Assessment of Salivary lipid profiles in patients with ischemic stroke and patients at risk of having stroke among Iraqi sample

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Citation

Abstract
Background: Altered lipid levels may occur in ischemic stroke, as well as stroke-related diseases like hypertension, diabetes and coronary heart diseases. The purpose of this study was to evaluate the reliability and accuracy of lipid profile assessment in saliva of patients with ischemic stroke and patients with some stroke-related diseases.

Patients & Method: This study was conducted on total of 150 individual to assess salivary and serum total cholesterol (TC), triglycerides (TG), high density lipoprotein- cholesterol (HDL-C), low density lipoprotein- cholesterol (LDL-C) and very low density lipoprotein (VLDL-C). They are divided into three group. The first group consists of fifty patients with recently diagnosed ischemic stroke (based on brain CT), the second group (risk group) consists of seventy five patients grouped into 3subgroups; 25 patients with hypertension, 25 patients with type 2 diabetes mellitus and other 25 patients with coronary heart disease. The last group (control group) consists of 25 individuals who are clinically healthy.

Results: Salivary TC, TG, LDL-C and VLDL-C concentrations were significantly high in patients with ischemic stroke when compared with risk and control groups. On the other hand, only serum TG, HDL-C and VLDL-C concentrations showed significant elevation (p< 0.001). Salivary optimum cut-off values of all lipid parameters (except for HDL-C)) were accurate in differentiating risk group patients from patients with ischemic stroke (ROC > 0.5).

Conclusion: Lipid profile can be assessed in saliva and can be used as simple, monitoring tool in stroke-prone individuals with reasonable accuracy.

INTRODUCTION
Stroke (CVA) is the third most common cause of death and a leading cause of severe disability in developed and developing countries. Surprisingly, little direct evidence is available to elucidate the role of lipids in the pathogenesis of ischemic stroke (1). In the Multiple Risk Factor Intervention Trial, mortality from ischemic stroke was greater among men with high total cholesterol levels and there was a continuous, progressive increase in thrombo-embolic stroke rates with increasing levels of total cholesterol (1). The association between total cholesterol and risk of ischemic stroke has been investigated in several prior observational studies, of which some found increased risk with increasing cholesterol levels (2,3,4,5), and some no clear association (6,7,8). It has been proposed that this may be due to the differing association with subtypes of stroke (1). An inverse association has been observed in hemorrhagic stroke (9) and a positive association with ischemic stroke (10). With regard to cholesterol components, there is a well-established relation between serum concentration of HDL-C and the risk of coronary heart diseases (11) but it is not well-documented risk factor for stroke. Several case-control studies noted an inverse relation between HDL-C and risk of stroke or transient ischemic attack (12,13) but few prospective studies have addressed this issue. The association between LDL-C and ischemic stroke is less studied and inconsistent (14,15,16). Saliva is increasingly used and well validated in diagnosing, monitoring systemic disease status and predicting disease progression. Biomarkers detected in saliva can be valuable in a wide range of clinical pathology, forensic medicine and sport medicine (17). Salivary assays present lot of advantages when compared to blood assay: the sampling is very easy to do especially in non-medical environment; it does not
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disturb intimacy when control is needed. Multiple samples could be collected providing more information than that of single blood sample (\textsuperscript{19}). Karjalainen et al (\textsuperscript{20}) assessed cholesterol in saliva of healthy adults; they concluded that salivary concentration levels reflect serum concentration to some extent. Saliva requires no special collection or storage equipments and can be used to detect some markers of many systemic diseases in non invasive manner (\textsuperscript{21}). The present study was conducted to determine the reliability and accuracy of measuring lipid profile concentration in saliva of patients with ischemic stroke and patients with stroke-related diseases and to find out the cut-off values of the studied parameters that may be used to discriminate between them.

PATIENTS AND METHODS

One hundred and fifty individuals were selected in this observational study. They were divided into 3 groups; the first group composed of 50 patients (24 males and 26 females) with recent ischemic stroke (diagnosis based on Neurologist clinical examination and brain CT scan). The second group (Risk group) composed of 25 patients (12 males and 13 females) with hypertension (systolic blood pressure≥160 mmHg or diastolic blood pressure ≥ 90mmHg, or receiving antihypertensive treatment); 25 patients (13 males and 12 females) with Non-insulin dependent diabetes (based on recall of physician diagnosis and Fasting blood glucose ≥120 mg/dl); other 25 patients( 11 males and 14 females) with coronary heart diseases (based on recall of physician diagnosis). The third group (healthy control) composed of 25 individual (12 males and 13 females) who were age and sex- matched with patients groups and served as control. Patients suffering from conditions that affects the lipid profile such as hypothyroidism, liver or kidney diseases, Cushing’s syndrome, obesity ( BMI >30), a history of familial dyslipidemia and advanced periodontitis were excluded, in addition, patients receiving medications affecting lipid metabolism, such as lipid-lowering drugs, beta- blockers, oral contraceptives, estrogen, progestin, thyroxin and vitamin E were also excluded from the study.

