AUTOANTIBODIES AGAINST MODIFIED LOW DENSITY LIPOPROTEIN IN PATIENTS WITH CORONARY HEART DISEASE AND NORMAL INDIVIDUALS

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Abstract - Different classes of autoantibodies against two antigenic forms of modified low density lipoprotein (mod-LDL) were detected in 140 patients by indirect ELISA method. Investigated autoantibodies included total immunoglobulins (IgT), immunoglobulin G (IgG), and immunoglobulin M (IgM) against oxidized LDL with copper ions (ox-LDL) and modified LDL with malondialdehyde (mal-LDL) (IgT-O), IgT against mal-LDL (IgT-M), IgG against ox-LDL (IgG-O), IgM against ox-LDL (IgM-O), and IgM against mal-LDL (IgM-M) were significantly ($p < 0.05$) higher in patients with coronary atherosclerosis than normal individuals, whereas titer of IgG against mal-LDL (IgG-M) didn't show any significan difference between these groups. In this study, no correlation was found between autoantibody titers and severity of coronary artery stenosis. The results indicate that titers of these autoantibodies are dependent on an active atherogenic process.

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Key Words: Atherosclerosis, autoantibodies, LDL modification, mod-LDL, ox-LDL, mal-LDL

INTRODUCTION

Atherosclerosis is the principal cause of death (1), although its pathogenesis is not fully understood. This disease is a multifactorial process in which different factors are involved. Recently, there has been considerable interest in the contribution of oxidative processes to the development of atherosclerosis (1-6). It is belived that oxidation of low density lipoprotein (LDL) has an important role in the pathogenesis of atherosclerosis. Folowing LDL oxidation, it is converted to oxidized-LDL (ox-LDL) which has important atherogenic properties, such as increased macrophage uptake and foam cell formation, chemotactic effect for macrophages, inhibition of tissue macrophage mobility, endothelium injuries and immunogenicity (1,4,6).

One of the most important changes during LDL oxidation is derivation of side ε- amino of lysine residue in apo B100 with malondialdehye and production of mlondialdehyde-LDL (mal-LDL). Malondialdeyde is a highly reactive dialdehyde. It is formed as a result of nonenzymatic lipid peroxidation of long-chain polyunsaturated fatty acids (7). It is also known to result from lipid peroxidation that occurs during phagocytosis by monocytes and is produced from normal arachidonic acid metabolism, for example during thromboxan metabolism, for example during thromboxan synthesis by platelets (7,8).

Ox-LDL and mal-LDL are immunogens and can stimulate autoantibodies production. These autoantibodies may have protective and physiologic function by inhanced uptake of modified LDL (mod-LDL) from circulation or may have pathologic role by formation immune complexes and induce endothelium injuries (9-11). Thus autoantibodies against mod-LDL have a critical role in atherogenic mechanisms. In order to assess this role, we detected these autoantibodies in patients with coronary artery stenosis and normal individuals.

MATERIALS AND METHODS

1) Sample

This study was carried on patients who had come to Shariati hospital for angiography and coronary artery bypass graft (CABG). Before angiography and CABG, 10 ml of venous blood was collected. After 2h, serum was seperated and stored at -20°C.

Angiography was carried on by cardiologist. In this study eight segment (Table 1) of coronary artery were evaluated for stenosis and amount of stenosis was assessed according to severity of stenosis and extent of stenosis. The results were expressed by coronary atherosclerosis score (CAS), adapted from Jenkins and co-workers (12).

2) Antigen Preparation

Antigen preparation for detection of autoantibodies against mod-LDL has been explained previously (1).

First, native-LDL (n-LDL) was isolated by sequential flotation ultracentrifugation (SFU) in density gravity between 1.020 to 1.055 g/ml, and in the presence of ethylenediaminetetraacetic acid (EDTA), phenylmethlsolfonylgloride (PMSF), butylated hydroxytoluene (BHT), sodium azid, chloramphenicol and gentamycin (1,7). Then n-LDL was converted to ox-LDL and mal-LDL in the presence of Cu^{2+} and malondialdehyde, respectively (13). Antigen quality was evaluated by protein electrophoresis in acetate cellulose, pH 8.6, by determination of optical density at 234 nm, and by measuring Thiobarbitoric acid Reactive Substances (TBRAS) (1). In addition, concentration of antigens was determined by LDL protein measuring with Lowry method (1,113,14).

