Drugs and Cell Therapies in Hematology (ISSN: 2281-4876)
Volume 10 Issue 3 (2021)

Effect of Vitamin D on Lipid Profile and Oxidative Stress in Rheumatoid Arthritis Patients

Zainab Qasim Muhammad Al-Yasiri [1 *] and Khalid Gatee Al-Fartosi [2]

[1] Dept. of Biology, College of Education for Girls, and [2] Dept. of Biology, College of Science, University of Thi Qar, Nasiriyah, Iraq.

Abstract

The current study was designed to investigate the level of vitamin D and its relationship with some biochemical parameters of rheumatoid arthritis patients in Thi Qar Governorate / Iraq. The study targeted 100 women diagnosed with rheumatoid arthritis, and their ages ranged between 30-80 years. As well as 50 healthy women of the same age as a control group, during the period from November 2020 to July 2021. Blood samples were used to obtain serum, which was used to measure biochemical tests, including Vitamin D (Vt. D), Lipid Profile, Oxidant-antioxidant system. The results showed a significant increase ($p \leq 0.05$) in the level of body mass index accompanied by a significant decrease ($p \leq 0.05$) in Vitamin D level in patients with rheumatoid arthritis compared to the control group. Also, The results showed that, the differences Vitamin D levels were not significant ($P>0.05$) when comparison of patients based on body mass index BMI.

Regarding the lipid profile, our results showed the presence of a significant increase ($p \leq 0.05$) in concentration of the cholesterol CHO, triglycerides TG, low-density lipoprotein LDL and very low-density lipoprotein VLDL, On the other hand, a significant decrease ($p \leq 0.05$) in the concentration of high-density lipoprotein HDL in the patients compared to the control group. On the other hand, patients within the third group for BMI showed a significant increase ($p \leq 0.05$) in all parameters of the lipid profile, except for HDL, which decreased significantly ($p \leq 0.05$) compared with the first group for BMI. As for the oxidant-antioxidant system, the results showed that there was a significant increase ($p \leq 0.05$) in MDA and ceruloplasmin concentration. While the albumin concentration was significantly lower for patients compared to the control group. When comparing based on BMI, despite the positive relationship between MDA and BMI, However, the difference in MDA value was not significant ($P>0.05$) among the three BMI groups for patients. In addition, albumin and ceruloplasmin did not record any significant differences ($P>0.05$) among patients.

1. Introduction:

Rheumatoid arthritis (RA) is a long-term inflammatory autoimmune disease that affects the joints, primarily causing : pain, stiffness, and difficulty in movements. Cartilage, synovial cells and some body systems are affected by the autoimmune and inflammatory processes of RA (Coutant, and Miossec, 2020). So, Early detection, diagnosis and treatment are the best ways to avoid joint destruction, organ damage and inefficiency (Alfatlawi *et al*., 2020).

It should be noted that, Inflammation in RA is caused by accumulation of autoreactive T cells and B cells in the synovial tissues of patients. T cells are immunologically tolerant to autoantigens; when self-tolerance is broken, autoreactive T cells are activated, and they stimulate B cells to induce production of autoantibodies "anti-citrullinated protein antibodies (ACPAs) and rheumatoid factor autoantibodies (RF)".(Ramwadhdoebe *et al*., 2019), RF levels were significantly higher in rheumatoid arthritis patients, this increase is likely to be attributed to the increased gene expression of High Mobility Group Box 1 (HMGB1), a nuclear protein that is a biomarker in many active

systemic autoimmune diseases such as rheumatoid arthritis. It has been proven that there is a positive correlation between the increase in the level of gene expression of HMGB1 and the levels of RF-IgG.(Alsaedi *et al.*, 2021).

On the other hand Silva *et al*., (2020), mentioned that Obesity is one of the most important diseases of the modern times, So that, Obesity defines as change in anti-inflammatory/ pro-inflammatory balance due to migration of monocytes to necrotic clusters in the center of adipose tissue. (Achari and Jain, 2017). in contrast, the reduction in physical activity is also associated with a greater development of chronic diseases such as rheumatoid arthritis. (Gonzalez *et al*. 2017) . In contrast ,Tissues with synovitis are characterized by angiogenesis or vasodilation, proliferation of synoviocytes, and accumulation of lymphocytes. In tissues with diffuse inflammation, the accumulation of memory T cells and B cells can lead to formation of lymphoid follicle - germinal center like structures.(Tanaka, 2019). As a result, Oxidative damage leads to the generation of free radicals, and causes endothelial cell damage by production of pro-inflammatory cytokines (IL-6, TNF-α) and adhesion molecules. (Cascão *et al*., 2020)

Vitamin D is a fat-soluble steroid hormone precursor that is mainly produced in the skin by exposure to sunlight. Vitamin D is biologically inert and must undergo two successive hydroxylations in the liver and kidney to become the biologically active 1,25‑dihydroxyvitamin D. (Glendenning and Inderjeeth, 2016). Vitamin D deficiency causes muscle weakness; in elderly the risk of falling has been attributed to the effect of vitamin D on muscle function. Vitamin D Insufficiency has been linked to many diseases including, autoimmune disases and innate immunity. (Berg *et al*., 2015).

1.2. Aim of the study

This study proposed to highlight the following points:

1 - Measuring the level of Vit.D for rheumatoid arthritis patients.
2 - Investigate the relationship of Vit.D with lipid profile parameters.
3- Evaluate the association of Vit.D with Oxidant- antioxidant system.
4 - Investigating the effect of body mass index on Vit.D and other parameters under study in RA patients.

