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***Full Length Research Paper***

# Oxidized lipids and lipoproteins in patients with stroke: A pilot study

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**ABSTRACT**

The objective of this pilot study was to investigate the status of oxidized lipid in Iraqi stroke patients. Blood samples were collected from subjects diagnosed with stroke and blood lipids and oxidized lipoprotein were analyzed using enzymatic and colorimetric methods. We found a significant ($p < 0.001$) increase in total lipids, and total lipid oxidation as measured by serum aldehyde malondialdehyde. We also found a significant increase in level of oxidized low density lipoproteins in patient with stroke in comparison with healthy individuals. In conclusion, our finding supports the use of oxidized LDL as a marker of stroke.

**Keywords:** Blood, colorimetric methods, malondialdehyde, ischemic stroke, cholesterol.

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## INTRODUCTION

Stroke is a serious life threatening condition which has significant consequences on patient's life quality and wellbeing. Many chronic diseases are behind the pathophysiology and incidence of stroke. Among those are hypertension (Wolf et al., 1991; Wolf et al., 1986), cardiovascular disease (Davis and Hachinski, 1992), and diabetes mellitus (Santos-Lasaosa et al., 2000). One of the major etiological factors associated with stroke incidence is obesity and changes in patient's lipid profiles (Weycker et al., 2007; Goldberg, 2014).

Several studies reported that a combination of low level of high density lipoprotein-cholesterol (HDL- c) and high total cholesterol and low density lipoprotein-cholesterol (LDL-c) is, almost always, associated with risk of stroke (Tirschwell et al., 2004). This phenomenon highlights the question of what type of lipid increases prior to or during stroke. Brain synthesised its own cholesterol which is metabolized into 24S- hydroxycholestrol and released into circulation. Cholesterol transports in the body via different classes of lipoproteins. The lipoprotein family includes five classes, chylomicrons, very low density lipoprotein-cholesterol (VLDL), LDL, intermediate density lipoprotein-cholesterol (LDL-c) and HDL-c.

During ischemia and reperfusion, significant amounts of free radicals are formed by several mechanisms (Mc Cord, 1985). First, disturbances in brain blood flow during stroke and reperfusion of oxygen is responsible about most of generated species of free radicals (Mc Cord, 1985). Second, reduction in antioxidant defence capacity and increase in oxidative play a pivotal role in the pathogenesis of stroke-associated neuronal injury (Braughler and Hall, 1989). The aim of this pilot study is to investigate the status of oxidized lipoproteins in Iraqi patients presented with stroke.

## MATERIALS AND METHODS

The average age of subjects recruited in this study was 60 ± 9.3 years. Two groups of subjects were enrolled, Group I: (control, n = 23), an apparent healthy individuals of both sexes (18 males and 5 females) were allocated randomly from AL-Yarmouk Teaching Hospital for this study. Exclusion criteria: subjects diagnosed with hypertension, diabetes mellitus, hypothyroidism and ischemic heart disease.

Group II is the stroke test patients that included 23 subjects. The inclusion criteria for the stroke group included: Patients with cerebrovascular accident (stroke) who were admitted to the Medical Ward at AL-Yarmouk Teaching Hospital within 24 h of stroke event. The diagnosis of stroke is based on radiological investigations including computerized tomography (CT-scan) and magnetic resonance image (MRI) of brain.

Ischemic stroke was reported in twenty one cases (91.3%) and hemorrhagic stroke in two cases (8.7%), occlusion of middle cerebral artery was observed in ten cases (43.47%) and one of these cases also shown occlusion of anterior cerebral artery. Posterior artery was occluded in two cases. Stroke patients were thoroughly examined and managed in hospital by consultant neurology. Data were obtained from each patient or his/her accompanied relatives in respect to the designed consent forums constructed to fulfill the requirements of the purpose of this work. The study design and its protocol were approved by the Scientific Committee of AL-Mustansiriya University and samples were collected after the informed consent signed. All procedures have been performed in accordance with the principles of Declaration of Helsinki.

#### Blood sampling and lipid profile

Ten millilitres of fasting venous blood sample were collected from each subject included in this study. Serum samples were separated from each blood sample and processed for determination of the following lipid profile using enzymatic method (Deeg and Ziegenhorn, 1982; Young et al., 1975) and commercially available kits: serum total cholesterol (TC), high density lipoprotein (HDL-C), and low density lipoprotein (LDL-C).

#### Lipid oxidation assessment

Lipid peroxidation was assessed as thiobarbituric acid reactive substance by spectrophotometer following the methods of Buege and Aust (1978), and Allain et al. (1974). Susceptibility of lipoproteins to oxidation was assessed by copper induced oxidation method. Oxidized low density lipoprotein (ox-LDL) level was measured followed the method described by Harris et al. (1998).

#### Statistical analysis

Student t-test was used to determine mean values and the significance different of lipid profile and oxidized LDL-c and HDL-c results for stroke patients and normal healthy control groups.

P values < 0.05 were considered significant. Prism software package was used for statistical analysis of the results.

## RESULTS

In this study, the stroke test group included 14 patients diagnosed with hypertension, 3 diagnosed with diabetes, one patient diagnosed with familial hyperlipidemia, one patient diagnosed with atrial fibrillation and 9 smokers. As mentioned above, the control group exclusion criteria ensured that all control subjects are free of any of the stroke risk factors.