3) ELISA Method

Autoantibodies against mod-LDL were detected by an indirect ELISA method (13,14).

Three antigen preparations, including n-LDL, ox-LDL, and mal-LDL, in phosphate buffered saline (PBS, with 10 mM phosphate and pH 7.2), were added to seperate wells of Costar 96-well microplates (10 μg in 100 μl) (13). After incubation for 20 h at 4°C, the antigen solutions were emptied and plates were washed three times with PBS buffer containing 0.5 ml/l tween 20 and 1 g/l bovin serum albumin (BSA). Wells were blocked by adding 200 μl of 1 g/l BSA per well and incubation at 37°C for 24 (14). After incubation, plates were emptied and washed as above. Subsequently, 100 μ/l of patient serum in duplicate was added at a dilution of 1:50 in 10g/l BSA. The plates were incubated at 37°C for 2H. After washing as above, 100μl of horseradish peroxidase (HRP) - conjugated antihumanimmunoglobulins (DAKO Ltd.), including antibodies against (total immunoglobulins (IgT), immunoglobulin G (IgG), and immunoglobulin M (IgM), diluted and 1:500, respectively, in 10 g/l BSA, was added to seperate wells.

Table 1. Coronary arteries which were studied by angiography

1) MLCA: Main left coronary artery.
2) LAD : Left anterior descending artery up to junction of middle and distal third of vessel.
3) S1: The proximal third of major septal branch of LAD
4) D1: The proximal third of major diagonal branch of LAD
5) CFX : Cicumflex coronary artery up to junction of middle and distal third of vessel.
6) OM1 : Proximal third of the major obtuse marginal branch of the CFX
7) RCA: Right coronary artery up and including the origin of PDA
8) PDA: Proximal third of the posterior descending coronary artery.

The plates were left for 1h at 37°C and again washing was performed four times as above. After last washing, colour was developed by incubating the plates 20 min at room temperature with 100 μl/well freshely prepared HRP substate, including four 2 mg O-phenylenediamine dihydrochloride (OPD, DAKO Ltd.), 50 μl of 30% hydrogen peroxide in 12 ml distilled water (14). The reaction was stopped with 0.5 M sulfuric acid, 100 μl/well (13). Finally optical density (OD) was measured by ELISA reader at 492 nm (13). Concentration results of autoantibodies against ox-LDL and mal-LDL were expressed as $OD_{ox\text{-}LDL}$-$OD_{n\text{-}LDL}$ and $OD_{mal\text{-}LDL}/OD_{ir\text{-}LDL}$, respectively.

4) Statistical Methods

Data analysis was performed with SPSS for windows 7.5 software. In this study, we used t-test, Mann-Whitney, Pearson coefficient, and Spearman coefficient. Quantitative results were expressed as mean ± SD and qualitative results as number or percent.

RESULTS

1) Subjects' Groups

The 140 subjects in the study were categorized according to clinical characteristics (table 2). Group 1 consisted of 32 subjects undergoing coronary angiography but with no evidence of atherosclerotic diseases; group 2 consisted of 79 patients undergoing coronary angiography and with evidence of anatomic coronary disease; and group 3 consisted of 29 patients whom had undergone CABG because of coronary artery stenosis.

2) Autoantibody titers

Autoantibody titers against mod-LDL are shown in table 3. Significant differences ($p<0.05$) were obtained between total immunoglobulin against ox-LDL (IgT-O), total immunoglobulin against mal-LDL (IgT-M), immunolobulin G against ox-LDL (IgG-O), immunolobulin M against ox-LDL (IgM-O), and immunolobulin M against mal-LDL (IgM-M) titers in group 1 and the same titers in groups 2 and 3. These results indicate that titers of autoantibodies against mod-LDL, except immunoglobulin G against mal-LDL or IgG M, are higher in patients with coronary artery stenosis(groups 2 and 3) than normal subjects (group 1).

