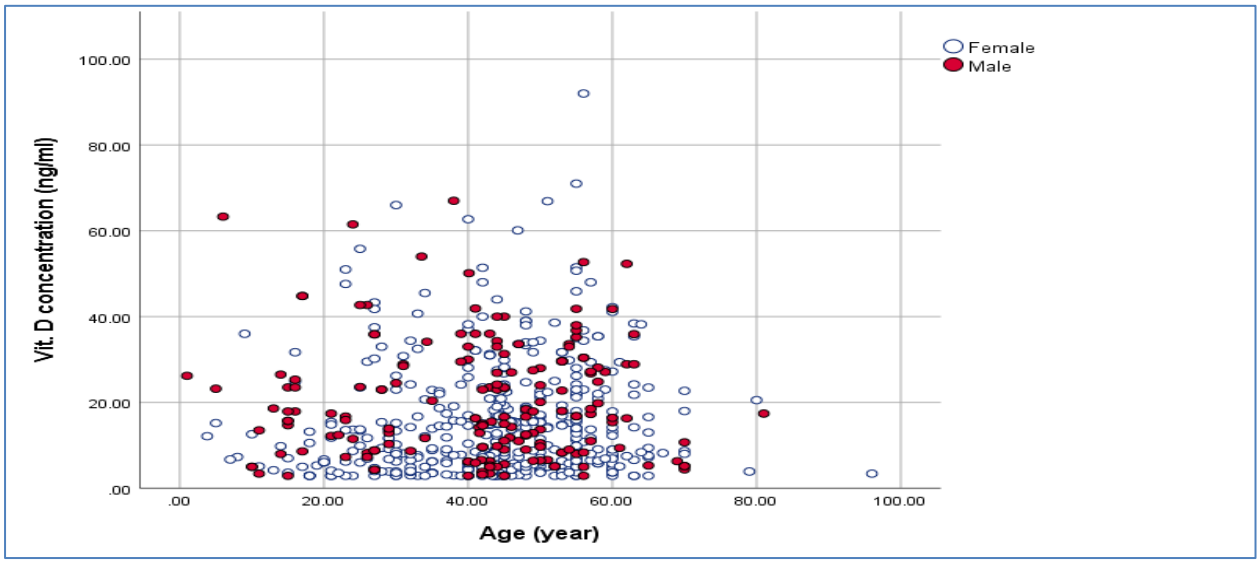

Figure 5: Scatter plot of vitamin D concentration versus age.

Upon looking at the extent of vitamin D severity among the 43.5% of the vitamin D deficient detected among the total RA patients enrolled in the current study (Table 1). The concentration of vitamin D was less than 0-5 ng/mL in 41.2% of them, while the rest, 58.8%, had a concentration of vitamin D in the range of 5.1-10 ng/mL, as illustrated in Table 3.

Table 3 The percentages of RA incidence and vitamin D concentration in both genders of the vitamin D severe deficient group.

Severe Vitamin D Deficiency			Gender		Total
			Female	Male	
Vitamin D concentration (ng/ml)	(0-5)	N	101	16	117
		% of Total	35.6%	5.6%	41.2%
	(5.1-10)	N	131	36	167
		% of Total	46.1%	12.7%	58.8%
Total		N	232	52	284
		% of Total	81.7%	18.3%	100.0%

From the above results, it is also clear that the female RA patients again have a higher percentage (81.7%) than male patients (18.37%), where 35.6% of them suffer very severe vitamin D deficiency (< 5 ng/mL).

