Oxidized LDL Antibodies (OLAB) in Patients with β-Thalassemia Major

Patrizia Brizzi1, Teresa Isaja2, Alfonso D’Agata2, Lucia Malaguarnera2, Mariano Malaguarnera4, and Salvatore Musumeci5

1 Department of Internal Medicine, University of Sassari, Sassari, Italy.
2 Department of Pediatrics, University of Catania, Catania, Italy.
3 Department of Biomedical Sciences, University of Catania, Catania, Italy.
4 Department of Internal Medicine, University of Catania, Catania, Italy.
5 Department of Pediatrics, University of Sassari, Sassari, Italy and Institute of Population Genetic, Italian National Research Council, Alghero, Sassari, Italy.

Thalassemic (TM) patients are subjected to peroxidative tissue injury because of continuous blood transfusions. It has been documented that circulating LDL from TM patients show marked oxidative modification, that could represent an event leading to atherogenesis. We investigated in 75 β-TM patients the levels of oxidized LDL antibody (OLAB) to assess their correlation with total cholesterol, LDL and HDL cholesterol, triglycerides Apo A-1 and Apo B. OLAB/mg chol-LDL is greater in TM patients than healthy controls (p<0.001). No correlation was found between OLAB and age, sex of patients, mean blood consumption, mean serum ferritin, mean transaminases, PT, PTT, and fibrinogen. A significant positive correlation was found between OLAB and triglycerides in TM patients (p<0.001). Also a significant correlation was found between OLAB/mg chol-LDL and level of triglycerides in TM patients, but not with total cholesterol, LDL and HDL chols, Apo A-1 and Apo B. On the contrary in the healthy controls this correlation between OLAB and OLAB/mg chol-LDL versus triglycerides was negative and not significant. High levels of OLAB/mg chol-LDL in patients with β-thalassemia, in absence of evident signs of atherosclerosis, suggest some regulatory mechanisms on the lipid peroxidation which modulate the deposition of ox-LDL in the macrophages and support the hypothesis that both serum iron and triglycerides are involved in the pathogenesis of LDL oxidation. J Atheroscler Thromb, 2002; 9: 139–144.

Key words: Thalassemia Major, Oxidized LDL Antibody, Atherogenesis

Introduction

The oxidative modification of low density lipoproteins (LDLs) plays a central role in the pathogenesis of the atherosclerotic lesions (1). Patients with β-thalassemia major (TM) are subjected to continuous blood transfusions and show peroxidative tissue injury through secondary iron overload. This is documented by a net drop in the concentrations of ascorbate, vitamin A, beta-carotene and lycopene, while serum level of vitamin E inversely are correlated with ferritin (2, 3). The alteration of the oxidant/antioxidant balance might affect the susceptibility of LDL to oxidation (4). In TM patients LDL oxidation has been proposed as one of the critical factors in promoting atherogenesis and this process results from the balance between the pro-oxidant stimuli and the endogenous antioxidants present in LDL (5). Thromboembolic events, frequent in TM patients are associated with known risk factors such as diabetes, complex cardiopulmonary abnormalities, hypothyroidism, altered liver function and post-splenectomy thrombocytosis (6-8), but not to atherosclerotic lesions of artery wall. This observation is in contrast with the role of oxidized LDL in the development and progression of vascular lesions. In fact, oxidation of LDLs generates a variety...
of oxidized modified lipids and proteins that represent highly immunogenic neo-determinants for the immune system in serum of animals including in man (9, 10). The consequence is the development of autoantibodies, which seem to correlate with the progression of atherosclerotic complication and reflect the oxidation process taking place in vivo (11). IgG can be isolated from the atherosclerotic lesions with specificity for epitopes of ox-LDLs and they are present in lesions as part of the immune complexes with ox-LDLs (12). Up to now the ox-LDL antibodies (OLAB) have been studied in different pathologies, but we did not find evidence of such study in TM patients. The aim of this paper was the determination of OLAB in a group of TM polytransfused patients and attribute a value to OLAB through a correlation with clinical data and laboratory indices.

Patients and Methods

Seventy-five TM patients 38 males and 37 females were studied at the Thalassemic Center of Pediatric Department of Catania University. Age range was between 2.5 and 41 (median 21). Twenty patients were splenectomized at a mean age of 8±/-5.5. All TM patients received regular blood transfusions at interval of 20-30 days, in order to maintain their pre-transfusion baseline haemoglobin levels above 9.5 g/dl. All TM patients were daily treated with subcutaneous desferrioxamine infusion. Data regarding the time of the first diagnosis, pretransfusional Hb level, yearly blood consumption, transfusional iron, serum ferritin level, serum transaminases, compliance with chelating therapy, associated pathologies (hypothyroidism, diabetes, reduced glucose tolerance, cardiomyopathy, other endocrinopathies or chronic hepatitis) were obtained from the clinical case sheet in the Thalassemic Center. Total cholesterol (T Chol), HDL-cholesterol (HDL-C), Apo-A, Apo-B and triglycerides (TG) were determined in all patients. LDL-cholesterol (LDL-C) was calculated by the Friedewald formula (13). Forty eight healthy subjects, 24 males and 24 females, aged 18 to 34 years (median 24), going for the first time to the Transfusion Center for blood free donation, were matched with the TM patients randomly and used as healthy controls. Blood samples for OLAB determination were collected the morning after a over night fasting before transfusion.

