

## PLASMA LIPID PEROXIDE LEVELS IN TYPE II DIABETICS : RELATIONSHIP WITH LONG TERM DIABETIC COMPLICATIONS

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### SUMMARY

In order to explore the relationship of lipid peroxidation and long-term complications in diabetes mellitus, we tested LPO (lipid peroxides), FPG (fasting plasma glucose), cholesterol, triglycerides, and HDL (High Density Lipoprotein) levels of 44 healthy controls and 83 type II diabetics. Mean plasma LPO value of healthy controls was found to be  $2.55 \pm 0.51$  nmol/ml. This value seemed to increase by age, but no sexual difference was observed (in women  $2.46 \pm 0.53$ , in men  $2.66 \pm 0.48$  nmol/ml, mean  $\pm$  S.D.). Comparison of plasma LPO values showed a significant difference between healthy controls and total diabetics ( $4.05 \pm 1.24$  nmol/ml), complicated group ( $4.46 \pm 1.32$  nmol/ml) and non-complicated group ( $3.49 \pm 0.80$  nmol/ml), ( $p < 0.001$ ). According to these data we concluded that increased lipid peroxidation in diabetes mellitus may induce the onset of long-term complications.

**Key Words :** Lipid peroxide, diabetics, complications.

### INTRODUCTION

Free radicals are reactive species that can react with macro-molecules and thus change their structures (1). Because of their implications on carcinogenic, diabetogenic, hemolytic and immune phenomena as well as aging, free radicals have recently been one of the most striking subjects (2-4).

In recent studies it has been observed that more lipid peroxidation occurs in diabetes than in healthy people, that more malondialdehyde (MDA) arises from the erythrocyte membranes of diabetics in case of oxidative stress (5-7) and that diabetic erythrocytes contain less reduced glutathione (8). Besides, lipid peroxidation is found to increase in plasma membranes of diabetics with retinopathy (8). Rabbits with Streptozocin-induced diabetes and retinopathy, when compared with controls, are found to have elevated retinal MDA levels (9). When experimental diabetes is induced in rats, in vivo lipid peroxidation and in vitro cytotoxic changes in VLDL and LDL fractions occur (10). Based on these data some investigators supported the role of increased oxidative stress while the others emphasized that

defence mechanisms are inadequate in diabetes (10,11). In our study, the effects of free radicals and lipid peroxidation on the onset of diabetic complications have been studied. If the data continue to support this hypothesis it seems to be possible to prevent the tissue damage occurring in diabetes by use of antioxidants in addition to conventional insulin therapy (12).

### MATERIALS AND METHODS

We worked on 44 healthy controls (23 women, 21 men, mean age  $31.9 \pm 14.7$ ) and 83 type II diabetics (50 women, 33 men, mean age  $58.1 \pm 9.6$ ). Diabetic group was formed by ambulatory patients attending our clinics or by patients hospitalized at our services. Fifty-two of these 83 diabetics were complicated patients. We accepted albuminuria as a positive evidence for nephropathy, electroretinogram or fundus oculi signs for retinopathy. Arteriosclerosis was confirmed by electrocardiographic signs.

After a 12-hour fasting, heparinized plasma was used for LPO's and sera for FPG, cholesterol, triglycerides and HDL's.

Plasma LPO's were studied by the method-defined first by Yagi in 1976- in which lipid peroxides are precipitated by means of phosphotungstic and sulphuric acids. Lipid peroxides in the precipitant when reacted with thiobarbituric acid (TBA) in a hot medium, first MDA reacting with TBA forms a pink coloured product. The intensity of this colour is proportional to the plasma lipid peroxide concentration.

### RESULTS

Mean LPO values of healthy controls were found to be  $2.55 \pm 0.51$  nmol/ml: in men  $2.66 \pm 0.48$ , and in women  $2.46 \pm 0.53$  nmol/ml, showing no significant difference owing to sexes. When control group is classified according to individuals' ages, the mean LPO value of over 50s ( $3.32 \pm 0.57$  nmol/ml) was significantly higher than younger's. In type II diabetic group LPO values were found to be minimum 2.00, maximum 7.30, mean  $4.05 \pm 1.24$  nmol/ml, significantly higher than healthy controls.

