AGE-RELATED CHANGES IN THE LIPID PEROXIDATION AND ANTIOXIDANT SYSTEMS OF MALE SUBJECTS

Dr. Saad Abid Al-rahmman Hussien* **Dr. Hameed Mahmood Majeed****
Layth Abid Al- Kareem***

Abstract

The effect of aging on the lipid peroxidation and antioxidant systems was evaluated in this study. For this purpose, we determined MDA as a marker of lipid peroxidation in blood serum, haemoglobin in whole blood, reduced glutathione (GSH) , total protein, albumin, and uric acid in blood serum as well as catalase and glutathione-s-transferase in serum of 84male healthy individuals (non-smokers).

These subjects were divided in to three groups: group1 (n=28;25-34years old); group2(n=28;35-44 years old); group3(n=28;45-63 years old).

The result showed increased levels of MDA and were observed as the stage of age increased P>.05). Whereas there is a depletion in GSH levels related to age increase (P < 0.05), which indicate that the low levels of GSH in the circulation may be due to their increased utilization to scavenge lipid peroxide. There was higher levels of catalase and GST in group3 compared with others.Group2 showed lower level in haemoglobin and higher level in albumin compared with other groups(P < 0.05).No meaningful differences were found in terms of serum total protein and uric acid levels.

المستخلص

لتقييم التغير الحاصل في مضادات الأكسدة في النظام الحيوي خلال مراحل مختلفة من العمر، أخذت عينات من الدم الوريدي لـــ 84 شخص من الذكور ممثلة لثلاثة مجاميع ، كل مجموعة مكونة من 28 شخص تراوحت أعمارهم بين (25-34)، (35-44)و (45-63)سنة للمجاميع 1، 2 و 3 على التوالي.

تم فصل مصل الدم لأجراء الاختبارات التالية :

MDA كمقياس دال على اكسدة اللبيدات، تقدير مضادات الاكسدة الانزيمية مثل (الكاتاليز و الكلوتاثايون ترانسفيريز) ، وغير الانزيمية مثل (الكلوتاثايون ، البروتين الكلي ، الالبومين وحامض اليوريك) بالاضافة الى تقدير الهيموغلوبين في الدم الكامل.

اظهرت النتائج وجود زيادة ملحوظة في مستوى اختبار الـــ MDA على الرغم من عدم معنوية هدا المقياس ، مع وجود استنزاف في مستوى الكلوتاثايون ترافق معنوياً مع عدم تقدم العمر (مستوى احتمالية٠,٠٥) ، وربما يعود هدا لدوره الفعال في ازالة البيروكسيدات.

كما أظهرت المجموعة الثالثة ارتفاع في مستوى الكاتاليز والكلوتاثايون ترانسفير مقارنة ببقية المجاميع، المجموعة الثانية أشارت إلى مستوى منخفض من الهيموكلوبين ومرتفع من الألبومين مقارنة

* **Asis. Prof /pharmacy college – Baghdad University**
** **Lecturer / Technical Institute - Baquba**
*** **trainer / Technical Institute - Baquba**

ببقية المجاميع ، في حين لا توجد أية فروق معنوية في مستوى البروتين الكلي وحامض اليوريك بين المجاميع عند مستوى احتمالية 0.05

Introduction

Free radicals are highly reactive species characterized by an unpaired electron in their outer orbital. Free radical reactions, including lipid peroxidation, are considered to be important factors in the pathogenesis of a variety of diseases.[1]

Free radicals can damage protein, lipids, carbohydrates, and nucleic acids. plasma membranes are critical targets of free radical reactions. Oxygen-derived free radicals can easily produce injuries to cell membranes (fig.I) by imitation of polyunsaturated fatty acid peroxidation, inactivation of membrane enzyme and receptors, and protein cross-linking and fragmentation. [2]

Figure (I) Lipid peroxidation (classic lipid peroxidation)