2. Materials and Methods

- **Subjects**

This study included 100 women with rheumatoid arthritis, and 50 healthy women as a control group. Their ages ranged between 30-80 years. This study was conducted in specialized outpatient clinics in thi Qar Governorate / Iraq, during the period from November 2020 to July 2021. The cases of rheumatoid arthritis were diagnosed by a medical specialist. The other group represents apparently healthy individuals as a control group. Depending on the body mass index (BMI), patients were divided into three groups (GI-Normal weight , GII- Over weight, and GIII- Class I obesity).

- **Blood collection**

Approximately (5 ml) of venous blood sample was withdrawn for both RA patients and the control group, Each sample was placed in anticoagulant-free tube, allowing it to clot to obtain serum, which was separated after centrifugation at 3000 rpm for 10 minutes. the serum was used for biochemical tests intended for this study or stored at -20°C unless used immediately.

2.1. Determination of body mass index (BMI)

The body mass index (BMI) value was obtained by dividing weight in kilograms by the square of height in meters (kg/m2) according to the equation below used by (Barlow and Dietz, 1998).
BMI = Weight (kg) / Height (m^2).

Depending on the classification established by the World Health Organization (WHO) regarding body mass index, a weight less than 18.5 is underweight, between 18.5 and 24.9 is normal weight, from 25 to 29.9 is overweight, between 30 and 34.9 is considered Class 1 obesity, From 35 to 39.9 it is class II obesity, and from 40 upwards it is class III obesity (pathological) (Girgin, 2018).

2.2. Biochemical parameters:

2.2.1. Assessment of Vitamin D level (Vit. D):

The electrochemiluminescence binding assay is intended for use on cobas e 411 immunoassay analyzer, to quantitative determination of total 25‑hydroxyvitamin D in human serum and plasma. This assay is to be used as an aid in the assessment of vitamin D sufficiency.(Heijboer *et al*., 2012). Competition principle. Total duration of assay: 27 minutes. The Elecsys Vitamin D total II assay employs a vitamin D binding protein (VDBP) labeled with a ruthenium complexa) as capture protein to bind 25‑hydroxyvitamin D3 and 25‑hydroxyvitamin D2. Cross‑reactivity to 24,25‑dihydroxyvitamin D is blocked by a specific antibody.

2.2.2. Determination of lipid profile:

- **Cholesterol (CHO)**

The enzymatic method described by Allian *et al*., (1974), as shown in the reaction scheme below:

$$Cholesterol\ \ esters \xrightarrow{CE} cholesterol + free\ fatty\ acids$$

$$Cholesterol + O2 \xrightarrow{CO} cholesten\ 4\ one\ 3 + H2O2$$

$$2H2O2\ + Phenol + PAP \xrightarrow{POD} Quinoneimine\ (pink) + 4H2O.$$

- **Triglyceride (TG).**

Test triglyceride brooded with lipoproteinlipase (LPL), liprate glycerol and free fatty acids . Glycerol is conversion to glycerol - 3-phosphate (G3P) , and adenose-5-diphosphate (ADP) by glycerol kinase and ATP . Glycerol-3 phosphate (G3P) then changed over to dehydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2) by glycerolphosphate dehydrogenase (GPO) . in the last response hydrogen peroxide (H2O2) responds for – chlorophenole and P- aminophenazone (PAP) by peroxidase (POD) to give a pink Quinoneimine. Enzymetic way described by Fossati and Prencpe (1982), which reaction scheme is as follows :

$$Triglyceride \xrightarrow{LPL} Glycerol + free\ fatty\ acids.$$

$$Glycerol + ATP \xleftrightarrow{GK} Glycerol\ 3\ Phosphate + ADP$$

$$Glycerol\ 3\ Phosphate + O2 \xleftrightarrow{GPO} DAP + H2O2$$

$$H2O2 + 4\text{-}Chlorophenol + PAP \xrightarrow{pod} Quinoneimine\ (pink) + H2O$$

The absorbance of the coloured complex (Quinoneimine), proportional to amount of triglyceride in the specimen, is measured at 500 nm.

- **High density lipoprotein (HDL)**

This reagent is only for treatment of specimens before determination of HDL-cholesterol with reagent for total cholesterol. Low density lipoprotein (LDL), very low density (VLDL) and chylomicrons from specimens are precipitated by phosphotungstic acid (PTA) and magnesium chloride. HDL-cholesterol obtained in supernatant after centrifugation is then estimated with total cholesterol reagent (Tietz , 1999).

- **Low density lipoprotein (LDL)**

The LDL level was estimated based on the Friedewald formula (FF) as shown in the equation below: (Friedewald *et al* 1972)

L D L (mg/dL) = Cholesterol - H D L - (Triglycerides \ 5) .

- **Very low density lipoprotein (VLDL)**

The level of VLDL can be estimated depending on its relationship with triglycerides according to the following equation (Friedewald *et al.,* 1972):

VLDL (mg/dL) = (Triglycerides \ 5)

2.2.3. Oxidant-antioxidant system

- **Determining of Malondialdehyde (MDA) .**

Lipid peroxidation (LPO) is a complex process that leads to the formation of various aldehydes, including malonaldehyde (MDA), which can be measured by (TBA method): thiobarbituric acid assay. In this method, MDA is formed from the oxidation of polyunsaturated fatty acids. was identified as the product of lipid peroxidation, it serves as a convenient index of the peroxidation reaction, MDA reacts with thiobarbituric acid (TBA), in coexistence with trichloroacetic acid (TCA). to give a red chromophore which is retained, its absorbance was read at 532 nm. (Fong *et al*., 1973).