When we divided the total diabetics into two subgroups as diabetics with and without complications, in the group with complications (n=52, mean±SD=4.46±1.32 nmol/ml) LPO levels were significantly higher than the group without complications (n=31, mean±SD=3.49±0.80 nmol/ml). When we compared the LPO levels of diabetics with complications with healthy controls, the difference was very significant statistically. In the comparison of LPO levels of non-complicated diabetics with healthy controls, the significance -even got smaller than the former- was very clear.

Complicated diabetics, when classified as nephropathics, retinopathics and those with arteriosclerotic heart disease, the LPO values were 4.42±1.14, 4.29±0.77, and 4.59±1.37 nmol/ml, respectively. When compared to non-complicated diabetics all of these three groups had significantly higher LPO values (Table I).

In order to explore the relationship between lipid peroxidation and high cholesterol, triglycerides and low HDL levels of diabetics, we classified the patients according to their plasma lipid levels. In our study cholesterol normals of healthy controls were found to

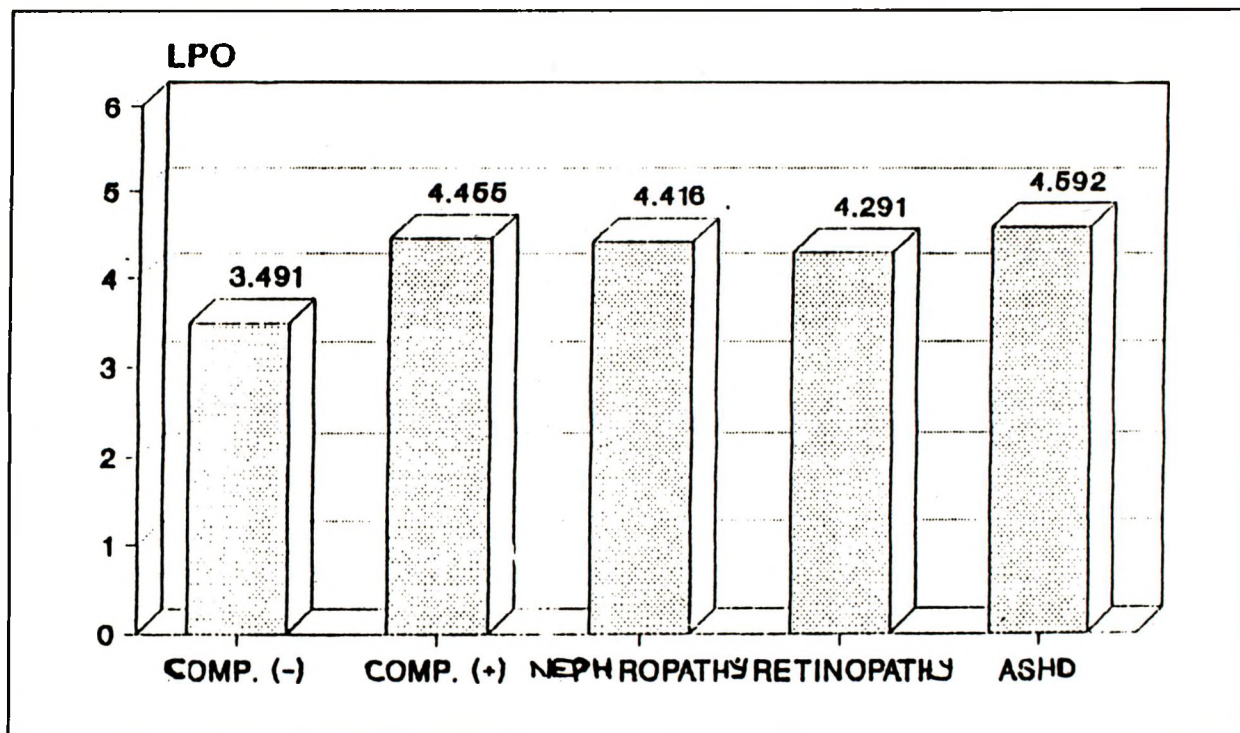
**Table I-** Minimum, maximum, mean and standard deviation values of plasma LPO levels of healthy controls, total diabetics, diabetics with complications, -nephropathics, retinopathics, arteriosclerotic cardiovascular disease (ASCVD)-, and diabetics without complications, in terms of nmol/ml.

