Vitamin D Status among Rheumatoid Arthritis Patients attending Benghazi Medical Centre Rheumatoid Out-patients Clinic: ACross-sectional Study

Abstract:
Rheumatoid arthritis is an autoimmune inflammatory disorder. Vitamin D deficiency has been linked to the occurrence of autoimmune illnesses such multiple sclerosis and type one diabetes millets. This study aims to evaluate the vitamin D status in RA patients, as well as the link between vitamin D levels and disease activity. The current study is a cross-sectional study carried on rheumatoid arthritis patients who attend Benghazi Medical Centre (BMC) rheumatoid clinic. The sample includes all age groups of RA patients who had a recent serum vitamin D test results giving a response rate of 95 %. The study consists of 248 patients, 39 (15.7%) are male and the remaining 209 (84.3%) are female. The overall mean ± standard deviation of age for men and women was in the range of 53 ± 4. 24% of patients had normal vitamin D levels, while 36% and 40% of patients had insufficiency and deficiency in vitamin D, respectively. Gender and marriage status were the only socio-economic factors associated with the subject's vitamin D status ($p < 0.05$). Body mass index was a nutritional factor associated with a subject's vitamin D status ($p < 0.05$) in RA patients. It is recommended that all RA patients have serum vitamin D as routine test.
Keywords: Vitamin D Status, Rheumatoid Arthritis, supplement, Nutrition

Introduction

"Rheumatoid arthritis (RA) is a long-term autoimmune and inflammatory disease. RA primarily attacks joints, but usually attacks many joints. In joints with RA, the lining of the joint becomes inflamed and damages the joint tissue. This damage can lead to long-term and/ or chronic pain, instability, imbalance, and deformation". [(1)]. "Stiffness and pain often worsen during rest. The disease can also affect other parts of the body, such as nerves, lungs, heart, skin, eyes, and blood. Consequently. It can lead to a low red blood cell count, inflammation around the heart, and inflammation around the lungs. Symptoms usually appear gradually over weeks to months. The main cause of RA is not clear, but it is believed to be related to a combination of genetic and environmental factors. The underlying mechanism is that the body's immune system attacks joints. It also affects the underlying bone and cartilage. Diagnosis is primarily based on a patient's symptoms and signs. X-rays and laboratory tests can support the diagnosis or rule out other diseases with similar signs". [(2, 3)]"During 2015, approximately 24.5 million people were affected by RA. This is 0.5 to 1% of adults in developed countries, and 5 to 50 out of 100,000 people develop the disease each year. Onset is most common in middle age, with women affected 2.5 times more often than men. There were 38,000 deaths in 2013 due to RA, compared to 28,000 in 1990. Most epidemiological studies of RA are conducted in the United States and Northern Europe. As a result, epidemiological estimates of RA and identification of risk factors come primarily from these nations. The incidence and prevalence of RA are much higher in some populations, including Pima Native American, where rates up to 10 times more than most of the population. A recent global exposure study estimated that the prevalence of RA in the MENA region was one of the lowest at 0.16%. Based on limited evidence from MENA studies in some regions, the severity and treatment of RA disease appears to be geographically different across regions". [(4, 5)]"There are three phases of progression of RA are an initiation phase (due to non-specific inflammation), an amplification phase (due to T cell activation), and chronic inflammatory phase",. [(1, 6)]"Factors allowing an abnormal immune include genetic disorders cigarette smoking is the most clearly defined risk factor.

