

**AUTO ANTIBODIES AGAINST OXIDIZED LOW DENSITY LIPOPROTEINS
AND LIPID PEROXIDATION IN PATIENTS WITH ESSENTIAL
HYPERTENSION**

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ABSTRACT: Lipid peroxidation has been suggested to play a key role in the oxidative modification of LDL (oxd-LDL) which stimulate the production of auto antibodies by B-cells and anti-oxd LDL antibodies are produced. These antibodies could represent a biological marker of oxidative stress and serve as markers of atherosclerosis. Essential hypertension is a major risk factor for atherosclerosis, recently border line hypertension also has been shown to be a risk factor for atherosclerosis. The aim of this study is to examine the correlation between oxd -LDL antibodies and lipid peroxidation in patient with essential hypertension. Blood samples were collected from patients with essential hypertension (n=155) and healthy individuals (n=160) levels of Malonaldehyde (MDA), Total cholesterol, Triglycerides, HDL and LDL were estimated by spectrophotometry and levels of Oxd- LDL antibodies were obtained by ELISA. Plasma levels of MDA, anti-oxdLDL antibodies, Total cholesterol and LDL Cholesterol is higher in patients than those in controls. Among patients concentration of MDA, total cholesterol and LDL cholesterol were not significantly different, however the concentration of anti-oxd LDL were higher in essential hypertensive patients (p=0.003). Significant positive correlation was observed between plasma levels of MDA, total cholesterol, LDL cholesterol and the concentration of anti-oxdLDL in patients but not in the controls. In conclusion High concentrations of anti-oxdLDL and MDA suggest an increase in oxidative stress that would contribute to the development of atherosclerosis. The observed correlation of MDA with anti-oxdLDL indicates the relationship between free radicals and atherosclerosis in essential hypertension.

Key words: Oxidized LDL auto antibodies, Malondialdehyde, atherosclerosis, essential hypertension

INTRODUCTION

Essential hypertension is a known risk factor for cardiovascular diseases (Iakka et al 1999). Hypothesis that associates hypertension and the underlying sub clinical hypothesis has been investigated (Ross R, 1933, and Raitakari OT, 1999). Studies shows the fact that atherosclerosis is not merely the result of disturbed lipid pattern and lipid deposition in arterial intima, but several other factors contribute to the process of atherosclerosis were immunological factors, atherosclerotic events, chronic low grade inflammation as well as oxidation stress play an important role (Adams MR et al, 2000). Regarding immune system role in atherogenic process, the concept that oxidized low density lipoprotein (ox LDL) may be a key antigen in this process has been demonstrated and supported (Hansson GK, 2001).

Oxidatively modified LDL is more atherogenic than compare to native form, as it stimulates LDL uptake by macrophage scavenger receptors, promoting the transformation of macrophages into foam cells and may the recruitment and migration of monocytes and leucocytes within arterial vessel (Mertens A, 2001 and Hansson GK, 2001). Oxidized LDL recognized as an immunogenic agent in the development of atherosclerosis (Stemme S, 1995). Several recent studies support the concept that oxidative stress enhance free radical formation and create oxidative neo-epitopes on apoB-100 moiety of LDL and its phospholipids by binding to malondialdehyde, terms immunogenic agents that may trigger the formation of auto antibodies (Salonen JT et al, 1992, Steinerova A et al, 2001 and Virella G, 2003). Autoantibodies of ox LDL (oLAB) represent a marker of progression of atherosclerosis (Salonen JT et al, 1992 and Steinerova A et al, 2001).

Oxidative stress play key role in the initiation and progression of cardiovascular dysfunction associated with diabetes mellitus, hyperlipidemia, ischemic heart disease and heart failure (Taniyama Y, 2003). Essential hypertension is associated with enhanced oxidative stress (Kumar KV, 1993).

The present study aimed to find the correlation of serum oxidized low density lipoprotein auto antibodies (oLAB) and lipid per oxidation product malondialdehyde (MDA) as possible risk factor role in patient with essential hypertension.

MATERIALS AND METHOD

Three hundred and fifteen subjects were randomly selected from the out patient clinics of the Narayna Medical college and super specialty hospital, Nellore, Andhra Pradesh, India. The subjects of this study divided into two groups, group-I that consisted of fifty five patients with essential hypertension and group-II that consist of sixty normal healthy volunteers of comparable age, sex, and socioeconomic status to the patients group. Patients suffering from infectious, inflammatory diseases, diabetes mellitus, systemic lupus erythematosus, pre eclasyia hyperthyroidism, acute myocardial infarction, cerebrovascular stroke, liver and renal diseases were excluded from the study.

