Modulation of some cardiovascular risks factors with different doses of Quercetin in patient with rheumatoid arthritis treated with Azathioprine

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Abstract:

Cardiovascular diseases are increasingly recognized contributors to increased morbidity and mortality in rheumatoid arthritis (RA). Attention to this feature of RA has been drawn by recognition of the key role played by inflammation in atherogenesis. This may be related to an atherogenic lipid profile and endothelial dysfunction. Conventional treatments of RA patient does not greatly affect those risk factors . Moreover , they may aggravate them and carry a potential adverse effects. Quercetin is a versatile flavonoid and has several biological activities that can abolish most of the undesirable effects of the inflammatory process associated with RA. It has well known anti-inflammatory, anti-oxidant and cardioprotective properties. In this work we investigated the effect quercetin on lipid profile and sICAM-1 in **160** RA patient treated with azathioprine for 8 weeks. Patient are divided into 4 groups each group was treated either with azathioprine (Aza.) combined with different doses of quercetin(500, 1000, 1500mg/day) . In addition, **30** apparently healthy volunteers were participated and served as control group. Blood samples of the patients taken at zero time and after 8 weeks , converted to serum and analyzed for ICAM-1 and lipid profile Results shows a significant ($P>0.05$) reduction in serum level of sICAM -1 in all doses tried in this study . Significant reduction in both total cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) specially at a dose of 1500mg/day quercetin . High density lipoprotein cholesterol had increased in all doses quercetin as well as azathioprine only treated group. The atherogenic index TC/HDL-c ratio had improved in all groups with no significant effect on triglycerides.

From this study we conclude that although the treatment of RA patient with azathioprine improve some cardiovascular risk factors specially lipid profile , the addition of quercetin to such patients greatly and significantly improve lipid profile and reduce the level of ICAM-1 which are important risk factors for atherogenesis . It has also concluded that 1500mg/day of quercetin gave an attractive results compared with other doses used in the study.

Key words: Cardiovascular risk, Quercetin, Rheumatoid arthritis, Azathioprine.

تعديل بعض عوامل الخطورة للجهاز القلبي الوعائي باستخدام جرع مختلفة من عقار الكورستين في مرضى التهاب المفاصل المعالجين بعقار ازاثوبرن

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الخلاصة:

تعبر الأمراض القلبية الوعائية المساهم الرئيسي لزيادة الأضرار ونسبة الوفيات الناتجة عن التهاب المفاصل الرثوي. إن الانتباه لهذه الخاصية جاء نتيجة معرفة الدور الرئيسي الذي يلعبه الالتهاب في عملية تكون الجلطات.وهذا يعود لزيادة نسبة الدهون المجلطة والاختلال بوظيفة الخلايا المبطنة للأوعية.إن العلاجات الاعتيادية المعروفة لالتهاب المفاصل الرثوي لم توثر كثيرا على هذه العوامل وإنها قد تزيد من خطورتها أو تؤدي إلى أثار جانبية عكسية.يعتبر الكويرسيتين من الفلافونويدات الرائدة حيث يمتلك العديد من الفعاليات الحياتية التي تبطل اغلب التأثيرات الضارة التي تصاحب العملية الالتهابية.كما يمتلك فعاليات مضادة للالتهاب و مضادة للأكسدة وحامية للقلب. في هذا العمل تم دراسة تأثير الكويرسيتين على صورة الدهون والجزيئات اللاصقة للخلايا في 160 مريض بالتهاب المفاصل الرثوي والمعالجين بالازاثايوبرين. حيث قسم المرضى إلى أربعة مجاميع عولجت المجموعة الأولى بالازاثايوبرين فقط فيما عولجت بقية المجاميع بالازاثايوبرين مضافا إليه جرعة مختلفة من الكويرسيتين . هذا بالإضافة إلى الاستعانة ب 30 متطوع صحيح واعتبروا كمجموعة سيطرة. حللت عينات المرضى في بداية الدراسة أي قبل العلاج وبعد 8 أسابيع من العلاج.تم قياس الكولسترول الكلي وعالي الكثافة والدهون الثلاثية وتم حساب الدهون واطئة الكثافة ومعامل التجلط بصورة غير مباشرة . لوحظ ان المعالجة بالكويرسيتين أدت إلى تغير معنوي بمستوى الجزيئات اللاصقة للخلايا كما أدت الجرعات العالية إلى قلة معنوية في الدهون واطئة الكثافة فيما أدت جميع الجرعات المستخدمة إلى زيادة معنوية في الدهون عالية الكثافة مما انعكس إيجابا على تقليل معمل التجلط.

