

Effect of Methotrexete (MTX) Treatment on Lipid Profile In Female Patients With Active Rheumatoid Arthritis

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الخلاصة

التهاب المفاصل الرثوي من الأمراض الالتهابية المزمنة وغالبا ما يؤدي إلى الموت لأنه يؤثر على الأوعية القلبية ويعجل من الإصابة بمرض تصلب الشرايين إذ يلاحظ لدى مرضى التهاب المفاصل الرثوي ارتفاع مقاييس الدهون والتي تكون مقترنة مع التفاعلات الالتهابية التي يعاني منها هؤلاء المرضى. الميثوتريكسيت (MTX) هو علاج محوري يقلل الالتهاب عن طريق تحفيزه لبروتينات تنشيط الخلايا المناعية (cytokines) التي ربما تؤثر على تعديل مستويات الدهون وبالتالي تقلل من خطر الإصابة بتصلب الشرايين لدى مرضى التهاب المفاصل الرثوي النشط. تضمنت الدراسة الحالية ثلاثون مريضة من النساء اللاتي يعانين من مرض التهاب المفاصل الرثوي معدل أعمارهن (٤٠-٦٧) سنة وخمسة وأربعون امرأة من الأصحاء. تم تقدير كل من الكوليسترول الكلي (TC) والكوليسترول عالي الكثافة (HDL-C) والكوليسترول واطئ الكثافة (LDL-C) والكليسيريدات الثلاثية (TG) إضافة إلى حساب نسبة الكوليسترول الكلي (TC) إلى الكوليسترول عالي الكثافة (HDL-C) ونسبة الكوليسترول واطئ الكثافة (LDL-C) إلى الكوليسترول عالي الكثافة (HDL-C) عند المريضات قبل أن يتعاطوا علاج أل (MTX) وبعد ذلك تم تقدير صور الدهون بعد سنة من بدء العلاج فوجد انه ليس هناك فروق معنوية بين المجموعتين ماعدا الكوليسترول عالي الكثافة (HDL-C) حيث كان هناك انخفاض معنوي. تم في الدراسة الحالية التحقق من تأثير علاج أل (MTX) بالاشتراك مع المقاييس الالتهابية مثل (ESR) و (CRP) على الصور المختلفة للدهون عند مرضى التهاب المفاصل الرثوي.

Abstract

Rheumatoid arthritis is a chronic inflammatory disease with an axes of cardiovascular morbidity and mortality due to accelerated atherosclerosis. patients with active RA frequently show an atherogenic lipid profile, which has been linked with the inflammatory reaction. Methotrexate, a pivotal pre-inflammatory cytokine implicated in pathogenesis of atherosclerosis in RA, may be involved in the development of the altered lipid profile observed in active RA. Thirty female patients with active RA (range of age 40-67 years) and 45 apparently healthy volunteers participated in the study .Total cholesterol(TC) , high density lipoprotein cholesterol (HDL-C) low density lipoprotein cholesterol (LDL-C), triglycerides (TG), the atherogenic index ratio (cholesterol/HDL cholesterol) and the ratio(LDL-C/HDL-C) were measured at base line and after 1 year of treatment, No differences were detected in serum TC,TG , LDL-C except HDL-C in non MTX users and subjects taking MTX . In our study investigate the effects of methotrexate treatment combination with inflammatory markers on lipid profile patients with active RA.

Key words: Rheumatoid arthritis, Methotrexate, cardiovascular, lipid profiles.

Introduction

RA is a chronic inflammatory polyarthritis that often leads to joint destruction, deformity and loss of function [1,2]. Patients with RA have a reduced life expectancy [3,4]. Early mortality

is largely due to cardiovascular disease (CVD), which is the commonest cause of death in patients with RA [5,6]. The increased prevalence of CVD is probably due to an increase in both the traditional risk factors for atherosclerosis and the presence of chronic inflammation[7]. Which is in line with the accumulating evidence that inflammation has a prominent role in the development of atherosclerosis [8,9]. The relevance of inflammation in the development of cardiovascular disease is shown by association between future cardiovascular events and raised C reactive protein (CRP) levels [10–13]. The underlying pathophysiological mechanism has not yet been completely elucidated and several possibilities have been suggested. Acute-phase proteins might: (a) deteriorate “fatty streaks” into (instable) plaques [9], (b) destabilise plaques and cause plaque ruptures [12], (c) give complement activation [13] or (d) facilitate deterioration of the lipid profile [14].

