# SALIVARY LIPID PEROXIDATION AND LIPID PROFILE LEVELS IN PATIENTS WITH RECENT ISCHEMIC STROKE

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**Abstract**

Abnormal lipid levels are an important risk factor in the development of atherosclerotic complications like stroke. Oxidative stress, lipid and lipoprotein Peroxidation are involved in neuronal damage induced by ischemia-reperfusion in stroke. Malondialdehyde (MDA) is widely utilized as a marker of lipid Peroxidation in state of elevated oxidative stress. The role of lipid in oxidative damage in saliva of patients with stroke is not yet completely elucidated. The aim of this study was evaluate the relationship of MDA as a marker of lipid Peroxidation with lipids and lipoprotein fractions and to find out the cut-off values of the studied parameters in saliva of patients with recent stroke .

We studied 50 patients with ischemic stroke and other 25 ages and sex matched health control. To evaluate the oxidative status we measured the levels of Malondialdehyde in saliva and serum of all participants. Lipid profile was also estimated by the total cholesterol, triglycerides, LDL-C and HDL-C.

MDA levels were significantly higher in patients with ischemic stroke than that of healthy control. Salivary critical value of triglycerides more than 0.5 mmol/L yields in highest accuracy rate (93%) to differentiate patients with ischemic from healthy control , followed by salivary MDA values which should be equal to or more than 0.38 µmol/L to be helpful in differentiation between two groups with (92% accuracy).

Assessment of salivary lipid Peroxidation together with salivary lipid profile may be useful in early detection and monitoring of patients with increased risk of stroke.

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## Introduction

Stroke (CVA) is a term that describes a clinical events caused either by occlusion or hemorrhage in the arterial blood supply to the CNS resulting in tissue infarction [1]. It is potentially the most common cause of severe disability. In term of mortality, stroke is the third most common cause of death in industrialized countries, following coronary heart disease and cancer [2]. As a matter of fact, cholesterol can be differently involved in stroke depending on the etiologic subtypes [3]. The elevated low density lipoprotein cholesterol (LDL-C) and reduced high density lipoprotein cholesterol (HDL-C) may increase the risk of athero- thrombotic brain infarction [4]. The higher levels of products of lipid and protein oxidation observed in plasma isolated from stroke patients compared to healthy subjects demonstrated that oxidative damage is involved [5-9].

Oxidative stress, lipid and lipoproteins Peroxidation and inflammation are involved in neuronal damage induced by ischemia-reperfusion [10]. The brain contains high levels of polyunsaturated fatty acids in membrane lipids; therefore, lipid Peroxidation is one of the major consequences of free radical-mediated injury to brain [11]. Malondialdehyde (MDA) level is widely

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utilized as a marker of lipid Peroxidation in state of elevated oxidative stress [12]. MDA is one of many low molecular weight end products of lipid Peroxidation [13]. The role of lipid in oxidative damage in saliva of patients with stroke is not yet completely elucidated .Saliva is increasingly used and well validated in diagnosing, monitoring systemic diseases status and predicting diseases progression [14]. It is an important physiologic fluid that contains a highly complex mixture of substances. Salivary assays present lots of advantages when compared to blood assay; the sampling is very easy to do especially in non-medical environment, it does not disturb intimacy when control is needed. Multiple samples could be collected providing more information than that of single blood sample [15].

Karajalainen et al [16] assessed cholesterol in saliva of healthy adults; they concluded that salivary concentration levels reflect serum concentration to some extent. The present study was undertaken to evaluate the relationship of MDA as a marker of lipid Peroxidation with lipids and lipoprotein fractions and to find out the cut-off values of the studied parameters in saliva of patients with recent stroke and compare it with healthy individuals.

## Materials and Methods

Seventy five individuals from Al-Diwaniya province in Iraq were enrolled in this observational study. They are grouped into two groups, the study group which consist of fifty patients (24 males and 26 females) having recent attack of ischemic stroke, the other twenty five (12 males and 13 females) were age and sex-matched healthy individuals and served as control group.

All individuals were evaluated by full medical history and clinical examination with laboratory investigations to exclude any other systemic and /or local diseases that may affect the parameters examined in this study.

Oral and periodontal examination was done for each individual and anyone with symptoms and signs of any active oral inflammation, advanced periodontitis or severe gingivitis were excluded from the study. The study was approved by the Institutional Ethical Committee of Al-Diwaniya Teaching hospital, prior signed consent was taken from all individuals participating in the study.