- **Ceruloplasmin Determination**

The concentration of serum ceruloplasmin (CP) was estimated by the method of Menden *et al*. , 1977, which based on the catalyzed Oxidation of ceruloplasmin for colorless para-phenylenediamine (PPD). to blue -violet oxidized form. then , followed Photometrically of the reaction , the blank value was determinned after enzyme inhibited with using of sodium azide at (0 ^{O}C).

$$Reduced\ PPD + 2H^{+} + \tfrac{1}{2}\ O2 \leftrightarrow Oxidized\ PPD + H2O$$

(colorless) (blue)

Then incubation the mixture of serum, substrate, and acetate buffer that its pH = 6.0 for 15 min. at 37 OC. stopped the reaction by adding sodium azide, The absorbance of purple formed (oxidized PPD), in the diluted test mixture has been read at 525 nm against the solution of blank . the corrected absorbance is related directly to the Cp concentration. (Ravin,1961).

- **Determining the albumin concentration :**

Serum albumin binds to bromocresol green in the buffered solution at PH 4.2, forming a colored compound which its absorbance reading at 630 nm is proportion with the concentration of albumin in the specimen. (Doumas *et al*., 1971).

2.3. Statistical Analysis

The results were statistically analyzed using the Statistical Package for Social Sciences (SPSS) for windows version 23. Using analysis of variance (ANOVA) and t-test. Means, standard deviations (SD) and LSD (least significant differences) were found. To find out the significance of the differences at the significance level ($p < 0.05$).

3. Results

3.1. Body mass index (BMI).

The results of the current study showed that the body mass index (BMI) of patients with rheumatoid arthritis was significantly increased, ($p \leq 0.05$), compared with the control group. As shown in (Table 3-1).

Table 3-1:
The BMI Level (Kg/m^2) in Rheumatoid arthritis patients and control subjects.

BMI (kg /m ^{2}) Mean ± SD		P- value
Control	Patients	
24.723^b ± 3.681	28.683^a ± 3.798	0.000

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences, (SD): standard deviation. ($P > 0.05$): no significant difference and ($p \leq 0.05$): are significant.

3.2. Biochemical parameters

3.2.1. Vitamin D (Vt. D):

It seems clear from (Table 3-2) that there is a significant decrease ($p \leq 0.05$) in the concentration of vitamin D in patients with rheumatoid arthritis compared to the control group.

Table 3-2:
The level of Vitamin D in Rheumatoid arthritis patients and control subjects

(Vt. D) ng/Ml Mean ± SD		P- value
Control	Patients	
22.958^{a} ± 3.897	8.882^{b} ± 1.818	0.000

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences, (SD): standard deviation. ($P > 0.05$): no significant difference and ($p \leq 0.05$): are significant.

Depending on the body mass index, the results showed that there were no significant differences ($P > 0.05$) in the concentration of vitamin D among the three groups for BMI of patients (GI Normal weight, GII Overweight and GIII Class I obesity), while there was a significant decrease ($p \leq 0.05$) in vitamin D concentration of patients in each group when compared with the control group assigned to it. This is indicated in (Table 3-3) .

Table 3-3:
The level of Vitamin D in Rheumatoid arthritis patients and control subjects according to BMI.

BMI Groups		**(Vt. D)** ng/mL Mean ± SD
(GI) Normal weight	Control	22.746^{a} ± 4.728
	Patients	10.001^{b}± 1.051
(GII) Over weight	Control	23.439^{a} ± 4.050
	Patients	9.027^{b} ± 1.941
(GIII) Class I obesity	Control	22.528^{a} ± 1.628
	Patients	8.372^{b} ± 1.764
LSD		2.733

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences , (LSD): least significant difference and (SD): standard deviation .

3.2.2. Lipid Profile

The results of the current study, through Table (3-4), showed that patients have a significant increase ($p \leq 0.05$) in concentration of the cholesterol CHO, triglycerides TG, low-density lipoprotein LDL and very low-density lipoprotein VLDL when compared with the control group .On the other hand, the patients recorded a significant decrease ($p \leq 0.05$) in the concentration of high-density lipoprotein HDL compared with the control.

Table 3-4:
Level of lipid profile in Rheumatoid arthritis patients and control subjects**.**

Parameters Mean ±SD	**Groups**		**P- value**
	Control	Patients	
CHO (mg/dl)	129.290 [b] ± 11.240	176.638 [a] ± 17.203	0.000
TG (mg/dl)	87.945 [b] ± 24.525	188.724 [a] ± 35.554	0.000
HDL (mg/dl)	44.492 [a] ± 2.424	36.623 [b] ± 3.827	0.000
LDL (mg/dl)	67.453 [b] ± 8.992	102.034 [a] ± 12.893	0.000
VLDL (mg/dl)	17.626 [b] ± 4.968	37.744 [a] ± 7.111	0.000

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences, (SD): standard deviation. ($P > 0.05$): no significant difference and ($p \leq 0.05$): are significant.

Depending on the body mass index, the third group of patients (GIII Class I obesity) recorded a significant increase ($p \leq 0.05$) in the concentration of CHO, TG, LDL and VLDL, when compared to the first group (GI Normal weight), while no significant difference ($P > 0.05$) was recorded compared to the second group (GII Over weight). as shown in (Table 3-5). In contrast, the third BMI group of patients (GIII Class I obesity) showed a significant decrease ($p \leq 0.05$) in HDL concentration compared with the first group (GI Normal weight), while the difference was not significant ($P > 0.05$) when compared with the second group (GII Over weight).

Table 3-5:
The level of lipid profile in Rheumatoid arthritis patients and control subjects according to BMI.