Dyslipidaemia may be responsible for the increased cardiovascular risk in patients with rheumatoid arthritis. Several investigators have shown that active rheumatoid arthritis is associated with an unfavorable lipid profile that is, a decreased total cholesterol and relatively lower high-density lipoprotein cholesterol (HDLc) levels [14–16]. The result is a less favorable atherogenic index, suggesting a relationship between inflammation and dyslipidaemia.

The lipid profile is a group of tests that are often ordered together to determine risk of coronary heart disease. They are tests that have been shown to be good of whether someone is likely to have a heart attack or stroke caused by blockage of blood vessels or hardening of the arteries (atherosclerosis)[17]. The lipid profile typically includes:

- Total cholesterol helps the body to form hormones ,vitamin D and other important substances ,but too much of it in the blood can clog and damage the blood vessels. Because it is a fat –link substance that doesn’t mix with blood , cholesterol has to combine with proteins to form lipoproteins .Lipoproteins can travel in the blood to all the organs and tissues of the body . Total cholesterol (lower is better).
- Low density lipoproteins(LDL, or bad cholesterol)build up in the blood and increase your risk of heart disease. LDL- cholesterol (lower is better).
- High density lipoprotein (HDL or good cholesterol) carry cholesterol to the liver ,where it is removed from the body. HDL- cholesterol (higher is better)

- Triglycerides store energy for your body to use when it is needed. If there is too much it can block blood vessels and cause other health problems such as abdominal pain and pancreatitis. Triglycerides (lower is better)
- Very low density lipoproteins (VLDL-cholesterol) is a type of lipoproteins and helps carry triglycerides to the liver and other parts of the body[18].
- Cholesterol to HDL Ratio: The Cholesterol to HDL ratio is a calculation of your risk for heart disease. It is optimal to have a low ratio. A low ratio indicates that total cholesterol is comprised mostly of HDL particles. This ratio is considered the most important indicator for atherosclerosis. Average risk for male (5.5-9.6) and for female (4.5-7.1) respectively.
- LDL to HDL Ratio: The LDL to HDL ratio is also a heart disease risk indicator. It is best to have a low ratio as this indicates there is sufficient HDL in relation to LDL to aid in prevention of atherosclerosis. Excessively high or low levels can indicate a problem. It is best to maintain these in proper balance to HDL. Average risk for male (3.7-6.3) and for female (3.3-5.0) respectively [19].

Methotrexate (MTX) is the most commonly prescribed disease-modifying antirheumatic drug (DMARD) for human rheumatoid arthritis. MTX is a folate antagonist that inhibits dihydrofolate reductase activity and is used for its anti-inflammatory and immunosuppressive properties in rheumatoid arthritis [20]. MTX blocks a number of enzymes involved in purine and pyrimidine metabolism. MTX promotes adenosine release and adenosine, acting at its receptors, mediates the immunological and anti-inflammatory effects of MTX in the treatment of rheumatoid arthritis [21-23]. Many studies demonstrated that clinical use of MTX for treating rheumatoid arthritis is associated with elevated expressions of atherogenic lipid profile (24-26), this is the first *in vivo* evidence in humans that commonly used low-dose MTX in rheumatoid arthritis might induce the mRNA expression of anti-atherogenic reverse cholesterol transporter expression in humans via the induction of these proteins, low-dose MTX treatment has the potential to protect against dyslipidemia through facilitation of cholesterol outflow in patients on this medication [24].

Present study designed to investigate whether – in Iraqi population of patients with stable rheumatoid arthritis on disease-modifying therapy (MTX) there was an association between inflammatory markers as ESR, CRP, and lipid levels specimens analyzed on the same day of collection.

Materials and Methods

Thirty female patients with active rheumatoid arthritis and forty five healthy age matched individuals (control group) were included in this study. The patient attended the AL-Hakeim hospital in AL- Najaf city. There ages ranged from 40 to 67 years. Active RA patients treated with methotrexate (0.2 mg/kg/week). The dose of methotrexate remained stable during the study. All patients were followed up every month for the first three months, and every three months thereafter. Overnight fasting blood samples were obtained at baseline and after twelve months follow-up from both the active RA patients and the control group. Disease activity was assessed by that patient had a minimum of three articulators involved, at least 9 sites of painful tenderness on digital compression, and had a morning rigidity exceeding 45 minutes and an ESR (erythrocyte sedimentation rate over 28 mm/hours [27]). Overnight fasting blood samples were drawn from rheumatoid arthritis patients and allowed to clot , then centrifuged for 15 min at a speed of 2500 Xg . Sera were separated to determine lipid profile, including total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C). Standard hematological and biochemical procedures were used to determine TC, TG, HDL-C and LDL-C of all subjects were evaluated using commercial analytical kits from BIOLABO (France). ESR estimation was performed by Westergen technique in each patients. Levels of c-reactive protein CRP determine by enzyme immunoassay [28] Virgo (USA).