نستنتج من هذا العمل إن الكويرسيتين يمتلك تأثيرات ايجابية في تحسين العوامل التي تزيد من نسبة حصول الأمراض الوعائية القلبية وقد تبين إن أفضل جرعة كانت 1500 ملي غرام في اليوم الواحد.

Introduction:

Cardiovascular diseases are increasingly recognized contributor to excess morbidity and mortality in rheumatoid arthritis(RA) (1).Although hypertension and age are potential additional contributors to cardiovascular events in this disease , markers of current and cumulative inflammation (WBC count and radiographic joint damage , respectively) are associated with ultrasonographically determined subclinical atherosclerosis , a predictor of cardiovascular events. Atherosclerosis often develops subclinically over prolonged periods of time; therefore, it may be too insensitive to show associations with recently acquired or temporarily active modifiable cardiovascular risk factors, such as systemic inflammation secondary to recent onset or uncontrolled RA. Clearly, other outcome variables that can identify patients at risk for cardiovascular disease at any point in time are needed in RA. One such potential marker is endothelial dysfunction , an essential step in atherogenesis(2). Most, if not all risk factors that are related to cardiovascular disease, are also associated with endothelial dysfunction, and

the process is reversible with effective treatment of operative risk factors(3). Endothelial status may be regarded as an integrated index of all atherogenic and atheroprotective factors present in an individual(2). A method to assess endothelial function involves the measurement of biomarkers of endothelial activation and dysfunction (circulating vascular cell adhesion molecule [VCAM]-1, intercellular adhesion molecule [ICAM]-1, and endothelial leucocyte adhesion molecule [ELAM]-1 [or selectin] (4). Elevated circulating adhesion molecules are associated with cardiovascular risk factors and predict atherosclerosis and cardiovascular events(5). It has been reported that such biomarkers play a more important role than traditional risk factors in cardiovascular disease in R A(6) .Important in this context is that high circulating adhesion molecule levels may not only reflect synovial inflammation but also indicate exposure of the systemic vascular endothelium to high circulating cytokine concentrations(3).

In general, and with some variations between different studies, the lipid profile of patients with active or untreated RA is primarily characterized by a decrease in serum levels of HDL-C whereas contrasting results have been published on the serum levels of TC and LDL-C .Importantly, the reduction in HDL-C has as a consequence the increase in the TC/HDL-C ratio. This ratio represents an atherogenic index, which is an important prognostic marker for cardiovascular disease (7). Indeed, the risk of myocardial infarction increases considerably when this ratio is higher than five, and it should ideally be four or less (8). The serum TC and HDL-C levels in RA are correlated with disease activity (9) suggesting a potential role for inflammation in the atherogenic profile and the higher atherosclerotic risk observed in RA.

Quercetin is a member of the class of flavonoids called flavanols and forms the backbone for many other flavonoids including the citrus flavonoids like rutin, hesperidins, naringenin and tangeritin(10,11). The best described property of quercetin is its ability to act as antioxidant. By scavenging free radicals, flavonoid; particularly quercetin can inhibit LDL oxidation in vitro. This action protects against atherosclerosis(12). Quercetin-induced suppression of TNF-α can result in the stimulation of anti-inflammatory cytokines via inhibiting the activation of NF-κβ, and therefore, one can anticipate that quercetin could be widely used as an anti-TNF-α therapy(13). Kaneuchi *et al.* (14) showed that quercetin has anti-proliferative activity and the mechanisms of quercetin action may be through modulation of cell cycle and cell growth regulatory genes. Quercetin has also been shown to limit the function of adhesion molecules on endothelial cells(15). In this study , we investigated the effect of different doses of quercetin on serum level of ICAM and lipid profile as a cardiovascular risk factors in patient with rheumatoid arthritis treated with conventional DMARD, azathioprine .