Other environmental and hormonal factors including onset after hormonal medications and childbirth". [7- 10] "RA is a multi-factorial disease, where in complex interactions between host and environmental factors determine the overall disease susceptibility".[11-13] "One cause of poor nutritional status in RA patients is weight loss and cachexia linked to cytokine production. On the other hand, the effects of arthritis medications that are frequently taken long-term may also compound these nutritional problems. The most observed vitamin and mineral deficiencies in patients with RA, are folic acid, vitamin C, vitamin D, vitamin B_6, vitamin B_{12}, vitamin E, calcium, magnesium, zinc, and selenium". [14, 15]"The non-classical actions of vitamin D are currently under discussion. Vitamin D deficiency has been implicated in the pathogenesis of autoimmune diseases, such as diabetes mellitus type 1 and multiple sclerosis. Reduced vitamin D intake has been linked to increased susceptibility to the development of rheumatoid arthritis (RA) and vitamin D deficiency has been found to be associated with disease activity in patients with RA. The objective was to evaluate vitamin D status in patients with RA and to evaluate the relationship between vitamin D levels and disease activity. Recently, the role of vitamin D deficiency in the pathogenesis of RA, as well as the relationship between vitamin D deficiency and the activity of RA is discussed. RA is an inflammatory disease characterized by flares and remissions; flares being characterized by pain. Vitamin D deficiency is also known to be associated with diffuse musculoskeletal pain. Thus, vitamin D deficiency may perturb immune tolerance and induce the development of autoimmune diseases, such as RA. Vitamin D has immunomodulatory properties, acting on the immune system both in an endocrine and in a paracrine manner. $1,25(OH)_2D_3$ suppresses proliferation and immunoglobulin production and retards differentiation of B-cell precursors into plasma cells. These data support a role for vitamin D deficiency in the development and progression of autoimmune inflammatory conditions in general, and RA in particular. Earlier data from animal models indicate that the $1,25(OH)_2D_3$ metabolite and its analogues may suppress collagen-induced arthritis. Other data suggest that vitamin D receptor agonists may also prevent and suppress established collagen-induced arthritis. Having said that, however, there are data showing that vitamin D may be negatively affected in acute response, that is, its levels may decrease in the setting of inflammation, such as in active RA. Despite that, treatment with rituximab in RA did not affect vitamin D levels, although it decreased indices of inflammation. Supplementation with vitamin D has been proposed to induce immune tolerance and thus prevent the development of autoimmune diseases. Recently, the combination of antirheumatic drugs with vitamin D has been suggested for RA. Patients with RA are prone to osteoporosis and suffer from pain when the disease is in flare. Vitamin D supplementation has been proposed for patients with RA for the prevention and treatment of osteoporosis as well as for its possible effects on disease activity". [16 -18] The current study aims to evaluate the vitamin D levels among rheumatoid arthritis patients attending Benghazi Medical Centre Rheumatoid Out-patients Clinic.

Subjects and methods

This is a cross-sectional study carried on rheumatoid arthritis patients who attend Benghazi Medical Centre (BMC) rheumatoid clinic. A total of 248 patients (Male and female) giving a response rate of 95 %. The inclusion criterion for enrolment in the present study was all age rheumatoid arthritis patients who had a recent serum vitamin D test result which suggests that vitamin D is an important predictor of RA causality and severity .Informed consent was obtained from the subjects who were also assured of the confidentiality of the information collected. The research was approved by the administration of the concerned hospital and University of Benghazi. In this study the questionnaire was divided into two basic sections : the first section covered socioeconomic information ;and the second section

covered medical and nutrition information like weight , height, BMI, disease duration ,food intolerance , nutritional supplements , pain severity , vitamin D level, ESR level and serum calcium level . All participants included in study should have RA and recent serum vitamin D test (3 months). Patients were divided into 3 diagnostic categories accordingly. To reduce bias of different vitamin D analysis techniques, ELISA was regarded as the accepted test, for being the most commonly used in Benghazi. [33, 34] All data was coded prior to being entered in (SPSS) version 22. Level of significance was set at p value < 0.05.