All patients and controls signed informed consent and the study protocol was approved by the Institutional Ethics Committee of AL-Diwaniya Hospital.

All participants were asked to fast for 12 hours before their morning clinic appointment; blood was drawn from an antecubital vein with minimal trauma. The serum was separated by centrifugation at 4°C. Supernatant were aspirated and stored frozen at -20°C until analyzed. Saliva samples were always collected in restful and quite circumstances, following flushing of mouth with 100 ml of tap water. The whole saliva was collected for 5 minutes by the subject leaning forward and spitting saliva into test tubes that were kept in crushed ice and immediately after collection the samples were cold centrifuged at 3000 rpm at 4°C for 5 minutes. The supernatant was aspirated and stored at -20°C until analyzed. Salivary and serum TC and TG concentration were measured by enzymatic method (\textsuperscript{22},\textsuperscript{23}). The HDL-C concentration was measured by the method of Warnick et al (\textsuperscript{24}). The concentration of LDL-C was calculated from the concentration of TC, HDL-C and TG by Friedwald formula (\textsuperscript{25}). VLDL-C concentration was estimated directly by dividing triglyceride value on 2.2(\textsuperscript{26}). All data were analyzed with SPSS-13 program, comparison between groups were made by Analysis of variance (ANOVA) test. The significance of difference in mean between each pair of group was performed by Benferonni 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

During the selection period (January to November 2007), 50 patients with ischemic stroke were recruited. Of these 41 had hypertension, 34 had diabetes, 26 were heavy smokers and only 8 had previous transient ischemic attack (table1).

Figure 1

Table 1: Distribution of risk factors in ischemic stroke patients.

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>HTN</th>
<th>DM</th>
<th>IHD</th>
<th>Smoking</th>
<th>Previous Ischemic Stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>22</td>
<td>17</td>
<td>0</td>
<td>6</td>
<td>2 (23.1%)</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>17</td>
<td>6</td>
<td>20</td>
<td>4 (23.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>81</td>
<td>34</td>
<td>16</td>
<td>26</td>
<td>4 (23.1%)</td>
</tr>
</tbody>
</table>

Lipid profile concentration in saliva and serum of all studied group did not vary with age and gender (data not shown), therefore results from males and females were grouped together in each group. All lipid values recorded in saliva followed that recorded in serum of all studied groups but in smaller values. Salivary concentrations of TC,TG, LDL-C and VLDL-C in patients with ischemic stroke were significantly higher than that of control group ( tables 2,3,5
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&6). Figure 2

Table 2: Mean levels of Total Cholesterol in serum and saliva of IS, Risk Group patients and healthy controls.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Serum Total Cholesterol (mmol/L)</th>
<th>Saliva Total Cholesterol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS (n=25)</td>
<td>6.54 ± 0.99</td>
<td>3.9 ± 0.75</td>
</tr>
<tr>
<td>IS (n=25)</td>
<td>6.54 ± 0.99</td>
<td>3.9 ± 0.75</td>
</tr>
<tr>
<td>Healthy control (n=25)</td>
<td>6.54 ± 0.99</td>
<td>3.9 ± 0.75</td>
</tr>
</tbody>
</table>

F (ANOVA) for differences between 3 study groups - 0.001

On the other hand, both salivary and serum HDL-C concentration were significantly lower in Stroke group in comparison to control group (table 4).

Figure 4

Table 4: Mean levels of HDL-C in serum and saliva of IS, Risk Group patients and healthy controls.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Serum HDL-C concentration (mmol/L)</th>
<th>Saliva HDL-C concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS (n=25)</td>
<td>0.84 ± 0.12</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>IS (n=25)</td>
<td>0.84 ± 0.12</td>
<td>0.67 ± 0.12</td>
</tr>
<tr>
<td>Healthy control (n=25)</td>
<td>0.84 ± 0.12</td>
<td>0.67 ± 0.12</td>
</tr>
</tbody>
</table>

F (ANOVA) for differences between 3 study groups - 0.001

Surprisingly, TC and LDL-C concentrations were not statistically different in stroke and control groups (table 2 & 5).

