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Original article

EVALUATION OF OXIDATIVE STRESS IN RENAL TRANSPLANT RECIPIENTS TREATED WITH CYCLOSPORINE/TACROLIMUS: ROLE OF HDL AND HEME OXYGENASE

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Abstract

Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality in patients receiving renal replacement therapy and the antioxidant defense system in patients with renal failure is deteriorated. In order to evaluate the oxidative stress of Cyclosporin /Tacrolimus treatment.The analysis of fatty acid composition and lipid peroxide levels of HDL and Heme oxygenase(HO) activity assays were performed within the study to evaluate oxidative stress in renal transplant recipients in this study. 18 renal transplantation patients treated with Cyclosporin(CsA,n=6) and Tacrolimus(FK506,n=12) were studied and monitored for 120 days following transplantation.12 healthy volunteers composed the control group.In current study fatty acid composition and lipid peroxide levels of HDL and Heme oxygenase activity of lymphocytes were measured. The levels of total cholesterol, tryglyceride, HDL- and LDL-cholesterol were also estimated.In FK506 group, C18:3 and C20:4 decreased significantly compared to control group($p<0.01$.)C18:1 and C18:2 levels at 60^{th} day in this group were also significantly higher than CsA group.Although the significant increase in HO activity at 60^{th} day compared to control and before transplantation in CsA group($p<0.05$), a significant decrease was observed at 120^{th} day. There is no significant difference in FK506 group.

The results showed that both groups were under oxidative stress after transplantation.Although the increase in HO activity sounds like it's the sign of antioxidant system is more favourable in CsA group, it was obvious that there was a relatively high oxidative stress in patients treated with CsA and it may be considered that the lipid profile of patients on FK506 therapy was more favorable than patients treated with CsA.The results show that oxidative stress is higher in Cyclosporin group.

Key words: *Transplantation, Oxidative stress, HDL, Heme oxygenase, Cyclosporine, Tacrolimus*

Siklosporin/Tacrolimus ile Tedavi Edilen Böbrek Nakli Alıcılarında Oksidatif Stresin Değerlendirilmesi: HDL ve Hemoksijenaz'ın Rolü

Aterosklerotik kardiyovasküler hastalık, böbrek yetmezliğinin son döneminde olan ve renal replasman tedavisi gören hastalardaki morbidite ve mortalitenin en önemli nedenidir ve bu hastalarda, antioksidan savunma sisteminin bozulduğu bilinmektedir.Bu çalışmada, Siklosporin/Tacrolimus tedavisi gören böbrek transplantasyon hastalarında ilaca bağlı oksidatif stresi değerlendirmek için HDL deki yağ asiti kompozisyonu ile lipit peroksit düzeyleri ile Hemoksijenaz aktivitesindeki değişikliklerin araştırılması amaçlanmaktadır.Böbrek transplantasyonu sonrasında Siklosporin(CsA,n=6) ve Tacrolimus(FK506, n=12) ile immunosupresif tedavi gören 18 hasta araştırmaya dahil edilmiştir ve transplantasyon sonrasında 120 gün takip edilmiştir. 12 sağlıklı gönüllü ise kontrol grubunu oluşturmaktadır. Araştırma kapsamında HDL yağ asiti bileşimi,lipid peroksit düzeyleri çalışılmış ve lenfositlerde hemoksijenaz aktivitesi saptanmıştır.Total kolesterol,trigliserit,LDL ve HDL kolesterol düzeyleri de ölçülmüştür. FK506 grubunda C18:3 ve C20:4 düzeyleri kontrol grubuna göre anlamlı derecede düşük bulunmuştur.

Bu grupta, transplantasyondan sonraki 60.günde C18:1 ve C18:2 düzeyleri, Siklosporin grubuna göre anlamlı derecede yüksek bulunmuştur.Siklosporin grubunda transplantasyon sonrası 60.günde hemoksijenaz aktivitesinde, transplantasyon öncesine ve kontrol grubuna göre anlamlı bir artış gözlenmesine rağmen($p<0,05$); transplantasyondan sonraki 120.günde anlamlı azalma saptanmıştır. FK506 grubunda anlamlı bir değişiklik görülmemiştir.

Sonuçlar, her iki grubun da transplantasyon sonrasında oksidatif stres altında olduğunu göstermektedir. Hemoksijenaz aktivitesindeki artış, antioksidan etkinin Siklosporin grubunda daha iyi olduğunu düşündürse de, FK506 tedavisi gören hastaların HDL lipid profili Siklosporin grubuna göre daha iyi bulunmuştur. Bulgular, Siklosporin grubunda oksidatif stresin daha fazla olduğunu göstermektedir.