### *Laboratory Analysis*

Saliva and blood samples were taken from each participant after overnight fasting (8.00-9.00 am). For serum isolation, 10 ml of blood sample was taken from each individual, centrifuged at 3000 rpm at 4 $^0$C for 5 minutes; the supernatant was aspirated and stored in tubes at -20 $^0$C until analyzed. Saliva samples were always collected in restful and quite circumstances following flushing of the mouth with 100 ml of distilled water. The whole saliva was collected for 5 minutes by the individual leaning forward and spitting saliva in test tubes that were cold centrifuged at 3000 rpm at 4 $^0$C for 5 minutes. The supernatant was aspirated and stored at -20 $^0$C until analyzed.

Salivary and serum lipid Peroxidation products, Malondialdehyde (MDA), was measured by the method outlined by Buege and Aust[17] where MDA react with Thiobarbituric acid (TBA) to yield a pink color product. The absorbance of 3 ml colored layer was measured at 335 nm spectrophotometrically.

Total cholesterol and triglycerides concentration in saliva and serum were measured by enzymatic methods[18, 19]. The HDL-C concentration was measured by the method described by Warnick et al[20]. LDL-C concentration was then calculated from the concentration of total cholesterol, HDL-C and triglycerides by Friedwald and Levy method[21].

All data were analyzed with SPSS-17 (Chicago, IL-USA). The significance of difference in the mean between groups was performed by Benferoni test. To compare the diagnostic performance of each test, Receiver Operating Characteristic (ROC) curve test was used. A p-value $< 0.05$ was considered statistically significant.

## Results

The mean age of patients with ischemic stroke was 58.2 years. All of them had history of one or more of the following underlying diseases: Hypertension (82%), diabetes mellitus (68%), ischemic heart diseases (30%). Regarding smoking habit, 52% of ischemic stroke patients were heavy smokers (table 1).

MDA and lipid sub fractions concentration in saliva and serum in study group did not vary with age and gender, therefore results of both gender were grouped together. All tested

parameter in saliva of ischemic stroke group has a significant direct relation to that recorded in serum, except for total cholesterol and LDL-C (table 2). Salivary concentration of MDA, triglycerides, LDL-C was significantly higher in patients with ischemic stroke when compared with that of control group. Salivary HDL-C concentration, on the other hand, was significantly lower in study group than that in control group (table 3).

Serum total cholesterol and LDL-C concentration did not show any significant differences between the study and control group (table 4).

Pearson correlation was applied for different parameters used in the present study; a highly significant direct correlation was observed between MDA with triglycerides and LDL-C in both saliva and serum of study groups, whereas a highly significant negative correlation was seen between MDA and HDL-C (table 5).

The atherogenic index as indicated by various risk ratios is shown in table (6). The risk ratio calculated as total cholesterol/HDL-C, LDL-C/HDL-C and Triglycerides/HDL-C. All ratios were significantly elevated in patients with stroke when compared to control. Since increased lipid Peroxidation is also risk factor for Ischemic stroke, it has been suggested that MDA values in saliva and serum multiplied by risk ratios may provide a new index which serves as better predictor of Ischemic stroke. All the ratios multiplied by MDA were significantly elevated in stroke patients when compared with controls.

ROC curve equation was applied for different cut-off values of the selected parameters to differentiate individuals with ischemic stroke from healthy one. The area under ROC curve for salivary triglycerides, LDL-C and MDA were significantly higher from 0.5 value of an equivocal test (table 7). Salivary critical value of triglycerides was ≥ 0.5 mmol/L yields in highest accuracy rate (93%) to differentiate patients with ischemic from healthy control , followed by salivary MDA values which should be equal to or more than 0.38 µmol/L to be helpful in differentiation between two groups with (92% accuracy). Both salivary LDL-C critical value (0.15 mmol/L) and serum HDL-C critical ratio (1.16 µg/L) can achieve 90.7% accuracy in discrimination between stroke group and control group (table 8).

| | Hypertension | Diabetes | Ischemic heart disease | Smoking |
|---|---|---|---|---|
| Females | 18 (44%) | 17(50%) | 9 (60%) | 6(23%) |
| Males | 23 (56%) | 17 (50%) | 6 (40%) | 20 (77%) |
| Total | 41/50 (82%) | 34/50 (68%) | 15/50 (30%) | 26 (52%) |

