The role of von Willebrand factor in determining the risk of ischemic stroke in Hawler city: a case-control study

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Abstract

Background and objective: Ischemic stroke is classically characterized as a neurological deficit attributed to an acute focal injury of the central nervous system by a vascular cause, it occurs as a result of obstruction by a blood clot (thrombus) or plugs within a blood vessel supplying blood to the brain. In this study, we investigated the association of von Willebrand factor-antigen and serum lipids with ischemic stroke.

Methods: The following retrospective study was conducted on 138 participants; 88 patients with ischemic stroke and 50 healthy controls.

Results: In crude analyses, Plasma von Willebrand factor antigen, fasting serum Total cholesterol, Triglycerides and LDL-C were significantly higher in patients with ischemic stroke than in controls ($P <0.001$, $P <0.001$, $P = 0.003$, $P <0.001$, respectively), while the difference in the level of serum HDL-C between patients with ischemic stroke and control was significantly lower ($P = 0.023$).

Conclusion: These data suggest that von Willebrand factor, total cholesterol, triglycerides, LDL-C and HDL-C gives some contribution to stroke risk even in the elderly and that von Willebrand factor antigen and lipid profile assessment must be taken into account in estimating the individual risks of stroke.

Keywords: Ischemic stroke; von Willebrand factor; Lipid profile.

Introduction

Ischemic stroke is sudden neurologic deficits that result from focal cerebral ischemia associated with permanent brain infarction. It is a major cause of disability and death worldwide which occurs as a result of atherothrombotic occlusion of large arteries; cerebral embolism; nonthrombotic occlusion of small, deep cerebral arteries; and proximal arterial stenosis with hypotension that decreases cerebral blood flow in arterial watershed zones.\(^1\) A multifunctional multimeric plasma glycoprotein von Willebrand factor (vWF), has long been known to be a key player in thrombus formation at sites of vascular damage, vWF act as a carrier protein for blood clotting FVIII and promotes platelet adhesion and aggregation at sites of vascular injury. The release of vWF is increased when endothelial cells are activated or damaged. Therefore, plasma vWF level is considered a marker of endothelial dysfunction, a condition that predisposes to atherosclerosis and thrombosis. Because of its direct role in hemostasis, and its indirect role as a marker of endothelial dysfunction, VWF is a potential risk indicator for cerebrovascular disease.\(^2\) Recent studies revealed that abnormal elevation of any or all lipids and/or lipoproteins which known as hyperlipidemia is considered a modifiable risk factors that play a crucial role in the pathogenesis of atherosclerosis and biological etiology of the ischemic stroke.\(^3\) Hyperlipidemia usually classified as either familial caused by specific genetic abnormalities, or acquired when resulting from endocrine, renal or hepatic diseases.\(^4\) Deposition of cholesterol and cholesteryl ester from the plasma lipoproteins into

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the artery wall, elevated levels of low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicron often cause premature or more severe atherosclerosis. Also, it may lead to several diseases like ischemic stroke, heart disease, hypothyroidism and renal dysfunction. Whereas high density lipoprotein (HDL) has the ability in reverse cholesterol transport and having an inverse correlation with the risk of coronary heart disease and ischemic stroke. We carried out a case-control study to determine whether von Willebrand factor (vWF) and lipids are associated with ischemic stroke.

Methods

1- Study population
The case-control study was carried out in Biochemistry Department, College of Medicine, Hawler Medical University from August 2015 to May 2016. Fifty participants (20 males and 30 females) who had no evidence for any blood diseases with the mean age 60 years were served as a control group (Group I). The remaining 88 patients (37 males, 51 females) previously diagnosed with ischemic stroke (Group II) who recruited from neurology department of Rizgary Teaching Hospital with the mean age 66 years were enrolled in this study (Table 1).

2- Sample collection
The specimen collection from both groups was carried out using venous blood, three milliliters of blood has been taken from participants and transferred into two different plain vacutainer tube with and without anticoagulant, the blood specimens left at room temperatures for 20 minutes, and then centrifuged at 3000 rpm for 10 minutes. The separated samples were either used immediately for the study of vWF-Ag test and lipid profile (T. Cholesterol (T.Ch), Triglycerides (TGs), HDL-C and LDL-C) or kept until further analysis.