Subjects and methods:

This study was performed on (190) subjects. 30 (10 male and 20 female) apparently healthy control and 160

randomly selected RA patients (55 males and 105 females) with active rheumatoid arthritis, at the out patient clinics in Al-Hakeem and Al-Sader hospitals in Najaf - Iraq during the period December-2008 - October-2009 **,** with age range (32 -71) years , mean age ± SEM; (52.9±2.2)**,** mean disease duration is 12 years (range 7-20 years) . (131) patient only completed the study.

All patients have active rheumatoid arthritis and fulfill the 1987 revised criteria for the diagnosis of rheumatoid arthritis, set by the American College of Rheumatology(ACR)[16]. All selected patient are informed about the nature and aim of the study . None of the patients had received any other specific anti-rheumatoid therapy during the three months prior to the present study. Some of them were on intermittent use of one or more NSAIDs , and those were informed to leave one week after the last dose of those medications to ensure complete clearance.

Exclusion criteria:

Smokers or patients suffering from conditions that affect the lipid profile, such as diabetes mellitus, hypothyroidism, liver or kidney disease, Cushing's syndrome, obesity and a history of familial dyslipidemia, were excluded. In addition, patients receiving medications affecting lipid metabolism, such as lipid-lowering drugs, beta-blockers, oral contraceptives, estrogen, progestin, thyroxin and vitamin E, were excluded from the study.

Study design:

The selected RA patient were allocated into 4 groups ,each of 40 patients , groups A , B , C and D that received azathioprine 50mg(Glaxo Wellcome Inc**.**) plus placebo(starch containing capsules), quercetin 250mg(Jarrow formulas, USA) plus azathioprine 50mg , quercetin 500mg plus azathioprine 50 mg and quercetin 750 mg plus azathioprine 50 mg, respectively . All treatments are given twice daily for eight weeks. In addition to 30 age- and sex- matched healthy subjects that did not received any medications including those used in the study. This group served as a control.

Fasting blood samples were taken from patients and control. Serum was obtained and analyzed for measurements of sICAM , total cholesterol l(TC), high density lipoprotein cholesterol (HDL-c), triglycerides (TG). All these parameters were analyzed using the standard kits available for this purpose. Low density lipoprotein cholesterol(LDL-c) level was determined indirectly using the Friedwald equation[17].Patients samples were analyzed at zero time (pre-treatment) and after 8 weeks (the end of the study).

Statistical analysis:

SPSS (version 14.0) software for windows was used to analyze the results of this study . All results are expressed as Mean ± SEM. Student t-test and ANOVA was used to examine the difference in the mean of parameters tested between studied groups. P value <0.05 was considered significant**.**

Results:

Results of this study showed a significant differences ($p<0.05$) in the pre-treatment level of sICAM of all RA groups with respect to the healthy

control values. These results showed that treatment with azathioprine alone did not significantly ($p<0.05$) affect sICAM level. However; the addition of 500, 1000 or 1500 mg/day of quercetin for patient treated with azathioprine results in significant reduction in sICAM level with the later dose was significantly different from other doses.(table 1 and fig. 1)

Results also indicate that only the highest quercetin dose (1500 mg/day) significantly ($p<0.05$) affect total cholesterol while both LDL-c and was significantly reduced in all doses of quercetin in addition to azathioprine only treated group. This reduction was independent on the dose of quercetin except for LDL-c in quercetin 1500 mg/day treated group which was significantly different from other doses. HDL-c significantly elevated by all doses of quercetin as well as the azathioprine only treated group. No significant ($p<0.05$) changes were seen regarding the level of TG in all studied groups. The atherogenic index (TC/HDL-c ratio) was significantly reduced in all treated groups ; however , all groups treated with quercetin were significantly different from azathioprine only treated group. Tables 2&3 and figures 2-6 show all these changes.

