Evaluation of Liver Function and Lipid profiles in Iraqi patients with Rheumatoid Arthritis

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Abstract. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease with a destructive pattern that affects the joints. It affects the synovial lining tissue of the joints. Females develop rheumatoid arthritis at a higher risk than males. The main treatment for rheumatoid arthritis is disease-modifying anti-rheumatic agents (DMARD) such as methotrexate (MTX) and Etanercept. In this study, ESR, CRP, liver function enzymes, renal function tests and lipid profile were evaluated for rheumatoid arthritis patients. The study includes 120 female patients with rheumatoid arthritis and 60 healthy individuals as a control group with an age range between (20-60) years. The obtained results indicated a significant increase in ESR, CRP activity (p <0.01) in patients compared to the control values. The results also indicated a substantial increase in liver enzymes activity, renal function and lipid profile parameters in rheumatoid arthritis compared to the control values. In conclusion, it appears that DMARD treatment such as methotrexate may be the cause of many complications such as renal impairment. On this basis, we can suggest lowering the weekly dose of methotrexate to patients when effects on liver enzymes activity, renal function, lipid profiles and/or changing treatment when MTX is no longer beneficial.

Keywords: Rheumatoid arthritis, liver enzymes activity, renal function, lipid profile.

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease with a destructive pattern that affects the joints [1]. It is caused by severe inflammation of the immune cellular reactions in the synovial lining tissue of the joints, and the interaction of the granulocytes with the immune complex in the synovial fluid [2].

RA can affect individual daily lifestyle and activity [3]. It affects the tissue of the synovial lining of the joints (the connective tissue membrane that lines the capsule of the joint and produces synovial fluid) [2]. It also affects the small arthrodial joints of the fingers, hands, wrists, knees, shoulders and feet [4], and is less common in the elbows, hips and neck [5].

Chronic inflammation causes pain and swollen, stiff joints which affects movement by limiting the individual’s range of motion [6]. Females are at a higher risk of developing rheumatoid arthritis than males. Although RA can develop at any age, it develops mostly in the middle years between the ages of thirty and sixty [7]. Laboratory tests to diagnose and evaluate rheumatoid arthritis activity included rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) to measure inflammation level [8], [9].

The main treatment for rheumatoid arthritis is anti-rheumatic drugs to slow the progression of joint destruction that called a disease-modifying anti-rheumatic agent (DMARD). The most common examples of DMARD are methotrexate (MTX) which is used as a form of chemotherapy, and Etanercept which is considered a biological treatment [10], [11]. Methotrexate is a folic acid antagonist widely used to treat tumor disorders. It has the ability to inhibit the synthesis of RNA, DNA and proteins by binding to dihydrofolate reductase [12]. While MTX is recommended for use as a
first-line drug by the American College of Rheumatology (ACR) and the European Society of Rheumatology (EULAR). MTX is often used as a first-line treatment due to its positive efficacy and low toxicity, compared to other disease-modifying anti-rheumatic drugs. [10], [13], [14]. Depending on the case, MTX is taken at a weekly dose of 7.5-10 mg and the dose may be increased to 25 mg / week [15]. Despite the treatment, patients often continue to experience pain and concomitant use of analgesics, including non-steroidal anti-inflammatory drugs (NSAIDs) such as paracetamol [16]. An interaction between NSAID and MTX may occur, and this was first described while using aspirin to treat tumors with high-dose MTX [17].

Rheumatoid arthritis has been associated with a variety of renal disorders due to drug exposure and chronic inflammation [18], [19]. NSAIDs may lead to acute kidney injury or end-stage renal disease (ESRD) due to tubular necrosis, vasoconstriction, and acute interstitial [20], [21]. Medicines used in rheumatology are often toxic to the liver. It is difficult to differentiate the hepatic manifestations of the underlying disease from the potential hepatotoxicity of the drugs [22]. Therefore, RA patients are regularly monitored for renal and other organ toxicity in connection with their DMARD therapy [23].