Parameters Mean ± SD	**BMI Groups**						**LSD**
	(GI) Normal weight		**(GII)** Over weight		**(GIII)** Class I obesity		
	Control	Patients	Control	Patients	Control	Patients	
CHO (mg/dl)	123.425[d] ± 10.369	163.769[b] ± 12.878	127.100[d] ± 6.861	175.667[ab] ± 14.537	143.367[c] ± 5.585	181.946[a] ± 18.286	14.367
TG (mg/dl)	79.590[d] ± 19.443	165.615[b] ± 27.268	77.952[d] ± 15.383	186.733[ab] ± 32.097	119.452[c] ± 19.117	198.457[a] ± 37.451	30.350
HDL (mg/dl)	45.244[a] ± 2.227	39.808[c] ± 1.548	45.173[a] ± 1.962	36.793[d] ± 3.611	42.033[b] ± 1.951	35.365[d] ± 3.939	3.199
LDL (mg/dl)	62.982[d] ± 9.014	90.838[b] ± 8.861	66.273[d] ± 6.207	101.526[ab] ± 10.708	77.364[c] ± 4.610	106.378[a] ± 13.467	10.762
VLDL (mg/dl)	15.923[d] ± 3.904	33.123[b] ± 5.453	15.587[d] ± 3.067	37.346[ab] ± 6.419	24.047[c] ± 3.907	39.689[a] ± 7.490	6.074

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences , (LSD): least significant difference and (SD): standard deviation .

3.2.3. Oxidant- antioxidant system

3.2.3.1. Lipid Peroxidation Status –Malondialdehyde (MDA)

The results presented in Table (3-6) indicated that there was a significant increase ($p \leq 0.05$) in MDA concentration for patients compared to the control group.

Table 3-6:

Level of MDA in Rheumatoid arthritis patients and control subjects.

MDA (µmol /L) Mean ± SD		P- value
Control	Patients	
$29.540^{b} \pm 4.514$	$54.721^{a} \pm 10.138$	0.000

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences, (SD): standard deviation. ($P > 0.05$): no significant difference and ($p \leq 0.05$): are significant.

The results shown in Table (3-7) indicate that there was no significant difference ($P > 0.05$) in MDA concentration among the three groups of patients (GI Normal weight, GII Overweight and GIII Class I obesity), depending on BMI.

Table 3-7:

The level of MDA in Rheumatoid arthritis patients and control subjects according to BMI.

BMI Groups		**MDA** (µmol /L) Mean ± SD
(**GI**) Normal weight	Control	$27.706^{b} \pm 5.119$
	Patients	$53.269^{a} \pm 11.907$
(**GII**) Over weight	Control	$29.953^{b} \pm 3.179$
	Patients	$54.003^{a} \pm 10.213$
(**GIII**) Class I obesity	Control	$31.889^{b} \pm 4.641$
	Patients	$56.273^{a} \pm 9.447$
LSD		8.871

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences , (LSD): least significant difference and (SD): standard deviation .

3.2.3.2. Antioxidant System

- **Serum Albumin and Ceruloplasmin**

The results presented in Table (3-8) showed a significant decrease ($p \leq 0.05$) in albumin concentration and a significant increase in ceruloplasmin concentration in the patients group compared to the control group assigned to each of them.

Table 3-8:
Level of Ceruloplasmin and Albumin in Rheumatoid arthritis patients and control subjects.

Groups	**Parameters** Mean ±SD	
	Ceruloplasmin (µmol/L)	**Albumin** (gm/dl)
Control	$0.798^{b} \pm 0.167$	$4.058^{a} \pm 0.377$
Patients	$1.743^{a} \pm 0.538$	$3.518^{b} \pm 0.276$
P- value	0.000	0.000

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences, (SD): standard deviation. ($P > 0.05$): no significant difference and ($p \leq 0.05$): are significant.

The results showed that there were no significant differences ($P > 0.05$) in the albumin concentration among patients in the three BMI groups (GI Normal weight, GII Overweight and GIII Class I obesity) when they were compared with each other depending on the BMI value. On the other hand, we note that the albumin concentration of patients in each group has decreased significantly ($p \leq 0.05$) compared to the control group which assigned to it. In contrast, the concentration of ceruloplasmin increased significantly in patient groups compared with their corresponding control groups, as shown below in (Table 3-9).

Table 3-9:
Level of Ceruloplasmin and Albumin in Rheumatoid arthritis patients and control subjects according to BMI.

BMI Groups		**Parameters** Mean ± SD	
		Ceruloplasmin (µmol/L)	**Albumin** (gm/dl)
(**GI**) Normal weight	Control	$0.923^{b} \pm 0.101$	$4.258^{a\pm} \pm 0.327$
	Patients	$1.816^{a} \pm 0.600$	$3.600^{b} \pm 0.270$
(**GII**) Over weight	Control	$0.805^{b} \pm 0.176$	$4.073^{a} \pm 0.302$
	Patients	$1.606^{a} \pm 0.427$	$3.539^{b} \pm 0.273$
(**GIII**) Class I obesity	Control	$0.748^{b} \pm 0.156$	$3.795^{a} \pm 0.294$
	Patients	$1.662^{a} \pm 0.560$	$3.483^{b} \pm 0.270$

LSD	0.336	0.289

The similar letters (a,a): no significant differences, The deferent letters (a,b): a significant differences , (LSD): least significant difference and (SD): standard deviation .

4. Discussion

4.1. Body mass index (BMI)

The results of the current study confirmed that the body mass index was significantly increased in patients with rheumatoid arthritis compared with the control group, and this is consistent with other studies that previously indicated that, Obesity and rheumatoid arthritis have been shown to be linked in different ways, Obesity appears to be associated with an increased risk of developing rheumatoid arthritis. It was proven that obese people with a BMI greater than 30 kg/ m2 have a higher risk of developing RA (Silva *et al*., 2020).