OLAB measurement was made using an enzyme immunoassay designed to determine autoantibodies directed to ox-LDL epitopes in human serum (OLAB-ELISA, Biomedica Gesellschaft MBH, Austria). The concentration of specific IgG in the samples was quantified by an enzyme colour change detectable on a standard ELISA reader. The OLAB concentration in the samples was quantified in milliunits/ml (mU/ml) based on a characterized serum with a high OLAB titre and a low cross reactivity with native LDL (<5%) and expressed as value absolute of OLAB or as OLAB/mg chol-LDL ratio, since this ratio indicate the real antibody response to ox-LDL in TM patients.

Statistical analysis

Results are expressed as medians and ranges or means ± standard deviation (SD). The comparison of means and SD between TM patients and healthy controls was made using the Student’s t-test. Statistical significance was considered when p was less than 0.05.

Results

TM patients showed significant lower levels of T-chol, LDL-C, HDL-C, TG, Apo A-1, Apo B than control group (Table 1).

The OLAB values in TM patients were on average 414±355 mU/ml (C.I.± 82, median 277 and range 15-1200). In healthy controls OLAB were on average 416±128 mU/ml (C.I.± 50, median 420 and range 25-520) (p<0.977). The distribution of OLAB values in 75 TM patients and in 48 healthy controls is reported in Figs. 1a, 1b. However when the OLABs were related to mg of LDL-C, the TM patients showed higher ratio, according to the lower levels of T-chol and LDL-C and the difference respect the control group was statistically significant (p<0.001). A significant positive correlation was found between OLAB and TG in TM patients (p<0.001), but on the contrary in the healthy controls this correlation was not significant (Figs. 2a, 2b). No correlation was found between OLAB and age, pre-transfusion Hb, yearly blood transfusions, iron transfusion, serum ferritin, Apo A-1, Apo B, HDL-C, LDL-C, T-

<table>
<thead>
<tr>
<th>Table 1. Serum levels of lipids in TM patients and healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>TM Patients</td>
</tr>
<tr>
<td>N. 75</td>
</tr>
<tr>
<td>Healthy Controls</td>
</tr>
<tr>
<td>N. 48</td>
</tr>
</tbody>
</table>

*Student’s t-test p<0.001 (TM patients versus controls)
chol. Also in the control group the correlation between OLAB and the other parameters of lipid metabolism was not significant. The correlation between OLAB/mg chol-LDL and TG was positively significant (<0.001) in TM patients (Fig. 3a) and not significant in control group (Fig. 3b), confirming the importance of considering the OLAB in relation with LDL-C concentration.

Discussion

The absolute levels of OLAB in TM are not statistically higher than healthy control, but the immune response should be valued related to antigen concentration. The method used for measure OLAB concentration is not specific for an single epitopes, but generic for oxidised LDL. Considering that TM have lower LDL-C than controls, also total concentration of LDL and of ox-LDL should be lower than controls. Consequently the OLAB/mg chol-LDL ratio indicate the real antibody response to ox-LDL in TM patients. In this prospective the TM patients show higher OLAB/mg chol-LDL ratio, suggesting that several mechanisms for lipid peroxidation are contemporaneoly active in this disease. In not TM patients LDL oxidation is the key step in the sequence of events leading to atherogenesis and related vascular alteration (14). LDL oxidation has been studied by measuring the in vitro formation of dienes, both the rate and the lag time (15). The levels of conjugated dienes indicate the actual LDL peroxidation, as occurs in the circulation, and the difference between various subjects may reflect differences in oxidative stress (16). Recent reports have indicate that circulating LDL of TM patients are more susceptible to the oxidation for the iron load secondary to continuous blood transfusions (5). Nevertheless, the elevation of iron alone may not carry out the free radical reaction causing oxidative stress to LDL, but other factors are required, such as depletion of antioxidant defences and decrease of HDL (3). Recently also an important role of unpaired haemoglobin chains and red blood cell hemolysis products are also under consideration, since it has been observed that the interaction between haemoglobin α-chains and LDL leads to the oxidative modification of LDL (17). In TM patients, here stud-
ied, no correlation was found between the level of OLAB and clinical and most common laboratory parameters, whereas highly significant correlation was found between OLAB and TG. Previous study showed in TM patients LDL richer in triglycerides (18), which are more sensitive to the action of lipase, with the formation of more dense LDL and then more sensitive to oxidation (19, 20).