Initiation

$$LH + X^{\cdot} \longrightarrow L^{\cdot} + XH$$

Propagation

$$L^{\cdot} + O2 \longrightarrow LOO^{\cdot}$$

$$LOO^{\cdot} + LH \longrightarrow L^{\cdot} + LOOH$$

Termination

$$2LOO^{\cdot} \longrightarrow \text{Non-radical products}$$

$$L^{\cdot} + LOO^{\cdot} \longrightarrow \text{Non- radical products}$$

$$L^{\cdot} + L^{\cdot} \longrightarrow \text{Non-radical products}$$

$$\text{Vitamin E} + L^{\cdot} \longrightarrow L^{\cdot} + \text{Vitamin E}^{\cdot} \quad \text{(scavenging)}$$

Termination may also occur with GSH or other Lipid antioxidants.
(LH) polyunsaturated fatty acid, ($X^{\cdot}$) Free radical atom.

We have evolved antioxidant defences to protect against free radicals. Superoxide dismutases (SOD) convert superoxide to hydrogen peroxide (H2O2) :

$$\text{Vitamin E} + L^{\cdot} \longrightarrow L^{\cdot} + \text{Vitamin E}^{\cdot} \quad \text{(scavenging)}$$

Termination may also occur with GSH or other Lipid antioxidants.
(LH) polyunsaturated fatty acid, ($X^{\cdot}$) Free radical atom.

We have evolved antioxidant defences to protect against free radicals. Superoxide dismutases (SOD) convert superoxide to hydrogen peroxide (H2O2) :

$$2O_2 + 2H \longrightarrow H_2O_2 + O_2$$

These enzymes are found in mitochondria and cytosol.[3]. catalases remove hydrogen peroxide ,are found in peroxisomes in most tissues, and probably serve to remove peroxide generated by peroxisomal oxidase enzymes.[4]

Glutathione peroxidases are major enzymes that remove hydrogen peroxide

generated by SOD in cytosol and mitochondria, by oxidizing the tripeptide glutathione (GSH) into its oxidized from (GSSG):

$$2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$$

GSH also has important roles in xenobiotic metabolism and leukotriene synthesis and it's found at millimolar concentrations in all human cells.[4]

The glutathione peroxidase that removes hydrogen peroxide contains selenium (essential for catalytic function) at its active site, as does a similar enzyme that can remove lipid hydroperoxides from membranes .[5]

Inborn defects in enzymes of glutathione metabolism can have sever clinical consequences. Since iron and copper ions are powerful promoters of free–radical damage, accelerating lipid peroxidation and causing formation of hydroxyl radical.[6].

We have evolved a complex system of transport and storage proteins to ensure that these essential metals are rarely allowed to be "free". Ferritin is the usual storage –form of iron .Iron with in ferritin will not stimulate free radical reaction [6],.since antioxidant defences are not completely effective, repair enzymes exist that destroy free radical-damaged proteins, remove oxidized fatty acids from membranes , and repair free radical damage to DNA (some of the oxidized bases removed are excreted in urine. All these defences are largely intracellular.

Other antioxidant defences are largely extracellular.They include the plasma iron-transport-protein transferring and the similar iron-binding-protein Lactoferrin found in many body secretions (eg,tears,nasal lining fluid); iron bound to these proteins can not catalyse free-radical damage. Caeruloplasmin is a safe transport form of copper and it assists loading of iron on to transferrin .Haemopexin and haptoglobin bind free heme and heme proteins to minimize their ability to catalyse free radical damage.[7]

Albumin,which has one sulphydryl group per molecule and is present at about 0.5 mmol/L in plasma, can scavenge several radicals and binds copper ions.[8]

GSH,Urate, & -tocopherol, and ascorbate remove free radicals by reacting directly with them non-catalytically.

Despite all these antioxidants, some free radicals still escape to do damage. Thus DNA undergoes constant "oxidative damage", and has to be repaired .Free radical-damaged proteins are degraded. End products of lipid peroxidation (eg, the isoprostanes) and of free –radical attack on urate are present in vivo.[9]

Materials and Methods:

To study the relationship between aging and the changes in serum lipid peroxidation and antioxidant status,84 healthy male subjects were used from different working places in baquba city, these subjects were divided into three groups: group1(n=28;25-34 years old); group2(n=28;35-44 years old); group3 (n=28; 45-63 yeas old).