Although liver injury is not common in rheumatoid arthritis, abnormalities in liver tests have been reported in 5% to 77% of patients with RA [24]. Liver damage during RA is more common in the form of abnormal liver tests (21). Liver injury is generally not recognized as an important feature of rheumatoid arthritis, but abnormal liver tests vary with disease activity [25]. Elevated levels of liver transaminases (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) have been associated with the use of DMARDs in rheumatoid arthritis (RA), especially methotrexate (MTX) [25], [26]. Moreover, NSAIDs may also affect cholesterol levels, as patients with RA frequently exhibit an atherogenic lipid profile that has been linked to an inflammatory reaction that can cause cardiovascular disease [27].

Consequently, this study aims to evaluate liver function, renal function, and lipid profile in Iraqi rheumatoid arthritis patients.

2. MATERIALS AND METHODS

2.1. Subjects and study design

This study includes 180 subjects whose ages ranged between (20-60) years old. Subjects were collected from a list of patients who attended the Consulting clinic Baghdad teaching hospital medical city, Baghdad, Iraq between September and November 2020. The subjects include 60 healthy individuals as a control group, female only, and 120 female patients with rheumatoid arthritis. The patients were diagnosed by a Joint specialist (rheumatologist) as rheumatoid arthritis patients with a positive Rf and were diagnosed for a long time period, minimum of 2 years. Patients were under chemotherapy and take MTX with folic acid or biologic therapy and take Etanercept which is sold under the brand name Enbrel. None of the patients were smokers or alcoholics. Also, none of the patients had a family history of the disease. Patients with other diseases besides rheumatoid arthritis such as diabetes, hypertension, hyperthyroidism and psoriasis were excluded.

2.2. Sample collection

From each individual, 10 ml of blood was drawn through a vein puncture using disposable syringes, 2 ml was collected in EDTA tube and 8 ml was collected in a gel tube. After collection, the whole blood samples were stored in a cooling fridge at 2-4 °C. The samples in the gel tubes were centrifuged at 3000 rpm for 10 minutes. The resulting serum was stored at -20 OC until the time of analysis.
2.3. Sample analysis

The body mass index (BMI) of the subjects studied was calculated using the following formula: 
\[
\text{BMI} = \frac{\text{weight (kg)}}{\text{length (m) }^2}
\]
ESR was measured manually using the Westergren method. This method measures the distance (in millimeters) that red blood cells in the anticoagulant whole blood drop to the bottom of a standard, straight, elongated tube under the influence of gravity and over one hour. The process of settling is called sedimentation, and will leave a clear, yellowish fluid at the top, which is blood plasma. The measurement will be in millimeters per hour (mm/hr). The normal reference range for ESR results is 1–20 mm/hr for females [28]. RF was determined according to latex slide agglutination method following the protocol of DIALAB kit supplied by DIALAB, Neudorf, Austria. CRP was determined according to latex slide agglutination method following the protocol of the commercial Biosystems kit supplied by Biosystems SA, Barcelona, Spain.

Serum levels of AST and ALT were determined by colorimetric method (Reitman and Frankel) by following the protocol of the commercially available Randox kits supplied by Randox Laboratories Ltd. (UK). ALP was determined by colorimetric method according to the protocol of the commercially available Diasys kit supplied by Diasys Diagnostic System GmbH, Holzheim, Germany. Urea and creatinine levels were measured spectrophotometry according to the colorimetric method following the protocol of Siemens kit supplied by Siemens Healthineers Erlangen, Germany. Uric acid was determined by colorimetric method following the protocol of the available kit supplied by linear chemicals, Spain. Levels of lipid profile (cholesterol, triglyceride, and HDL) were determined enzymatically using the colorimetric assay following the protocol of the available kits supplied by linear chemicals, Spain. Levels of LDL and VLDL were calculated according to the Friedewald equation.

2.4. Statistical analysis

Data was analyzed using SPSS statistical software, version 23. Independent-Samples Student t-test was performed between patients and control groups, and the resulting values were expressed as mean and standard deviation (SD). The statistical tests were significant at \( p<0.05 \) and highly significant at \( p<0.01 \) with a confidence interval of 95%.

