Lipid peroxidative damage in the erythrocytes and elevation of serum LDL-cholesterol, apolipoprotein-B, ferritin and uric acid with age and in coronary heart disease patients

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ABSTRACT

Objectives: To determine the normal serum levels of LDL-cholesterol, apolipoprotein-B, ferritin, uric acid, and the extent of erythrocytes lipid peroxidation in healthy control group subjects and to compare them with coronary heart disease patients. Secondly, to study the effects of age and sex on these parameters.

Methods: The blood samples from 150 healthy Libyan control group subjects (110 men and 40 women) were classified into 3 groups according to their age. Group I consisted of 76 subjects with an age range from 20 to 35 years. Group II consisted of 45 subjects with an age range from 36 to 50 years. Group III consisted of 29 subjects with an age range from 51 to 74 years. The blood samples from these groups were analyzed for LDL-cholesterol, apolipoprotein-B, ferritin and uric acid levels. Lipid peroxidation was compared in the erythrocytes of 56 selected healthy control group subjects (31 men and 11 women) of the aforementioned age groups.

Results: These parameters have shown age-dependent elevation in their levels. Meanwhile, LDL-cholesterol and Apolipoprotein-B levels in female subjects were higher than those of males. However, lipid peroxidation in the erythrocytes has revealed a statistically significant increase with increasing age. The comparison between 93 selected, sex and age matched, healthy control group subjects with 87 selected coronary heart disease patients (55 men and 45 women) with an age range from 30 to 74 years (49.6±13.25) has demonstrated significantly higher concentration of LDL-cholesterol, Apolipoprotein-B, ferritin and uric acid in coronary heart disease patients than those of healthy control group subjects. Meanwhile, lipid peroxidation was also significantly enhanced in coronary heart disease patients compared with healthy control group subjects.

Conclusion: Our study has revealed that an increase in the lipid peroxidation in erythrocytes with age and during coronary heart disease, makes red cell membranes more vulnerable to free radical damage via formation of reactive oxygen species. It is thus likely that peroxidative damage may be contributing to an increase in serum LDL-cholesterol, Apolipoprotein-B, probably after its oxidative modification, increase in ferritin and hyperuricemia in coronary heart disease patients.

Keywords: Lipid peroxidation, LDL-cholesterol, Apo B, ferritin, uric acid, coronary heart disease.

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Over the past 3 decades, great progress has been made in identifying cardiovascular risk factors and in developing and implementing measures to correct them.[1] A large number of reports have shown that the incidence of coronary heart disease is more common in men (male:female ratio = 2:1).[2] The

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incidence increases with age (males >45 years, females >44 years).[3,4] Numerous studies also provide the epidemiological, pathological and clinical evidence that incidence increases in smokers.[5] The incidence is also high in patients with hypertension, diabetes, hyperuricemia, hyperlipidemia (some plaques of atherosclerosis are derived from LDL-cholesterol (LDL-c)), and in patients with high serum ferritin.[6-11] Salonen and coworkers[12] presented a landmark study with the first empirical evidence that serum ferritin is a strong risk factor for acute myocardial infarction. Furthermore, cardiovascular diseases including atherosclerosis and cardiac tissue injury after myocardial infarction have been shown to result in part from lipid peroxidation (LPO) at the site of damage.[13] The lack of systematic investigation of the plasma lipid levels and lipoprotein fractions in selected groups in Libya and the great demand for surveillance of coronary heart disease (CHD) risk factors in such groups with low plasma cholesterol level,[14] and low incidence of CHD[15] has motivated us to investigate. Firstly, to study the common risk factors like: LDL-c, Apolipoprotein-B (Apo-B), ferritin and uric acid, and to study the erythrocytes LPO in healthy control group subjects and CHD patients in the Benghazi area of Libya. Secondly, to study the effect of age/sex on these risk factors. Thirdly, to correlate these risk factors with CHD among the Libyan population in the Benghazi area.

Methods.

The study group consisted of 150 healthy control group Libyan subjects (110 men and 40 women) ages ranging between 20-74 years (37.4 $\pm$SD 13.20) and 100 patients with CHD (55 men and 45 women) ages ranging between 30-74 years (49.46 $\pm$ SD 13.25). The CHD group comprised patients of myocardial infarction, angina pectoris and ischemic heart disease attending hospitals and polyclinics. The healthy control group subjects were selected from blood donors at the Central Blood Bank in Benghazi, Libya, and volunteered employees of Al-Arab Medical University. They had a normal resting electrocardiogram, had no history of CHD, hypertension or diabetes mellitus. They were divided into 3 age groups. In group I there were 76 subjects, ages ranging between 20 - 35 years. In group II there were 45 subjects, ages ranging between 36 - 50 years and in group III 29 subjects, ages ranging between 51 - 75 years.