Discussion

The current study explored the prevalence of vitamin D deficiency among Iraqi RA patients, where the participants were RA patients living in Waist Province in Iraq. The obtained results (Table 1 and Figure 1)) indicate that most of the patients enrolled in the present study have vitamin concentration lower than that reported as human sufficient concertation (less than 20ng/mL)

Substantial vitamin D insufficiency and deficiency are reported to occur among different healthy and diseased population groups, and such state of vitamin D sufficiency varies among and between populations [12, 25- 27]. Our results confirm the presence of an association between inadequate levels of vitamin D and RA disease. They are compatible with several studies' results that pointed to the presence of such an association, such as the study of [13, 17, 28-31] While disagreeing with others which reported the absence of such association[32-34]. The discrepancy in the reported results may be due to the differences in the study design, population, diet type, the supplement of natural food, the amount and the time of UV sunlight exposure, as well as the period of exposure and environmental pollution, which have synergistic effects [35, 36]. In addition to all these factors, Wang *et al.* confirmed the suggestion that there is a possible role of genetics in the etiology of vitamin D insufficiency where variants near genes involved in vitamin D synthesis and transport imply their effects on vitamin D status [37].

The results of the current study do not surprise us since RA is considered one of the autoimmune diseases [38]. Meantime, vitamin D has been reported to have immunomodulatory properties affecting the immune system by regulating both innate and adaptive immune responses [39]. A regulation that was suggested to be through a variety of mechanisms, including lowering the antigen presentation, suppressing proliferation and antibody production, and retarding β-cell precursors differentiation into plasma cells [40,41]. Therefore, hypovitaminosis may induce the development of diseases that are considered autoimmune, such as RA, by perturbing the immune tolerance of the body [42].

Furthermore, our results (Table 1) indicate that out of the 43.5% of the vitamin severely deficient group are female, which is much higher than that obtained in the male patients (8%). The observed percentage of patients with vitamin D severe deficiency in the present study is lower than that recorded by Baykal et al 2012 [13]. Meanwhile, our results disagree with what was reported in a study done in Slovenia, which reported that the prevalence of vitamin deficiency was 2.6% only, with no notable differences between adult males and females used in their study [29]. The observed high differences between both genders and a high incidence of deficiency among the female group (Table 2 and Figure 2) align with the results of [12, 29]. Meantime disagrees with what Alzarooni *et al*. reported in their study on the Abu Dhabi Emirates population [44].

The observed differences in the level of vitamin D between both genders may be due to the difference in the physiological state, the physical activity, and their hormonal variations state [45]. Moreover, women, in general, are more likely to be exposed to less UVB radiation since they spend more time indoors than men [45, 46]. Furthermore, the excessive and conservative clothing they wear for religious and sociocultural reasons is another reason for lowering their exposure to sunlight [46, 47]. Generally, it is well known that vitamin D can be provided to the human body by two sources; the main one is its cutaneous synthesis upon exposure to ultraviolet B radiation, which has been reported to provide about 80% of the body's requirement for this vitamin, the second source is the diet[24,30, 47, 48]. The amount of UVB radiation that reaches us is influenced by many factors such as the type of the climate, atmosphere pollution with ozone and smog, the darkness of the skin (the darker skin and the tanned one allow less amount of UVB to penetrate the body than the whiter one), also using sunblock leads to reduce the amount of this type of radiation[29,47]. The UVB amount is also influenced by the window glass used in the different buildings, which allows only about 5% of the UVB light to get inside the buildings [45]. Another factor that can play a role in the measured gender differences in vitamin D levels is the obesity that females suffer from more than males. Obesity has been reported to affect this vitamin bioavailability, and this can be explained as follows: vitamin D is fat soluble, and this property enables it to move into adipose tissues easily, resulting in lowering the serum level of this vitamin [40, 43].

The observed low vitamin D concentrations among the lower ages and elderly people (Figure 4) agree with the results of many types of research carried out on healthy populations and those with different diseases in many countries, such as the studies of[12, 23,25,26,29,46,48,49]. The factors that account for such results in elderly people are their insufficient cutaneous synthesis of vitamin D due to the that they spent most of their time at home, which results in their limited exposure to UVB light and thus insufficient synthesis of this vitamin in the skin, reduced renal synthesis of the vitamin, alteration of intestinal vitamin D and calcium absorption, reduced skin thickness, inadequate in addition to decreased dietary intake which means reduced vitamin D intake [12, 29, 30]. On the other hand, vitamin D deficiency during childhood and adolescence (mostly are students) is mostly a consequence of fast food and an unbalanced diet which is nutritionally poor that they have, in addition to the change in lifestyle nowadays where most people, especially children and younger female adults, spend most of their time in the door using computers and playing games

this probably lead to increase the number of cases with of hypovitaminosis among them in contrast to the males who spend more time outdoors [48- 50].