Results

The percentage of subjects from the age group less than 20 years with a total number of 12 constituted (4.8)%, while the percentage of people from the age group 20-40 years with a number of 98 made up (39.5)%, and the percentage of people from the group 41-60 years with a number of 110, and it is the highest percentage and constituted about (44.4%), and finally the percentage of the age group 61-80 years with a number of 28 constituted (11.3%). The total number of males was 39 (15.7%) and the total number of females was 209 (84.3%) .The mean ages of males mean ±SD about 55±3 and the mean±SD for females was about 52±6.2, while the general average ages of men and women mean ±SD about 53±4.6. The remaining Socio-economic characteristics details of the respondents were presented in Table (3) with the subject characteristics that have been mentioned in previous table. The marital status shows that 65% are married and 35% are not.Education level subjects where the percentage of people with a basic level was 25%, and the percentage of people with a secondary level was 10%, while the percentage of people with a high level was 58%, The place of residence of the participants, where 70% of the study participants live in Benghazi. People whose average income was 500-1000LD accounted for 56%. The percentage of people whose income was more than 1500LD was about 4%.Figure (1) shows duration of illness. The percentage of people who suffered from the disease for a period of less than 6 months constituted about 13%, the percentage of people who suffered from the disease for a period of 6 to 12 months about 5%, which is the lowest percentage, while the percentage of people who suffered from the disease for a period of 12 to 24 months was 10%. People who suffered from the disease for more than 24 months were about 72%. Figure (2) presents Body Mass Index (BMI). The percentage of people with normal weight was 35%, the percentage of people with underweight constituted about 41%, which is the highest for people with the disease, while the percentage of people with overweight and obese was 24%, which is the lowest percentage. Figure (3) shows food Intolerance. The percentage of patients who had food intolerance was 7.9%, and the percentage of patients who did not suffer from food intolerance was 92.1%.Figure (4) present the nutritional Supplement Use. The percentage of people who used iron supplement was about 5%, the percentage of people who used multivitamins and minerals accounted for about 18%, the percentage of people who used calcium and vitamin D supplements was about 25%, and the percentage of people who did not use nutritional supplements was about 52%.Figure (5) presents pain severity. The percentage of patients with light pain was about 10%; the percentage of patients with moderate pain was 43%, while the percentage of patients with severe pain was about 47%. Figure (6) shows vitamin D Level in three categories. The percentage of patients who had a normal vitamin D level was about 24%, the percentage of patients who had an insufficiency in the level of vitamin D was 36%, while the percentage of people who had a deficiency vitamin D level was the largest percentage of patients It reached about 40%. Figure(7) shows serum calcium level. The percentage of patients with a normal calcium level accounted for about 59%, and the percentage of patients with calcium deficiency was about 41%.Figure (8) shows erythrocyte sedimentation rate (ESR) test. The percentage of patients with a normal ESR was about 45%, the percentage of patients with an

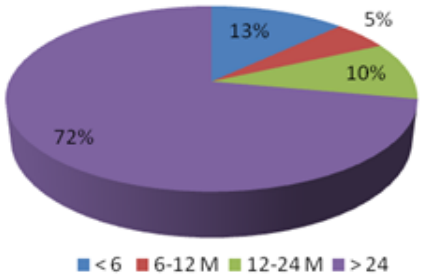
abnormal erythrocyte sedimentation rate was about 55%, which is the lowest percentage for patients who have active rheumatoid arthritis.

Table (1) Subject characteristics

Age (Years)		Total		Total
		Male	Female	
< 20	**No. (%)**	2(0.81)	10(4)	12(4.8)
20-40	**No. (%)**	12(4.84)	86(34.68)	98(39.5)
41-60	**No. (%)**	17(6.85)	93(37.5)	110(44.4)
61-80	**No. (%)**	8(3.23)	20(8.06)	28(11.3)
Total	**No. (%)**	39(15.7)	209(84.3)	**248(100)**
Age (Years) Mean ± SD		55±3	52±6.2	53±4.6

Table (2) socioeconomic characteristics of the respondents

Socioeconomic characters	No.	%
Gender		
Male	**39**	**15.7 %**
Female	**209**	**84.3 %**
Age (years)		
<20	**12**	**4.8 %**
20-40	**98**	**39.5 %**
41-60	**110**	**44.4 %**
61-80	**28**	**11.3 %**
Marital status		
Married	**161**	**65 %**
Not Married	**87**	**35 %**
Education Level		
Illiterate	**17**	**7 %**
Basic	**62**	**25 %**
Secondary	**25**	**10 %**
Higher	**144**	**58 %**
Address		
Benghazi	**174**	**70 %**
Out of Benghazi	**74**	**30 %**
Income level		
<500	**82**	**33 %**
500-1000	**139**	**56 %**
1000-1500	**17**	**7 %**
>1500	**10**	**4 %**



