Pertanika J. Sci. & Technol. 30(1): 123 - 140 (2022)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

The Relationships of Interleukin-33, Ve-Cadherin and Other Physiological Parameters in Male Patients with Rheumatoid Arthritis

Khalid F. AL-Rawi[1], Hameed Hussein Ali[1], Manaf A. Guma[2]*, Shakir F.T. Alaaraji[3] and Muthanna M. Awad[4]

[1]Department of Chemistry, College of Science, University of Anbar, Ramadi 31001 Iraq
[2]Department of Applied Chemistry, College of Applied Science- Hit, University of Anbar, Ramadi 31001 Iraq
[3]Department of Chemistry, College of Education for Pure Science, University of Anbar, Ramadi 31001 Iraq
[4]Department of Biology, College of Education for Pure Sciences, University of Anbar, Ramadi 31001 Iraq

ABSTRACT

Rheumatoid arthritis (RA) is a chronic joint inflammatory disease that involves various pro-inflammatory mediators and cytokines. This study explores the correlation among various biochemical and immunological parameters for the male patients with RA and performs a predictive equation that would correlate these parameters together. The study involved 44 male patients suffering from RA with the same number of healthy controls. Consent was achieved for all patients and controls, together with a general examination including complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and RF. Moreover, lipid profile, kidney function tests, specific enzymes and the following parameters have been detected, which were hypothesised to negatively impact RA disease such as TGF-β1, vitamin E, VE-cadherin, interleukin 33 and TIMP-1. Various enzymatic-linked immunosorbent assays (ELISAs), spectroscopic, serological, and haematological methods were used to quantify these parameters. Our results have revealed a significant positive correlation between ESR, RF, VE-cadherin and vitamin E, specifically type α-tocopherol that are associated with the non-biochemical parameters such as BMI, waist length, hip

ARTICLE INFO

Article history:
Received: 10 July 2021
Accepted: 15 September 2021
Published: 24 November 2021

DOI: https://doi.org/10.47836/pjst.30.1.07

E-mail addresses:
sc.kfwi72@uoanbar.edu.iq (Khalid F. AL-Rawi)
sc.dr.hameedh.ali@uoanbar.edu.iq (Hameed Hussein Ali)
manafguma@uoanbar.edu.iq (Manaf A. Guma)
esp.shaker.faris@uoanbar.edu.iq (Shakir F.T. Alaaraji)
muthanna.awad@uoanbar.edu.iq (Muthanna M. Awad)
*Corresponding author

ISSN: 0128-7680
e-ISSN: 2231-8526

length, thorax and age. The important parameters revealed correlated with RA were used to generate two predictive equations to help the physicians confirm whether a patient is diagnosed with RA directly. In addition, the study revealed some parameters that would have a positive effect on RA patients, such as TGF-β1, vitamin E and VE-cadherin, which have shown a decrease in their values compared to the controls. In contrast, other parameters showed an increase in RA patients, and therefore they can be useful biomarkers for RA disease.

Keywords: Immune system, ox-LDL, Rheumatoid arthritis (RA), TGF-β1, TIMP-1

INTRODUCTION

Autoimmunity is one of the most common and chronic disorders of the immune system. Rheumatoid arthritis (RA) is an autoimmune disorder that predominantly influences joints (Kumar et al., 2019). RA might affect many tissues and organs but mainly attacks flexible joints (Saraux et al., 2005). In autoimmune conditions, healthy cells are mistakenly targeted by the immune system. As a result, RA leads to inflammation and the destruction of cartilage and bone that show different symptoms such as warm, swollen, and painful joints and worsen after rest. Commonly, the symptoms include symmetrical arthralgia, mostly shown as loss of physical function in the hands and feet (Corada et al., 2001). Therefore, the dysregulation of the immune system results in damage in the soft and hard tissues, which is the main symptom of RA (Schmalz et al., 2019). Moreover, a decrease in inflammatory activity is the main goal for treating patients diagnosed with RA (Gonzalez-Juanatey et al., 2004). Studies showed that the prevalence of RA is within the range of 0.3% to 1% in the general population and usually affects the middle ages between 35 to 55 (Corada et al., 2001). Although the RA prevalence seems low, an increase of risk factors such as cardiovascular events correlates to RA increase in prevalence (McInnes & Schett, 2011).

Multiple clinical and biochemical methods are used to assess Rheumatoid arthritis: RA is frequently diagnosed by rheumatoid factors test (Shmerling & Delbanco, 1991). RF are antibodies (Ab) that bind specifically to gamma (γ) globulins and are known as auto-Ab. There are two well-known RF types: agglutinating RF and non-agglutinating RF. They can both be used to diagnose RA. Despite the fact that they are non-specific for RA, the valuation of agglutination of IgM-RF is still the most practical serological test for RA diagnosis. RF blood tests detect the amount of the RF antibody in the blood. Typically, The RF antibody can bind to normal body tissue and damage it. In contrast, antibodies generated by a healthy immune system can destroy and remove attacking bacteria and viruses, causing diseases (Renaudineau et al., 2005). Several factors could affect RA disorder, such as genetic factors, environmental factors and impairment in the autoimmune when the healthy body tissue is attacked by the immune system (McInnes & Schett, 2011).

For instance, Interleukin-33, a new member of the IL-1 family, has been reported to play a key role in arthritis (Iwahana et al., 1999). A recent study showed that IL-33 stimulates the emergence of group 2 innate lymphoid cells from the bone marrow (Riedel et al., 2017). Another study assessed the functions of IL-33 in fibroblast-like synoviocytes (FLS) in RA patients. The study found that IL-33 levels were higher in the plasma of RA patients than in patients with osteoarthritis OA. It suggested that an increase in plasma IL-33 in patients with RA might be a useful biomarker to diagnose RA. It would reflect the possible risks of bone erosion (Lee et al., 2016).