Results and Discussion

To understand the effect of treatment by methotrexate (MTX) on lipid test parameters, the enrolled individuals were categorized in three groups. Group (1) contained the control individuals. Group (2) consisted of patients that in baseline of disease without or prior use of disease modifying antirheumatic drugs (DMARDs) and /or corticosteroids. Group (3) comprised those patients that were subjected to the methotrexate treatment after 12 months follow-up.

The result of lipid test parameters determination revealed a significant($p < 0.05$) increases of TC, HDL-C, LDL-C, VLDL, TC/HDL ratio and LDL/HDL ratio in group (2) when compared with those of group(1). The data analysis of group (3) indicated significant ($p < 0.05$) elevation of TC, LDL-C, VLDL, TG, TC/HDL ratio and LDL/HDL ratio in comparison with those of group (1). In addition the levels of HDL was found to be significantly elevated ($p < 0.05$), but

other parameters were non-significant elevation in group (3) when compared with those of group (2) shown in **Table 1**.

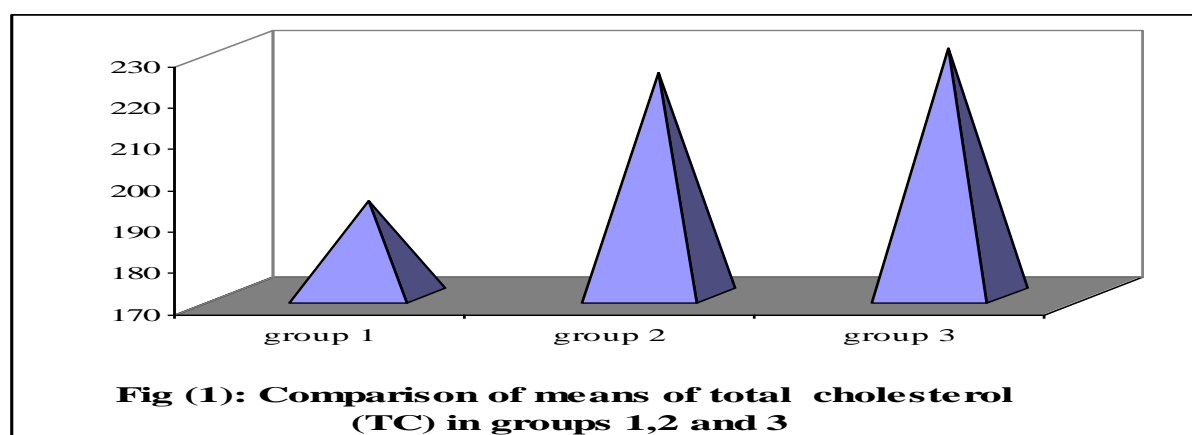
Table 1: Results Of Analysis Of Variance (ANOVA) For Lipid Test Parameters With The Status Of Treatment By Methotrexate.