Table(1): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level sICAM.

Group		**sICAM(pg/ml)**
Healthy control N=30	Untreated	106.4 ± 4.45
Azathioprine (100mg/day) N=40	Pre-treatment	446.3 ± 21.5*
	Post-treatment	431.7 ± 20.7*
Azathioprine+ Quercetin (100/500mg/day) N=40	Pre-treatment	485.7 ± 21.0*
	Post-treatment	388.0 ± 15.9*†
Azathioprine+ Quercetin (100/1000mg/day) N=40	Pre-treatment	435.5 ± 19.7*
	Post-treatment	310.6 ± 15.9*†
Azathioprine+ Quercetin (100/1500mg/day) N=40	Pre-treatment	448.6 ± 18.89*
	Post-treatment	279.6 ± 15.5*†[a,b]

Data are expressed as mean ±SEM
*P<0.05 with respect to healthy control group.
†P<0.05 with respect to pretreatment value.
[a]P<0.05 with respect to azathioprine treated group.
[b]P<0.05 with respect to azathioprine +Quercetin (100/500mg/day) treated group.

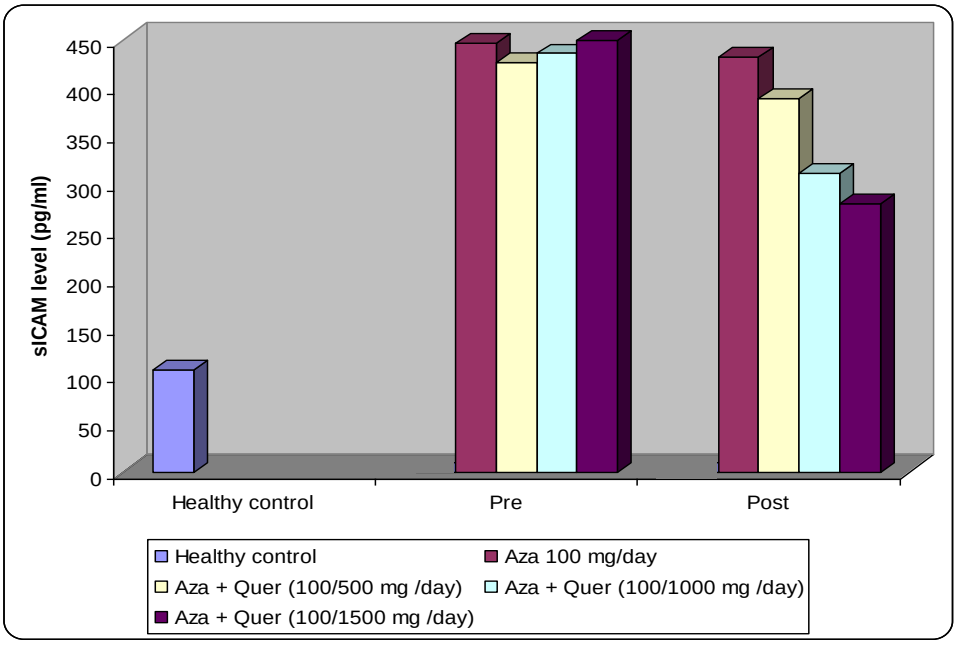
Table(2): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level TC, HDL-c and TC/HDL-c ratio.