Another factor that could affect RA disorder is VE-cadherin (vascular endothelial) cadherin. It is also known as Cadherin 5, type 2 or CD144 (Cluster of Differentiation 144). VE-cadherin is a classical cadherin which is a transmembrane protein belonging to the cadherin superfamily. Cadherins are the adhesion molecules in most cells families. VE-cadherin is the main endothelial-specific adhesion molecule responsible for the endothelial cell barrier's integrity and angiogenesis, forming new blood vessels located at connections between endothelial cells (Vestweber, 2008). VE-cadherin also controls and maintains the permeability of the blood vessel wall for cells and substances in the tissues (Vestweber, 2008). Since IL-33 is present in endothelial cells as a nuclear factor and VE-cadherin is an integrator of endothelial cell barriers, the correlation between IL-33 and VE-cadherin is influenced by other factors such as interleukin-4 and tissue inhibitor of metalloproteinases environments TIMP1 (Bokarewa et al., 2005). TIMP1 is a glycoprotein, and it is a subfamily of four protease inhibitors. TIMP1 is a crucial regulator for growth remodelling and apoptosis in different cells in physiological and pathological conditions. In addition, it is involved in the degradation of extracellular matrix molecules. Previous studies showed an autoimmune response to TIMP1 in RA patients (Verma & Hansch, 2007). In addition, VE-cadherin has been recently significantly correlated to C-reactive protein (Banse et al., 2017).

Another important factor that is associated with RA is Transforming Growth Factor β (TGF-β). TGF-β is defined as a multifunctional cytokine involved in numerous biological processes, as it plays fundamental functions in angiogenesis homeostasis, cell proliferation, migration and apoptosis (Gonzalo-Gil & Galindo-Izquierdo, 2014). However, the role of TGF-β in RA is a matter of debate (Schiller et al., 2004). Genetic studies showed that TGF-β includes three mammalian isoforms for TGF-β: TGF-β1, TGF-β2 and TGF-β3. These isoforms have a similar function, but they are expressed in different tissues (Battaglia et al., 2013; Schiller et al., 2004). A study showed that TGF-β1 plays a key role in the immune system, especially in controlling T cells' activation, proliferation, and differentiation (Nowak et al., 2016). However, non-classical risk factors might correlate to patients with RA indirectly, which have not been studied yet. For instance, oxLDL (oxidised low-density lipoprotein) fraction is associated with cardiovascular (CV) disease, indirectly affecting

RA patients. A study showed that a higher fraction of oxLDL was noticed in RA patients (Fernández-Ortiz et al., 2020). The study suggested that the elevated oxLDL is probably correlated to chronic inflammation in RA patients with the acceleration of atherosclerosis (Fernández-Ortiz et al., 2020). A recent study revealed that changes in the HDL-C and ox-LDL-C levels of the lipid profile are associated with high disease activity in early stages arthritis patients (Dai et al., 2018).

Another non-classical risk factor that might correlate indirectly to RA patients is vitamin E. It is a natural antioxidant that can regulate immune responses. It consists of two leading common families: tocopherols and tocotrienols. It has been reported that tocotrienol is an anti-inflammatory agent (Anderson et al., 2011). In addition, a study on neonatal rats showed that supplementation with vitamin E exhibited a synergistic effect with glucosamine against RA (Dai et al., 2018).

All the factors mentioned above seem to be correlated with RA. The diagnosis of the disease activity for RA is based routinely on C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) assessment (George et al., 2017). A study showed that a greater ESR in women with RA is associated with severe obesity, related to fat mass and greater BMI. However, men with RA have shown greater CRP and ESR with a lower BMI in the general population (Jain et al., 2000). Thus, all these factors appeared to play a part in rheumatoid arthritis collectively. Here, we aim to explore the biological roles of various immune and non-immune factors associated with obesity in Iraqi male patients with RA such as Interleukin-33, VE-cadherin, TIMP-1, TGF-β1, OX-LDL and Vitamin E. The reasons for choosing the male patients are to avoid the complication of women sex hormones that might involve RA formation. Also, males develop RA less than females due to genetics, and hormonal differences, which lead to consequently occuring RA symptoms were more severe in women than in men. Thus, we suggested studying the lowest level that could affect both genders. Also, we aim to reveal a predictor equation from statistical analysis for various parameters measured for RA patients to estimate whether a patient suffers from RA or not. The criteria are mentioned in the next section.

METHODS

Methods and Patients

A total of 88 patients had their blood drawn from the main venous line after admission. At least 3 mL of blood had been collected and placed into a serum separator tube and then centrifuged at 4 °C at 3000 rpm for 15 min. The serum was taken and then stored at −80 °C for later analysis. An additional 2 mL of whole blood were collected for ESR, and complete blood count (CBC) examinations were performed immediately. Patients were selected from the clinic of the main teaching hospitals in Ramadi city and Fallujah city in Iraq.

The study was conducted with critical criteria. First, only males' patients with RA were examined. It is to achieve our goal in this study. Another important criterion was that all patients with chronic and acute diseases such as diabetes and hypertension except RA were excluded in this study. It is to avoid any contradiction that would affect the results. In addition, only middle-aged, 40 to 60 years old, patients were chosen as there was a rare sample size for those out of middle age. The same things followed for control but with no RA disease.

Haematology

Full routine haematological values were obtained using a CBC blood test machine (Auto Haematology analyser MSLAB42 from Guandong, China) in the local governmental and private laboratories., Erythrocyte Sedimentation Rate (ESR) was determined following the procedure described by Sharma and Singh (2000) (as cited in Mu et al., 2010).

Serology

Multiple serological and immunological tests have been studied, such as rheumatoid factor, IL-33, VE-cadherin, TIMP-1, TGF-β1, ox-LDL and alpha-tocopherol vitamin E using commercial kits from My BioSource®.

Biochemical Study

Serum samples were analysed manually following commercial kits procedures from Cromatest® using Colorimetric Assay Kits to detect the following biochemical parameters; urea, creatinine, uric acid, lipid profile; Triglyceride (TG), Cholesterol, high-density lipoprotein HDL, and vitamin E specifically type α-tocopherol. All samples were processed and examined at the biochemistry laboratory of the main teaching hospitals in Ramadi city and Fallujah city in Iraq.