Preparation of serum samples. Fasting blood samples (serum) were drawn from seated individuals. The blood samples were placed into plain evacuated glass tubes and kept for 30 min at room temperature. The serum was separated by centrifugation at 300g for 15 minutes using bench centrifuge (Hettichi-Universal, Germany). Serum samples were separated into 3 different aliquots and stored at 30° till the day of analysis.

Serum analysis. Enzymatic determination of serum uric acid was performed according to the method of Kabasakalian[16] by using bioMerieux (France) Kit PAP 150. The concentration of serum LDL-c was evaluated by using bioMerieux (France) kit according to the procedure as described by Steinberg.[17] The Apo-B content in the serum samples was measured by immunoturbidimetric assay, as described by Rifai and King.[18] The serum ferritin levels were quantitatively determined by one step sandwich Enzyme Linked Immunoassay (ELISA). The assay was based on the method as described by Zuyderhoudt.[19]

Estimation of erythrocytes lipid peroxidation. Membrane peroxidation was carried out according to the procedure as described by Quinlan et al.[20] Fresh blood from 56 healthy control group subjects of either sex from 3 age groups (13-38 years, 40-55 years and 60-65 years) and from 28 CHD patients, age and sex matched, was collected in EDTA-containing sample collection tubes. The erythrocytes were spun down and plasma was discarded. The packed cells were washed thrice with an equal volume of 0.15M NaCl and spun down again. The supernatant was removed and cells were stored at -70°C in the deep freezer until analyzed within 1 hour. Packed erythrocytes were preincubated with phosphate-saline buffer (pH 7.4) containing sodium azide to inhibit catalase. Peroxidation was initiated by adding H_2O_2 (10mM) and the mixture was incubated at 37°C for 2 hours. The reaction was terminated by the addition of 28% (w/v) trichloroacetic acid. Supernatants were heated with thiobarbituric acid (1% w/v, in 0.05 M NaOH) at 100°C and the absorbance of the malondialdehyde-thiobarbituric acid adduct was measured at 532 nm.

Statistical analysis. The statistical analysis of data was carried out by using Statgraphics and Excel Computer Program Packages. The results were expressed as means $\pm$ SD. The difference between 2 means was calculated by students 't' test. The differences between the means of more than 2 groups was analyzed by One-Way Analysis of Variance. F ratio was calculated for homogeneity of variance, and the test of significance was calculated from percentage points of the F distribution table. Categorical variables were analyzed by Chi-square (X^2) test.

Results.

The serum samples (in duplicate) of healthy control group subjects were analyzed to determine the levels of LDL-c, Apo-B, ferritin, and uric acid. Furthermore, LPO of erythrocytes was measured. The levels of serum LDL-c ($P<0.001$); Apo-B ($P<0.001$), ferritin ($P<0.01$) showed an increasing tendency with age (Table 1). Table 2 shows that the increase in LDL-c was higher in females ($P<0.001$) than in males, while serum ferritin

Table 1 - The levels of serum LDL-cholesterol, Apolipoprotein B, Ferritin and Uric acid in the healthy control group subjects of different age groups.

Parameter	Group I Age 20 - 35 years (n = 76)	Group II Age 35 - 50 years (n = 45)	Group III Age 51 - 74 years (n = 29)	F ratio	P value
LDL-cholesterol (mg/dl)	82.67 ± 22.44	105.50 ± 7.61[a]	127.86 ± 23.46[a,b]	19.99	<0.001
Apolipoprotein B (mg/dl)	55.70 ± 21.60	103.70 ± 45.96[a]	105.31 ± 37.44[a]	8.44	<0.001
Ferritin (ng/dl)	70.34 ± 26.48	75.68 ± 38.88[a]	121.55 ± 52.19[a,b]	4.91	<0.01
Uric acid (mg/dl)	4.32 ± 0.93	4.46 ± 0.98	4.79 ± 1.38	2.22	NS

[a] significantly different from group I; [b] significantly different from group II;
The values represents the mean ± SD; n = number of samples in each group.

Table 2 - Effect of sex on LDL-cholesterol, Apolipoprotein B, Ferritin and Uric Acid in healthy control group subjects.

Parameter	Male Age 42.47 ± 4.71 years (n= 25)	Female Age 40.0 ± 2.12 years (n= 20)	P value
LDL-cholesterol (mg/dl)	102.06 ± 40.07	113.69 ± 30.84	> 0.05
Apolipoprotein B (mg/dl)	99.16 ± 40.60	107.28 ± 38.03	> 0.05
Ferritin (ng/dl)	97.56 ± 32.16	37.29 ± 12.70	< 0.001
Uric acid (mg/dl)	4.65 ± 0.80	4.03 ± 0.097	< 0.05

The values represents the mean ± SD; n = number of samples in each group.