3) Correlation

It was shown that there are significantly higher autoantibody titers against mod-LDL in patients with coronary artery disease. In order to evaluate the relation between these titers and severity of coronary stenosis (CAS), correlation coefficient between these titers in group 2 and their CAS was analysed. As indicated in table 4, no correlation was obtained between autoantibodies against mod-LDL and CAS.

Table 2. Patients groups

Groups	Subjects	n	%	age (year)
1	with normal angiograms	32	22.9	52.2 ± 11.6
2	with abnormal angiogrms	79	56.4	56.1 ± 9.4
3	undergoing CABG	29	20.7	55.0 ± 8.4

Table 3. Autoantibodies titers in different groups

Groups	IgT-O	IgT-M	IgG-O	IgG-M	IgM-O	IgM-M
1	1.04 ± 0.20	329 ± 110	1.08 ± 0.28	462 ± 240	1.08 ± 0.24	612 ± 234
2	1.17 ± .025*	411 ± 117*	1.21 ± 0.33*	492 ± 206	1.24 ± 0.37*	908 ± 260*
3	1.18 ± 0.25*	443 ± 119*	1.21 ± 0.30*	476 ± 188	1.33 ± 0.52*	974 ± 481*

* having significant difference ($p < 0.05$) vs group 1.

Table 4. Correlations between autoantibody titers and CAS in group 2

Autoantibodies	r	p
IgT-O	0.026	0.818
IgT-M	0.043	0.704
IgG-O	0.140	0.220
IgG-M	0.086	0.451
IgM-O	0.070	0.486
IgM-M	0.005	0.963

DISCUSSION

Results show that autoantibodies titers against mod-LDL are higher in patients with coronary artery disease than normal individuals. This is significant for IgT-O, IgT-M, IgG-O, IgM-O, and IgM-M, but not significant for IgG M. In addition, no significant difference was obtained between these titers and severity of coronary artery disease. This result is compatible with that of results of some groups (14-17), but is incompatible with others (18). Bui and co-workers (14) reported significant higher IgG-O in patients' serum with coronary artery disease than normal individuals. Maggi and co-workers (15) showed that titers of IgG-O, IgG-M, IgM-O and IgM-M were significantly higher in patients with severe carotid atherosclerosis than the compared group. Bergmark and co-workers (16) also reported significantly higher titers of IgT-O in patients with early - onset peripheral vascular disease than control group. However, Lucy and co-workers (17) found no significant difference in IgG M titers between patients with atherosclerosis and control group. The same results were obtained by Virella and co-workers (18) for IgG-O titers on normal and abnormal patients.

Our results about correlation between autoantibodies against mod-LDL and severity of coronary artery disease are also comparable with other studies (16-19). Maggi and co-workers (19) reported no significant correlation between IgG-O, IgG-M, IgM-O, and IgM-M titers and scores of clinical evidence of severity and extent of atherosclerotic lesions.

Existence or no difference between mod-LDL and severity of coronary artery disease and obtaining different results during different studies is not too surprising. As we know, one of the main factors that affects antibody titers in the body is immune system stimulation. Atherosclerosis is a vast spectrum of one disease which generally begins in childhood, progresses during early adulthood and finally presents the clinical complications during middle to late adulthood. Therefore, it is expected that active stages of atherogenesis and developing lesions, which are accompained with higher production of mod-LDL and immune system stimulation, occur before clinical presentation. In other words, at the testing time, atherosclerotic lesions are probably relatively stable and inactive, and also LDL oxidation and immune system stimulation have been decreased. As a result, patients might have had higher titers in the past, but their titers have been decreased by stabilization of atherosclerotic lesions. We conclude that patients with more severe stenosis are probably in the partially inactive stage of the disease, whereas patients having less severe lesion are at the early stage and have active lesions (17).

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