**Table 1.** Distribution of underlying diseases and risk factors in ischemic stroke group.

| **Serum versus Salivary Estimates** | **r** | **p** |
|---|---|---|
| **Total cholesterol concentration (mmol/L)** | **0.05** | **>0.05** |
| **Triglycerides concentration (mmol/L)** | **0.3** | **<0.01** |
| **HDL cholesterol concentration (mmol/L)** | **0.3** | **<0.01** |
| **LDL cholesterol concentration (mmol/L)** | **0.1** | **>0.05** |
| **MDA concentration (µmol/L)** | **0.28** | **<0.01** |

**Table 2.** Pearson correlation between serum and salivary estimates.

| | Control (n=25) | Ischemic stroke (n=50) | P value |
|---|---|---|---|
| MDA (µmol/l) | 0.23± 0.07 | 0.64± 022 | < 0.001 |
| Total cholestrol | 0.45± 0.08 | 0.84± 0.15 | NS |
| Triglycerides | 0.34± 0.09 | 0.8± 0.23 | < 0.001 |
| HDL-C | 0.19± 0.04 | 0.16± 0.04 | < 0.001 |
| LDL-C | 0.13± 0.16 | 0.31± 0.13 | < 0.001 |

**Table 3**. Salivary concentration of MDA and lipid fractions.

| | Control (n=25) | Ischemic stroke (n=50) | P value |
|---|---|---|---|
| MDA (µmol/l) | 1.32± 0.35 | 2.51± 1.1 | < 0.001 |
| Total cholestrol | 3.91± 0.66 | 4.44± 1.11 | NS |
| Triglycerides | 1.68± 1.03 | 2.96± 0.19 | < 0.001 |
| HDL-C | 1.59± 0.51 | 0.78± 0.26 | NS |
| LDL-C | 1.59± 0.56 | 2.32± 1.19 | < 0.001 |

**Table 4.** Serum concentration of MDA and lipid fractions.

| parameters | Saliva | | Serum | |
|---|---|---|---|---|
| | r | P value | r | P value |
| Total Cholesterol vs Triglycerides | 0.7 | p< 0.01 | 0.23 | p< 0.05 |
| Total Cholesterol vs LDL-C | 0.79 | p< 0.01 | 0.95 | p< 0.01 |
| Triglycerides vs MDA | 0.28 | p< 0.01 | 0.29 | p< 0.01 |
| Triglycerides vs HDL-C | -0.23 | p< 0.05 | -0.22 | p< 0.05 |
| HDL-C vs MDA | -0.32 | p< 0.01 | -0.36 | p< 0.01 |
| HDL-C vs LDL-C | -0.27 | p< 0.01 | -0.25 | p< 0.01 |
| LDL-C vs MDA | 0.28 | p< 0.01 | 0.29 | p< 0.01 |

**Table 5.** Pearson correlation between estimates.

| **SALIVA** | Control (n=25) | Ischemic stroke (n=50) | P value |
|---|---|---|---|
| Total cholesterol/HDL-C | 2.384± 0.552 | 5.476± 1.50 | <0.01 |
| LDL-C/HDL-C | 0.710± 0.838 | 2.078±1.062 | <0.01 |
| Triglycerides/HDL-C | 1.853± 0.766 | 5.271± 1.933 | <0.01 |
| Total cholesterol/HDL-C * MDA | 0.568 ± 0.243 | 3.417± 1.36 | <0.001 |
| LDL-C/HDL-C * MDA | 0.169 ± 0.182 | 1.27±0.759 | <0.001 |
| Triglycerides/HDL-C * MDA | 0.437±0.230 | 3.307±1.64 | <0.001 |
| **SERUM** | | | |
| Total cholesterol/HDL-C | 2.595± 1.00 | 6.73± 4.17 | <0.01 |
| LDL-C/HDL-C | 1.117± 0.601 | 3.66± 3.074 | <0.01 |
| Triglycerides/HDL-C | 1.336± 1.197 | 4.685± 2.96 | <0.01 |
| Total cholesterol/HDL-C * MDA | 2.88± 1.23 | 17.81± 13.03 | <0.001 |
| LDL-C/HDL-C * MDA | 1.22± 0.704 | 9.56±9.01 | <0.001 |
| Triglycerides/HDL-C * MDA | 1.474±1.307 | 12.61±9.88 | <0.001 |

