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Antibodies Against Oxidized Low Density Lipoproteins in Pregnant Women

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Summary

Oxidized low density lipoproteins (oxLDL) formed *in vivo* induce a humoral immune response. Oxidative modification of LDL renders it immunogenic and a heterogeneous population of specific anti-oxLDL antibodies is produced. These antibodies could represent a biological marker of oxidative stress and serve as markers of atherosclerosis. Autoantibodies against oxLDL (oLAb) have been detected in human subjects practically of every age. oLAb also appear in the blood of pregnant women. Some studies have shown that the levels of antibodies to oxLDL were elevated in women with established preeclampsia. The present study was aimed to estimate the oLAb IgG levels in the first and second trimester of pregnancy. Furthermore, we estimated the correlation between maternal serum (MS) levels of oLAb and alpha-1-fetoprotein (MS AFP), human chorionic gonadotrophin (MS HCG) and trophoblast-specific-beta-1-glycoprotein (MS SP1), because these proteins are determined as a part of prenatal biochemical screening for fetal congenital abnormalities. Our study deals with the oLAb changes in women with pregnancy-induced hypertension. We also investigated the correlation between oLAb IgG and anticardiolipin antibodies IgG (ACA) in the serum of pregnant women. We examined 40 pregnant women attending Institute for Mother and Child Care for their antenatal care as outpatients. Routine blood samplings between the 9-13th week of pregnancy and 16-18th week of pregnancy were performed as a part of biochemical prenatal screening for fetal congenital abnormalities (Group 1). Their mean age was 27±4.1 years. Furthermore, we examined 26 women in the second or third trimester with pregnancy-induced hypertension (Group 2). Group 2 was compared with 49 pregnant women in the second or third trimester who were normotensive (Group 3). We used commercial standardized ELISA kits for determination of oLAb IgG, ACA IgG, MS AFP and MS HCG, MS SP1 was analyzed by single radial immunodiffusion. We did not find any differences in the levels of oLAb IgG in the first and second trimester in the women of Group 1. The correlation between oLAb and ACA IgG was not statistically significant (Spearman coefficient $r=0.22$, $p=0.1$). The correlation between oLAb IgG with MS AFP, MS HCG and MS SP1 was not statistically significant. Weak negative correlation for AFP and HCG was suggested both in the first and in the second trimester. The levels of oLAb IgG in the group of women with pregnancy-induced hypertension were significantly lower than in the group of normotensive women (348±388 U/ml v.s. 579±400 mU/ml, $p<0.01$). We can conclude that the levels of oLAb do not differ in the first and second trimester of gravidity. However, we cannot exclude the possible influence of an inverse relationship between oLAb IgG titers and the synthesis of fetoplacental antigens. This finding is important especially in the context of the results of prenatal biochemical screening. Pregnancy-induced hypertension is associated with lower levels of oLAb. Weak cross-reactivity between oLAb and anticardiolipin antibodies may exist but there is a possibility that there are two different populations of antibodies reacting with various antigens.

Key words
Antibodies against oxidized low density lipoproteins • Pregnancy • Alpha-1-fetoprotein • Human chorionic gonadotrophin • Trophoblast-specific-beta-1-glycoprotein

Introduction

Oxidative stress develops in several pathological states and increased lipid peroxidation may be important in the etiology of many diseases. Low density lipoproteins (LDL) are one of the targets for lipid peroxidation which become oxidized as a consequence. LDL are particles, which are very susceptible to free radical oxidation. Oxidized LDL (oxLDL) are responsible for various effects. They may induce functional changes of endothelial cells by stimulating the expression of cell adhesion molecules, which have cytotoxic effects on cultures of vascular endothelial cells *in vitro*, and are chemoattractant for monocyte and induce macrophage secretion of proinflammatory mediators (Tatzber and Esterbauer 1995, Kaplan and Aviram 1999, Štípek 2000, Žák 2000).