3- Laboratory testing

a) Estimation of plasma vWF-Ag
The vWF-Ag assay is a quantitative, turbidimetric assay for indirect detection of plasma vWF, using (Sysmex CA 560, Japan). According to Ruggeri & Ware, 1992, when small polystyrene particles which is specific antibodies mixed with samples containing von Willebrand antigen, attached to the covalent bond of vW-Ag causes aggregation. This aggregation is then detected turbidimetrically via the increase in turbidity, which is proportional to the antigen level present in the test sample.

b) Estimation of fasting serum lipids and lipoprotein profiles
Readymade kits BIOLABO (France) were employed to the estimation of fasting serum T.Ch and TGs according to the method of Allain et al., 1974. While fasting serum HDL-C and LDL-C were determined in various samples by selective detergents without specimen pre-treatment using BIOLABO kit (France) by the direct method.

4- Statistical analysis
Data were analyzed using Statistical Package for Social Sciences (SPSS) version 19 software. The results were expressed as mean ± S. The significance of differences was evaluated by the Student's t-test, the levels of fasting serum lipid profile and plasma vWF-Ag of normal healthy subjects were compared with those

| Groups | No. of subjects | Age (year) Mean ± SE | Range | P value | BMI (Kg/m²) Mean ± SE | Range | P value |
|--------|----------------|----------------------|-------|---------|-----------------------|-------|---------|
| Group I | 50             | 60 ± 1.0             | 28-63 | 0.221   | 27 ± 0.4             | 20-37 | 0.140   |
| Group II | 88             | 66 ± 1.4             | 35-93 |         | 27 ± 0.6             | 18-60 |         |

Table 1: The baseline characteristics of the control group and patients with ischemic stroke.
of patients with ischemic stroke concerning the P value, statistical significance was set at P < 0.05.  

5- Ethical consideration

The study protocol was approved by the scientific research and Ethics Committee of the College of Medicine - Hawler Medical University. Permission was obtained from administrative authorities of Hawler neurology department of Rizgary Teaching Hospital and Central laboratory- Hawler Teaching Hospital. Informed consent was obtained from each participant before blood collection.

Results

1- Group I (Control group)

The laboratory parameters of the present study are presented in Table (2). The mean ± S.E value for plasma vWF-Ag was 112 ± 4.9 % and the range of variation was 58-161 %. The mean ± S.E value for serum T.Ch was 168 ± 3.4 mg/dl with a range of variation 114-203 mg/dl. The mean ± S.E value of serum TGs was 103 ± 5.0 mg/dl with a range of variation 40-211 mg/dl. The mean ± S.E value of serum LDL-C was 96 ± 2.9 mg/dl with a range of variation 54-128 mg/dl. The mean ± S.E value for serum HDL-C was 38 ± 1.0 mg/dl and the range of variation was 23-62 mg/dl.

2- Group II (Patients with ischemic stroke)

The biochemical and hematological results of the comparison between healthy controls and patients with ischemic stroke are expressed in mean ± SE and depicted in Table 2. The mean ± S.E value for plasma vWF-Ag was 300 ± 16 % and the range of variation was 121-678%. The mean ± S.E value for serum T.Ch was 172 ± 5.7 mg/dl with a range of variation 62-332 mg/dl. The mean ± S.E value of serum TGs was 126 ± 8.6 mg/dl with a range of variation 36-484 mg/dl. The mean ± S.E value of serum LDL-C was 102 ± 4.6 mg/dl with a range of variation 41-242 mg/dl. The mean ± S.E value of serum HDL-C was 35 ± 1.2 mg/dl and the range of variation was 7-63 mg/dl.

It has been observed that mean HDL-C levels were significantly lower (P < 0.05) in patients with ischemic stroke as compared to healthy controls.