Anahtar kelimeler: *Transplantasyon, Oksidatif Stres, HDL, Hemoksijenaz, Siklosporin, Tacrolimus*

INTRODUCTION

Renal transplantation is the preferred renal replacement therapy for patients with end-stage renal disease (1). It's known that the antioxidant defense system deteriorated in patients with renal failure and atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality in patients receiving renal replacement therapy (2).

High density lipoprotein (HDL) has a well defined protective influence against coronary disease. Current interest is focused on the under-exploited therapeutic potential of the lipoprotein. The attention has drawn into it's cardioprotective role (3). The ability of HDL to promote cholesterol efflux is thought to be important in its protection against cardiovascular disease. It is known that the concentration of cholesterol in HDL is an inverse predictor of future atherosclerotic cardiovascular disease (4).

It's been shown that dyslipidemia is frequent 1 year after renal transplantation (5) In addition, Zeljkovic et al. (6) observed that Renal transplant recipients had less HDL 2b, but more HDL 3a and 3b subclasses. Thus, renal transplant patients had impaired distribution of HLD and LDL particles. It's been demonstrated that a great extent of linoleic acid and polyunsaturated fatty acids enhances the oxidizability of LDL (7,8). However, there is no data about the lipid composition of HDL in renal transplant patients.

The role of lipoproteins in atherosclerotic cardiovascular disease is well known. In our previous study, we evaluated the fatty acid composition and the susceptibility to oxidation of LDL in renal transplant recipients (9). In the current study we aimed to investigate the fatty acid content of HDL and heme oxygenase (HO) as another enzyme of antioxidant system.

Heme oxygenases (HO) catalyze the rate-limiting step in heme degradation, resulting in the formation of carbon monoxide, iron and biliverdin that is subsequently reduced to bilirubin by biliverdin reductase. The products of this enzymatic reaction have important biological effects, including antioxidant, anti-inflammatory and cytoprotective functions (10). Bile pigments, biliverdine, and it's metabolite bilirubine were used in order to exploit the intrinsic antioxidant properties of these species at a cellular level (11). This hypothesis originated from a series of observations of bile pigment antioxidant activity in vitro (12,13). Under physiologic O_2 concentrations (2%), both bilirubine and biliverdine prevent the oxidation of polyunsaturated fatty acids in multilamellar liposomes at least as effectively as α-tocopherol (14).

In this study, it was aimed to evaluate oxidative stress in renal transplant patients treated with Cyclosporine (CsA) or Tacrolimus (FK506). Therefore the changes at fatty acid content of HDL, the levels of lipid peroxidation products of HDL and HO activity in lymphocytes were analysed in renal transplant recipients receiving CsA or FK506 therapy. The levels of total cholesterol, tryglyceride, HDL cholesterol and LDL cholesterol were also estimated.

EXPERIMENTAL

18 stable renal transplant recipients treated either with CsA (n=6) or FK506 (n=12) were evaluated by analyzing blood samples that was collected before transplantation (BT), and at the 60^{th} (AT60), and 120^{th} day (AT120) of tranplantation. None of the patients were treated with lipid-lowering therapy. 12 healthy donors were the control group. Patients were treated with doses of 4-6 mg/kg/day Cyclosporine or 0,07 mg/kg/day FK506. The patients who had another chronic disease history are kept out of the study.

HDL samples were isolated from venous blood by density gradient ultracentrifugation as described by Sykes et al (15). The measurement of fatty acid content and TBARS assay were estimated by using those isolated HDL samples and HO assay was performed via using the lymhocytes isolated from blood samples (16).

Fatty Acid Measurements in HDL

The fatty acid composition of HDL samples were analyzed by a modified method described by Sattler et al (17). Fatty acid methyl esters were chromatographed on a 60 m - 0.25 mm Supelco 24111 column with a film thickness of 0.2 m. The analysis was performed on an Agilent 6890N GC (injection temperature was 270 °C), which was equipped with a flame ionization dedector (FID; dedector temperature 280 °C). The fatty acid levels were calculated as % amounts by using standard methyl ester mixtures composed of C16:0 (palmitic acid), C18:0 (stearic acid), C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (linolenic acid), C20:4 (arachidonic acid), C22:4 (docosatetraenoic acid), and C22:6 (docosahexaenoic acid). Non-oxidized fatty acid levels were measured. The decrease in fatty acid levels defines the increase of oxidized amounts.