Oxidized LDL are immunogenic and can induce an autoimmune response leading to the formation of autoantibodies (Palinski *et al.* 1989, Steinerová *et al.* 2001). Autoantibodies against oxLDL (oLAb) have been detected in human subjects practically of all ages. They are mostly IgG 1 and 3 subclasses and may induce the formation of immune complexes between modified LDL and autoantibodies with inflammatory properties (Lopes-Virella *et al.* 1991, Mironova *et al.* 1996). oLAb have been proposed as a biological marker of oxidative stress and as a marker of atherosclerosis, such as oxidation of LDL, endothelial dysfunction and arterial inflammation (Salonen *et al.* 1992, Vaarala 2000).

oLAb also appear in the blood of pregnant women. It has been shown that the levels of antibodies to oxLDL are elevated in women with established pre-eclampsia and in pregnant women with a history of repeated abortion (Branch *et al.* 1994, Tulppala *et al.* 1995). There is also evidence that certain antiphospholipid antibodies (APA) crossreact with some common epitopes in oxidized lipoproteins (Vaarala *et al.* 1993). Antibodies to cardiolipin, which are usually examined, have been linked to adverse pregnancy events. Women with moderate-to-high levels of anticardiolipin antibodies had a significantly increased incidence of pregnancy loss (Brown 1991).

To our knowledge no study has investigated the changes of oLAb IgG during pregnancy and the relationship between levels of oLAb and fetoplacental antigens. The present study was therefore aimed to estimate the oLAb IgG levels in the 1st and 2nd trimester of pregnancy. Furthermore, we estimated the correlation between maternal serum levels of oLAb and alpha-1-fetoprotein (MS AFP), human chorionic gonadotrophin (MS HCG) and trophoblast-specific-beta 1-glycoprotein (MS SP1).

This is very important since these proteins are determined as part of prenatal biochemical screening for fetal congenital abnormalities (Fialová *et al.* 1993, Malbohan *et al.* 1994). If a certain association between serum levels of AFP, HCG, SP1 and oLAb does exist, it should be taken into account in the interpretation of prenatal biochemical screening. One part of our study deals with the oLAb changes in women with pregnancy-induced hypertension. We also investigated the correlation between oLAb IgG and anticardiolipin antibodies IgG (ACA) in the serum of pregnant women.

The aim of this study was to extend our knowledge about APA as a continuation of our previous report concerning the other antiphospholipid antibodies in pregnant women in which we found that 7.8 % and 9.8 % of pregnant women had a higher titer of APA IgG and IgM, respectively, and that one third of ACA IgG or IgM positive sera contained polyspecific autoantibodies reactive to at least two various phospholipids (Fialová *et al.* 2000).

Methods

Patients

We examined 40 pregnant women followed at the Institute for Mother and Child Care for their antenatal care. Routine blood sampling between 9-13th week of pregnancy and 16-18th week of pregnancy was performed as part of biochemical prenatal screening for fetal congenital abnormalities (Group 1). Their mean age was 27±4.1 years. Furthermore, we examined 26 patients in the 2nd or 3rd trimester with pregnancy-induced hypertension. Hypertension was defined as systolic blood pressure greater than 140 mm Hg and/or diastolic blood pressure greater than or equal to 90 mm Hg (Group 2). Group 2 was compared with 49 pregnant women in the 2nd or 3rd trimester who were normotensive (Group 3).

Venous blood was withdrawn in the morning after an overnight fast. Tubes with blood were

centrifuged at 2000 rpm for 15 min to separate the serum. Serum was stored at –20 °C until it could be assayed for oLAb IgG and ACA IgG levels. MS AFP, HCG and SP1 were determined in the fresh serum.

Determination of antibodies against oxidized low density lipoproteins IgG

For determination of oLAb we used commercial kit oLAb-ELISA (Biomedica, Wien). The microtiter strips are coated by Cu^{2+} oxidized LDL as the antigen. Prediluted serum samples were incubated 90 min at 37 °C. After washing, the anti-human IgG antibody conjugated with peroxidase was added into each well and incubated for 30 min at room temperature. The wells were washed, the substrate was added and the microplate was incubated for 15 min at room temperature. The enzymatic reaction was stopped by addition of sulfuric acid. The levels of IgG oLAb higher than 800 mU/ml were considered as positive.