These modifications of LDL induce the production of a more heterogeneous population of OLAB. It is known that OLAB of both IgG and IgM class may be also present in normal healthy individuals (21) and, similarly to circulating autoantibodies to self-protein (cardiolipin), reported in the atherosclerotic patient (22), OLAB can also be implicated in the atherogenesis process. Moreover it is known that OLAB may contribute to the development of atherothrombosis by interfering with blood coagulation (22), but the real role of this immune reaction in the atherogenesis remain up to now not clear.

Recently it has been suggested that OLAB may bind to ox-LDL, leading to the formation of immune complexes which are captured, at the enhanced rate, by Fc receptors on macrophages by scavenger pathway (23) and destroyed in the oxidative fire of lysosomes. Then we can hypothesize that the physiological function of OLAB is to participate in the removal of oxido-nitrogen compounds from the artery wall (9). On the other hand, several studies in not TM patients have demonstrated an association between the degree of atherosclerosis and OLAB (24, 25).

Recently the possible role of OLAB in the remotion of ox-LDL have been suggested by evidences showing that in vitro these antibodies enhance the uptake of ox-LDL by human macrophages through the Fc gamma receptor (23). In fact in vivo immunization with ox-LDL has been shown to protect from atherosclerosis ApoE deficient mice (26). At the light of these observations, the elevated levels of OLAB and the presence of immune complexes do not necessarily imply the appearance of a vascular damage, since they are commonly removed by macrophage and RES histiocyte cells (27).

In our TM patients three considerations may suggest a protective role of OLAB.

1) A study on the causes of death (7) and the pathology reports of TM patients (8), indicate only in 4% of patients the existence of thromboembolic events, which were localized in the venous districts, typical of liver abnormalities such as alteration of haemostatic balance and reduced antithrombotic proteins (ATIII, Protein C) (28).

2) The observation of altered lipoprotein profile in TM patients (29, 30) and of reduced levels of T-chol and LDL-C in patients with β-TM trait, due to increased uptake of LDL by macrophage and RES histiocyte cells, support the hypothesis that β-TM trait may have lesser risk of myocardial infarction (18-31, 32).

3) Recent evidences suggest that high levels of cholesterol have an important role in the immunoreactivity, since it has been shown that severe hypercholesterolemia induced a switch from Th1 to Th2 type reactivity, while moderate cholesterol levels were associate with Th1 type reactivity (33). These changes in the functional profile of immune response, modulating the activity of macrophage and RES histiocyte cells, may reduce the cardiovascular risk in TM patients.

At the end it was surprising to observe that the correlation between OLAB and triglycerides was different in TM patients in comparison with healthy controls. In TM patients there was a positive correlation (p<0.001), while in the healthy controls any correlation was found. We think that in TM patients since iron overload depletes the blood of antioxidant substances and HDL-C is lower than normal, then the LDL composition prevalently influenced by the trygliceride levels, became determinant in the oxidability of LDL. In this case the correlation between OLAB and triglycerides results significantly positive. On the contrary in healthy controls, where the oxidant effect of iron is not determinant, LDL composition and then tryglicerides are less important, because blood antioxidant substances such as HDL play better its role. Indeed in healthy controls the correlation between the OLAB and
triglycerides is not significant, confirming that in TM patients the iron load and LDL composition are determinant in the pathogenesis of LDL oxidation. In conclusion our data in TM patients show that low cholesterol levels are not sufficient condition to protect LDL by oxidation, where the triglycerides level seem determinant for the LDL composition and for their oxidation. Then it is possible that OLAB could affect atherosclerosis by preventing foam cell formation, through the enhanced clearance of ox-LDL from plasma, and reducing the deposition of ox-LDL in the endothelial cells of artery wall. This new role of OLAB in regulating ox-LDL uptake by macrophages could provide with new knowledge on atherosclerosis mechanism and may explain the slower progression of atherosclerosis in TM patients.

Acknowledgments: The authors thank the head of the Department of Chemistry of Catania University and particularly Rosanna Chillemi for her precious suggestions in the preparation of manuscript. The authors also thank all who helped and collaborated for the success of this work.

References

(10) Salomon RN, Underwood R, Doyle MV, Wong A, and Libby P: Increased apolipoprotein E and c-fms gene expression without elevated interleukin 1 or 6 mRNA levels indicates selective activation of macrophage functions in advanced human atheroma. Proc Natl Acad Sci USA, 89: 2814-2881, 1992
(20) De Graff J, Hek-Lemmers HLM, Hectors M, Denmarker PNM, Hendriks JCM, and Stalenhoef