**Table 6.** Atherogenic index as indicated by various risk ratios

| Parameters | ROC area | P value |
|---|---|---|
| | | |
| Salivary Triglycerides | 0.979 | < 0.001 |
| Salivary MDA | 0.969 | < 0.001 |
| Salivary LDL-C | 0.897 | < 0.001 |
| Serum HDL-C | 0.894 | < 0.001 |
| Serum MDA | 0.885 | < 0.001 |
| Serum triglycerides | 0.855 | < 0.001 |
| Salivary HDL-C | 0.760 | < 0.001 |
| Serum LDL-C | 0.685 | < 0.001 |
| Serum total cholesterol | 0.626 | 0.08 (NS) |

**Table 7.** ROC area for different cut-off values to diagnose cases with ischemic stroke from healthy control.

| Positive if ≥ cut-off value | Sensitivity % | Specificity % | Accuracy % | PPV at pretest probability =50% | PPV at pretest probability =90% | NPV at pretest probability =10% |
|---|---|---|---|---|---|---|
| **Salivary total cholesterol concentration (mmol/L)** | | | | | | |
| 0.47 | 100.0 | 64.0 | 88.0 | 73.5 | 96.2 | 100.0 |
| **0.61** | **94.0** | **96.0** | **94.7** | **95.9** | **99.5** | **99.3** |
| 0.64 | 90.0 | 100.0 | 93.3 | 100.0 | 100.0 | 98.9 |
| **Salivary Triglycerides concentration (mmol/L)** | | | | | | |
| 0.40 | 100.0 | 72.0 | 90.7 | 78.1 | 97.0 | 100.0 |
| **0.50** | **92.0** | **96.0** | **93.3** | **95.8** | **99.5** | **99.1** |
| 0.53 | 86.0 | 100.0 | 90.7 | 100.0 | 100.0 | 98.5 |
| **Salivary MDA concentration (µmol/L)** | | | | | | |
| 0.23 | 100.0 | 52.0 | 84.0 | 67.6 | 94.9 | 100.0 |
| **0.38** | **88.0** | **100.0** | **92.0** | **100.0** | **100.0** | **98.7** |
| **Salivary LDL cholesterol concentration (mmol/L)** | | | | | | |
| 0.05 | 100.0 | 24.0 | 74.7 | 56.8 | 92.2 | 100.0 |
| **0.15** | **96.0** | **80.0** | **90.7** | **82.8** | **97.7** | **99.4** |
| 0.57 | 2.0 | 96.0 | 33.3 | 33.3 | 81.8 | 89.8 |
| **Serum HDL-C concentration (µg/L)** | | | | | | |
| 0.61 | **28.0** | **100.0** | **52.0** | **100.0** | **100.0** | **92.6** |
| **1.16** | **98.0** | **76.0** | **90.7** | **80.3** | **94.7** | **99.7** |
| 1.27 | **100.0** | **68.0** | **89.3** | **75.8** | **96.6** | **100.0** |
| **Serum Triglycerides concentration (mmol/L)** | | | | | | |
| 1.40 | 100.0 | 52.0 | 84.0 | 67.6 | 94.9 | 100.0 |
| **1.85** | **96.0** | **68.0** | **86.7** | **75.0** | **96.4** | **99.4** |
| 4.66 | 2.0 | 96.0 | 33.3 | 33.3 | 81.8 | 89.8 |
| **Serum MDA concentration (µmol/L)** | | | | | | |
| 0.84 | 100.0 | 20.0 | 73.3 | 55.6 | 91.8 | 100.0 |
| **1.85** | **72.0** | **100.0** | **81.3** | **100.0** | **100.0** | **97.0** |

**Table 8.** Validity of some parameters to differentiate cases with ischemic stroke from healthy control.

## Discussion

To our knowledge, this is the first study to evaluate the salivary lipid Peroxidation and its relation to lipid fractions among patients with recent attack of ischemic stroke. Oxidative stress, lipid and lipoprotein Peroxidation and inflammation are involved in neuronal damage induced by ischemia reperfusion [22]. Lipid Peroxidation was measured by lipid hydroxyl peroxides [23] which are unstable and degrade to various secondary products like MDA and MDA-

like substances which were jointly called thiobarbituric acid reactive substance (TBARS) [24]. The brain contains high levels of polyunsaturated fatty acids in membrane lipid; therefore, lipid Peroxidation is one of the major consequences of free-radical mediated injury to brain. The assessment of lipid Peroxidation products (MDA) is important in measuring free radical- induced cerebral injury in patients with stroke. Lipid Peroxidation end products MDA has been observed in plasma from stroke patients compared to healthy subjects. MDA level is widely utilized as a marker of lipid peroxidation in states of elevated oxidative stress [12]. In the present study, MDA levels were measured in both saliva and serum which were significantly higher in patients with ischemic stroke than that of healthy control ($p< 0.001$). This finding means elevated oxidative stress as a result of free-radical- induced cerebral injury in patients with stroke as reported by other studies [12, 25, 26].

