The level of malondialdehyde-modified LDL and LDL immune complexes in patients with rheumatoid arthritis

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Abstract

Objectives: To explore possible associations of malondialdehyde-modified low-density lipoprotein (MDA-LDL) and LDL-immune complexes (LDL-IC) with atherosclerosis in rheumatoid arthritis (RA).

Design and methods: Plasma MDA-LDL, LDL-IC levels and mechanisms of the changes were investigated in RA patients with or without coronary artery disease (CAD), simple CAD patients and control.

Results: MDA-LDL and LDL-IC levels were found increased in all the studied patients, the RA patients with CAD exhibited the most significant changes. MDA-LDL levels were higher in the RA patients with CAD than those both in the simple RA and CAD patients. Multiple linear regression analysis showed that CAD, LDL-IC and erythrocyte sedimentation rate accounted for 36.5% of the variation in MDA-LDL levels; and age, activity, MDA-LDL and rheumatoid factors accounted for 34.5% of the variation in LDL-IC.

Conclusions: High levels of MDA-LDL and LDL-IC are risk factors for increased risk of atherosclerosis in RA patients and are associated with inflammation.

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Keywords: Low-density lipoprotein; Oxidative modification; Immune complex; Rheumatoid arthritis; Coronary artery disease; Atherosclerosis; Risk factors; Inflammation

Introduction

The autoimmune disease rheumatoid arthritis (RA) is associated with an unexplained increased cardiovascular risk, particularly due to excessive atherosclerosis [1]. Epidemiologic, clinical, and laboratory investigations have proven that the immune system and inflammation play a major role in all stages of atherosclerosis, and it has been established that these contribute considerably to the increased cardiovascular risk [2].

Oxidized low-density lipoprotein (ox-LDL) is thought to contribute to the development of atherosclerosis. Circulating ox-LDL and malondialdehyde-modified LDL (MDA-LDL) have been reported to be useful markers for identifying coronary artery disease (CAD) [3–5]. Additionally, it has been proposed that modified LDL might trigger an immune response leading to the production of autoantibodies and subsequently to the formation of immune complexes (IC). LDL-IC can induce foam cell formation more efficiently than any other known mechanisms, such as ox-LDL being taken up by scavenger receptor pathway, and its levels increase in patients with CAD [6,7]. Furthermore, it has been demonstrated that levels of ox-LDL and its autoantibodies are higher in RA patients than in healthy subjects [8–10], which suggests a possible link between autoimmune mechanisms and accelerated atherosclerosis in RA.

Studies investigating the possible contribution of the elevated ox-LDL autoantibodies towards atherosclerosis in RA patients are limited, and their results are contradictory [11–14]. To our knowledge, no study to date about the association of ox-LDL and LDL-IC levels with atherosclerosis in RA patients has been reported; it remains to be evaluated whether ox-LDL and LDL-IC are risk factors for atherosclerosis in patients with RA. So we detected the plasma MDA-LDL and LDL-IC levels in RA patients with or without CAD, and in simple CAD patients; their possible associations with lipid parameters, CAD, inflammatory markers, age and sex were also investigated in this study.

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Materials and methods

Subjects and blood collection

The present study included 55 patients with RA, 55 patients with simple CAD, as well as 56 healthy volunteers who were selected from routine healthy examination, physical and electrocardiography and laboratory tests without dyslipidemia, hypertension, diabetes mellitus, or any clinical evident sign of atherosclerosis. All the subjects were living in Nanjing of China, Han population, which is the major ethnic group in China.

The RA patients were selected from the outpatient rheumatology clinic of Jinling Hospital between January 2005 and December 2007, 13 of which had a history of CAD, 42 of which had simply RA. Inclusion criterion was: diagnosis of RA according to the 1987 American College of Rheumatology criteria [15]. The mean age was 45.58 years (range 21–72); the mean disease duration was 65 months (range 2–260). All patients were taking disease modifying antirheumatic drugs (DMARDs), while none was being treated with corticosteroids. They were considered to have activity if they had subjective symptoms, objective tender/swollen joints, erythrocyte sedimentation rate (ESR) ≥ 20 mm/h, or C-reactive protein (CRP) ≥ 10 mg/L. They were considered to have inactivity if they had no subjective symptoms or objective tender/swollen joints and met the criteria: ESR < 20 mm/h and CRP < 10 mg/L. Diagnoses of CAD were: myocardial infarction (MI), ischemic heart disease (anginal pain or anginal equivalent symptoms occurring with exercise, relief by rest or nitroglycerin). If occurring at rest, then symptoms relieved with nitroglycerin); or coronary angiography performed for evaluation of CAD. Exclusion criteria were: diabetes mellitus, hypertension, liver or kidney disease, a history of familial dyslipidemia, lipid-lowering therapy, or malignant disease. The patients were not on any other medications, except taking DMARDs, and did not have any other definitive diseases. The simple CAD patients in this study were selected from the inpatient in the department of cardiology of Jinling Hospital between January 2006 and December 2006, who were undergoing clinically indicated coronary angiography. The blood was sampled at least 12 h after fasting and collected into EDTA (1 mg/mL)-containing tube and plasma was separated immediately and stored at −70 °C until analysis and measured within 1 year.