Table 3 - Lipid peroxidation in erythrocytes of male and female healthy control group subjects and coronary heart disease patients.

Groups	Lipid peroxidation (malondialdehyde-thio-barbituric acid adduct) absorbance at 532 nm		P Value
	Control	CHD-Patients	
Male	0.190 ± 0.025 (n = 14)	0.280 ± 0.038 (n = 14)	< 0.05
Female	0.180 ± 0.036 (n = 26)	0.280 ± 0.030 (n = 14)	<0.05

Results are expressed as the mean + S.D.
n = number of samples in each group.

Table 4 - The levels of serum LDL-cholesterol, Apolipoprotein B, Ferritin and Uric acid in healthy control group subjects and coronary heart disease (CHD) patients.

Parameter	Control (n = 36)	CHD-Patients (n = 64)	Z value	P value
Male/Female ratio	2.09	2.10	X^2	> 0.5
Age (years)	44.70 ± 11.26	46.71 ± 11.56	1.18	< 0.001
LDL-cholesterol (mg/dl)	109.49 ± 34.42	160.85 ± 48.89	8.18	< 0.001
Apolipoprotein B (mg/dl)	94.75 ± 42.74	106.06 ± 38.34	1.35	< 0.05
Ferritin (ng/dl)	81.79 ± 76.67	111.95 ± 10.95	2.0	<0.05
Uric acid (mg/dl)	4.62 ± 1.02	6.29 ± 1.85	7.57	<0.001

The vales represent the mean ± SD
n = Number of samples in each group

and uric acid concentration in females was significantly lower than males (P<0.001; P<0.001). However, there was no sex difference for Apo-B levels. On the contrary, the erythrocytes LPO was greater (4%) in males than in females (Table 3). The second study (Table 1 and 2) was carried out after age/sex factors were eliminated between healthy controls and CHD patients of identical age/sex. In our study CHD patients (Table 4) have shown increased levels of uric acid, LDL-c, Apo-B and ferritin (P<0.001; P<0.05 and P<0.001) when compared to age/sex matured controls. Table 3 shows statistically significant enhancement (P<0.001) in the erythrocytes LPO in CHD patients (both male and female) compared to the healthy control group subjects.

Discussion. Coronary heart disease represents one of the most important health problems all over the world. During the past 4 decades a large number of studies have been performed concerning the effect of age and sex on CHD and its correlation with blood lipids and lipoprotein levels in several populations from Asia, Middle East, Africa, Europe and America.[21] The results of the present study have revealed that the levels of LDL-c significantly increase with age. Slightly higher levels of LDL-c in males than females in early age was also evaluated, while the LDL-c was significantly higher in females than the males in age groups more than 50 years. The results of our study are in agreement with an American population study and other reports in different populations.[22] On comparing the report by El-Fakhri et al on the Libyan population, with this study, it was evident that the level of LDL-c was higher than those reported by El-Fakhri et al.[14] After 1987, the lifestyle of people in Libya changed. Modernization, sedentary life, higher standards of living, increased intake of meat and fats. Our data shows that LDL-c increases with increasing age and more so in females. The increase is definitely associated with an increased risk of CHD. Mannies et al[23] also reported that LDL-c was a risk factor in CHD. West et al[24] suggested a strong correlation between elevated LDL-c levels and CHD. However, Wu et al[25] demonstrated no correlation between high LDL-c with CHD. Ecological studies have clearly shown that populations with low-fat intake have lower LDL-c and lower incidence of CHD than populations with high fat intake.[26] Indeed we have also found lower LDL-c levels in the Libyan population compared to that shown in populations of developed countries.

Iron overload is a major cause of greater occurrence of CHD in men than in women.[27] So far, no information is available on the iron paradigm of CHD among the Libyan population, and this study seems to be a singular attempt in this area. Our data has demonstrated that normal standard values of serum ferritin are consistent with previous reports.[27] The serum ferritin levels exhibited an age-dependent significant increase in healthy control group subjects (Table 1), but remarkably low levels of serum ferritin were elevated in females compared to male (Table 2) healthy control group subjects. Sullivan[27] has put forward the hypothesis that an increase in serum ferritin increases the risk of CHD, which is in agreement with our results. Substantially low levels of serum ferritin in young women in our study is especially relevant to the hypothesis that low iron stores of females in the reproductive age group (menstruation, pregnancy, and lactation reduce iron stores) protects against CHD.[27] Our investigation has revealed a significant elevation in serum ferritin levels in CHD patients compared to healthy control group subjects. The findings may be explained on the basis of the fact that cell injury after traumatic or ischemic insult has the potential to accelerate free radical reactions.[28] Superoxide radicals are known to liberate iron from ferritin, promoting LPO.[27,29-31] Interestingly, for the first time among the Libyan population, we have estimated a greater erythrocytes LPO in CHD patients compared to normal healthy control group subjects (Table 3). These findings predict that iron released from ferritin in CHD patients is stimulating LPO. It is thus likely that elevated serum ferritin levels in CHD patients in our study could be a greater risk factor for CHD among the Libyan population.