3. RESULTS AND DISCUSSION

These results obtained for the present study showed that the mean age of the patients group was 48.56 and the control group was 43.05 years with a non-significant \( p \)-value (\( p>0.01 \)). Weight, height, and BMI mean values were approximately similar with a non-significant \( p \)-value (\( p>0.01 \)) between the patient and the control groups, as presented in Table 1. These results give a valuable chance to do a case study between patients with rheumatoid arthritis and control.

Table 1: Measurement information for patients with rheumatoid arthritis and control groups.
The mean ± SD value of ESR of the patients was 35.84 ± 14.22 which are significantly higher compared to the values of the control group 6.67 ± 4.29, and the mean ± SD value of CRP was 17.33 ± 9.62 which also are significantly higher compared to the values of the control group 6.17 ± 1.87, as shown in Table 2.

These results are in agreement with results of another study reported that the ESR and CRP values are not completely equal. ESR tends to be given higher values in women and patients with long-term disease. It has also been suggested that CRP may be more accurate than ESR in the determination of RA activity, especially in long-term female patients [29]. It has been found that ESR is related to the patient's age. The older the patient the higher the ESR value [30]. Moreover, both ESR and CRP were reported to be associated with age, sex and BMI, although the association with BMI disappeared in the multivariate analyzes [31]. Some studies reported that CRP was a better marker of acute disease activity in RA than ESR [32], [33]. Indeed, the increased values of ESR and CRP in the current study may be due to age, disease duration, disease progression, inflammation, or doses and duration of treatment for each patient. All of these factors may be involved in disease activity.

Table 2: ESR and CRP activity for patient with rheumatoid arthritis and control groups.

| parameters | patient | control |
|------------|---------|---------|
|            | Mean    | Std. Deviation | Mean | Std. Deviation | P-value |
| Age (year) | 48.56   | 11.87    | 43.05 | 12.96         | 0.322 NS |
| Weight (kg)| 68.75   | 12.03    | 69.98 | 7.81          | 0.447 NS |
| Height (cm)| 161.55  | 5.39     | 160.91| 4.92          | 0.478 NS |
| BMI (kg/m2)| 26.50   | 4.04     | 26.85 | 3.07          | 0.565 NS |

* Significant at P<0.05, NS: Non-Significant.

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Table 2: ESR and CRP activity for patient with rheumatoid arthritis and control groups.

| parameters | patient | control |
|------------|---------|---------|
|            | Mean    | Std. Deviation | Mean | Std. Deviation | P-value |
| ESR        | 35.84   | 14.22    | 6.67  | 4.29          | 0.001* |
| CRP        | 17.33   | 9.62     | 6.17  | 1.87          | 0.001* |

* Significant at P<0.01.

The results of liver enzymes activity showed a significant increase in patients with rheumatoid arthritis compared to the control group, as shown in Table 3. These results are in agreement with another study that reported elevated levels of AST and ALT in rheumatoid arthritis patients [34]. Another study reported that a common complication of long-term treatment with MTX was the elevation of liver enzyme levels in patients with rheumatoid arthritis [35]. Moreover, other studies have reported that there was a slight increase in the levels of aminotransferase (AST and ALT) in the blood [36] [37]. However, these results are inconsistent with the findings of Thompson et al, who reported that serum transaminases are almost normal in RA patients [38]. The results of alkaline phosphatase (ALP) were found elevated in patients with RA compared with the control group, Table
3. These results are in agreement with the previous study by Carlo Selmi, who stated that the value of ALP was increased in 18 to 50% of patients with rheumatoid arthritis [39]. The elevation of liver enzymes in this study may be due to the presence of inflammation, which varies with disease activity, long-term MTX therapy, and different doses of therapy. Inflammation and MTX treatment may disrupt liver enzymes metabolism, so a comprehensive study should be undertaken to study mechanistic changes in enzymes activity.

**Table 3:** liver function enzymes activity for patient with rheumatoid arthritis and control groups.

| parameters | patient | control |
|------------|---------|---------|
|            | Mean    | Std. Deviation | Mean    | Std. Deviation | P-value |
| **AST**    | 30.78   | 7.47     | 27.23   | 9.35       | 0.008*  |
| **ALT**    | 33.41   | 8.31     | 28.87   | 5.43       | 0.127 NS|
| **ALP**    | 89.11   | 19.22    | 58.10   | 13.57      | 0.001*  |

* Significant at P<0.01, NS: Non-Significant.