This study protocol was approved by the Ethics Committee of Jinling Hospital, and all the subjects provided written informed consent.

Sandwich enzyme-linked immunosorbent assays (ELISA) for MDA-LDL and LDL-IC

MDA-LDL was measured by a sandwich ELISA according to Virella et al. [16], using polyclonal antibody against MDA-LDL as capture antibody and quantitating with monoclonal against apolipoprotein (apo) B enzyme conjugate as previously described [17]. MDA modification of proteins was performed according to Haberland et al. [18] by incubating equal volumes of freshly isolated LDL and 0.2 M MDA for 3 h at 37 °C, followed by extensive dialysis against 0.15 M PBS with 0.3 mM EDTA, pH 8.0. The degree of modification of the MDA-LDL preparations used to immunize rabbits was 62 mmol of MDA per mol of lysine, corresponding to the modification of 6.2% of lysine residues. Antibodies to MDA-LDL were obtained by immunization of New Zealand white rabbits with MDA-LDL. Cross-reactivity in the resulting antisera against LDL was removed by absorbing on a column of immobilized native LDL. Calculation of MDA-LDL level was based on the concentration of MDA-LDL as the standard. The coefficients of variation were 6.8% and 10.1% in intra- and inter-assay, respectively, and average recovery rate was 94%.

LDL-IC was measured by a sandwich ELISA, using anti-human IgG as the capture antibody and quantitating with monoclonal anti-apoB enzyme conjugate as previously described [7]. Soluble IC was precipitated with polyethylene glycol. A pooled fresh-frozen plasma sample (from 50 healthy subjects) was used as reference serum of LDL-IC and the value was expressed as 1 relative absorbance unit (AU).

Lipid level and inflammatory markers analysis

Total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) were measured on a HITACHI 7600 analyzer. LDL cholesterol (LDL-C) was calculated

Table 1

| Plasma lipids, inflammatory marker levels, age and sex in the studied group. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Variables       | RA with CAD (n=13) | Simple RA (n=42) | Simple CAD (n=55) | Control (n=56) |
| Age (y)         | 50.38±11.37      | 45.40±12.19      | 49.80±10.94      | 48.94±10.33     |
| Male/female (%) | 6/7              | 18/24            | 29/26            | 27/29           |
| Total cholesterol (mmol/L) | 4.65±1.37     | 4.82±1.21        | 5.13±0.97**      | 4.48±0.61       |
| Triglyceride (mmol/L)       | 1.50±0.80      | 1.55±0.83        | 1.83±0.86**      | 1.24±0.42       |
| HDL cholesterol (mmol/L)    | 1.41±0.30      | 1.58±0.30**      | 1.30±0.30**      | 1.47±0.24       |
| LDL cholesterol (mmol/L)    | 2.55±1.17      | 2.52±1.13        | 2.99±0.84**      | 2.43±0.50       |
| ESR (mm/h)        | 53.92±48.23†   | 36.05±33.28     | 19.69±26.17       |                 |
| CRP (mg/L)        | 33.48±49.81†   | 19.69±26.17      | 16.59±26.17       |                 |
| RF (IU/mL)        | 202.69±172.44  | 144.88±170.19    | 105.89±160.19     |                 |
| Activity/inactivity (n) | 8/5            | 22/20            |                  |                 |