To date, there is lack of information in the Libyan population on the interrelationship between uric acid and CHD patients. The normal standard serum uric acid levels in our study are well in congruence with the previous reports.[32] Furthermore, serum uric acid levels in healthy control group subjects were significantly lower in females than their corresponding males. Jossa et al[33] has shown that elevated serum uric acid levels are associated with the aging process in humans. Lee et al[34] has postulated that elevation in serum uric acid increases the risk of CHD, subsequently leading to atherosclerosis. We have also evaluated an increase in serum uric acid levels that seems to be associated with CHD. The association of uric acid with CHD may well be explained on the basis of the fact that when endogenous antioxidant defense capabilities are exceeded in reperfusion injury by oxidant influx that leads to tissue injury.[35] The enzyme xanthine oxidoreductase, that catalyzes terminal oxidation of purines to uric acid, exists in 2 catalytic active forms. The xanthine dehydrogenase form and xanthine oxidase form, which predominates in animal tissues. Xanthine oxidase uses molecular oxygen and generates superoxide anions. The hypothesis based on the cat intestine has revealed that ischemia results in an accumulation of purine (hypoxanthine and

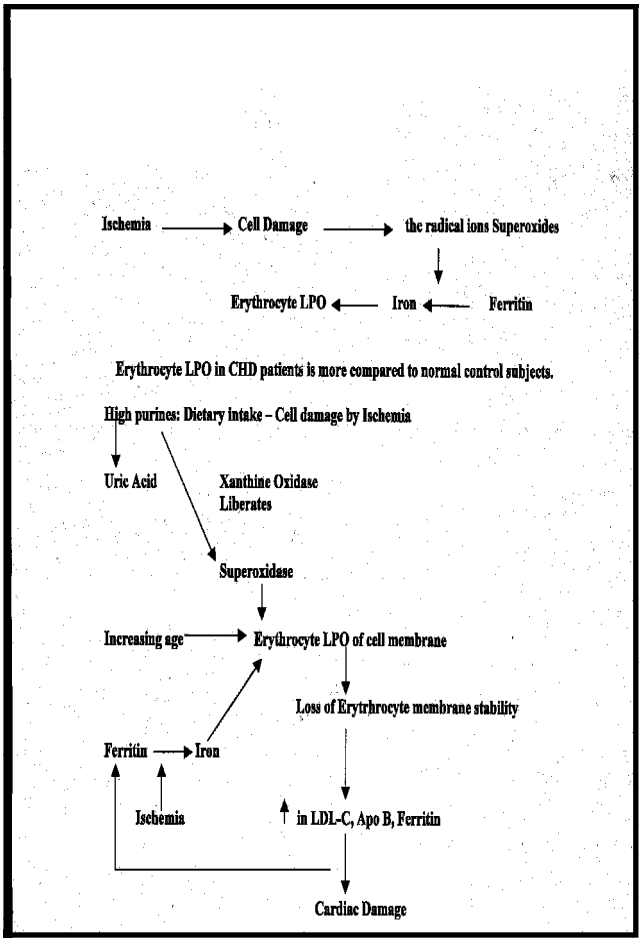

Figure 1 - Mechanism of erythrocytes lipid peroxidation.

xanthine) from the catabolism of ATP.[35] The abundance of O_2 rapidly drives purines oxidation by xanthine oxidase, thus generating the superoxide free radical.[35] It is thus likely in our study, as has been suggested by Rangan and Bulkley,[36] that the faster rate of oxidation of purine to uric acid has resulted in hyperuricemia.

As yet, no comparable report is available among the Libyan population on LPO of erythrocytes and CHD patients, and our study seems to be a singular attempt in this field of research. It has been shown that cardiovascular diseases, atherosclerosis and cardiac tissue injury after myocardial infarction are in part caused by generation of free radicals at the site of damage.[36,37] A possible mechanism of erythrocyte LPO in our study may be shown in Figure 1. Future studies are needed to assess the effect of antioxidant defense systems on erythrocytes LPO and CHD risk factors.

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