Group		TC (mg/dl)	HDL-c(mg/dl)	TC/HDL-c ratio
Healthy control N=30	Untreated	122.7 ± 4.91	50.2 ± 1.21	2.48 ± 0.11
Azathioprine (100mg/day) N=40	Pre-treatment	127.7 ± 4.63	28.4 ± 1.32*	4.67 ± 0.21*
	Post-treatment	123.3 ± 7.76	35.3 ± 0.89*	3.55 ± 0.16
Azathioprine+ Quercetin (100/500mg/day) N=40	Pre-treatment	134.2 ± 4.08	25.9 ± 1.07*	5.39 ± 0.24*
	Post-treatment	115.6 ± 2.79	43.3 ± 1.13†	2.72 ± 0.10
Azathioprine+ Quercetin (100/1000mg/day) N=40	Pre-treatment	136.0 ± 3.61	24.8 ± 0.86*	5.68 ± 0.24*
	Post-treatment	112.4 ± 2.67	45.6 ± 1.10†	2.50 ± 0.14†
Azathioprine+ Quercetin (100/1500mg/day) N=40	Pre-treatment	140.5 ± 3.36	23.3 ± 0.76*	6.18 ± 0.20*
	Post-treatment	104.4 ± 2.49†[b]	51.0 ± 1.68†	2.11 ± 0.15†[a]

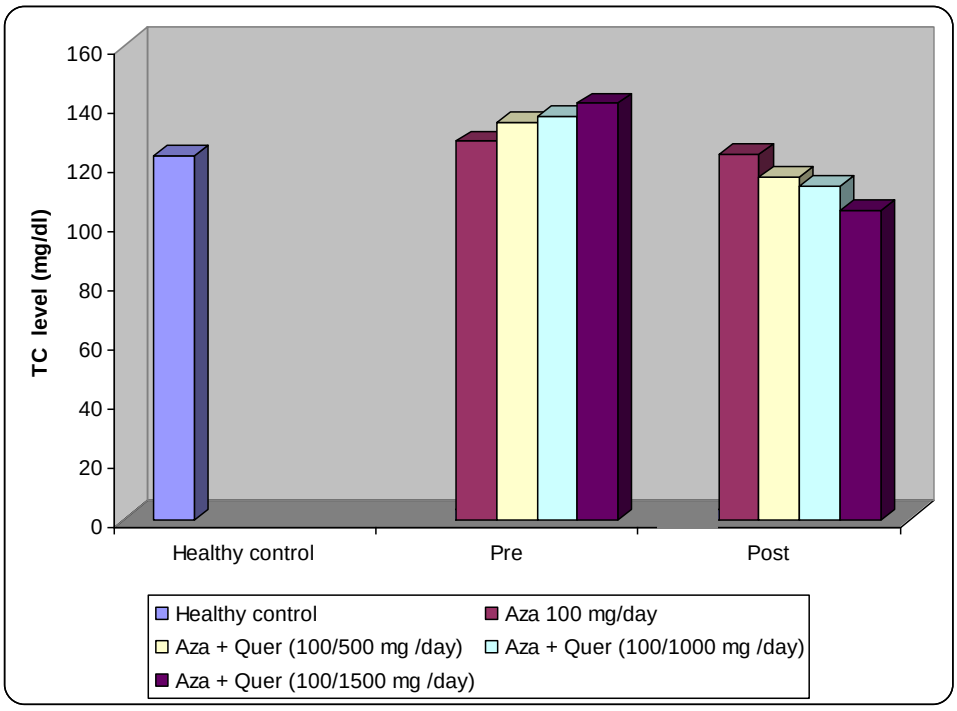
Data are expressed as mean ±SEM
*P<0.05 with respect to healthy control group.
†P<0.05 with respect to pretreatment value.
[a]P<0.05 with respect to azathioprine treated group.
[b]P<0.05 with respect to azathioprine +quercetin (100/500mg/day) treated group.

