

INTRODUCTION

Cerebrovascular stroke is the second most common cause of death (after ischemic heart diseases) and the leading cause of disability worldwide and one of the leading causes of death in Egypt, according to the WHO media center (***WHO media center May, 2014***).

Ischemic stroke is the most common type of stroke in Egypt and accounts for 43% to 79% of all stroke types as in other countries (***Khedr et al., 2013***).

Acute Ischemic stroke is diagnosed by full history, clinical examination including general and neurological examinations, emergent brain imaging for confirming the diagnosis including CT and MRI, and the conventional baseline laboratory testing that is often limited to blood glucose, coagulation studies, complete blood count (CBC) and lipid profile. Additional laboratory tests may include cardiac biomarkers, homocysteine level, antinuclear antibody (ANA), and rheumatoid factor. Emerging studies of the genetic basis of enzymes like matrix metalloproteinases and Angiotensin converting enzyme may be essential for early diagnosis and determining the prognosis of acute ischemic stroke (***Edward et al., 2014***).

There are some traditional factors that increase the risk of ischemic stroke such as hypertension and smoking, but

genetic risk factors, suggested by evidence from inheritance-based studies, might also contribute to a predisposition to ischemic stroke (*Flossmann et al., 2013*).

Inflammation is an essential process in the pathogenesis of ischemic stroke. Therefore, genes involved in inflammatory responses are under investigation to look for variants predisposing to ischemic stroke (*Libby, 2002*).

Genetic study of proteins, such as lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetylhydrolase (PAF-AH), shows that it is a calcium ion (Ca²⁺) independent phospholipase which belongs to phospholipase A2 superfamily and is composed of 441 amino acids (*Dada et al., 2002*).

The platelet-activating factor (PAF) is a phospholipid with a variety of biological functions. Lp-PLA2 was primarily recognized as an anti-inflammatory enzyme because of its capability of degrading platelet-activating factor. Intriguingly, this anti-inflammatory activity is gradually opposed by its pro-inflammatory action. Beside others, it is involved in the signaling and activation of proinflammatory cells such as platelets, neutrophils and macrophages (*Prescott et al., 2000*) and also it might inhibit the apoptosis of B cells (*Toledano et al., 2000*). Furthermore, it has been reported to alter vascular permeability and to induce hypertension, platelet-dependent

broncho-constriction, and smooth-muscle contraction (**Miwa et al., 2000, Hakkinen et al., 2000 and Stafforini et al., 2000**).

Lp-PLA2 also participates in the oxidative modification of low-density lipoprotein (LDL) by cleaving oxidized phosphatidylcholines, generating lysophosphatidylcholine (lyso-PC) and oxidized non-esterified fatty acids (oxNEFAs), which are two important pro-inflammatory mediators (**Shi et al., 2007 and Cai et al., 2013**).

Genetic study of the human Lp-PLA2 shows that it is encoded by the PLA2G7 gene, which is located on chromosome 6p21-p12 originally assigned as the human leukocyte antigen region (**Stafforini et al., 2000**).

One non-synonymous polymorphism A379V (Ala379Val) in exon 11, has previously been found to be associated with coronary artery disease and ischemic stroke in the Chinese and USA population (**Liu et al., 2006 and Sutton et al., 2008**).

However, little is known about whether the PLA2G7 gene polymorphism is associated with the risk of ischemic stroke in the Egyptian population or not.

AIM OF THE WORK

To investigate the role of A379V (Ala 379 Val) genetic variant of PLA2G7 gene in patients with acute ischemic stroke.

I- ACUTE ISCHEMIC STROKE (AIS)

A) Definition:

The traditional definition of stroke, devised by the World Health Organization in the 1970s, is a "neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours". This can be due to ischemia caused by thrombosis, embolism or hemorrhage. Approximately 79 percent of strokes are ischemic in nature (*Kidwell et al., 2003*). The 24-hour limit differentiates stroke from transient ischemic attack (TIA), which is a related syndrome of stroke symptoms that resolve completely within 24 hours (*Khedr et al., 2013*).

B) Epidemiology:

Cerebrovascular stroke is the most frequent cause of permanent disability in adults worldwide (*Donnan et al., 2008*) and the second most common cause of death (after ischemic heart diseases) and one of the leading causes of death in Egypt, according to the WHO (*WHO media center, 2014*). Annually, 15 million people worldwide suffer a stroke. Of these, 5 million are left permanently disabled. The World Health Organization (WHO) estimates that a stroke occurs every 5 seconds worldwide (*WHO Global burden of stroke, 2011*). Globally, the average 30-day case fatality following first ischemic stroke

is about 22.9% with the exception of Japan (17%) and Italy (33%) (*Feigin et al., 2005*).