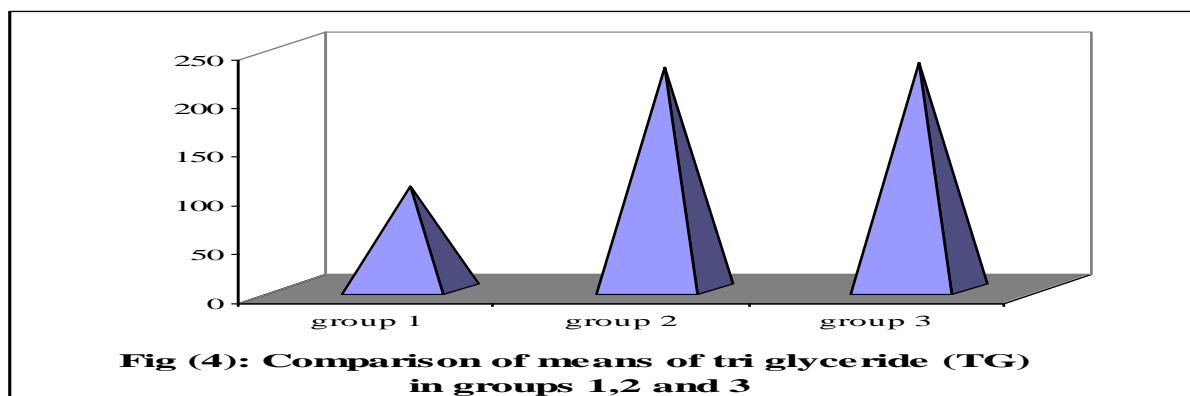
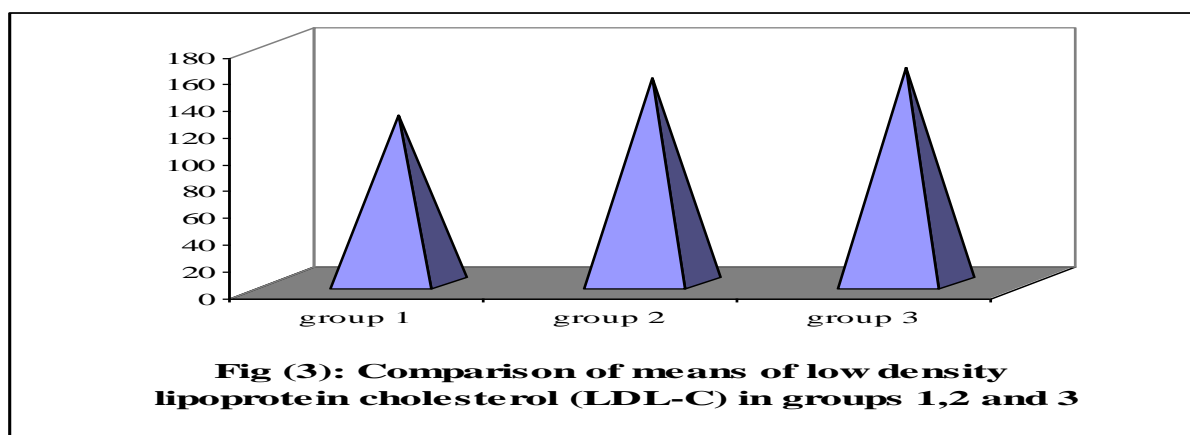
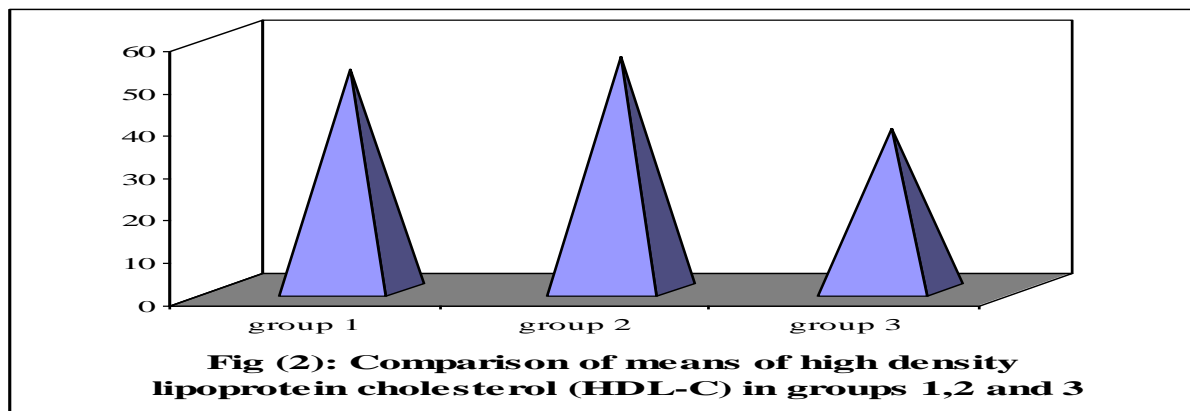
Groups	Parameters						
	TC	HDL	LDL	VLDL	TG	TC/HDL	HDL/LDL
1with 2	<0.05	<0.05	<0.05	<0.05	N.S	<0.05	<0.05
1with 3	<0.05	N.S	<0.05	<0.05	<0.05	<0.05	<0.05
2with 3	N.S	<0.05	N.S	N.S	N.S	N.S	N.S

The data of inflammatory markers determination pointed out significant ($p < 0.05$) increase of CRP and ESR in group (2) when compared with those of group (1). However patients of group (3) indicated non significant increase of ESR and CRP concentration when compared with those of group (1). One year of therapy with MTX in RA patients resulted in a significant ($p < 0.05$) decrease of the inflammatory markers CRP and ESR when compared with those of group(2) shown in **Table 2**.