The renal function of the rheumatoid arthritis patients was assessed by measuring blood urea, creatinine and uric acid, then the results compared with the control group, as shown in Table 4. The results showed that blood urea and creatinine values were significantly higher (P <0.01) than the control group, while a non-significant increase in uric acid was recorded for patients compared to the control group. These results contradict another study’s results that reported lower serum urea and creatinine in rheumatoid arthritis patients compared to controls [40]. While this research agrees with other studies that reported an observed increased level of serum uric acid in RA patients [41] [42]. In addition, another study reported that blood uric acid levels were particularly high after one year of treatment with MTX, and these increases were maintained during the second year of treatment [43]. Moreover, some studies have reported that elevated levels of uric acid may be related to inflammatory responses of the disease [44]–[46]. Uric acid is an ubiquitous by-product of purine metabolism and is believed to have a beneficial effect by acting as an antioxidant and has a protective effect against oxidative stress [47]. The levels of uric acid in the blood depend mainly on the balance of the production and the excretion of uric acid in the human body. The increase in serum uric acid in this study may be related to the pro-oxidative effect on the vascular system. These may be more effective risk factors for developing cardiovascular disease or kidney impairment.

**Table 4:** Renal function parameters for patient with rheumatoid arthritis and control groups.

| parameters    | patient | control |
|---------------|---------|---------|
|               | Mean    | Std. Deviation | Mean    | Std. Deviation | P-value |
| **BL. urea**  | 40.96   | 6.12     | 28.47   | 3.83       | 0.001*  |
| **s. creatinine** | 1.12 | 0.42     | 0.71    | 0.20       | 0.001*  |
Uric acid

6.56
1.45
4.58
1.32
0.922 NS

* Significant at P<0.01, NS: Non-Significant.

The lipid profile values appeared to be higher for rheumatoid arthritis patients compared to the control group, as shown in Table 5. These results are consistent with the result of a previous study that reported elevated levels of cholesterol, LDL-cholesterol, and HDL-cholesterol for those taking MTX drugs in addition to etanercept [48]. Moreover, another study reported that rheumatoid arthritis is linked to an abnormal lipoprotein pattern. Increased cholesterol levels from baseline associated with increased TG and HDL led to a greater development of the disease activity [49]. It has been reported that patients with rheumatoid arthritis may have a higher chance of developing early cardiovascular disease due to abnormal values of lipid profiles. Therefore, improvement of the lipoprotein profile of rheumatoid arthritis patients appears to be associated with suppression of inflammation [50]. Indeed, the effect of medications on the lipoprotein profile of rheumatoid arthritis patients showed that patients who underwent methotrexate therapy were more susceptible to dyslipidemia than those receiving other DMARDS treatments [51].

In this study, the results of the lipid profile analysis confirmed that it may depend on the progression of rheumatoid arthritis disease and response to treatment, as well as the specific treatment received. This increase may be due to aging, which is a risk factor for lipid profile disorders [52], as well as prolonged inflammation (prolonged disease duration) [53]. Therefore it can be assumed that aging and disease duration are potential risk factors for dyslipidemia in patients with RA.

Table 5: Lipid profiles parameters for patient with rheumatoid arthritis and control groups.

| parameters | patient Mean | Std. Deviation | control Mean | Std. Deviation | P-value |
|------------|--------------|----------------|--------------|----------------|---------|
| Cholesterol | 152.92       | 23.25          | 132.92       | 22.80          | 0.554 NS |
| TG         | 122.83       | 27.56          | 92.12        | 23.39          | 0.333 NS |
| HDL        | 58.96        | 5.49           | 47.30        | 9.49           | 0.001*  |
| VLDL       | 24.56        | 5.51           | 18.42        | 4.67           | 0.333 NS |
| LDL        | 68.66        | 24.52          | 66.51        | 21.35          | 0.257 NS |

* Significant at P<0.01, NS: Non-Significant.