Ischemic stroke is the most common type of stroke in Egypt and worldwide, is accounting for 43% to 79% of all stroke types (*Khedr et al., 2013*).

C) Causes and Classifications of Acute Ischemic Stroke (AIS):

In an ischemic stroke, blood supply to part of the brain is decreased, leading to dysfunction of the brain tissue in that area. There are many reasons why this might happen (*Stam, 2005*): (1) Thrombosis (obstruction of a blood vessel by a blood clot forming locally), (2) Embolism (due to an embolus from elsewhere in the body), (3) Systemic hypoperfusion (general decrease in blood supply, e.g. in shock) (*Stam, 2005*), (4) Stroke without an obvious explanation which is termed "cryptogenic" (of unknown origin); constitutes 30-40% of all ischemic strokes (*Donnan et al., 2008*).

There are various classification systems for acute ischemic stroke:

- 1- The Oxford Community Stroke Project classification (OCSP, also known as the Bamford or Oxford classification) relies primarily on the initial symptoms. Based on the extent of the symptoms, the stroke episode is classified as total anterior circulation infarct (TACI), partial anterior

circulation infarct (PACI), lacunar infarct (LACI) or posterior circulation infarct (POCI). These four entities predict the extent of the stroke, the area of the brain affected, the underlying cause, and the prognosis (**Bamford, 2000**).

- 2- The Trial of Organization 10172 in Acute Stroke Treatment (TOAST) classification is based on clinical symptoms as well as results of further investigations. On this basis, a stroke is classified as being due to (a) thrombosis or embolism due to atherosclerosis of a large artery, (b) embolism of cardiac origin, (c) occlusion of a small blood vessel, (d) other determined cause, (e) undetermined cause (no cause identified, or incomplete investigation) (**Goldstein et al., 2005**).
- 3- Thrombotic stroke can be divided into two types depending on the type of vessel the thrombus is formed on (**Donnan et al., 2008**): (i) Large vessel disease: involves the common and internal carotids, vertebral arteries, and the Circle of Willis. Diseases that may form thrombi in the large vessels include (in descending incidence): atherosclerosis, vasoconstriction (tightening of the artery), aortic, carotid or vertebral artery dissection, various inflammatory diseases of the blood vessel wall (Takayasu arteritis, giant cell arteritis, vasculitis), non inflammatory vasculopathy and fibromuscular dysplasia, (ii) Small vessel disease: involves the smaller arteries inside the brain: branches of the circle of

Willis, middle cerebral artery, stem and arteries arising from the distal vertebral and basilar artery. Diseases that may form thrombi in the small vessels include (in descending incidence): lipohyalinosis (build-up of fatty hyaline matter in the blood vessel as a result of high blood pressure and aging) and fibrinoid degeneration (stroke involving these vessels are known as lacunar infarcts) and microatheroma (small atherosclerotic plaques) (*Donnan et al., 2008*).

D) Pathophysiology of Acute Ischemic stroke (AIS):

1- Risk Factors of AIS:

a) Non modifiable risk factors:

- i. Age:** Atherosclerosis increases with age, subsequently increasing the risk of ischemic stroke. The prevalence of stroke for individuals older than 80 years of age is approximately 27%, compared with 13% for individuals 60 to 79 years of age (*Rosamond et al., 2008*).
- ii. Race differences:** The annual incidence of age- adjusted initial ischemic strokes per 100,000 in people 20 years of age in Northern Manhattan Stroke Study (NOMASS) was 88 in whites, 191 in blacks and 149 in Hispanics (*White et al., 2005*). The race differences are most probably related to atherosclerosis. According to Atherosclerosis Risk in Communities (ARIC) study data, the age-adjusted incidence of stroke per 100,000 population in people 45 to 84 years of age

is 360 in white males, 230 in white females, 660 in black males, and 490 in black females (*Rebbeca et al., 2008*).

iii. Sex: Men have a higher risk for stroke (1.25 times that of women), but more women die from stroke. Women account for three out of every five stroke deaths. This may be due in part to the fact that men do not live as long as women so they are usually younger when they have their strokes and therefore are better able to survive the trauma (*Mosca et al., 2007*). Some risk factors for stroke apply only to women. Primary among these are pregnancy, childbirth, menopause and the treatment with hormonal replacement therapy (HRT) (*Del Zoppo, 2010*).