Table(3): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level of LDL-c and TG

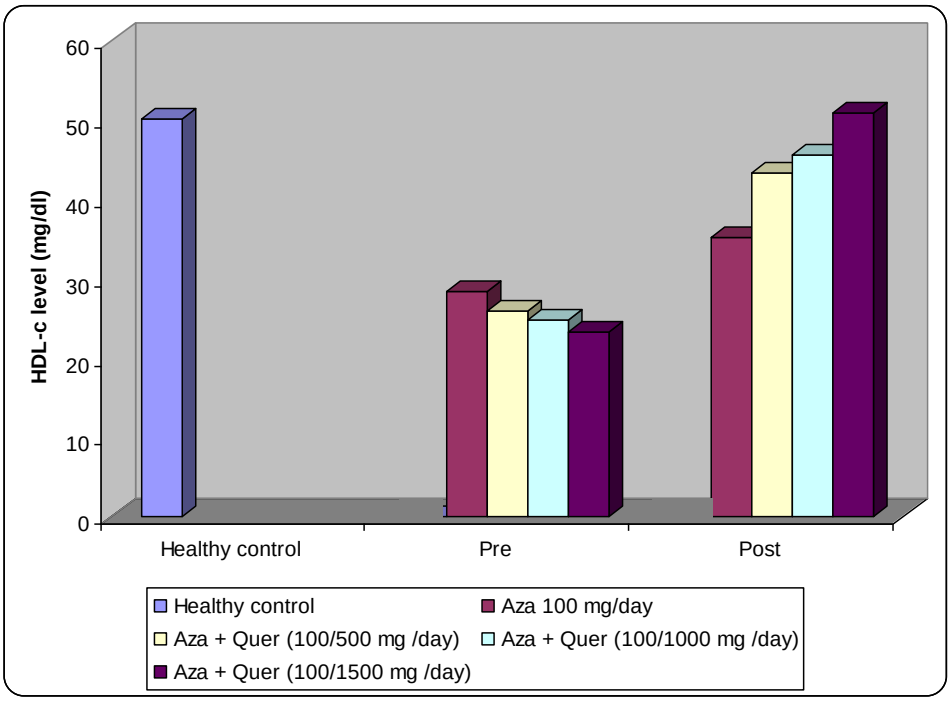
Group		LDL(mg/dl)	TG (mg/dl)
Healthy control N=30	Untreated	49.8 ± 4.98	113.7 ± 2.44
Azathioprine (100mg/day) N=40	Pre-treatment	82.2 ± 4.27*	119.6 ± 2.41
	Post-treatment	60.7 ± 4.70†	110.7 ± 2.35
Azathioprine+ Quercetin (100/500mg/day) N=40	Pre-treatment	90.03 ± 3.79*	120.2 ± 2.18
	Post-treatment	55.11 ± 2.92†	108.03 ± 2.09
Azathioprine+ Quercetin (100/1000mg/day) N=40	Pre-treatment	97.77 ± 3.36*	120.7 ± 1.98
	Post-treatment	58.52 ± 3.28†	104.4 ± 2.05
Azathioprine+ Quercetin (100/1500mg/day) N=40	Pre-treatment	92.7 ± 3.37*	124.1 ± 2.36
	Post-treatment	32.7 ± 3.33*†[a,b]	103.5 ± 2.28