Compared with control: *: P<0.05, **: P<0.01; compared with simple RA: †: P<0.05, ††: P<0.01; compared with CAD: ‡: P<0.05, ‡‡: P<0.01. RA=rheumatoid arthritis; CAD=coronary artery disease; HDL=high-density lipoprotein; LDL=low-density lipoprotein; ESR=erythrocyte sedimentation rate; CRP=C-reactive protein; RF=rheumatoid factor.
Table 2
Plasma MDA-LDL and LDL-IC levels in the studied group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>RA with CAD (n=13)</th>
<th>Simple RA (n=42)</th>
<th>Simple CAD (n=55)</th>
<th>Control (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-LDL (mg/L)</td>
<td>183.96±89.78**</td>
<td>101.60±52.66**</td>
<td>57.39±39.56**</td>
<td>33.05±27.83</td>
</tr>
<tr>
<td>LDL-IC (AU)</td>
<td>2.58±1.69**</td>
<td>1.87±0.74**</td>
<td>1.78±0.45**</td>
<td>1.22±0.39</td>
</tr>
</tbody>
</table>

Compared with control: *: P<0.05, **: P<0.01; compared with simple RA: †: P<0.05, ††: P<0.01; compared with simple CAD: ‡: P<0.05, §§: P<0.01.
RA=rheumatoid arthritis; CAD=coronary artery disease; MDA-LDL=malondialdehyde-modified low-density lipoprotein; LDL-IC=LDL-immune complexes.

Results

Plasma lipids, inflammatory marker levels, age and sex in the studied patients

The plasma lipids, inflammatory marker levels, age and sex in the studied group are shown in Table 1. TC, TG and LDL-C levels remained similar among the RA patients with CAD, simple RA patients and control; while their levels were all found higher in simple CAD patients than those in control. HDL-C levels remained unchanged among the simple RA, RA with CAD and the control, while its levels were lower in the simple CAD patients than both in control and the simple RA.

Table 3
Logistic regression of the relationship of RA and CAD with MDA-LDL and LDL-IC, controlling for plasma lipids, age and sex.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-LDL</td>
<td>20.84</td>
<td>5.06–85.75</td>
<td>0.000</td>
</tr>
<tr>
<td>CAD</td>
<td>10.53</td>
<td>1.18–93.64</td>
<td>0.035</td>
</tr>
<tr>
<td>LDL-IC</td>
<td>33.27</td>
<td>5.31–208.62</td>
<td>0.000</td>
</tr>
<tr>
<td>MDA-LDL+LDL-IC</td>
<td>52.47</td>
<td>11.32–243.23</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Cutoff values were ≥ 88.71 mg/L for MDA-LDL, and ≥ 2.00 AU for LDL-IC.
RA=rheumatoid arthritis; CAD=coronary artery disease; MDA-LDL=malondialdehyde-modified low-density lipoprotein; LDL-IC=LDL-immune complexes; OR=odds ratio; CI=confidence interval.

Plasma MDA-LDL and LDL-IC levels in the studied patients

Compared to the control, MDA-LDL and LDL-IC levels were found both significantly increased in all the studied patients, of which, the RA patients with CAD exhibited the most significant changes. Furthermore, MDA-LDL levels were found higher in the RA patients with CAD than those both in the simple RA and CAD patients, and significantly higher in the simple RA than in the simple CAD (Table 2).

We next performed logistic regression to examine the relationship of RA and CAD with MDA-LDL and LDL-IC in RA patients and control subjects (n=111), controlling for plasma lipids, age and sex. After controlling for these risk factors, the odds ratios (OR) of MDA-LDL on RA and CAD were 20.84 (95% confidence interval [95% CI] 5.06–85.75) and 10.53 (1.18–93.64), respectively, compared with controls; those of LDL-IC on them were 33.27 (5.31–208.62) and 0.99 (0.22–4.38), respectively. OR of the combined variables were also listed in Table 3.

Relationships between lipids and inflammatory markers, CAD, inflammatory markers, age and sex in RA patients

Table 4 shows the relationships between lipids and inflammatory markers in 54 RA patients. ESR and CRP levels were found both positively related with MDA-LDL and LDL-IC, while negatively related with TC and HDL-C levels, respectively. RF was only found negatively related with HDL-C.

Table 4
Relationships between lipids and inflammatory markers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CRP</th>
<th>ESR</th>
<th>RF</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA-LDL</td>
<td>−0.289  (P=0.032)</td>
<td>−0.286  (P=0.035)</td>
<td>−0.107  (P=0.439)</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>−0.041  (P=0.765)</td>
<td>0.041   (P=0.768)</td>
<td>0.100   (P=0.468)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>−0.140  (P=0.308)</td>
<td>−0.164  (P=0.232)</td>
<td>0.017   (P=0.904)</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.467  (P=0.000)</td>
<td>−0.437  (P=0.001)</td>
<td>−0.287  (P=0.034)</td>
</tr>
<tr>
<td>MDA-LDL</td>
<td>0.292   (P=0.030)</td>
<td>0.335   (P=0.013)</td>
<td>0.122   (P=0.374)</td>
</tr>
<tr>
<td>LDL-IC</td>
<td>0.381   (P=0.004)</td>
<td>0.430   (P=0.001)</td>
<td>0.178   (P=0.194)</td>
</tr>
</tbody>
</table>

HDL=high-density lipoprotein; LDL=low-density lipoprotein; MDA-LDL=malondialdehyde-modified LDL; LDL-IC=LDL-immune complexes; ESR=erythrocyte sedimentation rate; CRP=C-reactive protein; RF=rheumatoid factor.