Statistical Analysis

The data were collected and analysed using SPSS, version 25. Figures were plotted using JASP software version 0.14 and Prism Graph Pad 8.3.1. The descriptive statistic tables for patients and healthy controls were expressed as mean, minimum, maximum, and SD values. In addition, the concentrations of the following parameters (Ox-LDL, TGF-β1, VE-cadherin, vitamin E and IL-33) were plotted versus the duration of the disease, which was expressed in years.

RESULTS AND DISCUSSION

It is a descriptive and predictive study involving patients admitted to the clinic of the main teaching hospitals in both Ramadi and Fallujah cities in Iraq. The study was based on data

collected from 44 orally consented Iraqi male patients aged 40 to 60 years suffering from RA and joint pain. With equal numbers of healthy control. The samples were collected from patients who attended multiple laboratories and clinics at the main teaching hospitals in Ramadi and Fallujah cities in Iraq. All patients were interviewed, clinically examined, and approved according to the signed ethical forms of the responsible committee on humans in the Ramadi and Fallujah institutions that the Ministry of Health directs. Informed consent was obtained from all patients included in this study.

The study was conducted on a total of 44 male patients suffering from RA. An equal total of healthy controls was also included. The general examination included complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and RF. Most of the biochemical parameters have been examined as well, such as a lipid profile, kidney function, and specific molecules have been detected which were hypothesised to decrease RA diseases such as TGF-β1, vitamin E, VE-cadherin, interleukin 33, and TIMP-1. Some measurable parameters such as waist, age, hip and neck width and blood pressure have also been recorded. In addition, other parameters assumed to affect RA have also been recorded, such as smoking, employment duration, disease duration, academic achievements, and social conditions (Tables 1 & 2). The age range for the patients was from 42 to 62 years with a mean of 50.66 ± 6.035 years, and the duration of their disease ranged from 1 to 10 years, with a mean of 6 ± 2 years (group 2). For the control, the age range was between 40 to 60 years, with a mean of 48.48 ± 5.663 years.

Table 1

Descriptive statistical analysis of the biochemical, serological, immunological and social measured parameters for males' healthy controls.

The Parameters	N	Minimum	Maximum	Mean	Std. Deviation
OX-LDL ng/ml	44	5	15	10.36	3.033
TGF-B1 pg/ml	44	61	196	119.72	42.691
VE- Cadherin ng/ml	44	5	20	12.4	5.016
Vitamin E µg/ml	44	2	4	3.1	0.579
IL-33 pg/ml	44	22	175	94.43	46.22
TIMP1pg/ml	44	52	288	150.95	72.915
Age years	44	40	60	48.48	5.663
BMI kg/m^2	44	21	29	24.82	2.222
Hip length cm	44	80	105	94.35	7.367
Waist length cm	44	74	104	92.5	6.882

Table 1 *(Continued)*

The Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Thoracic cm	44	82	114	101.81	7.06
Neck length cm	44	34	45	39.29	2.645
SBP mmHg	44	92	133	119.09	8.263
DBP mmHg	44	66	96	78.64	5.714
ESR (mm/hr)	44	10	26	16.91	3.094
RF (IU/mL)	44	8	16	11.09	3.94
CRP (mg/L)	44	6	24	9.41	4.91
S. Creatinine (mg/dL)	44	1	1	0.89	0.224
S. Uric Acid (mg/dL)	44	3	7	5.11	0.892
S.Urea (mg/dL)	44	13	38	25.89	5.654
T. Cholesterol (mg/dL)	44	97	203	160.45	22.733
Triglycerides (mg/dL)	44	52	136	100.52	23.291
HDL (mg/dL)	44	34	63	48.7	5.381
LDL (mg/dL)	44	27	122	91.65	22.223
VLDL (mg/dL)	44	10	27	20.1	4.658
Hb (g/dL)	44	11	16	13.2	1.123
WBC (10^3/L)	44	3	10	7.1	1.349
Smoking	44	0	1	0.07	0.255
Rural OR Urban	44	0	1	0.5	0.506
Employment	44	0	1	0.82	0.39
Academic achievement	44	0	4	1.41	1.127
Valid N (listwise)	44				

Some useful abbreviations : IL-33; interleukin 33, SBP mmHg; Systolic Blood Pressure, DBP; Diastolic blood pressure, Hb; Haemoglobin, WBC; White Blood Cells, Smoking: Yes(1) OR No(0), Employment; Yes (1) Or No(0), Rural (1) OR Urban (0), Academic achievement : Ph. D(4); Master (3); BA(2); Sec(1); Pri (0)

Table 2

Descriptive statistical analysis of the biochemical, serological, immunological and social measured parameters for males' patients with RA

The Parameters	N	Minimum	Maximum	Mean	Std. Deviation
OX-LDL ng/ml	44	12	55	31.69	13.007
TGF-B1 pg/ml	44	32	99	60.16	19.521
VE- Cadherin ng/ml	44	2	10	5.33	1.951
Vitamin E µg/ml	44	2	3	2.16	0.471
IL-33 pg/ml	44	49	499	284.57	117.156
TIMP1pg/ml	44	61	300	163.04	70.742
Age years	44	42	62	50.66	6.035
BMI kg/m2	44	23	36	29.74	2.98
Hip length cm	44	90	138	108.17	8.795
Waist length cm	44	90	129	106.58	9.158
Thoracic cm	44	85	117	99.04	7.521
Neck length cm	44	30	46	37.21	3.811
SBP mmHg	44	2	9	5.3	2.268
DBP mmHg	44	115	161	136.55	10.513
ESR (mm/hr)	44	66	109	91.34	10.751
RF (IU/mL)	44	37	111	60.8	14.866
CRP (mg/L)	44	8	32	22.18	8.942
S. Creatinine (mg/dL)	44	6	48	26.32	13.768
S. Uric Acid (mg/dL)	44	1	14	1.81	1.909
S.Urea (mg/dL)	44	5	9	7.1	1.097
T. Cholesterol (mg/dL)	44	27	53	40.55	6.815
Triglycerides (mg/dL)	44	154	291	204.11	30.388
HDL (mg/dL)	44	115	1399	194.18	189.545
LDL (mg/dL)	44	24	59	35.64	9.791
VLDL (mg/dL)	44	-119	237	129.64	46.309
Hb (g/dL)	44	23	280	38.84	37.909
WBC (103/L)	44	10	14	11.67	1.044
Smoking	44	6	15	9.38	1.892
Rural OR Urban	44	0	1	0.16	0.37
Employment	0	0	0	0	0
Academic achievement	44	0	2	1.34	0.645
Valid N (listwise)	0				