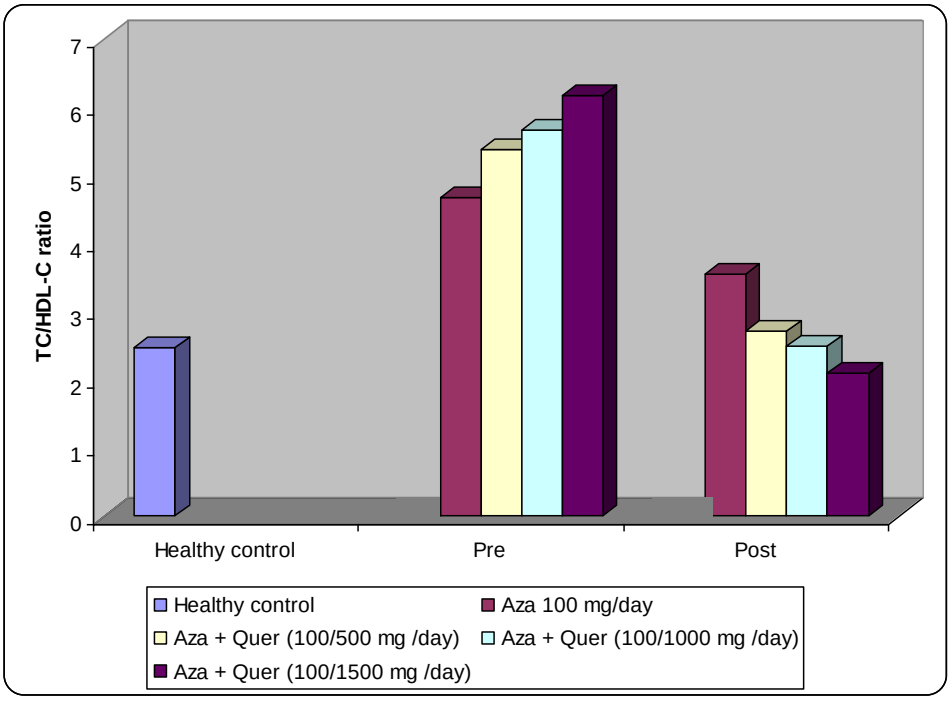
Fig(1): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level of sICAM



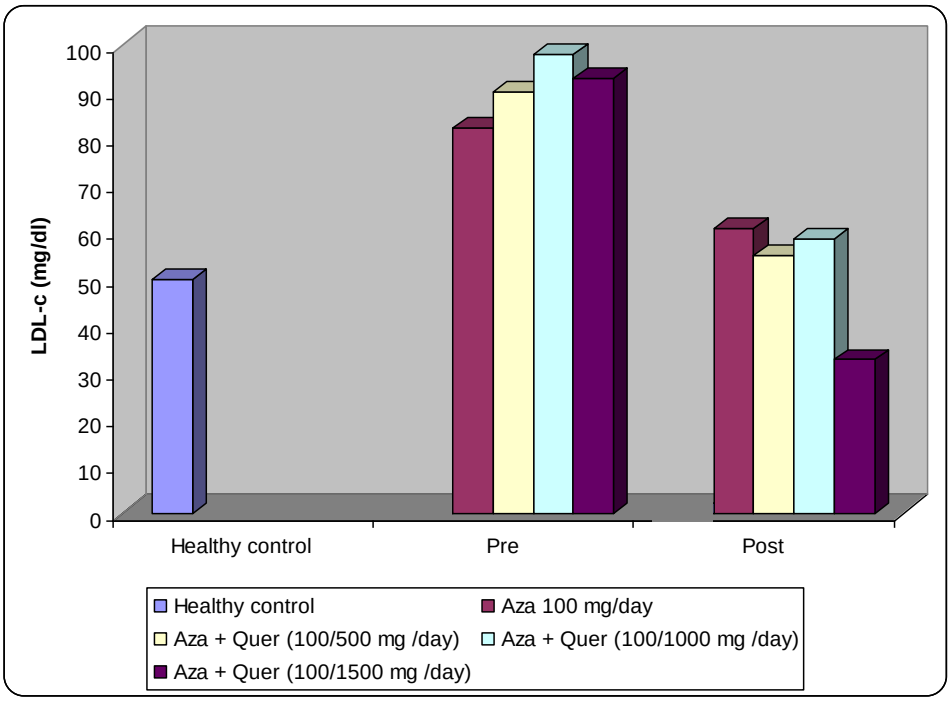
Fig(2): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level of TC