Statistical analysis

Statistical analysis were performed with SPSS 11.5. The values were expressed as mean±standard deviation. The differences between variants were analyzed by Student’s t-test or ANOVA, and the differences between groups were subsequently determined by Fisher’s LSD test when appropriate. Correlations between variables were calculated by the non-parametric Spearman’s rank coefficient test. Binary logistic regression was used to analyze the relationship of RA and CAD with only and combined variables of MDA-LDL and LDL-IC, controlling for plasma lipids, age and sex. A multiple regression test was performed, considering the levels of MDA-LDL or LDL-IC levels as dependent variables, with the independent variables consisting of lipid parameters, LDL-IC (or MDA-LDL), CAD, activity, inflammatory markers, age and sex. Values of P<0.05 were considered statistically significant.

ESR was measured by the Westergren method, CRP was measured by turbidometry, and rheumatoid factors (RF) were determined by nephelometry.
levels. Furthermore, according to the activity of RA, the RA patients were subdivided into 2 groups; the levels of MDA-LDL (140.97 ± 85.18 mg/L vs. 97.18 ± 41.43 mg/L, respectively; \( P < 0.05 \)) and LDL-IC (2.39 ± 1.26 AU vs. 1.62 ± 0.59 AU, respectively; \( P < 0.01 \)) in active RA group (\( n = 30 \)) were higher than in inactive RA (\( n = 25 \)).

We next performed the multiple linear regression analysis for MDA-LDL (or LDL-IC) vs. each factor. A backward elimination procedure of stepwise analysis was used; lipid parameters, LDL-IC (or MDA-LDL), CAD (RA patients with or without), activity (RA patients in active or inactive phase), inflammatory markers, age and sex were treated as independent variables, respectively. Consequently, CAD (\( \beta = 0.361, P = 0.003 \)), LDL-IC (\( \beta = 0.307, P = 0.010 \)) and ESR (\( \beta = 0.224, P = 0.052 \)) were found to account for 36.5% of the variation in MDA-LDL levels. Similarly, age (\( \beta = 0.358, P = 0.003 \)), activity (\( \beta = 0.350, P = 0.011 \)), MDA-LDL (\( \beta = 0.275, P = 0.027 \)) and RF (\( \beta = -0.229, P = 0.079 \)), were found to account for 34.5% of the variation in LDL-IC levels.

**Discussion**

This study showed that MDA-LDL and LDL-IC levels were higher in the RA patients with CAD, the simple RA and simple CAD patients than in control; among all the patients, the RA patients with CAD exhibited the most significant changes. To our knowledge, the data presented here are the first to investigate the possible contribution of the elevated ox-LDL and LDL-IC levels towards atherosclerosis in RA patients.

Traditional cardiovascular risk factors do not fully explain the excessive cardiovascular events in patients with RA or that they can be explained by adverse effects from antirheumatic treatment [20–22]. There is also evidence of an inverse relationship between lipid levels and the acute phase response [21,23]. Wallace et al. [24] reported a 15–20% decrease in serum TG, TC, and LDL concentrations and a reversal of the lipid-raising effects of corticosteroids in patients with systemic lupus erythematosus (SLE) and RA. Lazarevic et al. [25] also reported that the significantly decreased concentrations of total serum lipids, TC, LDL-C and HDL-C in RA patients increased to almost normal levels, as the disease activity decreased. The present study showed that plasma lipid levels in both the simple RA patients and RA patients with CAD were similar to those of control, while they all significantly changed in the simple CAD patients. Furthermore, ESR and CRP levels were found both negatively related with TC and HDL-C level, respectively. Normal antiinflammatory HDLs play several critical roles in the prevention of atherosclerosis. Proinflammatory HDLs are unable to prevent the oxidation of LDL and the recruitment of monocytes, and may enhance the inflammatory response and predispose to atherosclerosis [26, 27]. It has also been reported that proinflammatory HDLs increase in patients with RA or SLE and are associated with elevated levels of ox-LDL [28]. Besides inflammatory reactions and the effects from antirheumatic treatment, the increased proinflammatory HDLs may be one of the reasons that results in non-decreased HDL levels in the studied RA patients. Kim et al. [8] also reported unchanged HDL levels in RA patients, which is similar to ours. The present data support that inflammation causes the altered lipid metabolism in RA.