Conclusion

The results indicated the prevalence of severe hypovitaminosis among the studied Iraqi RA patients with a percentage of (43.5%) where some of them had vitamin D levels less than five ng/mL. RA disease and vitamin D deficiency seem to be more predominant in Iraqi women than men. The inadequate vitamin D concentration is among the RA patients with an age of less than 20 years and those with higher than 61 years.

In our opinion, the prevalence of hypovitaminosis among the Iraqi people will increase in the near future because of the increased environmental pollution as a result of the many wars Iraq passed through and all the bombs used during these wars. Such pollution causes a reduction in the amount of vitamin D cutaneous synthesis. Furthermore, the international sanctions imposed on Iraq and thus on Iraqi people during the nineteenth of last century for long time leads to an increase the unemployment (a situation which the Iraqi people have suffered until now) that led to an increase in the percentage of poor people and thus lack the food with good quality they can have. Therefore, the awareness to increase sunlight exposure, as well as vitamin D supplementation, are essential for appropriately managing this health problem and avoiding its bad consequences on the body.

References

1- Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911–30.

2- Lodder MC, de Jong Z, Kostense PJ, Molenaar E, Staal K, Voskuyl A, Hazes J, Dijkmans B, and Lems W . Bone mineral density in patients with rheumatoid arthritis: relation between disease severity and low bone mineral density. An Rheum Dis. 2004; 63:1576–1580.

3-Munns CF, Shaw N, Kiely M, Högler w. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab 2016; 101: 394–415.

4- Molina P, Carrero JJ, Bover J, Chauveau P, Mazzaferro S, Torres PU.. Vitamin D, a modulator of musculoskeletal health in chronic kidney disease. J. Cachexia Sarcopenia Muscle. 2017; 8: 686–701.

5. Autier P, Mullie P, Macacu A, Dragomir M, Boniol M, Coppens K, Pizot C, Boniol M. Effect of vitamin D supplementation on non-skeletal disorders: systematic review of meta- analyses and randomized trials. Lancet Diabetes Endocrinol. 2017; 5:986–1004.

6-Martínez-Zavala M, López-Sánchez GN, Vergara-Lopez A, Chávez-Tapia NC, Uribe M & Lámbarr N. Vitamin D deficiency in Mexicans have a high prevalence: a cross-sectional analysis of the patients from the Centro Médico Nacional 20 de Noviembre, Archives of Osteoporosis. 2020; 15:88.

7- Neve A, Corrado A, Cantatore FP. Immunomodulatory effects of vitamin D in peripheral blood monocyte-derived macrophages from patients with rheumatoid arthritis. Clinical and experimental medicine. 2014; 14(3):275–83.

8- Hwaidi AH and Hasan HR. The Impact of vitamin D levels on the oxidative stress in Iraqi rheumatoid arthritis patients Biochem. Cell. Arch. 2019; 19(2): 4139-4145.

9- Lawson. DE, Paul AA, Black A, Cole TJ. Sunlight and 25-hydroxy **vitamin D** in healthy children and adults. **Br Med J.1979**; 27, 1(6158):221–223.

10- Holick MF. Vitamin D: important for prevention of osteoporosis, cardiovascular heart disease type 1 diabetes, autoimmune diseases, and some cancers. South. Med. J. 2005 Oct; 98(10):1024-7.

11- Czernichow S, Fan T, Nocea G, Sen SS. Calcium and vitamin D intake by postmenopausal women with osteoporosis in France. Curr Med Res Opin. 2010 Jul; 26(7):1667-74.