HO Assay

The method described by Maines has been modified (18). The assay was performed with minimum light inorder to protect biluribin photosensitivity. Lymphocytes isolated from the blood by a slight modification of the method of Boyum (16) (1mg/ml) were added into a combined solution including cytochrome c reductase (20U/ml), biliverdine reductase (500U/mg), heme solution (1 mM), and buffer solution (0,1 M potassium phosphate buffer including 1 mM EDTA, 0,1% Triton X-100). The tubes were vortexed 10 sec and incubated in a circulating water bath at 37°C for 5 min in the dark. Following incubation, the tube content were divided into two tubes as blank and sample. 2.75 M NADPH solution was added into sample tube and the same amount of buffer solution was added into blank tube. The tubes were incubated in a circulating water bath at 37°C for 15 min. Reactions were then stopped on ice and the absorbance at 465 nm was monitored in 10 mm quartz cuvettes with Shimadzu UV-1700 spectrophotometer for 180 sec. The HO activity was calculated by the changes of absorbance during the formation of bilirubin.

HDL Oxidation and TBARS Assay

The measurement of lipid peroxide levels was performed spectrophotometrically. The HDL lipoprotein fraction was in vitro oxidized for 180 min at 37 °C by using freshly prepared $CuCl_2.5H_2O$ solution, and the final cupper concentration was adjusted to 8mM. TBARS measurement was estimated by the method of Puhl et al (19). After incubation of HDL samples, 0,67 umol/L EDTA (final concentration) was added and tubes were placed in ice for 10 min to stop the reaction. TCA (%0,1) and TBA (%0,67) solutions were added and the tubes were placed in boiling water for 20 min and than 2,5 ml n-butanol was added and the tubes were centrifuged at 3000 rpm for 10 min. Absorbance measurements at 532 nm were recorded.
Quantitation of TBARS was performed by comparison with a standard curve of malondialdehyde (MDA) equivalents generated by acid-catalyzed hydrolysis of

1,1,3,3-tetraetoxypropane and lipid peroxide levels were determined as nmol MDA/mg HDL protein.

Statistics

The data represented mean values ± standard errors from 3 independent experiments, which were performed in duplicate. Mann-Whitney U test was used for statistical comparisons.

This study was approved by the local ethics committee.

RESULTS

According to the results, total cholesterol level of CsA group increased significantly ($p<0,05$) at AT60 (254,80±30,63) compared to control (178,68±32,03) and we observed that the tryglyceride levels of both groups (CsA and FK506) increased significantly ($p<0,05$) at AT60 compared to control and BT. LDL-cholesterol level of CsA group also increased significantly ($p<0,05$) at AT60 compared to control and BT. decreased at the 60th day compared to control, whereas it was higher in CsA group. There was no significant change at HDL-cholesterol level in the groups.

In FK506 group, the levels of C16:0 increased significantly at AT60 compared to control and BT. C18:3 and C20:4 decreased significantly compared to control (Table 1). In CsA group (Table 2), there was not any significant change after transplantation except C18:0 level at AT60. In addition to this, C18:1 and C18:2 levels at AT60 in FK506 group were significantly higher than CsA group.

Table 1. Characteristics of the study subjects treated with CsA (n=6) and control ((n=12).

	Control	BT	AT60	AT120
Total Cholesterol (mg/dl)	178,68±32,03	177,00±12,77	254,80±30,63 [a]	223,50±27,22
Tryglyceride (mg/dl)	55,00±10,56	147,67±56,92	211,50±36,97 [a]	204,50±60,48
LDL cholesterol (mg/dl)	90,00±5,00	109,50±9,19	144,40±27,23 [a,b]	125,33±24,11
HDL cholesterol (mg/dl)	57,00±3,00	38,00±4,24	65,20±8,26	57,00±18,47

* Data are presented as means ± standard error. Results are expressed as mg/dl.
[a] $p < 0.05$ (compared control) [b] $p < 0.05$ (compared BT)

Table 2. Characteristics of the study subjects treated with FK506 (n=12) and control (n=12).