The reason for the weight gain may be attributed to the lack of physical activity and the consequent decreased secretion of the hormone irisin in patients with arthritis, given the role that irisin plays in the fight against obesity By increasing energy expenditure and browning of white adipose tissue, and as a result, high levels of triglycerides begin to provide energy substrates for the organism. (Tsai *et al*., 2020). In addition to the above, the decrease in estrogen concentration may be the reason for the high BMI in the second age group of patients, as this hormone plays a major role in fat oxidation and weight loss, and its concentration decreases after menopause, which leads to weight gain. The accumulation of fat in the body, As reported in the study by Ko and Kim, (2020).

On the other hand, It may explain the secret of the obese association with taking medications that are used to treat people with rheumatoid arthritis, because they contain glucocorticoids and their effects on weight gain and loss of muscle mass. (Wilson *et al*.,2019). In addition to the above, an increase in the concentration of the Leptin hormone may be one of the causes of obesity in patients with rheumatoid arthritis. Leptin as an indicator of obesity, it was found that blood levels of leptin in rheumatoid arthritis patients are higher compared to those in the control group. (Al-Garawi *et al*., 2021).

4.3. Biochemical parameters

4.3.1. Vitamin D (Vt. D):

The results of the study showed that the concentration of vitamin D decreased significantly in patients with rheumatoid arthritis compared with the control group. In contrast, there were no significant differences between patients when comparing based on BMI. These results are in agreement with the findings of (Mouterde *et al*., 2019), It was reported that the level of vitamin D in the blood was significantly lower in the rheumatoid arthritis group compared to the control group, in addition, Vitamin D deficiency has been linked to autoimmune disease, and its considered a risk factor in the development of rheumatoid arthritis (Harrison *et al*., 2020).

Furthermore, Vitamin D inhibits B-cell proliferation prior to differentiation into immunoglobulin-secreting cells and consequently reduces immunoglobulin production, and also contributes to immune tolerance by affecting both innate and adaptive immune responses. The use of vitamin D supplements in the treatment of rheumatoid arthritis may have a clear logical evidence to prove what was mentioned above. (Littlejohn and Monrad, 2018).

With age, arthritis patients may develop osteoporosis, which is associated with a deficiency of both calcium and vitamin D, according to studies indicated by (Kashat and Ali, 2021) and that vitamin D deficiency in female patients may be due to insufficient exposure to sunlight, not consuming enough of vitamin D in food. On the other hand, The ability of the kidneys to manufacture vitamin D decreases with age, which leads to vitamin D deficiency.

In contrast, the low concentration of vitamin D in RA patients is likely to be due to the Obesity, It was found that Fat cells extract vitamin D from the bloodstream in the human body, so people with a BMI of 30 or more often suffer from a vitamin D deficiency. (Philippou and Nikiphorou, 2018).

4.3.2. Lipid Profile

Concerning the lipid profile, the results of this study showed that the concentrations of cholesterol, triglycerides, low-density lipoprotein and very low-density lipoprotein, were significantly increased in patients with rheumatoid arthritis compared to the control. While HDL recorded a significant decrease in patients compared to control. In contrast, when comparing patients on the basis of BMI, the third group GIII recorded a significant increase compared to the first group GI, while the difference was not significant when compared with the second group GII. However.These results are in agreement with those found by Dessie *et al*., (2021) The result of his study showed that there is a significantly higher CHO, TG, and a lower value for HDL among RA patients compared to the control group (P value < 0.05). This abnormality in lipids may be attributed to higher systemic inflammation in rheumatoid arthritis patients compared with the control group, through higher ESR and C-reactive protein indices. (Parveen *et al*., 2017).

It was found that there is a positive relationship between cholesterol levels and RA, and that high cholesterol levels are among the risk factors for the development of rheumatoid arthritis in women. early menopause, and a high body mass index (BMI) all contribute to an increase in cholesterol. (Turesson *et al*., 2015).

It was shown that the plasma HDL levels were significantly decreased in RA patients compared to the healthy groups, indicating that the inflammatory status and severity of RA are inversely related to the plasma HDL levels. While the high level of VLDL in the group of patients is due to the increase in the concentration of triglycerides, as there is a positive correlation between them. Depending on the body mass index, the lipid profile values in the third group of patients with rheumatoid arthritis appeared to be significantly higher compared to the first group. It is in the first group of patients. Furthermore, another study reported that RA is associated with an abnormal lipoprotein phenotype. Increased cholesterol levels are associated with increased TG and led to greater progression of disease activity (Dessie *et al*., 2021).

It is likely that there is a role for adiponectin in these findings, It was found that, In patients with severe rheumatoid arthritis, who were undergoing to DMARD drugs, adiponectin concentrations inversely correlated with triglycerides, total cholesterol and body mass index. (Taghadosi *et al*., 2020). In contrast, evidence suggests that lipid metabolism is altered in RA due to inflammation (Ferreira *et al*., 2021), Furthermore, this lipid profile results may be associated with the decrease in melatonin, It is mentioned that melatonin is secreted in response to darkness, while preventing its secretion when exposed to light during the night. Among its effects, it reduces body mass during fat oxidation, lowers cholesterol, lowers leptin and triglycerides, raises HDL, and serves

as antioxidant against oxidative stress, all of which prove its effect in reducing obesity. (Xu *et al*., 2020)

4.3.3. Oxidant-antioxidant system

4.3.3.1. Lipid Peroxidation Status –Malondialdehyde (MDA)

The results of the current study recorded a significant increase in the concentration of MDA for patients with rheumatoid arthritis compared with the healthy group, and to explain the reason for this, it must be noted that, oxidative status as a potential contributor to establishment the inflammatory environment in individuals with RA. In fact, the role of oxidative stress in the pathogenesis of rheumatoid arthritis has been confirmed (Fonseca *et al*., 2019). In this regard, A positive correlation was observed between lipid peroxidation (assessed by Malondialdehyde "MDA") and the degree of disease activity. (Bordy *et al*., 2018).