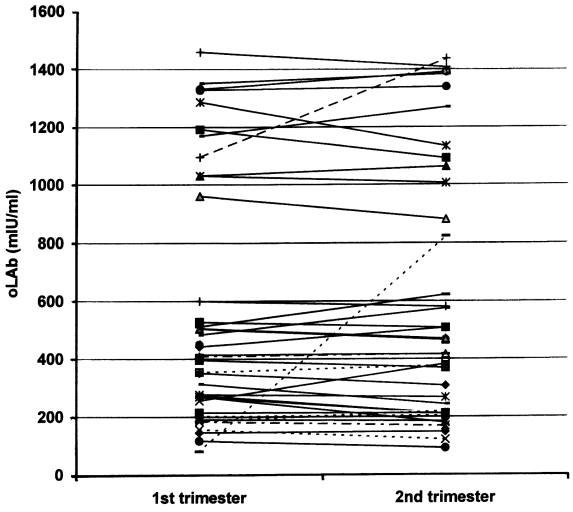

Fig. 1. *Individual maternal serum levels of oLAb IgG in pregnant women. The lines join the values of oLAb IgG in the 1st and 2nd trimester in different women.*

Determination of other antibodies

Anticardiolipin antibodies IgG were examined by standardized ELISA utilizing cardiolipin as the antigen (ORGenTec, Germany). The results were expressed in international units of IgG anticardiolipin antibodies GPL and were interpreted as positive if the levels exceeded 19 GPL.

Maternal serum levels of AFP and HCG were assayed by ELISA kits (Sevapharma a.s., CR). MS SP1 were analyzed by single radial immunodiffusion using Q-antiserum (SwAHu SP1, Sevapharma, a.s. CR). The results were expressed as multiples of the median (MoM) for each week of pregnancy.

Statistical methods

The interdependence of oLAb IgG serum levels and IgG ACA and MS AFP, MS HCG and MS SP1 was assessed by calculating the Spearman correlation coefficients. The changes of oLAb levels in the 1st and 2nd trimester were calculated using the paired nonparametric Wilcoxon test. The difference between oLAb IgG titer in women with hypertension during pregnancy and normotensive women was calculated by the unpaired t-test.

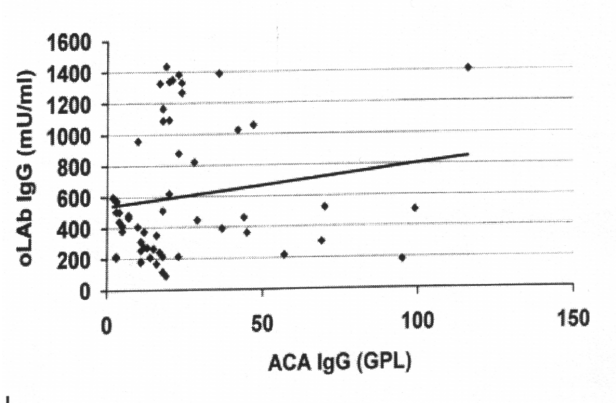

Fig. 2. *Correlation between oLAb IgG and ACA IgG. Spearman coefficient r=0.22, p=0.1.*

Results

We did not find any differences in the levels of oLAb in the 1st and 2nd trimester in women of Group 1. Their levels were 561.5±424 mU/ml in the 1st trimester and 581±434 mU/ml in the 2nd trimester (Fig. 1). The correlation between oLAb and ACA was not statistically significant (Spearman´s coefficient r=0.22, p=0.1, see Fig. 2). Some positive samples for oLAb and negative for ACA and *vice versa* were encountered. In the 1st trimester, 19.2 % and in the 2nd trimester 16.1 % of autoantibodies only react with one antigen were detected (Table 1). Table 2 shows the correlation coefficients of oLAb IgG with MS AFP, MS HCG and MS SP1. For AFP and HCG a weak negative correlation not statistically significant was suggested both in the 1st and in the 2nd trimester.