The correlation between all variables included in our study was examined for RA patients using Pearson correlation analysis, and the results were represented in Table 6. The results revealed the presence of a positive correlation between the levels of ALP and creatinine, with the ESR level. The analysis also revealed a positive correlation between the levels of AST, ALT and creatinine, with the level of CRP in patients with RA. In addition, a positive correlation was observed between the level of AST and the levels of ALT and creatinine, and between the levels of ALT and creatinine, as shown in Table 6. Moreover, the results obtained showed the presence of a positive correlation between the high level of ALP and the level of creatinine, and between the levels of urea and HDL of patients with RA. Also showed the presence of positive correlation between the level of cholesterol and LDL, opposite
to a negative correlation between triglyceride and LDL, and between HDL and LDL, and between VLDL and LDL. While showed a positive correlation between the levels VLDL and TG of patients with RA.

**Table 6:** Correlations between variables in the RA patients group.

|                | ESR  | CRP  | AST  | ALT  | ALP  | UREA | Creatinine | Uric acid | Cholesterol | TG   | HDL  | VLDL | LDL  |
|----------------|------|------|------|------|------|------|------------|-----------|-------------|------|------|------|------|
| ESR            | 1.00 | -     | -    | -    | -    | -    | -          | -         | -           | -    | -    | -    | -    |
| CRP            | .188 | 1.00 |     | -    | -    | -    | -          | -         | -           | -    | -    | -    | -    |
| AST            | .152 | .266 | 1.00 | -    | -    | -    | -          | -         | -           | -    | -    | -    | -    |
| ALT            | .136 | .235 | .958 | 1.00 | -    | -    | -          | -         | -           | -    | -    | -    | -    |
| ALP            | .353 | .132 | .106 | .116 | 1.00 | -    | -          | -         | -           | -    | -    | -    | -    |
| UREA           | -.108| -.005| -.03 | -.116| 1.00 | -    | -          | -         | -           | -    | -    | -    | -    |
| Creatinine     | .523 | .359 | .472 | .471 | .387 | -.064| 1          | .000      | -.046       | -.29 | -.01 | -.029| -.086|
| uric acid      | .051 | -.007| -.033| -.018| -.042| .104 | .000       | 1         | .070        | -.006| -.112| -.06 | .073 |
| Cholesterol    | .080 | -.007| -.113| -.050| .177 | -.164| -.046      | .070      | 1           | .002 | -.096| .002 | .903 |
| TG             | -.035| -.050| .005 | -.001| .014 | -.077| -.029      | -.006     | .002        | 1    | .160 | 1.00 | -.256|
| HDL            | .013 | .026 | -.007| -.080| -.086| .195 | -.001      | -.112     | -.096       | .160 | .1   | .16 | -.308|
| VLDL           | -.035| -.050| .005 | -.001| .014 | -.077| -.029      | -.006     | .002        | 1.00 | .160 | 1   | -.256|
| LDL            | .062 | .024 | -.112| -.045| .178 | -.157| -.086      | .073      | .903        | -.256| -.308| -.256| 1    |

4. Conclusion

This study evaluates some important biomarkers for rheumatoid arthritis patients. Rheumatoid arthritis patients were under methotrexate chemotherapy (MTX) or biological therapy (Etanercept). The results of this study showed an increase in the activity of liver function enzymes, in addition to an increase in indicators of kidney function. Lipid profiles were observed and characterized by increased cholesterol, LDL cholesterol, VLDL cholesterol, and HDL cholesterol in rheumatoid arthritis patients. This allows for the conclusion that DMARDs may be the cause of increased activity of liver function enzymes, kidney function and lipid profile.

Methotrexate may affect the body's organs after a while and may be considered a negative effect and may cause many complications such as renal dysfunction. Patients treated with DMARDs should be followed closely to monitor treatment effectiveness and any side effects. On this basis, we can suggest lowering the weekly dose of methotrexate to patients when this affects liver enzyme activity, renal function parameters and lipid profile, and/or changing treatment when MTX is no longer beneficial. Indeed, the implications of treatment against RA should be taken into account and studied in detail. Therefore, more research is needed to determine the effect of specific treatment of DMARDs on liver function, kidney function and lipid profile.

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