Table 2: Details of biochemical and hematological parameters of the studied groups.

| Biochemical and hematological parameters | Group I (Control) | Group II (Ischemic stroke) | P value |
|----------------------------------------|-------------------|-----------------------------|---------|
|                                        | Mean ± SE         | Range                       | Mean ± SE | Range         |         |
| vWF-Ag (%)                             | 112 ± 4.9         | 58-161                      | 300 ± 16  | 121-678       | <0.001  |
| T. Ch (mg/dl)                          | 168 ± 3.4         | 114-203                     | 172 ± 5.7 | 62-332        | <0.001  |
| TGs (mg/dl)                            | 103 ± 5.0         | 40-211                      | 126 ± 8.6 | 36-484        | 0.003   |
| LDL-C (mg/dl)                          | 96 ± 2.9          | 54-128                      | 102 ± 4.6 | 41-242        | <0.001  |
| HDL-C (mg/dl)                          | 38 ± 1.0          | 23-62                       | 35 ± 1.2  | 7-63          | 0.023   |
In an attempt to bring to light the most important pathophysiological effect of vWF, serum lipids and lipoprotein profile on ischemic stroke, we assayed a series of samples of clinically defined ischemic stroke and healthy controls. Abnormal coagulation and fibrinolytic factors have been suggested as possible predictors of hyperthrombosis and the key mechanistic event in ischemic stroke.\(^\text{10}\) vWF has long been known to be a key player in thrombus formation at sites of vascular damage. Knowledge about the role of vWF in stroke is much more limited. However, in recent years, an increasing amount of clinical and preclinical evidence has revealed the critical involvement of vWF in stroke development.\(^\text{11}\) Current study observed that the mean vWF-Ag in patients with ischemic stroke was significantly higher (\(P < 0.01\)) compared to healthy controls. This result was in agreement with the other researchers.\(^\text{2,12,13}\) The mechanism responsible for increase plasma vWF levels and the occurrence of ischemic stroke is due to inflammation resulting in endothelial cell activation, which leads to increase vWF secretion and subsequent reduce clearance of it. When vWF more release, it facilitates over platelet adhesion leading to secretion of excess active substances and excess generation of platelet plug which leading to thrombosis.\(^\text{12-14}\) It has long been known that lipids abnormalities are a major risk factor for the onset of ischemic stroke.\(^\text{15}\) Our data reveal that the mean fasting serum T.Ch level in patients with ischemic stroke was significantly higher (\(P < 0.01\)) compared to healthy controls. This result was in agreement with results obtained by other investigators.\(^\text{16,19,22}\) Whereas the mean fasting serum HDL-C level was significantly lower (\(P < 0.05\)) in patients with ischemic stroke compared to the control group. Similar results have been reported by other studies.\(^\text{17,23,24}\) The exact mechanism responsible for ischemic stroke in hyperlipidemia is multi factorial and are not completely understood yet. It has enormous clinical, social, and economic implications and demands a significant effort from both basic scientists and clinicians in the quest for understanding the underlying pathogenetic mechanisms, and thereby adopting suitable preventive measures and successful therapies.\(^\text{24}\) Ischemic stroke can be caused by a number of monogenic disorders in lipid metabolism, while the most important factor is abnormal lipid metabolism and oxidation of LDL.\(^\text{25}\) Oxidized LDL stimulate the adhesion of monocytes to the endothelium. Monocytes penetrate into the arterial intima, then differentiate into macrophages and eventually become foam cells by binding and endocytosing oxidized LDL (especially cholesteryl esters which accumulate as insoluble residues) through scavenging receptors, ultimately leads to the generation of atherosclerosis plaque.\(^\text{26,27}\) Foam cells produce matrix metalloproteinase 9 (MMP-9), which contributes to the degradation of the fibrous cap of atheromatous plaques, resulting in its rupture and formation of a blood clot. If the blood clot dislodges from the arterial wall, bloodstream can carry it to the brain, where it lodges in a cerebral artery and causes an embolic ischemic stroke.\(^\text{28}\) Furthermore, HDL has antiatherogenic properties, it has the ability in (transporting cholesterol from the peripheral cells to the liver, preventing lipid peroxidation (antioxidant effects) and inhibiting platelet activation and aggregation). Therefore HDL has an inverse correlation with the risk of stroke.\(^\text{23}\)