Salivary MDA levels are directly affected by systemic oxidative stress. These findings was also supported by the results of ROC test which revealed that salivary MDA was significantly accurate parameter in predicting patients at risk of stroke with 92% accuracy rate and 100% specificity for the optimum cut-off value ≥0.38µmol/L. The low level of salivary glutathione (GSH) recorded in stroke patients [27]indicated that salivary GSH was not consumed, to a considerable extent, in scavenging or detoxification of free radicals or lipid peroxidation products, this lead to significant increase in salivary MDA levels among stroke patients.

The association of blood cholesterol with the risk of stroke, a very important clinical and public health issue appears to be in dispute. Some studies found increased risk of ischemic stroke associated with increases total cholesterol levels [28-32], while other studies found no clear association [33-36]. Regarding cholesterol fractions, an association between LDL-C and Ischemic stroke is less studied and inconsistent [36-42].

In the present study all lipid parameters (except for total cholesterol) measured in serum and saliva of patients with stroke showed significant differences when compared with control group. Total cholesterol concentration alone in saliva and serum is considered as non sensitive parameter since it did not show any significant differences between disease and control group. Salivary and serum triglycerides concentrations were 2-3 times higher in ischemic stroke group than in control group , moreover , both salivary and serum levels of triglycerides were directly correlated with MDA, LDL-C and inversely correlated with HDL-C .This finding may support the evidence that triglycerides concentration had positive risk factor-adjusted association with the risk of cerebral stroke and patients with highest levels of triglycerides were 2-7 times more likely to suffer from atherosclerotic stroke than those with lower levels[43]. Few prospective population-based studies have examined the association between HDL-C and stroke [44]. HDL-C concentration is markedly reduced in patients with stroke in comparison to that in healthy control. HDL-C are particles with numerous athero-protective functions including facilitation of reverse cholesterol transport, improvement of endothelial function, protection of LDL-C against oxidation, limitation of hemostasis and retardation of inflammatory activity related to the vascular wall [45]. There is a well established inverse relationship between serum HDL-C concentration and the risk of coronary heart diseases [31], but it is not well documented risk factor for strokes, although few case-control studies have noted an inverse relationship between HDL-C and risk of stroke or TIA [39-41]. The highly significant inverse relationship between HDL-C with MDA and LDL-C in saliva and serum of stroke group in the present study reflect the deficiency of athero-protective function of HDL-C in patients with stroke . The association between LDL-C and risk of ischemic stroke has only been evaluated in few studies. A large study of over 11000 patients with coronary heart diseases showed a 14% increase in relative risk of verified ischemic stroke per 1.03 mmol/L[42]. In contrast, a large cohort study of over 14000 middle aged men and women found no consistent association between LDL-C and ischemic stroke during 10 years follow up [32].

The present finding comes in accordance with the abovementioned study, since no significant statistical difference was found between the tested groups. On the other hand, salivary LDL-C levels were significantly higher in stroke group in comparison to healthy group. Therefore, a combination formula like ***MDA multiplied by atherogenic indecies*** was used to test their significance in determining patients at increased risk to ischemic stroke which was

several times more sensitive than atherogenic index alone. Therefore, this index can be used as a valuable salivary marker for screening individuals who are at risk of cardio and / or cerebrovascular diseases. Total cholesterol hazard value was (3.45 mmol/L) which was greater than that recorded in other study [43] which was ((5.85 mmol/L). HDL-C hazard value in Kurth et al study [44] was (0.20 mmol/L) and was not significant. However, the hazard value (cut-off value) of HDL-C recorded in this study was significant and greater than that recorded by others [44, 45].

The highest validity of salivary lipid parameters in predicting ischemic stroke was clearly seen in the present investigation. Analysis of saliva may therefore provide effective, non-invasive approach for screening large population [46]. The constituents are derived from the local vasculature of salivary glands. The accuracy of salivary lipid peroxidation and lipid profile may help physicians and other health professional to pay much attention for the use of saliva as screening tool for patients at risk of cardio and /or cerebrovascular diseases.

## Conclusion

Lipid peroxidation (indicated by MDA) as well as lipid fractions particularly triglycerides can be assessed in saliva and may be used alone or in combination with other lipid parameters for monitoring patients at increased risk of ischemic stroke .

## Declaration of Interest

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