	Control	BT	AT60	AT120
Total Cholesterol (mg/dl)	178,68±32,03	165,20±57,14	213,11±49,09	187,75±17,67
Tryglyceride (mg/dl)	55,00±10,56	111,86±42,89	195,50±49,21 [a]	143,14±42,13
LDL cholesterol (mg/dl)	90,00±5,00	95,67±43,15	108,67±39,40	95,89±17,83
HDL cholesterol (mg/dl)	57,00±3,00	45,00±15,10	61,44±19,65	54,67±12,80

* Data are presented as means ± standard error. Results are expressed as mg/dl.
[a] $p < 0.05$ (compared control)

Table 3. Fatty acid composition of control and FK506-treated patients.*

Fatty Acid	Control	FK506 Group		
		BT	AT60	AT120
C16:0	2.65±1.80 n=3	1.02±0.62 n=5	10.48±1.02[ae] n=5	5.23±2.42[f] n=6
C18:0	0.67±0.40 n=3	0.10±0.04[a] n=7	0.13±0.07[a] n=6	0.13±0.08 n=5
C18:1	2.49±1.61 n=5	3.50±1.69 n=7	3.77±0.97 n=6	3.04±1.18 n=6
C18:2	2.70±1.36 n=4	9.11±2.09[d] n=7	7.86±1.09[d] n=7	2.19±0.92[f] n=5
C18:3	4.92±1.09 n=4	1.89±0.61[a] n=4	2.97±1.47[a] n=8	4.26±0.79[c] n=5
C20:4	60.87±6.67 n=5	48.73±8.14[d] n=7	57.89±6.52 n=8	36.63±16.88[ac] n=7
C22:4	14.04±7.24 n=4	6.09±3.22 n=5	8.08±3.11 n=7	23.19±13.35[c] n=6
C22:6	1.74±0.80 n=3	3.84±1.51 n=9	3.20±1.94 n=6	2.05±0.49 n=4

* Data are presented as means ± standard error. Results are expressed as percentages of total fatty acid.
[a] $p < 0.05$ (compared control) [b] $p < 0.05$ (compared BT) [c] $p < 0.05$ (compared AT60)
[d] $p<0.01$ (compared control) [e] $p< 0.01$ (compared BT) [f] $p<0.01$ (compared AT60)

Table 4. Fatty acid composition of control and CsA-treated patients.*

Fatty Acid	Control	CsA Group		
		BT	AT60	AT120
C16:0	2.65±1.80 n=3	0.41±0.14 n=3	1.09±0.65 n=3	3.25±1.01 n=4
C18:0	0.67±0.40 n=3	0.25±0.02 n=3	0.11±0.04[ab] n=5	0.18±0.10 n=3
C18:1	2.49±1.61 n=5	2.40±1.37 n=4	1.21±0.47 n=4	1.19±0.56 n=5
C18:2	2.70±1.36 n=4	2.73±1.71 n=4	4.50±2.12 n=3	4.02±1.91 n=4
C18:3	4.92±1.09 n=4	2.93±1.67 n=4	3.40±1.71 n=4	2.26±1.38 n=4
C20:4	60.87±6.67 n=5	53.91±12.63 n=5	59.20±7.16 n=3	35.99±19.94 n=4
C22:4	14.04±7.24 n=4	9.80±3.47 n=3	16.91±8.74 n=3	11.09±2.39 n=3
C22:6	1.74±0.80 n=3	3.60±1.28 n=4	1.99±0.33 n=3	2.54±1.42 n=4

* Data are presented as means ± standard error. Results are expressed as percentages of total fatty acid.
[a] $p < 0.05$ (compared control) [b] $p < 0.05$ (compared BT)

In FK506 group, after in vitro HDL oxidation, the lipid peroxide levels at AT60 decreased significantly compared to BT. However there was a significant increase at the 120th day's results when compared to AT60. In CsA group, there were no significant changes after transplantation. On the other hand the lipid peroxide levels of FK506 group at AT60 were significantly lower than CsA group (Table 3).

In CsA group, HO activity has significantly increased at the 60th day compared to control and BT. On the other hand no significant change was observed in FK506 group and the activity measured at AT60 in FK506 group was significantly lower than CsA group ($p<0.05$) (Table 4).

Table 5. The results of HO activity and HDL lipid peroxidation measurements of control and FK506-treated patients*.

		FK506 Group		
	Control	**BT**	**AT60**	**AT120**
HO Activity (µmol/dk/mg protein)	10.68±6.26 n=8	14.55±4.72 n=7	10.16±6.05 n=6	18.36±7.14 n=7
HDL TBARS (nmol MDA/mg protein)	15,70±9,63 n=4	20,13±1,94 n=4	11,25±5,86[b] n=7	19,07±5,43[c] n=7

* Data are presented as means ± standard error.
[b] $p < 0,05$ (compared BT) [c] $p < 0,05$ (compared AT60)

Table 6. The results of HO activity and HDL lipid peroxidation measurements of control and CsA-treated patients*.