Blood samples were obtained from all subjects and used in the analysis in Baquba hospital laboratory. Venous blood samples were taken in morning which divided in to two parts part in the EDTA-anticoagulated samples for haematological test and the other part in test tube centrifuged at 3000 rpm for 5min at 4C° to obtain the serum.

Samples were analyzed for malondialdehyde (MDA) by mixed 1ml serum and 2ml TBA reagent (0.375gm thiobarbituric acid,15gm trichloroacetic acid in 100ml of 0.25N HCL). Light absorbance of supernatant solution is determined at 535 nm against blank.

Assay of Serum GSH activity:

Glutathione contents in serum (measured as total sulfhydryl groups) were measured according to the method of [10], which is based on the reaction of GSH with

dithionitro benzene at PH 8 to form yellowish color chromophore which absorbs light at 412nm. The concentration of GSH was calculated using standard curve prepared for this purpose.

Assay of Serum GSH Catalase Activity:

Serum catalase activity was assayed according to the method of [11],based on spectrophotometric follow-up for the decomposition of 1ml(30mM) H_2O_2 after rapid mixing with 100UL of serum at Ph7.0 after 15 and 30 seconds, relative to control sample containing 1ml of phosphate buffer instead of H_2O_2.

Assay of Serum GST Activity:

Glutatione-s-trasferase activity was determined according to the spectrophotometric method of [12].The enzyme activity was estimated by monitoring the change in absorbance at 340nm,as a result of conjugating glutathione with1-chloro-2,4-dintrobenze (CDNB). Specific enzyme activity is defined as the amount of enzyme that catalyzes the formation of one mole of product/minute (which is termed 1 unit)/liter, and can be calculated from the molar absorptivity of the (CDNB)conjugate formed (9.6mole/L/cm).

Selected serum biochemical parameters were also determined in serum (table1): total protein and uric acid by colorimetric methods; albumin by a photometric method using bromcresol green. Haemoglobin by MSq instrument.

Statistical Analysis:

All data expressed as the mean value ± SE. Statistical analysis of the results were performed using ANOVA table. Duncan's test was used to compare the results between the groups.

Results:

The table shows elevated levels of serum MDA related to age but not significantly ($P > 0.05$). There were a significant elevated of the catalase and glutathione-s-transferase in group3 compared with other groups. Whereas there were a significant lowered in GSH between the groups related to aging. There wa a significant decrease in Haemoglobin and significant elevated levels in albumin in group2 compared with other groups , whereas total protein and uric acid were not significant different among groups.

Table (1) : Selected biochemical parameters in the blood of male subjects related to age

45-63		35-44		25-34		Age (year)
28		28		28		Numbers
SE	M	SE	M	SE	M	
0.161	1.576	0.16	1.759	0.156	1.318	MDA Umol/ L
0.22	13.971[a]	0.28	12.325[b]	0.315	13.6[a]	Haemoglobin Conc.(g/dl)
0.14	7.125	0.11	7.008	0.142	7.15	Total protein (mg/dl)
0.09	3.47[b]	0.11	3.88[a]	0.134	3.51[b]	Albumin (mg/dl)
0.25	5.99	0.22	5.78	0.196	5.63	Uric acid (mg/dl)
0.015	0.178[c]	0.02	0.22[b]	0.016	0.274[a]	Glutathione (GSH)

0.508	7.2^{a}	0.46	4.617^{b}	0.532	5.721^{b}	Catalase
4.338	38.629^{a}	3.11	29.264^{b}	2.267	29.464^{b}	Glutathione-S-Transferase

Values represent mean ± SE , a,b, c represent significant different (P<.05)

Discussion:

Under normal conditions, there is a steady State balance between the production of oxygen free radicals and their destruction by the antioxidant systems .The oxygen free radicals, which accumulate via an imbalance between generation and scavenging, are believed to induce many disease states .[13]

Elevated MDA serum levels might be the result of increased lipid peroxidation which an-intermediate product of the oxidation of polyunsaturated fatty acids .[14]

Our data show that there is an age related increase in lipid peroxidation expressed as MDA and low levels of GSH in the circulation which may be due to their increased utilization to scavenge lipid peroxide: GSH acts as a substrate in the detoxification of peroxides like H_2O_2 and lipid peroxides , a reaction involving GPX . This generates oxidized GSH(i.e GSSG),which is subsequently reduced by GR in a reaction mtilising .