	n	Min.	Max.	Mean	S.D.
Healthy controls	44	1.71	3.78	2.55	0.51
Total diabetics	83	2.00	7.30	4.05	1.24
With complications	52	2.78	7.30	4.46	1.32
Nephropathics	15	2.99	7.30	4.42	1.14
Retinopathics	10	2.97	5.21	4.29	0.77
ASCVD	27	2.78	6.87	4.59	1.37
Without complications	31	2.00	5.10	3.49	0.80

\* p < 0.001, when compared with healthy controls.

. p < 0.001, when compared with non-complicated diabetics.

.. p < 0.01, when compared with non-complicated diabetics.



**Fig 1.** Plasma LPO values (nmol/ml) and complication relationship in type II diabetes mellitus.

be  $204 \pm 50$  mg/dl, triglycerides normals  $103 \pm 42$  mg/dl, and HDL normals  $54.9 \pm 16.4$  mg/dl. Total diabetics were divided into two groups each time referring to the cut-off points 250 mg/dl for cholesterol, 150 mg/dl for triglycerides and 38.5 mg/dl for HDL-cholesterol. Based upon this classification mean LPO values of high-cholesterol and high-triglycerides groups were greater than the mean LPO levels of low-cholesterol and low-triglycerides groups, but both differences were not statistically significant. Nevertheless, mean LPO of low-HDL group was significantly higher than high-HDL group. We did not find any correlation between cholesterol ( $r=0.32$ ), triglycerides ( $r=0.34$ ), and HDL ( $r=0.58$ ) concentrations and LPO values.

## DISCUSSION

Lipid peroxidation, as a factor of aging, has long been studied and increased lipid peroxides have been identified in plasmas of elderly people in many studies (13,14). In our study also, LPO values increased by age. Especially healthy people over fifty years of age had higher values.

Lipid peroxidation is thought to be responsible of micro and macro-vascular complications occurring in diabetes in many cases. Blackman et al (15) found that in diabetics and cigarette smokers plasma LPO values were higher than normal individuals. Uzel et al. found high LPO values in both group of diabetics with and without retinopathy (8). Oberley (16) said that type II diabetic and angiopathic patients have 90% more "TBA-reactive material" in their plasmas than controls do, but patients without angiopathy do not show such an increase. According to Baynes (17), LPO levels in a human's plasma is related to hypertriglyceridemia and vascular disease rather than diabetic metabolism. Based on our data we can say that increased lipid peroxidation in diabetic patients may be a factor of long-term complications but the subject still needs further investigation. At least, it can be said that plasma lipid peroxide level may be useful to judge the prognosis of a diabetic. In literature, there are also a few data concluding that there is no difference between diabetic and normal controls' plasma LPO levels (18,19). In one of these, Arshad (19) said that in diabetic plasma lipid peroxidation occurred much more faster than normal plasma -in the presence of  $H_2O_2$  and  $Cu^{++}$  - and thus emphasized that in diabetics there is an increase in plasma peroxidation potential.

Whether altered lipid profile in diabetics has an effect on lipid peroxidation has also been investigated and different ideas are generated. Altomare et al (20) worked on MDA/cholesterol and MDA/triglycerides relationship and found these ratios higher in poorly controlled diabetics and interpreted that increase of lipid peroxidation in diabetes mellitus is a mechanism free from the increase of any of the lipid fractions. According to Yagi, "TBA-reactive material" in normals and diabetics is concentrated in LDL fraction, but increase of this material in diabetics occurs in HDL

fraction (21). Blackman (15) did not find any relationship between plasma LPO levels and plasma lipids. In our study, increased lipid peroxidation does not seem to happen as a result of increased plasma cholesterol and triglycerides, but somehow it seems to be related with the decrease of antiatherogenic HDL fraction.

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