12- Bouillon R. Vitamin D status in Africa is worse than in other continent. The Lancet Global health. 2019; 7.

13- Baykal T, Senel K, Alp F, Erdal A, Ugur M. Is there an association between serum 25 hydroxyvitamin D concentrations and disease activity in rheumatoid arthritis? Bratisl Lek Listy. 2012; 113:610–611

14- Chen J, Liu W, Lin Q, Chen L, Yin J, Huang H. Vitamin D deficiency and low bone mineral density in native Chinese rheumatoid arthritis patients. Int J Rheum Dis.2014; 17(1):66–70.

15- Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol 2014; 2: 76–89

16- Aletaha,D, Smolen JS, Diagnosis and Management of Rheumatoid Arthritis: A Review. JAMA 2018 October 2, 320 (13): 1360-1372

17- Brance MI, Brun LRS, Liol A, Sanchex M, Adala, Oliver B. Vitamin D levels & bone mass in arthritis Rheumatoid Int, 2015, 35:299-507.

18- Vallbrachi I, Rieber J, Oppemann M, Forger F, Siebert U. Diagnostic & clinical value of anti-cyclic citrullinated peptide antibodies compared with rheumatoid factor isotypes in rheumatoid arthritis. Ann Rheum Dis.2004; 63:1079-1084.

19- Alkazzaz AMH. Incidence of Rheumatoid Arthritis [2001 to 2011]. Iraqi Academic Scientific Journal 2013; 12(4): 568-572.

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

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

22- Hatem A K & Lateef H F. The state of Vitamin D in Iraqi Patients with Parkinson Disease Al-Kindy College Medical Journal. 2017; 13(.1): 137-141.

23- Al daoseri HA and Zuhair MB. Vitamin D deficiency and treatment in Iraqi patients with primary fibromyalgia syndrome. The Egyptain Rheumatologist. 2020; 42(1):47-50.

24- Al-Sharifi ZAR, Al-Ammar HAJ, Mahmood HG, Turki KM, Al-Karkhi II. Lack of Vitamin D in Iraqi Children with Asthma. Biomed Pharmacol J. 2017; 10(1): 89-93.

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

26- Spiro A and Buttriss J. Vitamin D: An overview of vitamin D status an intake in Europe. Nutrition Bulletin. 2014; 39(4), 322-350.

27- Craig SM, Yu F, Curtis JR, Alarcon GS, Conn DL, Jonas B, Caliahan LF, Smith ED, Moret, Bridges Jrs & Mikulis TR: Vitamin D status and its associations with disease activity and severity in African Americans with recent-onset rheumatoid arthritis. J Rheumatol. 2010; 37(2):275-81.

28- Song GG, Bae SC, Lee YH. Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. Clin Rheumatiol. 2012. 31:1733–1739.

29. Lin J, Liu J, Davies ML, Chen W Serum Vitamin D Level and Rheumatoid Arthritis Disease Activity: Review and Meta-Analysis. PLOS ONE 2016, January 11:1- 17.

30. Hribar M, Hristov H, Gregorič M, Blaznik U, Zaletel K, Oblak A, Osredkar J, Kušar A, Žmitek K, Rogelj I and Pravst I. Nutrihealth Study: Seasonal Variation in Vitamin D Status among the Slovenian Adult and Elderly Population.Nutrients 2020; 12: 1838

31- Rossini M, Maddali Bongi S, La Montagna G, Bongi SM, La Montagna G Minisola G, Malavolta N, Bernini L, Cacace E, Sinigagalia L, Di Munno O & Adami S . Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. Arthritis Res Ther, 2010; 12:R216.