Table 2: Results Of Analysis Of Variance (ANOVA) For Inflammatory Markers With The Status Of Treatment By Methotrexate.

Groups	Parameters	
	ESR	CRP
1 With 2	<0.05	<0.05
1 With 3	N.S	N.S
2 With 3	<0.05	<0.05





Patients with rheumatoid arthritis have an increased mortality from cardiovascular disease, and untreated patients have an atherogenic lipid profile which can be positively influenced by the use of disease-modifying antirheumatic drugs (DMARD) therapy[29,30]. There is, however, some doubt as to the significance of these changes, since it has been shown previously that the increased cardiovascular risk is not completely explained by traditional risk factors [31]. The mechanism by which MTX may reduce the risk of CVD in RA is likely to be complex. Multiple factors have been implicated in increasing the risk of CVD in RA. These include the effects of inflammation, an increase in traditional risk factors for CVD,

drug therapies used in RA, hyperhomocystinaemia and the presence of RF. Although the primary site of inflammation in RA is in the synovium, release of cytokines, including TNF and IL-6, produce chronically elevated cytokine levels [32]. This can induce changes in the vasculature that accelerate the process of atherosclerosis including endothelial dysfunction, secondary dyslipidaemia and activation of the coagulation cascade [33]. By reducing the disease activity and systemic inflammation, MTX would be expected to reduce the progression of atherosclerosis. However, in our review several studies showed reductions in CVD events after controlling for measuring of systemic inflammation and disease activity, suggesting an additional benefit of MTX use. Indeed, in patients treated with TNF antagonists dramatic improvements in inflammation have not been mirrored by striking reductions in CVD events, such as MI [34]. This may suggest either that other factors, such as increases in traditional factors, may be equally important in contributing to the increased CVD risk or that inflammation in RA, despite being reduced by drug therapy, is not suppressed enough to prevent the progression of atherosclerosis. Indeed an elevated HsCRP is associated with an increased CVD risk in the general population [35]. In the healthy population without hyperlipidaemia, but a raised HsCRP rosuvastatin significantly reduces the HsCRP and the risk of major CVD events [36]. HsCRP is not measured in routine clinical practice, and therefore cannot be controlled for in the studies included in our review. It is also interesting to consider how different therapies used in the treatment of RA may have an effect on the different parts of the immune system, and therefore the different effects on CVD. MTX appears to reduce the disease activity in RA but has little or no effect on RF levels. RF has recently been associated with CVD in individuals with inflammatory arthritis (Norfolk arthritis register) and in the normal population [37, 38].

Patients with RA have an increase in traditional risk factors for CVD. Treatment with MTX improves physical function and mobility, which may subsequently increase exercise levels leading to a reduced CVD risk [21-23]. Treatment with some DMARDs and biologics may improve the lipid profile and insulin resistance in patients with RA [39,40]. One of studies did demonstrate an improvement in lipid profile in patients with early RA after treatment with MTX and prednisolone [41]. However, this improvement could have resulted from decreases in inflammation rather than MTX use. Currently, there is not enough evidence to determine the effect of MTX on traditional risk factors.

Previous studies suggests that the use of MTX in RA is associated with a decreased risk of clinical CVD morbidity and mortality. With the exception of one study, all the studies demonstrated either a significant reduction or trend towards a reduction in CVD events in patients treated with MTX [39-42]. The evidence is insufficient to draw conclusions on the association between MTX use and risk factors for CVD or pre-clinical atherosclerosis. Interestingly, the association between the reduction in CVD events and MTX use may occur very early in the disease course, potentially before the diagnosis of RA. This raises the exciting prospect that MTX use very early in the disease course may not only delay the onset of RA but may also reduce the risk of collateral damage such as atherosclerosis [42]. It is, therefore, possible that a window of opportunity exists early in the disease process not only for suppressing the disease activity but also for limiting the effect of inflammation on atherosclerosis. Atherosclerosis in itself is an inflammatory disease. It is unclear from the published literature whether the effects of MTX on reducing CVD are due to direct effects on atherosclerotic lesions or as a result of a reduction in rheumatoid-driven systemic inflammation.

Conclusions

- The result suggesting a relationship between inflammatory markers and atherogenic lipid profile.
- Dyslipidemia may be responsible for the increased cardiovascular disease CVD risk in patients with rheumatoid arthritis.
- Low dose of methotrexate MTX treatment was the potential to protect against dyslipidemia.
- The use of MTX in rheumatoid arthritis is associated with a decrease risk of clinical CVD morbidity and mortality.

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