Ox-LDL is known to play a central role in the pathogenesis of atherosclerosis. Oxidation affects both the lipid and protein components of LDL. Reactive aldehyde products formed during the oxidation of polysaturated fatty acids, such as MDA and 4-hydroxynonenal (HNE), are capable of attaching covalently to the ε-amino groups of lysine residues of apoB [29]. MDA-LDL can be taken up by macrophages through its scavenger receptors [18]. MDA-LDL levels have also been reported to be useful markers for identifying CAD [4]. Circulating MDA-LDL levels have been detected by using a few monoclonal or polyclonal antibodies against MDA-LDL [30,31,16]. These antibodies were all specific for MDA-LDL and had little reactivity with native LDL, while they also had low reactivity with copper-oxidized LDL. Similar, the present assay for MDA-LDL showed specificity for the MDA-LDL, copper-oxidized LDL could also be detected with low efficiency, just as Virella et al. reported [16]. Studies investigating the possible contribution of the elevated ox-LDL autoantibodies towards atherosclerosis in RA patients are limited, and their results are contradictory [11–14]. Peters et al. reported that autoantibodies against ox-LDL were independently associated with intima thickening, but not associated with prevalent cardiovascular disease [11], while Cvetkovic et al. described increased titers of the autoantibodies among RA patients with a history of myocardial infarction [12]. Two other studies investigated the relation between autoantibodies against ox-LDL and carotid atherosclerosis, and these showed opposite results [13,14]. Only 2 studies have investigated in vivo levels of oxidized lipoproteins in RA patients and found that the levels of ox-LDL, oxidized lipoprotein(a) [Lp(a)] and Lp(a)-IC in active RA patients are higher than those in both control subjects and inactive RA patients [8,32]. Circulating IC levels have also been reported significantly increased in RA patients [33]. The new findings in the current study were that MDA-LDL and LDL-IC levels increased in both simple RA patients and RA patients with CAD. Interestingly, MDA-LDL levels were found higher in the RA patients with CAD than those both in the simple RA and simple CAD patients, and significantly higher in the simple RA than in the simple CAD. Similarly, the OR of MDA-LDL reached statistically significant on both RA and CAD; and the OR of LDL-IC and combination of MDA-LDL and LDL-IC reached statistically significant only on RA, while did not on CAD. In addition, no association of CAD was found with RA activity in the studied RA patients (data not shown). These increases may contribute to the high risk of cardiovascular disease in RA patients.

There is growing evidence that inflammation plays an important part in the pathogenesis of cardiovascular disease particularly in atherosclerosis. An association between inflammation and cardiovascular disease has been observed in RA patients. Memon et al. [34] demonstrated that the host response to infection and inflammation increased oxidized lipids in serum and induced LDL oxidation in vivo. They suggested that increased LDL oxidation during infection and inflammation
might promote atherogenesis and could be a mechanism for the increased incidence of CAD in patients with chronic infections and inflammatory disorders. Therefore, we analyzed the relationships between LDL-IC, MDA-LDL levels and inflammatory markers. ESR and CRP levels were found both positively related with MDA-LDL and LDL-IC. The levels of MDA-LDL and LDL-IC were also found higher in active RA patients than in inactive RA. The multiple linear regression analysis showed that CAD, LDL-IC and ESR accounted for 36.5% of the variation in MDA-LDL levels; and age, activity, MDA-LDL and RF accounted for 34.5% of the variation in LDL-IC levels. The above data suggest that CAD and inflammation be associated with elevated levels of MDA-LDL in RA patients. The association between MDA-LDL and LDL-IC is similar to what we analyzed previously[7,32], which seems that increased levels of MDA-LDL results in the strong inflammation be associated with elevated levels of MDA-LDL and LDL-IC in patients with rheumatoid arthritis. Further-relationships between LDL-IC, MDA-LDL levels and inflammatory markers. ESR and CRP levels were found both inflammatory disorders. Therefore, we analyzed the importance role in the pathogenesis of cardiovascular disease particularly in atherosclerosis.

Acknowledgments

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References