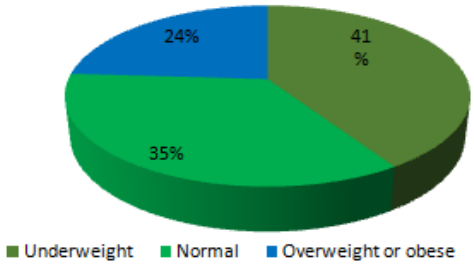
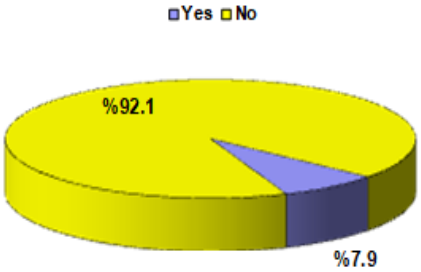


Figure (1): Duration of illnessFigure (2): Body Mass Index of the respondents



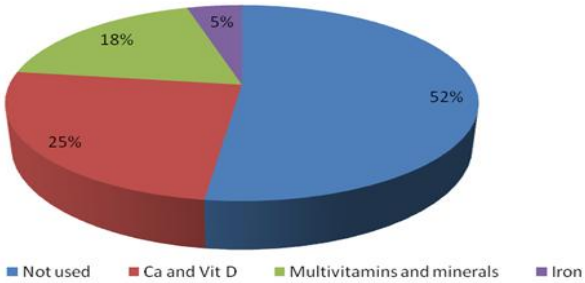
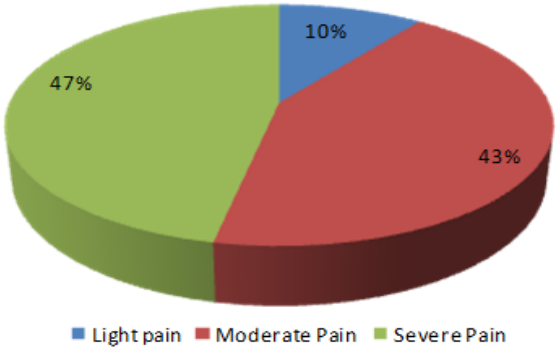


Figure (3): Food Intolerance Figure (4): Nutritional Supplement Use



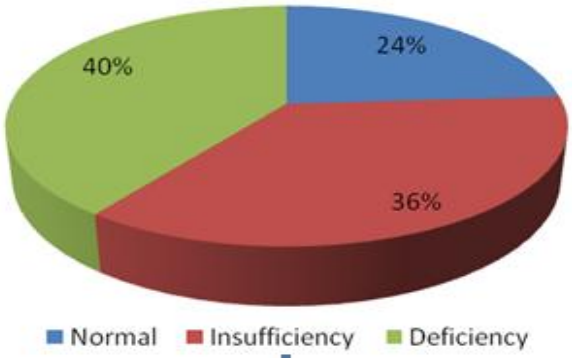
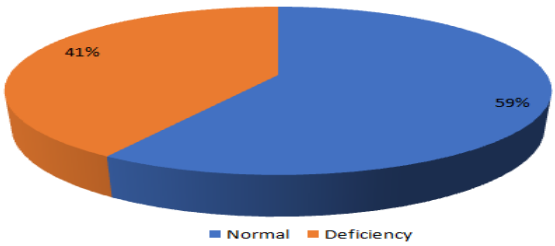