Figure 3

Table 3: Mean levels of TG in serum and saliva of IS, Risk Group patients and healthy controls.

<table>
<thead>
<tr>
<th>Risk group</th>
<th>Serum Triglycerides concentration (mmol/L)</th>
<th>Saliva Triglycerides concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS (n=25)</td>
<td>2.96 ± 0.68</td>
<td>2.52 ± 0.68</td>
</tr>
<tr>
<td>IS (n=25)</td>
<td>2.96 ± 0.68</td>
<td>2.52 ± 0.68</td>
</tr>
<tr>
<td>Healthy control (n=25)</td>
<td>2.96 ± 0.68</td>
<td>2.52 ± 0.68</td>
</tr>
</tbody>
</table>

F (ANOVA) for differences between 3 study groups - 0.001
Regarding risk group, salivary and serum concentration of TG and VLDL-C were significantly higher in hypertensive patients, diabetic patients and patients with coronary heart diseases when compared with healthy control (table 3 & 6).

Hypertensive patients as well as diabetic patients have high total cholesterol concentration in their saliva and serum when compared with that of control group (table 2). When comparison of salivary and serum estimates between stroke group and risk group done, all serum estimates did not show any significant differences, however; salivary TG, VLDL-C concentrations were significantly different between them (p < 0.01) (table 3 & 6). Salivary TC and LDL-C concentration is only statistically different between stroke group and hypertensive patients (table 2 & 5). HDL-C concentration did not differ in the saliva of the two groups (table 4).

ROC curve equation was applied for different cut-off values of selected lipid parameters when used to diagnose cases with ischemic stroke and differentiating it from risk group. As seen in table (7) the area under ROC curve for salivary TC, TG, LDL-C, VLDL-C in addition to serum HDL-C were significantly higher from 0.5 value of an equivocal test in differentiating patients with stroke from risk group patients. The most valid lipid parameter was salivary triglycerides (optimum cut-off values ≥ 0.71 mmol/L), followed by salivary VLDL-C (optimum cut-off value ≥ 0.38 mmol/L), then salivary total cholesterol (optimum cut-off value ≥ 0.84 mmol/L), followed by salivary LDL (optimum cut-off value ≥ 0.44 mmol/L). On the other hand, the only valid serum lipid in this prediction...
was serum HDL-C with optimum cut-off value ( < 0.66 mmol/L).

**Figure 7**

Table 7: Tested variables ordered according to their significance in separating Ischemic Stroke patients from the whole Risk Group patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cut-Off Value</th>
<th>ROC area</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary Triacylglycerol</td>
<td>Positive if ≥ 0.71</td>
<td>0.771</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Salivary VLDL-Cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 0.38</td>
<td>0.766</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Salivary Total cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 0.84</td>
<td>0.759</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Salivary LDL-Cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 0.44</td>
<td>0.659</td>
<td>0.009</td>
</tr>
<tr>
<td>Serum HDL-Cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 0.46</td>
<td>0.62</td>
<td>0.023</td>
</tr>
<tr>
<td>Serum VLDL-Cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 3.54</td>
<td>0.597</td>
<td>0.07 [NS]</td>
</tr>
<tr>
<td>Serum LDL-Cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 1.04</td>
<td>0.562</td>
<td>0.24 [NS]</td>
</tr>
<tr>
<td>Serum Triglycerides</td>
<td>Positive if ≥ 2.19</td>
<td>0.533</td>
<td>0.54 [NS]</td>
</tr>
<tr>
<td>Salivary HDL-Cholesterol concentration (mmol/L)</td>
<td>Positive if ≥ 0.20</td>
<td>0.527</td>
<td>0.61 [NS]</td>
</tr>
</tbody>
</table>

**DISCUSSION**

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 increased total cholesterol levels (3–5, 7), while others found no clear association (8, 9, 10, 11).