32- Turhanoglu AD, Guler H, Yonden Z, Aslan F, Mansuroglu A, Ozer C. The relationship between vitamin D and disease activity and functional health status in rheumatoid arthritis Rheumatology international. 2011; 31(7):911–

33- Baker JF, Baker DG, Toedter G, Shults J, Von Feldt JM, Leonard MB. Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis. Clin Exp Rheumatol. 2012; 30 (5):658–64

34- Haga HJ. Vitamin D in rtheumatoid arthritis; Expert Rev Clin Immunol. 2013; 9(7): 591-593

35- Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clinical and experimental immunology. 2010; 160(1):1–9.

36- Attar SM. Vitamin D deficiency in rheumatoid arthritis. Prevalence and association with disease activity in Western Saudi Arabia. Saudi medical journal. 2012; 33(5):520–5.

37- Wang TJ, Zhang F, Richards JB, Kestenbaum B, Van Meurs JB, Berry D, Kiel DP, Streeten EA, et al. Common genetic determinants of vitamin D insuffienc : a genome- wide association study. Lancet 2010; 376: 180-188.

38- Andjelkovic Z, Vojinovic J, Pejnovic N, Popovic M, Dujic A, Mitrovic D, Pavlica L, Stefanovic D. Disease modifying and immunomodulatory effects of high dose 1α (OH) D3 in rheumatoid arthritisnpatients. Clin Exp Rheumatol.1999; 17:453– 456.

39- Rosen CJ, Adams JS, Black DM, Demay MB, Manson JE, Murad MH, and Kovacs CS. The nonskeletal effects of vitamin D: an Endrocrine Society Scientific Statements. Endocr. Rev 2012; 33:456-492.

40- Chen W, Dawsey SM, Qiao YL, Mark SD, Dong ZW, Taylor PR, Zhao P, Abnet CC. Prospective study of serum 25(OH)-vitamin D concentration and risk of oesophageal and gastric cancers. Br J Cancer 2007; 97:123-128.

41- Bartels M, Saviouk V, de Moor MHM. Willemsen G. Heritability and Genome-Wide Linkage Scan of Subjective Happiness; Twin Research and Human Genetics: 2010; 13 (2) 135–142.

42- Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A & Kaldrymides P Vitamin D and glycemic control in diabetes mellitus type 2 Ther Adv Endocrinol Metab. 2013 Aug; 4(4): 122–128.

43- Hollis BW, Wagner CL. Assessment of dietary vitamin D requirements during pregnancy and lactation, The American Journal of Clinical Nutrition, 2004; 79 (5): 717–726.

44- Al Zarooni AAR, Al Marzouqi FI, Al Darmaki, Prinsloo EAM and Nagekerke N. Prevalence of vitamin D deficiency and associated comorbidities among Abu Dhabi Emirates population. BMC Research Notes.2019; 12:503-508.

45- Waldron JL, Ashby HL, Cornes MP, Bechevaise J, Razavi C,Thomas OL, Chugh S, Deshpande S,Ford C, Gama R. Vitamin D: a negative acute phase reactant. J Clin Pathol 2013; 66:620-622.

46- Botros RM, Sabry IM, Abdelbaky RS, Eid YM, Nasr MS, Hendawy LM.Vitamin D deficiency among healthy Egyptian females. Endocrinology Metabolism, 2015; 62(7): 314-321

47- Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Thomsen J, Charles P, Eriksen: Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. J Intern Med 2000; 247: 260- 268.

48- Atli T, Gullu S, Uysal AR, Erdoganf G. The prevalence of Vitamin D deficiency and effects of ultraviolet light on Vitamin D levels in elderly Turkish population. Archives of Gerontology and Geriatrics. 2005; 40: 53–60.

49- Abdulrahman RM and Abdul Rahman BM. Prevalence of vitamin D level in the serum of patient living in Erbil city, Iraq, referred to private clinical laboratory and effect of age and sex on it. Journal of Biological Research. 2018; 91:6916.

50- Al-Horani H, Abu Dayyih W, Mallah E, Hamad M, Mima M, Awad R and Arafat T. Nationality, gender, age, and body mass index influences on vitamin D concentration among elderly patients and young Iraqi and Jordanian in Jordan. Biochem Res Int. 2016: 8920503.