Figure (5): Pain Severity Figure (6): Vitamin D Level



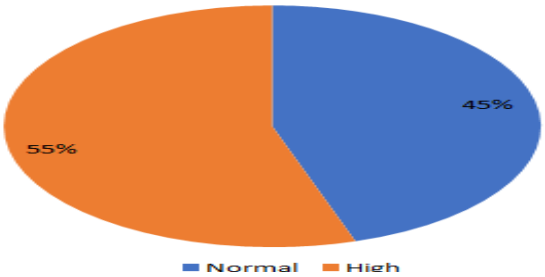


Figure (7): Serum calcium LevelFigure (8): ESR Level

The current study will analyse various socioeconomic factors, disease duration and severity of RA patients and trying to find out associations between these variables and vitamin D levels.A Chi Square test was carried out to see if there was any statistically significant association between the vitamin D levels and various socio-economic variables, disease duration and severity, body mass index, calcium levels, and ESR levels, within this RA patients who attending BMC Rheumatoid Clinic.Genderand marital status was the only

socio-economic factors associated ($p< 0.05$) with the vitamin D status of the subjects. Female gender was associated ($p< 0.05$) with deficientvitamin D status. There was a shift of patients from the normal and insufficient to deficiency as the genderbecome female in table (3).Marital status was associated ($p< 0.05$) with deficient vitamin D status. There was a shift of patients from the normal and insufficient to deficiency as the status becomes married in table (3).Body mass index was the nutritional factors that associated ($p< 0.05$) with the vitamin D status of the subjects as shown in table (3). As body weight increase the serum vitamin D level decreases among the RA patients. After adjustments of most risk factors, disease pain severity and disease duration remained significantly related with 25(OH) D levels ($P = 0.002$ and 0.001) respectively. No significant relation between supplement and disease pain severity and disease duration.

Table (3) Association of gender with the vitamin D status

Gender	Vitamin D status		
	Sufficient	**Insufficient**	**Deficiency**
Male	7	47.4	45.6
Female	2.6	28.1	69.3
Unmarried	15	45.4	39.6
Married	9.6	25.1	65.3
Underweight	15.9	36.1	48
Normal	20.7	30	49.3
Overweight and obese	9	28.6	62.4

5. Discussion

Vitamin D deficiency has been implicated in the pathogenesis of autoimmune diseases. Reduced vitamin D intake has been linked to increased susceptibility to the development of rheumatoid arthritis (RA) and vitamin D deficiency has been found to be associated with disease activity in patients with RA. The aim of the current study is detecting and evaluating the vitamin D levels among rheumatoid arthritis patients attending Benghazi Medical Centre Rheumatoid Out-patients Clinic. A total of 248 patients who answered the complete questionnaire clearly were finally enrolled for the study giving a response rate of 95 %. The total number of males were 39 (15.7%) of the total number, and the total number of females was 209 (84.3%) of the total number.Our subjects, however, and unlike patients reported in studies from North America and Europe, are about 10 years younger at the time of study. A potential explanation could be related to the lower average age of the population in the Middle Eastern countries. However, genetic and environmental factors cannot be excluded.The mean ages of males mean ± SD about 55±3 and the mean ± SD for females was about 52±6.2, while the general average ages of men and women mean ±SD about 53±4.6. The current mean age is similar to mean age of Benghazi RA patients whom studied by Elfagi et al 2021.Gender and marital status was the only socio-economic factors associated ($p< 0.05$) with the vitamin D status of the subjects. Female gender was associated ($p< 0.05$) with deficient vitamin D status. There was a shift of patients from the normal and insufficient to deficiency as the gender become female. “Similarly, to other autoimmune pathologies, rheumatic diseases show a significant female bias”. [(20)]“This sexual dimorphism seems, in part, to rely on the different sex hormone-induced regulation on male and female immune systems. Females, in fact, retain greater immune reactivity and competence likely due to estrogens, which, at variance with androgens, are associated with a greater resilience to infections but also to a higher risk for autoimmunity. In this scenario, there is growing interest on vitamin D supplementation for prevention or therapy in rheumatic diseases in relation to gender and sexual hormones. Some effects of vitamin D appear to be different in men and women and strictly related to its interplay with estrogens.