A t-test was performed to examine the significant difference between the means of the biochemical parameters in RA patients and control, as seen in Table 3. Moreover, one sample t and Wilcoxon test experiment were performed to examine the significant difference between the variation of the concentrations for the biochemical parameters versus the disease duration for RA patients, as seen in Table 4. The study revealed that five parameters only amongst others had shown significant differences in RA patients compared to the healthy control, which are TIMP-1, TGF-β1, ox-LDL, vitamin D and IL-33. In addition, the concentrations of these parameters are independent of the duration of RA disease, which can be seen in Figure 1.

Table 3

The statistical analysis of the biochemical and immunological parameters performed using t-test experiment for males' patients with RA verses the healthy control as shown in Figure 1.

Unpaired t test	Ox-LDL	TGF	VE	Vit E	IL-33
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P value summary	****	****	****	****	****
Significantly different (P < 0.05)	Yes	Yes	Yes	Yes	Yes
One- or two-tailed P value?	Two-tailed	Two-tailed	Two-tailed	Two-tailed	Two-tailed
t, df	t=10.59, df=86	t=8.416, df=86	t=8.708, df=86	t=8.424, df=86	t=5.386, df=86
Mean of Patients (B)	10.36	119.7	5.332	2.157	163.0
Mean of Control (A)	31.69	60.16	12.40	3.105	94.43
Difference between means (B - A) ± SEM	21.32 ± 2.014	-59.56 ± 7.077	7.066 ± 0.8114	0.9477 ± 0.1125	-68.61 ± 12.74
95% confidence interval	17.32 to 25.33	-73.63 to -45.49	5.453 to 8.679	0.7241 to 1.171	-93.93 to -43.28
R squared (eta squared)	0.5660	0.4516	0.4686	0.4521	0.2522
F test to compare variances					
F, DFn, Dfd	18.39, 43, 43	4.783, 43, 43	6.611, 43, 43	1.513, 43, 43	2.343, 43, 43
P value	<0.0001	<0.0001	<0.0001	0.1784	0.0062
P value summary	****	****	****	ns	**
Significantly different (P < 0.05)?	Yes	Yes	Yes	No	Yes

Table 4

The statistical analysis of the biochemical and immunological parameters was performed using a one-sample t and Wilcoxon test experiment affected by the duration of RA disease, as shown in Figure 1.

One sample t test	Ox-LDL	TGF	VE	Vit E	IL-33
t, df	t=16.16, df=43	t=20.44, df=43	t=18.13, df=43	t=30.39, df=43	t=15.29, df=43
P value (two tailed)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Discrepancy	31.69	60.16	5.332	2.157	163.0
SD of discrepancy	13.01	19.52	1.951	0.4707	70.74
SEM of discrepancy	1.961	2.943	0.2941	0.07096	10.66
95% confidence interval	27.73 to 35.64	54.22 to 66.09	4.739 to 5.925	2.014 to 2.300	141.5 to 184.5
R squared	0.8586	0.9067	0.8843	0.9555	0.8446

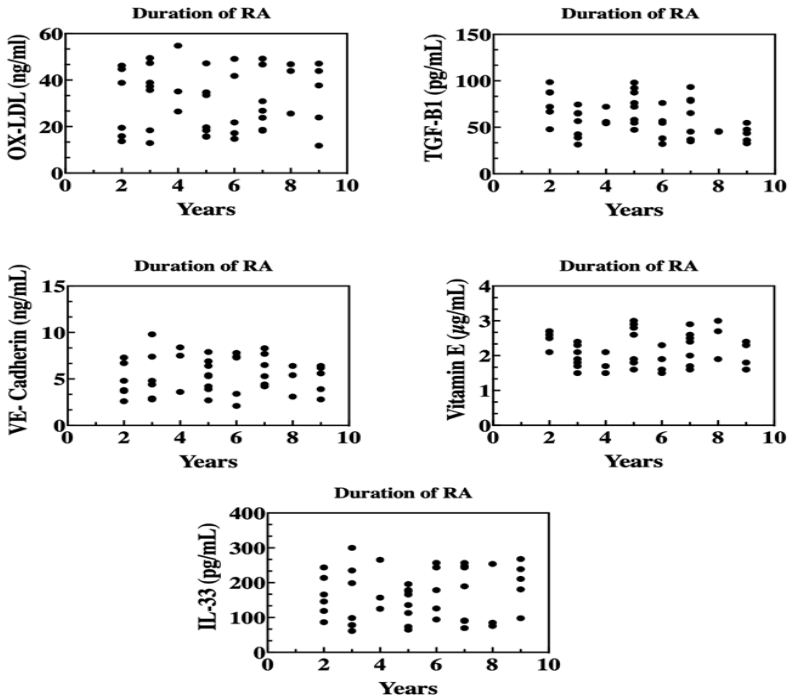

Figure 1. The descriptive statistical analysis of the following quantified biochemical and immunological parameters; TIMP-1, TGF-β1, ox-LDL, vitamin D and IL-33 versus the durations of RA diseases (expressed by years).