**Discussion**

In an attempt to bring to light the most important pathophysiological effect of vWF, serum lipids and lipoprotein profile on ischemic stroke, we assayed a series of samples of clinically defined ischemic stroke and healthy controls. Abnormal coagulation and fibrinolytic factors have been suggested as possible predictors of hyperthrombosis and the key mechanistic event in ischemic stroke.\(^\text{10}\) vWF has long been known to be a key player in thrombus formation at sites of vascular damage. Knowledge about the role of vWF in stroke is much more limited. However, in recent years, an increasing amount of clinical and preclinical evidence has revealed the critical involvement of vWF in stroke development.\(^\text{11}\) Current study observed that the mean vWF-Ag in patients with ischemic stroke was significantly higher (\(P < 0.01\)) compared to healthy controls. This result was in agreement with the other researchers.\(^\text{2,12,13}\) The mechanism responsible for increase plasma vWF levels and the occurrence of ischemic stroke is due to inflammation resulting in endothelial cell activation, which leads to increase vWF secretion and subsequent reduce clearance of it. When vWF more release, it facilitates over platelet adhesion leading to secretion of excess active substances and excess generation of platelet plug which leading to thrombosis.\(^\text{12-14}\) It has long been known that lipids abnormalities are a major risk factor for the onset of ischemic stroke.\(^\text{15}\) Our data reveal that the mean fasting serum T.Ch level in patients with ischemic stroke was significantly higher (\(P < 0.01\)) compared to healthy controls. This result was in agreement with results obtained by other investigators.\(^\text{16,19,22}\) Whereas the mean fasting serum HDL-C level was significantly lower (\(P < 0.05\)) in patients with ischemic stroke compared to the control group. Similar results have been reported by other studies.\(^\text{17,23,24}\) The exact mechanism responsible for ischemic stroke in hyperlipidemia is multi factorial and are not completely understood yet. It has enormous clinical, social, and economic implications and demands a significant effort from both basic scientists and clinicians in the quest for understanding the underlying pathogenetic mechanisms, and thereby adopting suitable preventive measures and successful therapies.\(^\text{24}\) Ischemic stroke can be caused by a number of monogenic disorders in lipid metabolism, while the most important factor is abnormal lipid metabolism and oxidation of LDL.\(^\text{25}\) Oxidized LDL stimulate the adhesion of monocytes to the endothelium. Monocytes penetrate into the arterial intima, then differentiate into macrophages and eventually become foam cells by binding and endocytosing oxidized LDL (especially cholesteryl esters which accumulate as insoluble residues) through scavenging receptors, ultimately leads to the generation of atherosclerosis plaque.\(^\text{26,27}\) Foam cells produce matrix metalloproteinase 9 (MMP-9), which contributes to the degradation of the fibrous cap of atheromatous plaques, resulting in its rupture and formation of a blood clot. If the blood clot dislodges from the arterial wall, bloodstream can carry it to the brain, where it lodges in a cerebral artery and causes an embolic ischemic stroke.\(^\text{28}\) Furthermore, HDL has antiatherogenic properties, it has the ability in (transporting cholesterol from the peripheral cells to the liver, preventing lipid peroxidation (antioxidant effects) and inhibiting platelet activation and aggregation). Therefore HDL has an inverse correlation with the risk of stroke.\(^\text{23}\)
Conclusion
This study demonstrates a statistically significant difference in mean concentrations of fasting serum T.Ch., TGs., and LDL-C between normal healthy subjects and patients with ischemic stroke, and a significant decrease in the level of fasting serum HDL-C in ischemic stroke patients. The results obtained in the present study indicate a significant increase in the plasma level of vWF-Ag in patients with ischemic stroke. Our results suggest that lipids and vWF give some contribution to stroke risk. Lipid profile and vWF-Ag assessment must be taken into account in estimating the individual risk of stroke.

Competing interests
The authors declare that they have no competing interests.

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