Interestingly, studies in humans and in animal models showed that Estrogen is able to decrease the expression of CYP24A1, the cytochrome P450 component of the 25-hydroxyvitamin D(3)-24-hydroxylase enzyme, which inactivates vitamin D. This effect leads to vitamin D accumulation, thus resulting in a more potent anti-inflammatory response in females than in males. Interestingly, the anti-inflammatory effects mediated by E2 in females could be reproduced treating immune cells from male subjects with this hormone. In addition, E2 increases the expression of VDR gene in diverse human and rat tissues and, in particular, in $CD4^+$ T cells from mice".[35]"The relationship between E2 and vitamin D is further supported by (i) the significant increase of 25(OH) D levels observed in women assumingestrogen containing contraceptives, and (ii) the association between low 25(OH) D levels and low E2 levels in women. As observed in most autoimmune diseases, RA shows a higher incidence in females especially in the post-menopausal period (female:male ratio of 3:1). In addition, disease severity seems to be worse in women than in men. Women are more likely to display conditions like depression, fibromyalgia, osteoporosis, and thyroid dysfunctions than males. A key role in the pathogenesis of RA is played by Th1 and Th17 cells, which contribute to maintaining a chronic inflammatory state at the level of the joint synovium". [36]"The etiology of RA is still unknown but, also in this instance, the interaction between genetic, epigenetic, hormonal, and environmental factors is believed to be fundamental in the development of the disease. Regarding sex hormones, a multifaceted role in RA onset and severity has been revealed. The female to male ratio of 3:1 may suggest that estrogens increase the risk of RA. However, some data, such as (i) the peak incidence at age 45–55, which coincides with the peri-menopausal years, (ii) the lack of association between hormonal therapy and the risk of developing RA, and (iii) the reduction of disease activity during pregnancy, support a systemic anti-inflammatory effect of estrogens. On the other hand, at local level, a pro-inflammatory role for estrogens in peripheral tissues of RA patients has been suggested. In both male and female RA patients, estrogens are strongly upregulated in synovial fluid due to the increased aromatase activity in monocyte-derived macrophages, induced by local inflammatory cytokines".[3, 6, 36]The results of the current study are similar to the results of Dupuis ML et al 2021, Yan X et al 2019, Craig SM et al 2010, Thambiah SC et al 2018.[24,37,39]In Arab world, the current results are similar to results from Kingdom Saudi Arabia, Qatar, United Arabic Emirates, Lebanon and Jordan. [40]Regarding marital status; about 65% of the subjects were married. The current results close to the findings of Elfagi et al 2021. Elfagi et al 2021 indicated that Most of the respondents were married (70%).Marital status was associated ($p< 0.05$) with deficient vitamin D status. There was a shift of patients from the normal and insufficient to deficiency as the status becomes married. "Several studies have found that married patients with RA exhibit greater disease progression and disability than their unmarried counterparts. Close relationships such as marriage are increasingly recognized as important to health and functioning in RA and other chronic pain conditions. Our first hypothesis to justify this result is that married participants havemoresocial responsibility, heavy works, psychological disability and marginally more affective pain than unmarried subjects. Thus, married people may be at risk for psychological disability and higher pain relative to those who have high levels of rest and stability socially and economically. Research has traditionally noted that being married confers responsibilities for one's mental and physical health. Among those with RA, poorer quality of the marital relationship has been linked to higher pain and psychological distress. Conversely, patients with RA who experience positive interpersonal relationships report less pain and psychological distress. Consistent with prior findings, some studies suggests that that the association between marital status and health status depends on the quality of the marriage; only being in a well adjusted marriage is linked with better health status". [1, 41, 42]These findings are consistent with prior studies showing that