Moreover, there seems to be a significant positive correlation between ESR, RF, VE-cadherin, vitamin E with the non-biochemical parameters such as BMI, waist length, hip length, thorax, and age. Other biochemical examined parameters have not shown a direct impact on RA disease (Table 1). Therefore, a predictive equation that links these parameters collectively to calculate rheumatoid factor RF was generated (Equation 1). Standard values were obtained from the statistical analysis to link the most significant parameters in the predictive Equation 1. Another predictive equation was also generated to calculate ESR or any other unknown parameter belonging to the RA (Equation 2). It should be noted that these two equations are only valid if the range of the collected data is within the same conditions of these data's range.

$$RF= -57.63+ 0.5\ ESR+ 1.26\ BMI+ 0.33\ waist. \quad (1)$$

$$ESR= -7.44 + 0.39\ Ox\text{-}LDL- 0.08\ TGF\text{-}\beta1- 129 VE\text{-}Cadhrin + 1.75\ hip- 1.59\ Thorax+0.06\ IL33-7.02\ vitamin\ E +0.39\ waist +0.52\ age. \quad (2)$$

The results showed that serum IL-33 concentrations were two folds higher in RA patients than in healthy controls. These results confirm previous studies which have shown similar output (Hidayat et al., 2019; Salama et al., 2017). Other recent studies have reported that serum IL-33 is linked to RA disease, indicating a brilliant diagnostic performance (Albertsen et al., 2013; Harris & Nelson, 2010). The results indicate that IL-33 is probably involved in the RA pathogenesis, and it might suggest a direct therapy for IL-33 to treat RA patients who have been assessed with high levels of IL-33 (Chen et al., 2019). In contrast, the statistical analysis between the duration of RA disease for each patient and the concentrations of IL-33 showed that IL-33 concentrations remain abnormally high in all the durations. In other words, IL-33 concentration was not back to normal once it had changed. Thus, serum IL-33 assessment can be considered a useful biomarker for evaluating RA disease activity (Figure 1). However, an earlier study found a significant negative association with IL-33 level with disease duration in years (Gonzalo-Gil & Galindo-Izquierdo, 2014). Other biochemical parameters such as ox-LDL showed a significant increase in RA patients than in controls. Ox-LDL concentrations were shown to be about three times higher in RA patients than controls (Figure 2).

However, the concentrations of Ox-LDL remained elevated in all durations of RA patients. Ox-LDL stimulates inflammations, cell damage, generating cytokines, cell adhesion, apoptosis (Bašić et al., 2019). Therefore, it can be said that Ox-LDL is a valuable biomarker for RA disease. Therefore, it was involved in the predictive equation (Figure 2 and Table 3). TGF-β1 was shown to be significantly decreased in RA patients than in controls. The maximum concentration of TGF-β1 was 100 pg/ml in RA patients, while

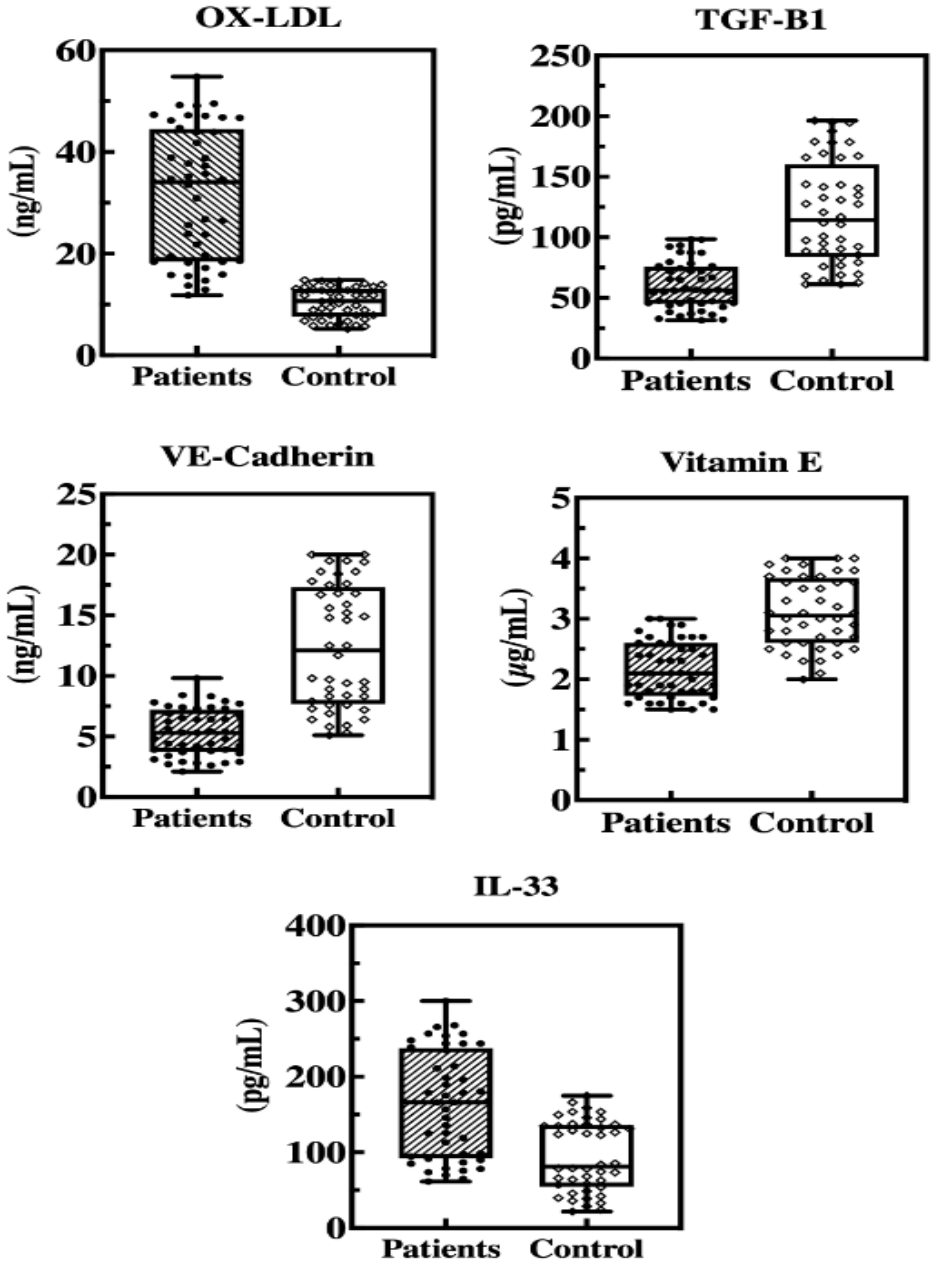

Figure 2. The biochemical and immunological quantification of TIMP-1, TGF-β1, ox-LDL, vitamin D and IL-33 in the serum of RA patients and healthy controls.

the maximum concentration was 200 pg/ml in the healthy controls. Moreover, people who have struggled with RA for 8 and 9 years have shown less TGF-B1 concentrations than the control (Figure 2 and Table 4). These findings are compatible with a recent study that showed that TGF-β is an effective therapeutic target (Pap et al., 2000). Another study suggests that blocking the TGF-β pathway is correlated with immune tolerance defeat and natural autoimmunity, defective tissue repair, and other effectors (Sakuma et al., 2007). Therefore, an increase in TGF-β would reduce RA.