Table 1. Comparison of oLAb IgG and ACA IgG in the 1st trimester and the 2nd trimester

	1st trimester (n = 26)	**2nd trimester** (n = 31)
oLAb + and ACA +	15.4 %	25.8 %
oLAb + and ACA -	7.7 %	0 %
oLAb - and ACA +	11.5 %	16.1 %
oLAb - and ACA -	65.4 %	58.1 %

The levels of oLAb IgG in group 2 (women with pregnancy-induced hypertension) were significantly lower than in group 3 of normotensive women (348±388 mU/ml v.s. 579±400 mU/ml, $p<0.01$). oLAb IgG showed a wide dispersion within the group and there was a considerable overlap of oLAb titer values between both groups.

Discussion

The importance of oxidative stress in pregnant women is beginning to be studied to an increasing extent. Lipid peroxides are important because their uncontrolled production may result in oxidative stress with significant damage to cell integrity. There is general agreement that the level of lipid peroxides in the blood is generally higher in pregnant women in comparison with nonpregnant women (Morris *et al.* 1998). The high levels seen even in physiological pregnancies have made new baseline studies a priority. Lipid peroxides can be produced in the placenta, but the pattern of their changes during pregnancy is not clear. The elevation of lipid peroxides appear by the second trimester and may further increase in gestation and subsequently decrease after delivery (Little and Gladen 1999).

Oxidized LDL formed *in vivo* induces a humoral immune response. Oxidative modification of LDL renders it immunogenic and a heterogeneous population of specific oLAb is produced. Autoantibodies binding to various epitopes of oxLDL have been described in man, rabbits and mice (Palinski *et al.* 1989, 1990, Vaarala 2000, Steinerová *et al.* 2001). Besides being considered as markers of accelerated atherosclerosis antibodies to oxidized LDL, they represent an independent marker of impaired endothelium-dependent and endothelium-independent vasodilatation. This was recently detected in the forearm vasculature in a series of patients with coronary heart disease (Sinisalo *et al.* 2000).

Table 2. Spearman correlation coefficients between oLAb IgG titer and levels of fetoplacental antigens

Correlation oLAb IgG (mU/ml) with	**1st trimester**		**2nd trimester**	
	r	**p**	**r**	**p**
AFP (MoM)	–0.13	0.40	–0.09	0.59
HCG (MoM)	–0.26	0.10	–0.07	0.68
SP1 (MoM)	0.08	0.62	0.11	0.49

The occurrence of antibodies to oxidized LDL was described in systemic lupus erythematosus (SLE) and the antiphospholipid syndrome (Vaarala *et al.* 1996, Amengual *et al.* 1997). It may be associated with enhanced oxidative stress in these autoimmune conditions. The production of autoantibodies to these proteins may thus reflect a focal inflammatory reaction, and the autoantibodies serve as markers for thrombotic risk due to their association with vascular inflammatory changes. Some anticardiolipin antibodies bind exclusively to the peroxidized molecule, indicating that these antibodies recognize neo-epitopes derived from phospholipid oxidation (Hörkkö *et al.* 1996). A subpopulation of oLAb to oxidized lipids in the LDL molecule is likely responsible for cross-reactivity with phospholipids such as cardiolipin (Vaarala *et al.* 1993). Another subpopulation of antibodies to oxidized LDL recognize oxidized apolipoprotein B of LDL which is modified during oxidation. We detected only weak positive correlation between oLAb IgG and ACA IgG in our pregnant women. Similar weak correlation was found by Amenguel *et al.* (1997) in patients with the antiphospholipid syndrome.

In our study, we followed the course of oLAb levels in pregnancy. We did not find any significant changes in the levels of oLAb in the 1st and 2nd

trimester. This is possible because the increase of lipid peroxides did not appear until the 2nd trimester.