higher marital quality is associated with better health in RA. That marital status was related most strongly to psychological disability and affective pain, a construct that assesses pain unpleasantness or emotional qualities of patients' pain, suggests that the marital relationship may play a particularly strong role in influencing patients' suffering; that is, the affective or emotional experience of distress associated with their pain. The results of the current study aresimilar to the results from Bermas BL et al 2000, Danoff-Burg S, et al 2005, and Waltz M, et al 1998. [41- 43]A bidirectional relationship between marital distress and functioning is most likely. This study did not examine gender differences in how marital status or marital quality might be related to health status. The relatively small number of men, particularly unmarried men, in this sample precluded such analyses. There are a number of studies showing that simply being married may be more protective of health for men than women. Yet, marital quality, rather than just marital status, may be more important to women, and women have been found to have greater physiological reactivity to marital stress than do men. Still other studies have found no gender differences in health benefits of marriage. Thus, this complicated picture of the role of gender, marital status, marital quality and health needs further study.Longitudinal studies could clarify how marital status and marital adjustment affect disease and health status. [44]The percentage of people with normal weight was 35%, the percentage of people with underweight constituted about 41%, which is the highest for people with the disease, while the percentage of people with overweight and obese was 24%. Although overweight and obese is the lowest percentage; however, as body weight increase the serum vitamin D level decreases among the RA patientsA possibleexplanation is the sequestration of vitamin D as afat-soluble micronutrient, in the adipose tissue. Associations between BMI and vitamin Dscores were confirmed in the GeneticInvestigation of Anthropometric Traits (GIANT)consortium, each 1 kg/m2 increase in BMI wasaccompanied with 1.15% lower 25(OH)D.In a study included 11 406 patients with RA and 54 701 controls. The proportion of obese subjects among RA patients with vitamin D deficiency was higher in comparison with controls, (33.4% versus 31.6%, respectively). In multivariate regression model, obesity were found to be associated with RA patients vitamin D level , whereas male gender was found as inversely related to RA patients vitamin D level. Obesity is a civilization disease that is a growing problem. It is also a more and more frequent phenomenon in the RA population and carries particular consequences for RA patients. Increased BMI is a known risk factor for developing RA. In a systemic review of 13 studies involving 13,562 RA patients and 400,609 participants in total, it was confirmed that both obesity and overweight increase RA risk (RR = 1.21, 95% CI 1.02–1.44 and RR = 1.05, 95% CI 0.97–1.13, respectively). There was a 13% increase in RA risk for every 5 kg/m^2 increase in BMI. Interestingly, a positive correlation between BMI and RA was found in women and not in men. Among examined subgroups, another significant association between body weight and RA patients deficiency serum vitamin D (RR = 1.47, 95% CI 1.11–1.96 for obesity and RR = 1.21, 95% CI 1.06–1.39 for every 5 kg/m^2 increase in BMI). [18, 33,45]After adjustments of most risk factors, disease pain severity and disease duration remained significantly related with 25(OH) D levels (P = 0.002 and 0.001) respectively. No significant relation between supplement and disease pain severity and disease duration. At a first glance, the most obvious justification for these results is that patients with very active disease are at higher risk of vitamin D deficiency rather than the other way around. (46) This indicates that patients with uncontrolled RA and/or with severe functional impairment are less prone to spend time outdoors in sunshine and are, therefore, at higher risk of vitamin D deficiency. Thus, the conclusions drawn in previous cross-sectional studies regarding the immunomodulatory role played by vitamin D in inflammatory arthritis, should be interpreted with caution, if 25(OH)D values are not adjusted for the known risk factors for vitamin D deficiency (16, 17). However, when the

correlations between disease activity scores and vitamin D deficiency were reanalysed by adjusting the 25(OH)D levels for sun exposure and BMI, the association remained statistically significant for Steinbrocker's functional state, DAS28, treatment response, HAQ score and mobility ADL. These results indicate that patients with very active RA are at higher risk of vitamin D deficiency for similar BMI and sun exposure, for reasons that remain unknown.

Conclusion

A total of 248 patients who answered the complete questionnaire clearly were finally enrolled for the study. Gender and marital status was the only socio-economic factors associated ($p< 0.05$) with the vitamin D status of the subjects. Body mass index was the nutritional factors that associated ($p< 0.05$) with the vitamin D status of the subjects. As body weight increase the serum vitamin D level decreases among the RA patients.It is recommended that all RA patients have serum vitamin D as routine test.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

CONSENT

As per international standards or university standards, Participants' written consent has been collected and preserved by the author(s).

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