RA patients with active disease have increased levels of reactive oxygen species and decreased antioxidant potential, which ultimately leads to increased oxidative stress compared to healthy controls . Thus, a greater degree of lipid peroxidation could be found (Phull *et al*., 2018). This explains the significant increase in MDA level for patients compared to the control group. Conversely, lower levels of antioxidants are also found in the serum and synovial fluid of RA patients (Karagülle *et al*., 2018).

Oxidative stress is described as a harmful condition characterized by an imbalance between oxidants and antioxidants, in which oxidizing molecules predominate (Smallwood *et al*., 2018), In line with the previously mentioned, the current study showed a significant increase in the MDA value of arthritis patients compared to the control group, It is believed that the reason for this is due to the increase in inflammation and this was confirmed by the results of this study through the high values of the inflammatory indicators that were measured and the consequent increase in the levels of reactive oxygen species. This is also linked to high triglycerides and their inflammatory effects on the one hand, and low HDL and their antioxidant effects on the other. (Fonseca *et al*., 2019)

In addition, the cause of the high level of MAD may be the increase in the level of leptin, the study conducted by Al- Garawi *et al*. (2021) showed a significant increase in the hormone leptin in rheumatoid arthritis patients compared to the control group, and this study highlights that leptin may act As a pro-inflammatory mediator in rheumatoid arthritis, on the other hand, there is a link between inflammation and an increase in reactive oxygen species that contribute to increased oxidative stress, and this is demonstrated by comparing MDA between patients depending on the BMI value, It was observed that MDA increases with the increase of BMI, although the differences were not significant.

4.3.3.2. Antioxidant System.

- **Serum Albumin and Ceruloplasmin**

The results of our current study indicated a significant decrease in albumin concentration, while there was a significant increase in ceruloplasmin concentration in patients with rheumatoid arthritis compared to healthy controls. However, there were no significant differences in the concentration of albumin and ceruloplasmin among the patients, depending on body mass index. Perhaps the increase in the ESR value explains these results, as it was found that the ESR value is

positively correlated with serum ceruloplasmin levels, while it has a negative correlation with albumin concentration. (Mohamed *et al*., 2017).

The reason for the low albumin in patients with rheumatoid arthritis may be attributed to the presence of inflammation. It was found that Inflammatory cytokines, increase C-reactive protein production and decrease serum albumin production and transferrin synthesis, So that, Low serum albumin has been used as a marker of inflammation (Belinskaia *et al*., 2020). albumin acts as a major and predominant anti-oxidant in plasma exerting more than 80% of the free radical-trapping activity of serum. On the other hand, The decreased albumin levels in our RA patients were confirmed by many other study. (Nakashima *et al*., 2020).

At the same time, the presence of inflammation may explain the increase in the level of ceruloplasmin (Cp), because, there were Several physiologic functions of (Cp) had been proposed including : bactericidal activity as an acute-phase reactant by iron metabolism and transport (Mohamed *et al*., 2017). Infectious agents use iron for growth, One of the defense mechanisms against infections consists in activating the metabolic pathways that increase intracellular iron, which decreases serum iron, Cp permits the incorporation of iron into transferrin without the formation of toxic Fe products (Beaumont and Karim, 2013). The reason for the increase in the concentration of ceruloplasmin may be the rise in the value of MDA, a significant positive correlation was observed between serum MDA and plasma Cp concentration, It is possible to conclude that increased oxidative stress in RA patients evidenced by increased serum MDA, resulted in compensatory changes in the levels of some antioxidants, such as SOD, CAT, GSH, GST and Cp. These changes, in turn, may provide additional protection against LPO. (El-barbary *et al*., 2011).

5. Conclusions and Recommendation

5.1.Conclusions

1 -High level of body mass index in RA patients due to lack of physical activity.
2 -Decrease Vitamin D level in RA patients and there is a negative association of vitamin D with the body mass index.
3 - Negative association of vitamin D with lipid profile parameters except for HDL.
4 -Negative relationship between vitamin D and oxidative stress in RA patients.

5.2. Recommendations:

1 -We strongly advise the necessity of maintaining weight within the health standards for patients with rheumatoid arthritis.
2 -We recommend eating foods rich in vitamin D and exposure to the sun, due to the positive correlation between it and HDL.
3 -Investigating the effect of vitamin D supplementation on lipid profile parameters and oxidative stress in RA patients.
4 -Studying the effect of weight loss on the level of vitamin D and its relationship to oxidative stress in patients.

References:

1. Achari AE and Jain SK (2017) Adiponectin, a therapeutic target for obesity, diabetes, and endothelial dysfunction. International Journal of Molecular Sciences 18(6): 1321.