Another important factor that has shown a similar impact to TGF-β on RA is VE-cadherin. It has shown a significant decrease in RA patients than in controls. The average range concentration for VE-cadherin was observed between 2-10 ng/ml in RA patients, while it was between 5-20 ng/ml in the healthy controls. Moreover, the duration of the disease was not shown to affect the VE-cadherin concentration for RA patients. It is probably due to the multiple functions of VE-cadherin in the immune system but also due to its behaviour in different cell types: various cellular processes such as apoptosis and cell proliferation are regulated by VE-cadherin, which also regulates vascular endothelial growth key receptor functions (Chen et al., 2019). Therefore, VE-cadherin also regulates cell dynamics and cell cycle progression (Hidayat et al., 2019). The studies have described the correlation between IL-33 and VE-cadherin with other cytokines as follows; IL-33 is released when tissue is injured. IL-33 then warn the immune system, which releases Th2 cells. Thus, increased IL-33 with the association of the growth factors (TGF-β) showed significantly reduced work as a beneficial biomarker for RA disease. Therefore, both measured parameters with IL-4 could reduce endothelial integrity and stimulates permeability, as has been shown previously. It also could escort the downregulation of VE-cadherin mRNA expression. In addition, IL-33 might also lead to less-controlling of endothelial barrier down-regulation process, which is performed to regulate the release of the proteins, solutes and the leukocytes entry into injured tissue that secretes the IL-33 to alarm the immune system (Chalubinski et al., 2015).

A study has suggested that TGF-β signalling could be blocked to become a useful strategy for RA treatment (Sakuma et al., 2007). Due to these crucial functions of VE-cadherin, an impairment on such a molecule could result in diseases such as RA. VE-cadherin is associated with an increase in the vasculature, which is one of the most characteristic changes in the synovial tissue of the joints in RA (Jain et al., 2000). Studies suggested that an increase in vasculatures could result from VE-cadherin-dependent angiogenesis and a potential therapeutic target of the disease. VE-cadherin is expressed by synovial fibroblastic cells and form tube-like structures. Recently, non-endothelial cells have expressed VE-cadherin, which mature vasculatures in placental tissue and malignant tumours (Fernández-Ortiz et al., 2020; Nowak et al., 2016).

The last measured biochemical parameter which has shown less impact on RA patients than others is vitamin E. However, it is still a beneficial biomarker that could be taken with other mentioned parameters for RA diagnosis, as in Equation 2. The total average concentration for vitamin E was observed at 3 g/ml in RA patients, while the average concentration was observed at 4 g/ml in the healthy controls. It indicates that a decrease in vitamin E in the blood might increase the probability of rheumatoid arthritis. A preclinical study on a rat model of collagen-induced arthritis showed that the tocotrienol-rich fraction from palm oil containing vitamin E has a beneficial therapeutic and anti-inflammatory activity against the disease (Zainal et al., 2019). Moreover, we have shown that the duration of the disease has a remarkable effect on the concentration of vitamin E (Figure 1). These findings demonstrate the crucial role of vitamin E on the immune system itself, not only during the disease period but also compared to the healthy control. Another study on neonatal rats showed supplementation with vitamin E exhibited a synergistic effect with glucosamine against RA (Hinds et al., 2013).

CONCLUSION

Rheumatoid arthritis (RA) is a chronic joint inflammatory disease that involves various pro-inflammatory mediators and cytokines. There are various biochemical and immunological parameters associated with RA patients. This study aims to find the most important biochemical and immunological parameters correlated to RA disease and drive an equation that directly guides the diagnosis. Various enzymatic-linked immunosorbent assays (ELISAs), spectroscopic, serological, and haematological methods were used to examine these parameters. Our results have revealed a significant positive correlation amongst ESR, RF, VE-cadherin and vitamin E, associated with non-biochemical parameters such as BMI, waist length, hip length, thorax, and age. All these mentioned parameters were shown to be significantly abnormal in RA patients in comparison to healthy control. The statistical study between the durations of the disease for each patient and the concentrations of the following parameters: TIMP-1, TGF-β1, ox-LDL, vitamin D and IL-33 revealed that their concentrations remain abnormal in all the duration of the disease, which can highlight the importance of these parameters in RA diagnosis.

Thus, we suggest all of the parameters be examined as biomarkers using the two predictive equations generated in this study to confirm whether a patient is diagnosed with RA directly. In addition, the study revealed that the concentrations of most parameters that showed significant differences are independent of the duration of the disease. Moreover, our findings indicated that some parameters that showed significant differences are inversely proportional to each other for RA patients, which need further investigation in the future. Also, the study revealed some useful parameters that would positively affect RA patients and could be targeted therapeutically to obtain an increase in TGF-B1, vitamin E, and VE-

cadherin. In contrast, other biochemical and serological parameters showed an increase with RA patients, and they can be useful biomarkers for RA disease.

ACKNOWLEDGMENT

We are immensely thankful for:

1. Bilal Altaha, Department of Applied Chemistry, College of Applied Sciences Hit (head), University Of Anbar, Iraq; and
2. Dr Lorenza Francescut, Champion Mental Health First Aider, Department of Molecular and Cell Biology, College of Sciences, Henry Welcome Building, University of Leicester, UK.