With regard to cholesterol components, an association between LDL-C and ischemic stroke is less studied and inconsistent (11-17). The underlying risk factors reported in stroke group of this study were hypertension in 87% of cases followed by diabetes in 50% of cases. All lipid parameters measured in serum and saliva of stroke patients and risk group patients showed significant differences in comparison with healthy control, however, no apparent significant differences were found in concentration of lipid fractions between risk group and stroke group. This may be explained on the basis that both stroke and stroke-related diseases sharing the same vascular process of arteriosclerosis acceleration that lead to stenosis and embolism (17). For this reason, early detection and control of risk factors such as hypertension, diabetes and ischemic heart diseases are thought to be crucial in reducing the risk of stroke through providing effective care (18). Saliva is considered as an ultra filtrate of plasma. It can easily collect with fewer compliance problems as compared with the collection of blood. Analysis of saliva may provide effective, non-invasive approach for screening large population (19). The constituents are derived from the local vasculature of salivary glands. The total lipid content of whole saliva was 2-3µg/ml. This consisted of cholesterol, fatty acids and triglycerides (20). Salivary lipids are mostly glandular in origin, but some believed to diffuse directly from serum. Unfortunately, little attention has been paid for salivary lipid analysis. Karajalinen et al (20) recorded the normal concentration of total cholesterol in saliva which ranged from 0.02-5.46 mmol/L, the value is higher than that reported in this study (0.3-0.61 mmol/L). This difference may be due to different collection methods used or may be due to the direct influence of local factors like periodontitis that may affect the results. Salivary and serum triglycerides concentration were also assessed in this study, its levels were 2-3 times higher in patients with ischemic stroke when compared with control group, this 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 (18). Few prospective population-based studies have examined the association between HDL-C and stroke (19). HDL-C are particles with numerous athero-protective functions, including facilitation of reverse cholesterol transport, improvement of endothelial function, protection of LDL-C from oxidation, limitation of hemostasis and retardation of inflammatory activity related to the vascular wall (20). There is well-established inverse relation between serum HDL-C concentration and the risk of coronary heart diseases (21), but it is not well-documented risk factor for stroke, although few case-control studies have noted an inverse relation between HDL-C and risk of stroke or transient ischemic attack(TIA) (22, 23). Salivary and serum HDL-C concentration recorded in this study may reflect the protective function of HDL-C, since it was marked reduced in patients with stroke as well as patients with stroke-related diseases. The association between LDL-C and risk of ischemic stroke has only been evaluated in few studies. A large study of over 11,000 patients with coronary heart diseases showed a 14% increase in the relative risk of verified ischemic stroke or TIA per 1.03 mmol/L in LDL-C (24). In contrast, a large cohort study of over 14,000 middle aged men and women found no consistent association between LDL-C and ischemic stroke during 10 years follow up (25), our results come in accordance with that study, where serum LDL-C concentration did not show any significant difference when patients with stroke were compared with healthy control. On the other hand, salivary LDL-C concentrations in patients with ischemic stroke were
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significantly higher than that of healthy control. This conflicting results between and serum and saliva could be attributed to small sample number used in this study. High VLDL-C values can cause build up of cholesterol in arteries and increases the risk of heart diseases and stroke (33). The present study demonstrated two-fold increase in salivary and serum VLDL-C values in patients with ischemic stroke. This increase could be due to excess levels of triglycerides which increases the concentration of two types of fat particles “chylomicrons and VLDL-C”. These fat particles may contribute to the fatty deposits that obstruct blood flow leading to ischemic stroke (33). In women health study, ischemic stroke risk for the highest versus lowest quintile of total cholesterol and cholesterol fractions have been studied (35). Total cholesterol hazard ratio was (5.85 mmol/L), which is higher than that recorded in this study (3.45 mmol/L). HDL-C hazard ratio in Kurth et al study (35) was (0.20 mmol/L) and was not significant. The cut-off value for HDL-C in this study was statistically significant and was greater than that recorded by others (32, 35). This difference may be due to different life styles and different dietary habits between two communities. All salivary cut-off values of lipid parameters (except for HDL-C) were significant in differentiating patients with ischemic stroke from risk group. These cut-off values can be considered as potential values for stroke risk. The high validity of salivary lipid parameters in predicting ischemic stroke was clearly seen in this study. The accuracy of salivary lipid profile estimation may help physician and other health personnel to pay more attention to use saliva as monitoring tool for patients at risk of having stroke.

CONCLUSION

Lipid profile assessment in whole saliva (particularly triglycerides) can be used alone or in conjunction with serum for monitoring patients at increased risk of ischemic stroke. To best of our knowledge, this study is the first that assess lipid fractions in saliva of patients with stroke and stroke–related diseases.

References
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