Clinical examination

The patients and healthy subjects were subjected to measurement of blood pressure, complete physical examination, calculating body mass index and detailed history obtained regarding drug intake, smoking and vitamin therapy. All the patients involved in the study were on ant hypertensive medication (β -blockers and calcium channel blockers).

Blood sample collection

5ml of venous fasting blood (10-12 hours of last meal) were collected fro each individual. Portion of the blood was taken on EDTA, and the resulting plasma was stored for MDA determination the rest was left to obtain serum.

Biochemical analysis

Routine biochemical parameters included the determination of serum concentration of glucose (both fasting and post prandial glucose levels), urea, creatinine, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides and liver specific enzyme alanine amino transferase (ALT), and aspartate amino transferase (AST) activities were measured. Analyses were conducted on the humaster 300 (GmbH) autoanalyser.

Estimation of Plasma Lipid Peroxidation

Lipid per oxidation in plasma was estimated calorimetrically by measuring malondialdehyde by the method of (albro et al, 1986 and das et al, 1990).In brief 0.1ml of plasma was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCL reagents (TBA 0.37%, 0.25N HCL and 15% TCA) and placed in water bath for 15min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank. Results were deduced from a standard curve of serially diluted 1,1,3,3 tetra hydroxyl propane standard simultaneously treated as the sample.

Estimation of Serum Anti-Oxidized LDL Antibodies (oLAB)

Enzyme linked immunosorbant assay (ELISA) technique was used in the determination of serum concentration of oxidized low density lipoprotein auto antibodies (oLAB) (human GmbH Germany)

Statistical Analysis

Statistical analysis was done by using the SPSS software package (15.0), to obtain the mean and standard deviation for comparison between different groups involved in this study, students (t) test was used. Pearson correlation coefficient (r) was applied to the test of hypothesis of linear relation between the studied variables. A p-value < 0.05 was considered statistically significant.

RESULTS

Table 1 shows that systolic and diastolic blood pressure were significantly higher in the patient group when compared to the control group (p=0.003, and p=0.002).

TABLE-1: CLINICAL DATA OF THE PATIENTS AND CONTROLS INCLUDED IN THE STUDY

Variables	Group –I (n=155)	Group –II (n=160)	P- value
Age (years)	46.6±5.6	44.78±5.7	0.229
BMI (Kg/m ²)	29.12±3.2	27.3±4.1	0.728
WC (cm)	78.6±6.37	77.9±5.3	0.801
HC (cm)	96.6±6.2	97.2±5.5	0.813
WHR	0.81 ±1	0.80 ± 0.96	0.302
SBP (mmHg)	151±9.8	123.3±1.8	0.003*
DBP (mmHg)	92.56±8.7	69.89±5.0	0.002*

Abbreviations

BMI= Body mass Index, WC= Waist circumference, HC=Hip circumference, WHR=Waist to Hip ratio, SBP=systolic blood pressure, DBP= diastolic blood pressure. (p- value <0.05 was considered statistically significant*)

Table 2 shows serum total cholesterol, LDL cholesterol and serum triglyceride levels were significantly higher in patient group when compare to the control group (p=0.004, p=0.02, p=0.0001 and p=0.03).

TABLE-2: ROUTINE BIOCHEMICAL PARAMETERS OF BOTH GROUPS

Variables	Group-I (n=155)	Group-II (n=160)	p-value
Fasting glucose (mg/dl)	103±112	99±9.1	0.010*
Post prandial glucose (mg/dl)	110±8	103±6	0.039*
Urea (mg/dl)	28±4.2	21.8±5.8	0.381*
Alanine amino transferease (U/L)	19±7	17±5	0.102*
Aspartate amino transferase (U/L)	20±4	19±3	0.203*
Total cholesterol (mg/dl)	207±4.6	157±6.8	0.004*
HDL cholesterol (mg/dl)	38.2±4.3	53±1.2	0.001*
LDL cholesterol (mg/dl)	125±6.3	85±4.5	0.02*
Triglycerides (mg/dl)	168±8.3	133±6.2	0.03*

Abbreviations:

HDL= High density lipoprotein, LDL= Low density lipoprotein
(p -value <0.05 was considered statistically significant*)

Table 3 shows the mean value of the oLAB and MDA were significantly higher in the patients group when compare to the control group (p=0.0003 and p=0.0001)

Table 4 shows significant correlation in the patient group.oLAB correlated positively with both cholesterol (r=0.401, p=0.031) and LDL- cholesterol fraction (r=0.382, p=0.037) MDA levels are correlated positively with total cholesterol (r=0.422,p=0.02) and its LDL fraction (r=0.433, p=0.018) among patients. oLAB and MDA level shows positive correlation with each other (r= 0.411, p=0.029)