Serum lipid profile results in patents diagnosed with stroke showed significant changes (Table 1). Although the mean cholesterol levels was within the normal reference value, it was significantly ($p < 0.05$) higher than the mean serum levels of healthy individuals. Mean serum level of LDL-c of patients with stroke was significantly higher ($P < 0.01$) than that of healthy individuals. There was a significantly ($P < 0.01$) lower level of HDL-c in stroke patients in comparison to healthy control subjects.

HDL-c: High density lipoprotein-cholesterol; LDL-c: Low density lipoprotein-cholesterol. The above results are expressed as mean ± SD of number of observation. All comparison are between the study group and the control group *$P < 0.05$, ** $P < 0.01$.

We found a significantly higher ($p < 0.001$) level of malondialdehyde (MDA), as marker of total lipid peroxidation in serum of stroke patients when compared to normal healthy individuals (average 6.061 ± 2.490 versus 0.504 ± 0.123 nmol/ml respectively) (Table 2).

The measured value of ox- LDL (or modified MDA-LDL) in patients with stroke was significantly ($p < 0.01$) higher than that of the healthy individuals (Table 2). We found strong correlation of ischemic stroke with the level of ox-LDL and weak association with haemorrhagic stroke (Table 3).

## DISCUSSION

In this study, we investigated the changes in serum lipids and oxidized lipid profile in Iraqi patients diagnosed with stroke. Lipids are significant etiological factors in stroke incidence and mortality. Analysis of specific population profile is quite significant in understanding of population health and well being. Although we had small population ($n = 23$), it was possible to conclude that male stroke patients numbers (78.3%) was significantly ($p < 0.001$) higher than females stroke numbers (21.7%). In regard to stroke risk factors, as stated in the above results, 19 of the subjects recruited in this study were diagnosed with hypertension. Hypertension is well known risk factor in stroke (Wolf et al., 1991). HDL-c concentrations were much lower in stroke patients when compared to matching control values. This result is in agreement with the previously published studies which listed lower levels of HDL as a stroke risk factor (Sridharan, 1992).

In this study, total serum cholesterol and LDL-c concentration in stroke patient was significantly higher ($P < 0.01$) than those of the control. At the time of this study, none of the recruited patients were receiving dyslipidemia treatments. Our result is in agreement with a previously published study which reported a similar result in Iraqi population (Ewadh et al., 2005). It is worth mentioning that average level of cholesterol in stroke patients falls at the lower spectrum in comparison to published guidelines of dyslipidemia (Stone et al., 2013; Slemmer et al., 2008). In this study, we found that serum level of LDL in patients with stroke was significantly ($P < 0.01$) higher than that of healthy individuals (144.93 ± 50.63 vs. 110.5 ± 32.98). Our result is a further conformation that LDL is a significant risk factor in stroke.

As shown in Table 2, serum level of MDL was about 12 times higher in stroke patients in comparison to control subject values. MDL elevation is a strong indicator of overall free radicals generation and cellular stress. In

**Table 1.** Lipid profile of stroke patients and normal subjects.

| Lipid profile variable | Group I (n = 23) | Group II (n = 23) |
|---|---|---|
| Total cholesterol (mg/dl) | 169.77 ± 35.65 | 196.41 ± 49.83* |
| HDL-c (mg/dl) | 44.24 ± 6.12 | 40.23 ± 15.59** |
| LDL-c mg/dl) | 110.5 ± 32.98 | 144.93 ± 50.63** |

The above results are expressed as mean ± SD of number of observation. All comparison are between the study group and the control group * P < 0.05, ** P < 0.01.

**Table 2.** Oxidative stress markers.

| Serum MDA (nmol/ml) | | Ox-LDL nmol/ml | |
|---|---|---|---|
| Group I | Group II | Group I | Group II |
| 0.50 ± 0.12 | 6.061 ± 2.49** | 0.23 ± 0.11 | 5.9 ± 0.65** |

The above results are expressed as mean ± SD of number of observation. All comparison are between the study group and the control group * P < 0.05, ** P < 0.01.

**Table 3.** Association of type of stroke with ox-LDL.

| Type of stroke | Within normal ox-LDL | Higher than control ox-LDL |
|---|---|---|
| Ischemic stroke | 3 | 18 |
| Haemorrhagic stroke | 1 | 1 |

Results are analyzed using the mean with Yates correction: 14.24, Df = 1, (p < 0.001).

stroke, brain cell injury and death occur in two stages. In the first stage, oxygen and glucose deprivation take place, and in the second stage, free radical generation peaks during the re-oxygenation period (Stone et al., 2014).

The results presented in Table 2 show that ox-DL in stroke patient was significantly (P < 0.01) higher than of the control subjects. Our results re-confirmed the role of free radicals in stroke process. Our calculation of the association of ischemic stroke with abnormal high level of ox- LDL had shown that it is highly significant (P < 0.01) in comparison to association of ox-LDL with hemorrhagic stroke. The role of LDL in stroke etiology is quite critical as it has pro-inflammatory effect on the vasculature and it is an integral part of the pro-atherogenic mediators. Recently, it has been demonstrated that circulatory oxidized LDL has strong association with incidence of stroke, coronary diseases, diabetes and metabolic syndrome (Bernabé García et al., 2014; Md Sayed et al., 2014). As shown in Table 3, we found a strong correlation between oxidized LDL level and ischemic stroke. Our results support the recent suggestion to use serum level of oxidized LDL as a marker for stroke and coronary heart disease (Tsai et al., 2014).

## Conclusion

Our study demonstrated that LDL is a significant risk factor in stroke. In this study, oxidized LDL showed strong correlation to ischemic stroke in Iraqi patients. The current work supports the suggestion to use ox-LDL level as marker for stroke diagnoses and treatment.

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