		CsA Group		
	Control	**BT**	**AT60**	**AT120**
HO Activity (µmol/dk/mg protein)	10.68±6.26 n=8	10.36± 3.77 n=6	30.69±18.05[ab] n=3	10.35±5.67 n=5
HDL TBARS (nmol MDA/mg protein)	15,70±9,63 n=4	22,20±4,60 n=4	20,93±6,71 n=5	21,70±3,39 n=4

* Data are presented as means ± standard error.
[a] $p < 0,05$ (compared control) [b] $p < 0,05$ (compared BT)

DISCUSSION

Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality at the late terms of renal transplantation. Oxidative stress is detrimental to blood lipid metabolism and accorded as a major role in the atherosclerotic process (20).

A recent study showed the existence of dyslipidemia in renal transplant patients (21) (Suleimani, 2009). We also observed that lipid profile altered in transplanted subjects and the levels of total cholesterol and tryglyceride increased after transplantation. HDL cholesterol levels were also seemed altered at the 60th and 120th days compared to before transplantation results.

HDL is the major carrier of extremely low concentrations of lipid hydroperoxides in human plasma, and initially, HDL lipids are oxidized in preference to those in LDL when human plasma is exposed to aqueous peroxyl radicals. Moreover, in comparison to LDL, HDL has a weak complement of antioxidants such as vitamins (22). The lipoprotein can bolster its antioxidant capacity by acquiring peptides capable of such activity.

The study of Rabini et al (23) showed that VLDL and HDL from NIDDM patients showed a decrease in the saturated fatty acid content with a concomitant increase in unsaturated fatty acid contents. They observed that basal peroxide levels increased proportionally with unsaturated

fatty acid content. Another study of Solakini et al (24) also showed that oxidation rate of lipoproteins was positively associated with polyunsaturated fatty acid content of lipoprotein.

At present study we investigated the oxidative state of HDL in renal transplant recipients. Although most of the polyunsaturated fatty acid levels at AT60 in FK506 group were higher than the pre-transplant term's results, the further decrease at AT120 may be the indicator of vulnerability of HDL unsaturated fatty acids to oxidation.

In FK506 group, following a significant decrease at AT60, the TBARS levels again increased at AT120 and the high TBARS levels in CsA group didn't change in terms of time. Considering the inequality of lipid peroxide levels of FK506 group, it may give rise to the thought of the oxidation at this group may be controlled.

HO is the rate controlling enzyme of the predominant pathway of hepatic heme degradation (25). A proposed functional role of HO is to produce the bile pigments biliverdine and its metabolite bilirubine, in order to exploit the antioxidant properties of these species at a cellular level (26). Under physiological O_2 concentrations (2%) both biliverdine and bilirubine prevent the oxidation of polyunsaturated fatty acids in multilamellar liposomes at least as effectively as α-tocopherol (14) and the inhibition of bilirubin formation leads to oxidative damage in the organism.

In CsA group, HO activity has significantly increased at the 60th day compared to control and BT. On the other hand we didn't observe any significant changes in FK506 group.

CONCLUSION

The results show that, both groups were under oxidative stress after transplantation. The increase of HO activity may sound like it's the sign of the antioxidant system being more favourable in CsA treated group at the late terms of transplantation. However when the results are completely evaluated, it's obvious that there is a relatively high oxidative stress in patients treated with CsA and it may be considered that the lipid profile of patients on FK506 therapy was more favorable than patients treated with CsA.

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REFERENCES

1. Papadopoulos O, Konofaos P, Chrisostomidis C, Lionaki S, Georgiou P, Vlasis K, Kostakis A, Reconstructive surgery for kidney transplant recipients, Transplant Proc 37, 4218-4222, 2005.
2. Chisolm GM, Steinberg D, The oxidative modification hypothesis of atherogenesis: an overview, Free Radic Biol Med 28, 1815–1826, 2000.
3. Deakin S, Moren X, James RW, HDL oxidation compromises its influence on paraoxonase-1 secretion and its capacity to modulate enzyme activity, Arterioscler Thromb Vasc Biol 27, 1146-1152, 2007.
4. Sviridov D, Mukhamedova N, Remaley AT, Chin-Dusting J, Nestel P, Antiatherogenic functionality of high density lipoprotein: how much versus how good, J Atheroscler Thromb 15(2), 52-62, 2008.