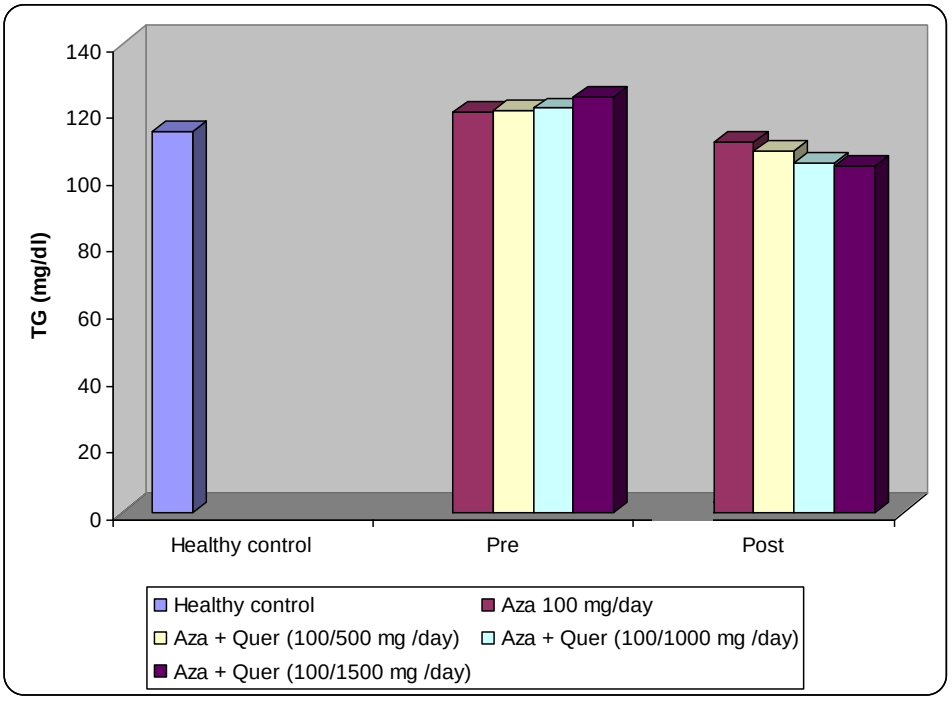
Fig(3): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level HDL-c



Fig(4): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on TC/ HDL-c ratio



Fig(5): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level LDL-c



Fig(6): Effect of eight weeks treatment of RA patient with azathioprine alone or its combination with different doses of quercetin on serum level TG

Discussion:

Interest in cardiovascular (CV) disease among patients with rheumatoid arthritis (RA) is growing, and several key studies have been published during the past several years. Attention to this feature of RA has been drawn by recognition of the key role played by inflammation in atherogenesis .The role of antirheumatic drugs promoting atherosclerosis and CV disease has also been the subject of considerable interest. Risk factors for atherosclerotic events and cardiovascular disease include male sex, increased age, elevated plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C) and endothelial dysfunction(18). Graf et al., 2005 showed that 21% decrease in cardiovascular disease mortality is seen in humans ingesting 4 mg or more of quercetin per day(19). This reduction in cardiovascular events seems to be results from its improving on lipid profile and endothelial dysfunction by reducing the level of ICAM-1(18). An increased resistance to LDL oxidation was seen in humans after 14 days of supplementation with 30 mg quercetin per day(20). ICAM1 and VCAM1 are molecules which bind white cells to the endothelium and reflect the state of the health of the endothelium, particularly in relationship to atherosclerosis(21). If the endothelium is damaged by oxidized lipid, these markers increase(22). An antioxidant might be expected to lower the level of these markers(23) as does a statin which lowers plasma lipid level(24). quercetin reduce adhesion molecules in endothelial cells *in vitro*(25). A variable dosage had used by many researchers to study effect of quercetin. Some studies used 300-500 mg daily as an anti-inflammatory dose (15)while others use even larger doses**(26)** . For this reason , several doses has been used to determine the best dose that satisfy the objectives of this work.

Conclusions:

From this study we conclude that although the treatment of RA patient with azathioprine improve some cardiovascular risk factors specially lipid profile , the addition of quercetin to such patients greatly and significantly improve lipid profile and reduce the level of ICAM-1 which are important risk factors for atherogenesis . It has also concluded that 1500mg /day of quercetin gave an attractive results compared with other doses used in the study.

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