2. Alfatlawi R, Al-Mashhadi H, Hameed W and Aljazaeri T.(2020). Comparative study between anti-ccp and rheumatoid factor as diagnostic value of rheumatoid arthritis patients. Department of Clinical Laboratory Sciences, Faculty of pharmacy, university of Kufa,23(S6):604-608.DOI: http://doi.org/10.36295/ASRO.2020.2366.
3. Al-Garawi Z S, Tahir N T, Al- Tabatabai Z M and Salman A T (2021). The mechanism of leptin and IGF-1 in the diabetic rheumatoid arthritis Iraqi patients. The International Conference of Chemistry . IOP Publishing. J. Phys. : Conf. Ser. 1853 012003. doi:10.1088/1742-6596/1853/1/012003.
4. Allian, C.C.; Poon, L.S.; Chan, C.S.; Richmond, W. and FU, P.C. (1974). Enzymatic Determination of Total Serum Cholesterol.‖ Clinical Chem. 20, (4): pp.470–75.
5. Alsaedi A.A., Al-Ali S. J. and Waheed S. (2021). The Association of The High -Mobility Group Box 1 Gene and Its Product with Rheumatoid Arthritis in Basra Province – Iraq, University of Thi-Qar, Journal of Science (UTsci). ISSN Onlin:2709-0256, 1991-8690 Volume (8), No.1
6. Barlow, S. E., and Dietz, W. H. (1998). Obesity evaluation and treatment: expert committee recommendations. Pediatrics, 102(3), e29-e29.
7. Beaumont C and Karim Z (2013). [Iron metabolism: State of the art]. Rev Med Interne, 34(1):17-25.
8. Belinskaia, D.A.; Voronina, P.A.; Shmurak, V.I.; Vovk, M.A.; Batalova, A.A.; Jenkins, R.O.; Goncharov, N.V.(2020). The Universal Soldier: Enzymatic and Non-Enzymatic Antioxidant Functions of Serum Albumin. Antioxidants 2020, 9, 966. [CrossRef]
9. Berg AH, Powe CE, Evans MK, et al.(2015). 24,25-Dihydroxyvitamin d3 and vitamin D status of community-dwelling black and white Americans. Clin Chem 2015;61(6):877-884.
10. Bordy R., Totoson P., Prati C., Marie C., Wendling D., and Demougeot C.,(2018). "Microvascular endothelial dysfunction in rheumatoid arthritis," Nature Reviews Rheumatology, vol. 14, no. 7, pp. 404–420, 2018.
11. Cascão, R.; Moura, R.A.; Perpetuo, I.; Vieriea-Sousa, E.; Mourao, A.F.; Rodrugues, A.M.; Polido-Pereira, J;.Queiroz, M.V.; Rosario, H.S.; Souto-Carneiro, M.M.; et al.(2020). Identification of a cytokine network sustaining neutrophil and Th17 activation in untreated early rheumatoid arthritis. Arthritis Res. Ther. 2020. [CrossRef]
12. Coutant, F.and Miossec, P.(2020). Evolving concepts of the pathogenesis of rheumatoid arthritis with focus on the early and late stages. Curr. Opin. Rheumatol., 32, 57–63. [CrossRef]
13. Dessie G, TadesseY, Demelash B and Genet S, (2021). Assessment of Serum Lipid Profiles and High-sensitivity C-reactive Protein Among Patients Suffering from Rheumatoid Arthritis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia: A Cross-Sectional Study. Open Access Rheumatology: Research and Reviews downloaded from https://www.dovepress.com/ by 37.237.175.24 on 14-Nov-2021
14. Doumas B. T. , Watson W.A. and Biggs H.G. (1971). Albumin standard and the measurement of serum albumin with bromcresol green, Clin. Chim. Acta.,31,p.87-96.
15. El-barbary A M, Abdel Khalek M A, Elsalawy A M and Hazaa S M, (2011). Assessment of lipid peroxidation and antioxidant status in rheumatoid arthritis and osteoarthritis patients, The Egyptian Rheumatologist. Production and hosting by Elsevier ,doi:10.1016/j.ejr.2011.07.002.
16. Ferreira H B , Melo T , Artur Paiva A and Domingues M R (2021). Insights in the Role of Lipids, Oxidative Stress and Inflammation in Rheumatoid Arthritis Unveiled by New Trends in Lipidomic Investigations. https://www.mdpi.com/journal/antioxidants, 10, 45. https:// doi. org/ 10.3390/antiox10010045.

17. Fong, K. L., McCay, P.B. and Poyer, J. L. (1973).Oxidative stress. Free Radic. J. Biol. Chem, 248(22),7792- 7797.
18. Fonseca L , Nunes-Souza V, Goulart M O F and Rabelo L A.(2019). Oxidative Stress in Rheumatoid Arthritis: What the Future Might Hold regarding Novel Biomarkers and Add-On Therapies. Hindawi, J. Oxidative Medicine and Cellular Longevity.Volume 2019, Article ID 7536805, 16 pages. https://doi.org/10.1155/2019/7536805
19. Fossati, P and Prencipe, L.(1982). Clin.Chem.28,p.2077-2080.
20. Friedewald W. T., Levy R. I., and Fredrickson D. S. (1972) "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," Clinical Chemistry, vol. 18, no. 6, pp. 499–502 .
21. Girgin E (2018). The effect of emotional eating behavior on nutritional status in obese individuals. Master's Thesis, Medipol University, Institute of Health Sciences, Istanbul.
22. Glendenning P and Inderjeeth CA.(2016). Controversy and consensus regarding vitamin D: Recent methodological changes and the risks and benefits of vitamin D supplementation. Crit Rev Clin Lab Sci 2016;53(1):13-28.
23. Harrison S, Jutley G, Li D, Sahbudin I, Filer A, Hewison M, and Raza K, (2020). Vitamin D and early rheumatoid arthritis, BMC Rheumatology. https://doi.org/10.1186/s41927-020-00134-7.
24. Heijboer AC, Blankenstein MA, Kema IP, et al.(2012). Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clin Chem 2012;58:543-548.
25. Karagülle M., Kardeş S., Karagülle O. et al., (2017). "Effect of spa therapy with saline balneotherapy on oxidant/antioxidant status in patients with rheumatoid arthritis: a single-blind randomized controlled trial," International Journal of Biometeorology, vol. 61, no. 1, pp. 169–180.
26. Kashat H. H. and Ali B.R. (2021). The Role of Some Hormones and Interleukins and Their Relationship with Vitamin D3 Concentration in Osteoporosis Patients. Department of pathological analyzes/ College of Science/ , University of Thi-Qar, Journal of Science (UTsci). ISSN Onlin:2709-0256, 1991-8690 Volume (8), No.1.
27. Ko S.H. and Kim H.S.(2020). Menopause-Associated Lipid Metabolic Disorders and Foods Beneficial for Postmenopausal Women, www.mdpi.com/ journal/nutrients. 12, 202; doi:10.3390/nu12010202.
28. Littlejohn, E.A. and Monrad, S.(2018). Early Diagnosis and Treatment of Rheumatoid Arthritis. Prim. Care: Clin. O. Pr., 45, 237–255. [CrossRef] [PubMed].
29. Menden, E. E.; Boiano, J. M.; Murthy, L., and Petering, H. G. (1977). Modification of a P-Phenylenediamine Oxidase Method to Permit Non-Automated Ceruloplasmin Determinations in Batches of Rat Serum or Plasma Microsamples. Analytical Letters, 10(3), pp.197-204.
30. Mohamed M, Khelil S, Dbibis M, Khalifi L, Chahed H, Ferchichi S, Bouajina E and Mild A. (2017). Hepatic Proteins and Inflammatory Markers in Rheumatoid Arthritis Patients, Iran J Public Health, Vol. 46, No.8, Aug 2017, pp.1071-1078 Original Article, Available at: http://ijph.tums.ac.ir.
31. Mouterde, G.; Gamon, E.; Rincheval, N.; Lukas, C.; Seror, R.; Berenbaum, F.; Dupuy, A.M.; Daien, C.; Daurès, J.P.; Combe, B.(2020). Association Between Vitamin D Deficiency and Disease Activity, Disability, and Radiographic Progression in Early Rheumatoid Arthritis: The ESPOIR Cohort. J. Rheumatol. 2020, 47, 1624–1628. [CrossRef] https://doi.org/10.3899.