TABLE 3 -MEAN VALUE OF MDA AND oLAB

Variable	Group -I (n=155)	Group-II (n=160)	p-value
oLAB (mg/dl)	41.4±9.3	26.8±5.7	0.0003*
MDA (nmol/ml)	7.5±2.5	3.74±0.9	0.0001*

Abbreviations:

oLAB= Oxidized low density lipoprotein auto antibodies, MDA= Malondialdehyde.
(p-value <0.05 was considered statistically significant*)

TABLE 4-SIGNIFICANT CORRELATION IN THE PATIENTS GROUP

Parameters	r-value	p-value
oLAB with		
Total cholesterol	0.401	0.031*
LDL cholesterol	0.382	0.037*
MDA with		
Total cholesterol	0.422	0.02*
LDL cholesterol	0.433	0.018*
MDA with oLAB	0.411	0.029*

(p- value <0.05 was considered statistically significant*)

DISCUSSION

Hypertension plays an important role in the pathogenesis of atherosclerosis. Border line hyper tension (BHT) has been shown to be a risk factor for atherosclerosis(Adaikkappan et al,2002). Many pathological factors have been implicated in the genesis of hypertension. One of the novel concept that involve in the structural and functional abnormalities in the `vasculature is lipid per oxidation which may antedate hypertension and contribute to its pathogenesis has gained support in recent years.

In the present work the serum oLAB level in the hypertensive patient group was significantly higher than its level in the corresponding control groups ($p=0.003$).this results agrees with similar studies reporting a high oLAB titer in atherosclerosis and its related complications (Hulthe et al,2001 and Wu R, de Faire U et al 1999) High oLAB titers were reported to be independent predictors of the progression of carotid atherosclerosis.(Salonen JT ,1991and Hulthe J et al 2001). On the other hand, failure to confirm such a relation between both parameters was also reported (Wu R, de Faire U et al 1999).

It has been postulated that the physiological function of oLAB is to participate in the removal of oxLDL from the removal of oxLDL from the artery wall and circulation, thus having a protective role similar to the defense mechanism in infectious diseases (Wu R, de Faire U et al 1999). This leads to a reduction in the atherogenic lipoproteins in arterial lesions(Hansson GK. Et al 2001). Studies on experimental animal demonstrated an association between the degree of atherosclerosis and oLAB. The study hypothesized an enhanced antibody level may simply reflect chronic inflammation in artery wall(Nilsson J et al 1997). Indeed in later stage of essential hypertension, increased oLAB levels have been reported (. Maggi E et al 1995).

Significant positive correlation existed between oLAB and each total cholesterol ($r = 0.401$, $p=0.031$) and its LDL fraction ($r=0.382$, $p=0.037$). These correlations can be attributed to the protective effect of oLAB to the oxLDL which is a modified form of LDL (table-4).

The process of rapid and total lipid peroxidation starts by free radicals that initiate a peroxidation cascade where polyunsaturated fatty acids are gradually changed to conjugated dienes, hydro peroxides and other products leading finally to their fragmentation and formation of alkanes and reactive aldehyde compounds, of which malondialdehyde (MDA), 4-hydroxy neonenal and hexanal constitute the main products. These can react with lysine residues of apoB-100 containing lipoproteins (Esterbauer H et al 1996)

Conflicting results are reported concerning the lipid peroxidation and antioxidants in hypertension. This may be primarily due to the different nutritional habits of the studied groups, as well as the distinct analytical methodologies used to evaluate these variables (Kumar KV et al 1993 and Moriel P et al 2000)

Different methods can be used to appraise the damage due to lipid peroxidation. In the present work, plasma MDA were chosen as one of the markers of lipid peroxidation, where their mean plasma value in the hypertensive patients was significantly higher than their corresponding mean plasma value in the control group ($p=0.0001$). The present study demonstrated a significant positive correlation between the plasma MDA level and each of total cholesterol ($r=0.422, p=0.02$) and its LDL cholesterol ($r=0.433, p=0.018$). This agrees with results of Moriel et al (2000). Positive correlation between MDA and oLAB shows ($r=0.411, p=0.029$) increase in lipidperoxidation stimulates oxLDL formation and as a protective function oLAB levels were elevated. Limitation of the present study is the carotid media intima thickness was not measured in the study group.

The present study concludes increased concentration oLAB may shows the progression of atherosclerosis, where as lipidperoxidation (free radical generation) in the essential hypertensive patients may act as pro atherogenic factor.

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