REFERENCES

Albertsen, H. M., Chettier, R., Farrington, P., & Ward, K. (2013). Genome-wide association study link novel loci to endometriosis. *PloS One*, *8*(3), Article e58257. https://doi.org/10.1371/journal.pone.0058257

Anderson, J. K., Zimmerman, L., Caplan, L., & Michaud, K. (2011). Measures of rheumatoid arthritis disease activity: Patient (PtGA) and provider (PrGA) global assessment of disease activity, disease activity score (DAS) and disease activity score with 28-joint counts (DAS28), simplified disease activity index (SDAI), cl. *Arthritis Care & Research*, *63*(S11), S14-S36. https://doi.org/10.1002/acr.20621

Banse, C., Polena, H., Stidder, B., Khalil-Mgharbel, A., Houivet, E., Lequerré, T., Fardellone, P., Le-Loët, X., Philippe, P., & Marcelli, C. (2017). Soluble vascular endothelial (VE) cadherin and autoantibodies to VE-cadherin in rheumatoid arthritis patients treated with etanercept or adalimumab. *Joint Bone Spine*, *84*(6), 685-691. https://doi.org/10.1016/j.jbspin.2016.10.012

Bašić, J., Vojinović, J., Jevtović-Stoimenov, T., Despotović, M., Sušić, G., Lazarević, D., Milošević, V., Cvetković, M., & Pavlović, D. (2019). Vitamin D receptor gene polymorphism influences lipid profile in patients with juvenile idiopathic arthritis. *Clinical Rheumatology*, *38*(1), 117-124. https://doi.org/10.1007/s10067-018-4264-2

Battaglia, A., Buzzonetti, A., Baranello, C., Fanelli, M., Fossati, M., Catzola, V., Scambia, G., & Fattorossi, A. (2013). Interleukin-21 (IL-21) synergizes with IL-2 to enhance T-cell receptor-induced human T-cell proliferation and counteracts IL-2/transforming growth factor-β-induced regulatory T-cell development. *Immunology*, *139*(1), 109-120. https://doi.org/10.1111/imm.12061

Bokarewa, M., Dahlberg, L., & Tarkowski, A. (2005). Expression and functional properties of antibodies to tissue inhibitors of metalloproteinases (TIMPs) in rheumatoid arthritis. *Arthritis Research & Therapy*, *7*(5), 1-9. https://doi.org/10.1186/ar1771

Chalubinski, M., Wojdan, K., Luczak, E., Gorzelak, P., Borowiec, M., Gajewski, A., Rudnicka, K., Chmiela, M., & Broncel, M. (2015). IL-33 and IL-4 impair barrier functions of human vascular endothelium via different mechanisms. *Vascular Pharmacology*, *73*, 57-63. https://doi.org/10.1016/j.vph.2015.07.012

Chen, Z., Bozec, A., Ramming, A., & Schett, G. (2019). Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nature Reviews Rheumatology*, *15*(1), 9-17. https://doi.org/10.1038/s41584-018-0109-2

Corada, M., Liao, F., Lindgren, M., Lampugnani, M. G., Breviario, F., Frank, R., Muller, W. A., Hicklin, D. J., Bohlen, P., & Dejana, E. (2001). Monoclonal antibodies directed to different regions of vascular endothelial cadherin extracellular domain affect adhesion and clustering of the protein and modulate endothelial permeability. *Blood, The Journal of the American Society of Hematology*, *97*(6), 1679-1684. https://doi.org/10.1182/blood.V97.6.1679

Dai, W., Qi, C., & Wang, S. (2018). Synergistic effect of glucosamine and vitamin E against experimental rheumatoid arthritis in neonatal rats. *Biomedicine & Pharmacotherapy*, *105*, 835-840. https://doi.org/10.1016/j.biopha.2018.05.136

Fernández-Ortiz, A. M., Ortiz, A. M., Pérez, S., Toledano, E., Abásolo, L., González-Gay, M. A., Castañeda, S., & González-Álvaro, I. (2020). Effects of disease activity on lipoprotein levels in patients with early arthritis: can oxidized LDL cholesterol explain the lipid paradox theory? *Arthritis Research & Therapy*, *22*(1), 1-12. https://doi.org/10.1186/s13075-020-02307-8

George, M. D., Giles, J. T., Katz, P. P., England, B. R., Mikuls, T. R., Michaud, K., Ogdie-Beatty, A. R., Ibrahim, S., Cannon, G. W., & Caplan, L. (2017). Impact of obesity and adiposity on inflammatory markers in patients with rheumatoid arthritis. *Arthritis Care & Research*, *69*(12), 1789-1798. https://doi.org/10.1002/acr.23229

Gonzalez-Juanatey, C., Testa, A., Garcia-Castelo, A., Garcia-Porrua, C., Llorca, J., & Gonzalez-Gay, M. A. (2004). Active but transient improvement of endothelial function in rheumatoid arthritis patients undergoing long-term treatment with anti-tumor necrosis factor α antibody. *Arthritis Care & Research*, *51*(3), 447-450. https://doi.org/10.1002/art.20407

Gonzalo-Gil, E., & Galindo-Izquierdo, M. (2014). Role of transforming growth factor-beta (TGF) beta in the physiopathology of rheumatoid arthritis. *Reumatología Clínica (English Edition)*, *10*(3), 174-179. https://doi.org/10.1016/j.reumae.2014.01.006

Harris, E. S., & Nelson, W. J. (2010). VE-cadherin: At the front, center, and sides of endothelial cell organization and function. *Current Opinion in Cell Biology*, *22*(5), 651-658. https://doi.org/10.1016/j.ceb.2010.07.006

Hidayat, R., Isbagio, H., Alwi, I., Soewondo, P., Setiabudy, R., Jusman, S. W., Immanuel, S., Harimurti, K., & Kalim, H. (2019). The effect of hydroxychloroquine on endothelial dysfunction in patients with rheumatoid arthritis: A double-blind randomized clinical trial. *International Journal of Clinical Rheumatology*, *14*(2), 59-64.