5. Spinelli GA, Felipe CR, Park SI, Mandia-Sampaio EL, Tedesco-Silva H Jr, Medina-Pestana JO. Lipid profile changes during the first year after kidney transplantation: risk factors and influence of the immunosuppressive drug regimen, Transplant Proc 43(10), 3730-7, 2011.
6. Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Peco-Antic A, Kostic M, Vasic D, Spasic S. Characteristics of low-density and high-density lipoprotein subclasses in pediatric renal transplant recipients, Transpl Int 24(11), 1094-102, 2011.
7. Reaven P, Parthasarathy S, Grasse BJ, Miller E, Steinberg D, Witztum JL, Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects, J Clin Invest, 91, 668, 1993.
8. Bonanome A, Pagnan A, Biffanti S, Opportuno A, Sorgato F, Dorella M, Maiorino M, Ursini F, Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification, Arterioscler Thromb 12, 529, 1992.
9. Bakar F, Keven K, Dogru B, Aktan F, Erturk S, Tuzuner A, Erbay B, Nebioglu S, Low-density lipoprotein oxidizability and the alteration of its fatty acid content in renal transplant recipients treated with cyclosporine/tacrolimus, Transplant Proc 41(5), 1630-3, 2009.
10. Bach FH, Heme oxygenase-1 as a protective gene, Wien Klin Wochenschr 114(4), 1-3, 2004.
11. Stocker R, Induction of heme oxygenase as a defense against oxidative stress. *Free* Rad Res Commun 9, 101-112, 1990.
12. Neuzil J, Stocker R, Bilirubin attenuates radical-mediated damage to serum albumin, FEBS Lett 331, 281-284, 1993.
13. Neuzil J, Stocker R, Free and albumin bound bilirubin are efficient co-antioxidants for alpha-tocopherol, inhibiting plasma and low density lipoprotein lipid peroxidation, J Biol Chem 269, 16712-16719, 1994.
14. Stocker R, Yamamoto Y, McDonagh A, Glazer AN, Ames BN,Bilirubin is an antioxidant of possible physiological importance, Science 235, 1043-1045, 1987.
15. Sykes E, Meany M, Schulz V, Kessel D, Separation of plasma lipoproteins with a tabletop ultracentrifuge, Clin Chim Acta 205, 137-144, 1992.
16. Boyum A, Separation of leukocytes from blood and bone marrow, Scand J Clin Invest 21, 77–90, 1963.
17. Sattler W, Puhl H, Hayn M, Kostner GM, Esterbauer H, Determination of fatty acids in the main lipoprotein classes by capillary gas chromatography: BF3/methanol transesterification of lyophilized samples instead of Folch extraction gives higher yields, Anal Biochem 198, 184-190, 1991.
18. Maines MD, Carbonmonoxide and nitric oxide homology: differential modulation of heme oxygenases in brain and detection of protein and activity, Methods in Enzymology 268, 473-488, 1996.
19. Puhl H, Waeg G, Esterbauer H, Methods to determine oxidation of low-density lipoproteins, Methods in Enzymology 233, 425-441, 1994.
20. Chisolm GM, Steinberg D, The oxidative modification hypothesis of atherogenesis: an overview, Free Radic Biol Med 28, 1815–1826, 2000.
21. Suleiman B, El Imam M, Elsabigh M, Eltahir K, Eltahir A, Miskeen E, Lipid Profile in post transplant renal patients treated with Cyclosporine in Sudan, Saudi J Kidney Dis Transpl 20 (2),312-317, 2005.
22. Bowry VW, Stanley KK, Stocker R, High density lipoprotein is the major carrier of lipid hydroperoxides in human blood, Proc Natl Acad Sci 89, 10316–10320,1992.
23. Rabini RA, Tesei M, Galeazzi T, Dousset N, Ferretti G, Mazzanti L, Increased susceptibility to peroxidation of VLDL from non-insulin-dependent diabetic patients: A possible correlation with fatty acid composition, Molecular and Cellular Biochemistry 199, 63–67, 1999.

24. Solakivi T, Jaakkola O, Salomaki A, Peltonen N, Metso S, Lehtimaki T, Jokela H, Nikkari ST, HDL enhances oxidation of LDL in vitro in both men and women, Lipids in Health and Disease 4(25), 1-7, 2005.
25. Lincoln BC, Bonkovsky HL, Hepatic heme catabolism in cultured-hepatocytes, Fed Proc 46, 2264, 1987.
26. Stocker R, Induction of haem oxygenase as a defence against oxidative stres, Free Radic Res Commun 9, 101-112, 1990.

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