Furthermore, we became interested in the interrelationship between the titers of oLAb IgG and the levels of fetoplacental antigens commonly used in prenatal screening of chromosomal abnormalities. A weak negative correlation between maternal serum levels of fetoplacental antigens and oLAb IgG was found. HCG and AFP are known to possess immunosuppressive effects (Adcock *et al.* 1973, Murgita 1976). As an immunoregulatory protein placental HCG and fetal AFP are involved throughout gestation in pregnancy-associated immunosuppression. It is possible that their high levels during pregnancy is one of the immunosuppressive factors contributing to the decrease of oLAb levels. On the contrary, low levels of HCG and AFP may result in the elevation of oLAb or may reflect the oxidative stress in pregnancy resulting in a decrease of synthesis of HCG and AFP in association with increased production of oLAb. Because the increased levels of MS HCG and decreased levels of MS AFP are associated with increased risk of Down´s syndrome in the fetus (Malbohan *et al.* 1994), the high levels of oLAb may indirectly lead to an incorrect interpretation of the results of prenatal biochemical screening for chromosomal abnormalities.

The data available about oLAb in the preeclamsia are controversial. Lipid hydroperoxides and malondialdehyde were significantly raised in pre-eclampsia compared with nonpregnant women. Some studies reported high levels of oLAb, but in others normal titers were found (Branch *et al.* 1994, Kurki *et al.* 1996, Uotila *et al.* 1998). Increased lipid peroxidation in preeclamsia apparently plays a role in its etiology. There is evidence that oxidative stress occurs in preeclampsia. Oxidative stress may indicate which fetoplacental and maternal factors converge, resulting in the protean manifestations of preeclamsia (Hubel 1998).

Preeclampsia is currently considered a two stage disorder: reduced placental perfusion usually secondary to abnormal implantation and a consequent maternal disorder characterized by endothelial dysfunction. But reduced perfusion and abnormal implantation occur in other conditions without the maternal syndrome. This may be explained by the fact that reduced placental perfusion must interact with maternal constitutional factors to develop preeclampsia. The risk factors and metabolic alterations are similar in preeclampsia and atherosclerosis and this might suggest a common pathophysiology. Roberts (2000) proposes that oxidative stress secondary to reduced placental perfusion leads to endothelial dysfunction, linking the two stages of the syndrome.

In our study, lower levels of oLAb were detected in women with hypertension in pregnancy. We found similar results in patients with hyperlipoproteinemia and hypertension, who had lower titers of oLAb (Zeman *et al.* 2000). Wu *et al.* (1999) also observed significantly lower oLAb levels IgG and IgM in patients with borderline hypertension. The possible explanation of this finding may be based on a decreased immune reaction to oxLDL or on an increased consumption of oLAb due to binding to early atherosclerotic lesions.

We also must take into account that the antibody titer represents a balance between antibody formation and "consumption", and that conditions affecting the function of the immune system are also likely to influence the titer. It has been reported that oLAb exist both in free and antigen-antibody complexes. Measuring the binding of antibodies to plated antigens may be misleading, because some of the antibodies may be bound to circulating antigens, e.g. oxidation-specific epitopes present in circulating LDL. It is even possible that increased formation of immune complexes due to acute increases in antigen lead to apparent decreases in antibody titers so that the presence of immunocomplexes may interfere with the assay of oLAb. The levels of immunocomplexes may correlate better with the course of disease than the levels of free oLAb (Festa *et al.* 1998, Lopes-Virella *et al.* 1999, Palinski and Witztum 2000).

We can conclude that the levels of oLAb do not differ in the 1st and 2nd trimester of gravidity. We cannot exclude the influence of an interrelationship between oLAb IgG titers and the synthesis of fetoplacental antigens in an inverse manner. This finding is important especially in connection with the interpretation of results of prenatal biochemical screening. Pregnancy-induced hypertension is associated with lower levels of oLAb and the oLAb examination should therefore be considered especially in this group of pregnant women. Weak cross-reactivity may exist between oLAb and anticardiolipin antibodies, but it is possible that two different populations of antibodies may react with non-identical antigens.

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