Hinds, D. A., McMahon, G., Kiefer, A. K., Do, C. B., Eriksson, N., Evans, D. M., St Pourcain, B., Ring, S. M., Mountain, J. L., & Francke, U. (2013). A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. *Nature Genetics*, *45*(8), 907-911. https://doi.org/10.1038/ng.2686

Iwahana, H., Yanagisawa, K., Ito-Kosaka, A., Kuroiwa, K., Tago, K., Komatsu, N., Katashima, R., Itakura, M., & Tominaga, S. (1999). Different promoter usage and multiple transcription initiation sites of the interleukin-1 receptor-related human ST2 gene in UT-7 and TM12 cells. *European Journal of Biochemistry*, *264*(2), 397-406. https://doi.org/10.1046/j.1432-1327.1999.00615.x

Jain, S., Sharma, N., Singh, S., Bali, H. K., Kumar, L., & Sharma, B. K. (2000). Takayasu arteritis in children and young Indians. *International Journal of Cardiology*, *75*, S153-S157. https://doi.org/10.1016/S0167-5273(00)00180-7

Kumar, R., Attri, A., Rozera, R., Verma, S., Yadav, A. K., Singh, S. K., & Sudhakar, C. K. (2019). A review on pathophysiology of rheumatoid arthritis in conjunction with modernistic approaches of treatment. *Plant Archives*, *19*(2), 2201-2206.

Lee, E. J., So, M. W., Hong, S., Kim, Y. G., Yoo, B., & Lee, C. K. (2016). Interleukin-33 acts as a transcriptional repressor and extracellular cytokine in fibroblast-like synoviocytes in patients with rheumatoid arthritis. *Cytokine*, *77*, 35-43. https://doi.org/10.1016/j.cyto.2015.10.005

McInnes, I. B., & Schett, G. (2011). The pathogenesis of rheumatoid arthritis. *New England Journal of Medicine*, *365*(23), 2205-2219.

Mu, R., Huang, H. Q., Li, Y. H., Li, C., Ye, H., & Li, Z. G. (2010). Elevated serum interleukin 33 is associated with autoantibody production in patients with rheumatoid arthritis. *The Journal of Rheumatology*, *37*(10), 2006-2013. https://doi.org/10.3899/jrheum.100184

Nowak, B., Madej, M., Łuczak, A., Małecki, R., & Wiland, P. (2016). Disease activity, oxidized-LDL fraction and anti-oxidized LDL antibodies influence cardiovascular risk in rheumatoid arthritis. *Advances in Clinical and Experimental Medicine*, *25*(1), 43-50.

Pap, T., Müller-Ladner, U., Gay, R. E., & Gay, S. (2000). Fibroblast biology: Role of synovial fibroblasts in the pathogenesis of rheumatoid arthritis. *Arthritis Research & Therapy*, *2*(5), 1-7. https://doi.org/10.1186/ar113

Renaudineau, Y., Jamin, C., Saraux, A., & Youinou, P. (2005). Rheumatoid factor on a daily basis. *Autoimmunity*, *38*(1), 11-16. https://doi.org/10.1080/08916930400022574

Riedel, J. H., Becker, M., Kopp, K., Düster, M., Brix, S. R., Meyer-Schwesinger, C., Kluth, L. A., Gnirck, A. C., Attar, M., & Krohn, S. (2017). IL-33–mediated expansion of type 2 innate lymphoid cells protects from progressive glomerulosclerosis. *Journal of the American Society of Nephrology*, *28*(7), 2068-2080. https://doi.org/10.1681/ASN.2016080877

Sakuma, M., Hatsushika, K., Koyama, K., Katoh, R., Ando, T., Watanabe, Y., Wako, M., Kanzaki, M., Takano, S., & Sugiyama, H. (2007). TGF-β type I receptor kinase inhibitor down-regulates rheumatoid synoviocytes and prevents the arthritis induced by type II collagen antibody. *International Immunology*, *19*(2), 117-126. https://doi.org/10.1093/intimm/dxl128

Salama, A. A., Mahmoud, A. B., Al-Sharaki, D. R., & Gomah, A. F. (2017). Role of interleukin-33 in rheumatoid arthritis patients from Menoufia University Hospitals. *Menoufia Medical Journal*, *30*(3), 952-957. https://doi.org/10.4103/1110-2098.218272

Saraux, A., Guillemin, F., Guggenbuhl, P., Roux, C. H., Fardellone, P., Le Bihan, E., Cantagrel, A., Chary-Valckenaere, I., Euller-Ziegler, L., & Flipo, R. M. (2005). Prevalence of spondyloarthropathies in France: 2001. *Annals of the Rheumatic Diseases*, *64*(10), 1431-1435.

Schiller, M., Javelaud, D., & Mauviel, A. (2004). TGF-β-induced SMAD signaling and gene regulation: Consequences for extracellular matrix remodeling and wound healing. *Journal of Dermatological Science*, *35*(2), 83-92. https://doi.org/10.1016/j.jdermsci.2003.12.006

Schmalz, G., Davarpanah, I., Jäger, J., Mausberg, R. F., Krohn-Grimberghe, B., Schmidt, J., Haak, R., Sack, U., & Ziebolz, D. (2019). MMP-8 and TIMP-1 are associated to periodontal inflammation in patients with rheumatoid arthritis under methotrexate immunosuppression - First results of a cross-sectional study. *Journal of Microbiology, Immunology and Infection*, *52*(3), 386-394. https://doi.org/10.1016/j.jmii.2017.07.016

Shmerling, R. H., & Delbanco, T. L. (1991). The rheumatoid factor: An analysis of clinical utility. *The American Journal of Medicine*, *91*(5), 528-534. https://doi.org/10.1016/0002-9343(91)90190-9

Verma, R. P., & Hansch, C. (2007). Matrix metalloproteinases (MMPs): Chemical-biological functions and (Q) SARs. *Bioorganic & Medicinal Chemistry*, *15*(6), 2223-2268. https://doi.org/10.1016/j.bmc.2007.01.011

Vestweber, D. (2008). VE-cadherin: The major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *28*(2), 223-232. https://doi.org/10.1161/ATVBAHA.107.158014

Zainal, Z., Rahim, A. A., Radhakrishnan, A. K., Chang, S. K., & Khaza'ai, H. (2019). Investigation of the curative effects of palm vitamin E tocotrienols on autoimmune arthritis disease in vivo. *Scientific Reports*, *9*(1), 1-11. https://doi.org/10.1038/s41598-019-53424-7