There were significant increased in catalase NADPH and GST in group3 compared with other groups.

Several enzymatic system can detoxify free radicals copper/zinc-superoxide dismutase (SOD) catalyzes the conversion of the superoxide anion to hydrogen peroxide and works concomitantly with hydroperoxide, removing enzymes such as catalase and glutathione peroxidase.

The conversion of H_2O_2 to hydroxyl radical may be enhanced by iron, these mechanisms may contribute to the increased serum lipid peroxidation.

GST provide protection from membrane lipid peroxiation, the increase in GST induction confers resistance to the cells against oxidative stress.[15]

Albumin ametal-binding proteins is shown to possess free radical scavenging properties, and may be selective antioxidants, in our study serum albumin concentrations showed significant increased in group2 compared with other groups while haemoglobin in this group showed significant lowered with other groups.

No significant differences in serum total protein and uric acid with age.

Conclusion:

Determination of lipid peroxidation and antioxidant status ,may be useful test to evaluate oxidative stress.

The depletion of GSH seems to be a major determination of age effect, whereas increased catalase and GST provide a suitable mechanisms for detoxification.

Further studies are needed to clarify the relations between lipid peroxidation and antioxidative function.

References:

1-Mc Call MR, frei B. can antioxidant vitamins materially reduce oxidative damage in

humans? Free radical Biol Med; 26(7/8); 1034-1053. 1999
2-Dasgupta, A , Hussain S , Ahmed S, increased lipid peroxidation in patients on maintenance hemodialysis , Nephron; 60:56-59. 1992
3-Halliwell, B; Free radicals , antioxidants , and human disease: curiosity, cause , or consequence? The Lancet; No 1.344. September 10:721-724. 1994
4-Babior, B M, woodman RC .chronic granulomatous disease. Semin Hamatol ; 27:247-59. 1990
5-Maiorino, M ; Chu FF ; Ursini F ; Davies K ; Doroshow JH: Essworthy RS. phospholipid hydroperoxide glutathione peroxidase is the 18 K Daseleno protein expressed in human tumor cell lines. J Biol. chem.;266;7728. 1991
6-Halliwell, B ; Gutteridge JMC. Free radicals in biology and medicine. 2nd cd. oxford. clarendon press ,1989.
7-Halliwell, B ; Gutteridg JMC.The antioxidants of human extracellular fluids. Arch Biochem. Biophys; 280, 1-8. 1990
8-Evans, P. and Halliwell B. , Micronutrients: oxidant/antioxidant status. British Journal of Nutrition, 85, suppl. 2, 567-574 ,2001
9-Kaur. H, Halliwell B. Action of biologically. relevant oxidizing species up on uric acid. chem. Biol. interact; 73; 235-47 ,1990
10-Godin, D.V. ; Wohaieb, S.A. ; Garnett , M.E.etal: antioxidant enzyme alterations in experimental and clinical diabetes. Mol cell Biochem; 84:223-231. 1988
11-Aebi , H. :Erythrocyte catalase . Methods in enzymatic analysis. Bergmeyer, H.V.(ed), Velay chemie, weinheim. P.673,1974.
12-Habig, W.H. ; Pabst, M.J. and Jakoby , W.B. : colorimetric method for determination of glutathione-s-transferase in the serum. J Biol chem; 249:7130-7136. 1974
13-Gonen, G A. :Yesim A talk ; Mohmet N ; et al: Lipid peroxidation and antioxidant systems in haemodialyzed patients (Dialysis an transportation) : Vol. 31.No2. 2002
14-Baliga, R ; Meda N; shan sv. oxidant mechanism in glomerular disease. In ;Current Nephrology; Vol. 20, P. 135-151. 1997
15-Montserrat , M ; Arthur 1; cederbaum ; American association for the study of liver disease. 2001.