32. Nakashima, F.; Shibata, T.; Uchida, K.(2020). A unique mechanism for thiolation of serum albumins by disulphide molecules. J. Biochem. 2020, 167, 165–171. [CrossRef]
33. Parveen S, Jacob R, Rajasekhar L, Srinivasa C, Mohan IK.(2017). Serum lipid alterations in early rheumatoid arthritis patients on disease modifying anti rheumatoid therapy. Indian J Clin Biochem. 2017;32 (1):26–32. doi:10.1007/s12291-016-0566-9
34. Philippou, E.; Nikiphorou, E.(2018). Are we really what we eat? Nutrition and its role in the onset of rheumatoid arthritis. Autoimmun. Rev. 2018, 17, 1074–1077. [CrossRef].
35. Phull, A.-R.; Nasir, B.; ul Haq, I.; Kim, S.J.(2018). Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem. Biol. Interact. 2018, 281, 121–136. [CrossRef]
36. Ramwadhdoebe, T.H.; Van Baarsen, L.; Boumans, M.J.H.; Bruijnen, S.; Safy, M.; Berger, F.H.; Semmelink, J.F;. Van Der Laken, C.J.; Gerlag, D.M.; Thurlings, R.M.; et al.(2019). Effect of rituximab treatment on T and B cell subsets in lymph node biopsies of patients with rheumatoid arthritis. Rheumatology, 58, 1075–1085. [CrossRef]
37. Ravin, H. A. (1961). An improved colorimetric enzymatic assay of ceruloplasmin. The Journal of Laboratory and Clinical Medicine, 58(1), pp.161-168.
38. Silva, G. M. D., Sandes, M. D. O., Vasconcelos-Filho, F. S. L., Rocha, D. S., Rocha-e-Silva, R. C. D., Silva, C. A. D. and Brito, I. R. (2020). Responses of plasma adipokines to high intensity interval training :systematic review. Revista Brasileira de Medicina do Esporte, 26(3), 262-266.
39. Smallwood M. J., Nissim A., Knight A. R., Whiteman M., Haigh R., and Winyard P. G., (2018). "Oxidative stress in autoimmune rheumatic diseases," Free Radical Biology & Medicine, vol. 125, pp. 3–14.
40. Taghadosi M, Samimi Z, Assar S, et al. (2020) Plasma leptin does not reflect the effect of high body mass index on disease activity in rheumatoid arthritis. Immunological Investigations 49(1–2): 32–45.
41. Tietz ,N.W. (1999). Text book of clinical chemistry, 3rd Ed. C.A. Burtis, E.R. Ashwood, W.B. Saunders p. 819-861.
42. Tsai, Y. C., Wang, C. W., Wen, B. Y., Hsieh, P. S., Lee, Y. M., Yen, M. H., and Cheng, P. Y. (2020). Involvement of the p62/Nrf2/HO-1 pathway in the browning effect of irisin in 3T3-L1 adipocytes. Molecular and Cellular Endocrinology, 110915.
43. Turesson G , Ulf Bergström U, Pikwer M, Nilsson JA, and Jacobsson L (2015). High serum cholesterol predicts rheumatoid arthritis in women, but not in men: a prospective study, J. Arthritis Research & Therapy 17:284, DOI 10.1186/s13075-015-0804-1
44. Wilson JC, Sarsour K, Gale S, et al. (2019). Incidence and risk of glucocorticoid associated adverse effects in patients with rheumatoid arthritis. Arthritis Care Res (Hoboken);71(4):498–511.
45. Xu, Z.; You, W.; Liu, J.; Wang, Y.; Shan, T. (2020). Elucidating the regulatory role of melatonin in brown, white, and beige adipocytes. Adv. Nutr., 11, 447–460. [CrossRef] [PubMed]