iv. Family history: Family history of stroke in one of the two parents, transient ischemic attacks (TIA), or myocardial infarction is associated with 1.4 to 3.3 fold increased risk for stroke. The increased prevalence of stroke between monozygotic and dizygotic twins is almost fivefold. In addition, cerebral autosomal dominant arteriopathy with sub cortical infarcts and leukoencephalopathy (CADASIL), has been reported to cause recurrent strokes with typical onset between the ages of 30 to 50 years (*Rebbeca et al., 2008*).

b) Well-established modifiable risk factors:

i. Hypertension: The risk is increased because hypertension accelerates the development of atherosclerosis, ultimately leading to an increased number of atherothrombotic events

(*Chobanian et al., 2003*). The mortality of heart disease and stroke double with each increment of 20 mmHg systolic blood pressure (*Sacco et al., 2006*).

- ii. Diabetes:** It is estimated that nearly 40% of all ischemic strokes can be attributed to the effects of diabetes either alone or in combination with hypertension (*Kissela et al., 2005*). In addition, more clinical studies have identified impaired glucose tolerance as an independent risk factor for recurrent stroke in patients who have TIA or minor ischemic stroke. This can be explained by the high glucose concentrations that increase the formation of toxic products and reactive oxygen species that damage vessel walls. In addition, glucose and its products in cells increase osmotic pressure which contribute to vessel injury leading to death of cells in eyes, kidneys and nerves (*Vermeer et al., 2006*).

c) Potentially Modifiable Risk Factors:

These factors are less documented than well-established risk factors. They include cardiac diseases, inflammation and other factors (*Rebecca et al., 2008*).

- i. Asymptomatic carotid stenosis:** Asymptomatic carotid stenosis greater than 50% is detected in 5% to 10% of adults 65 years of age or older, and stenosis greater than 80% is found in approximately 1% (*Goldstein et al., 2006*) that can be treated by carotid angioplasty (*Brooks et al., 2004*).

- ii. Dyslipidemia:** Dyslipidemia is a major risk factor for coronary heart disease (CHD), its role in the pathogenesis of ischemic stroke is not clear, however, some epidemiological studies have provided the association of dyslipidemia and ischemic stroke. Overall elevated LDL cholesterol level appears to increase the risk of atherosclerosis and thereby ischemic stroke. Low level of HDL cholesterol also appears to be associated with a greater risk. Whereas the importance of high triglyceride level is not clear (*Vasilios, 2009*).
- iii. Diet:** Studies have shown that there is a protective relationship between citrus fruit and green leafy vegetable consumption and ischemic stroke risk. There is 6% reduction in risk of ischemic stroke with each one-serving increase of fruits and vegetables daily (*Sauvaget et al., 2003*). On the contrary, overweight individuals, higher sodium intake which already increase blood pressure are associated with increased risk for stroke mortality (*Nagata et al., 2004*). However, potassium supplementation has shown a decrease in mean systolic and diastolic blood pressure and this is associated with reduction in stroke mortality (*Rebbeca et al., 2008*).
- iv. Obesity:** Obesity is a risk factor for ischemic stroke in women and men. Men who have body mass index (BMI) greater than 30 kg/m², have a (1.95) relative risk for ischemic stroke (*Kurth et al., 2002*). In addition, there is a

relation between abdominal adiposity and stroke risk. Abdominal adiposity is defined as highest quartile of waist circumference or hip/waist ratio. It is associated with increase in atherosclerotic risk and subsequently it can be found as a risk factor for ischemic stroke (*Hu et al., 2007*).

- v. **Hormone replacement therapy (HRT):** In clinical trials, women who received estrogen replacement therapy (HRT) had a 2.9 relative risk for strokes, and the non-fatal strokes that occurred were associated with increased functional deficits. This potential risk can be attributed to hypercoagulable state occurring by HRT and subsequent risk of cardiac embolism or thrombosis (*Rebbeca et al., 2008*).
- vi. **Hyperhomocysteinemia:** Hyperhomocysteinemia is associated with vasculopathy. Its role in the pathogenesis of ischemic stroke is not clear, however, some epidemiological studies had found that some patients with stroke have homocysteine level 1.5 time more than those of age and sex matched controls (*Rebbeca et al., 2008*). In addition, prospective and case control studies have found that incidence of stroke increases with increasing homocysteine level (*Rebbeca et al., 2008*). It is poorly understood how homocysteine might exert damaging effects. A hypothesis is that homocysteine has a toxic effect on the cells that make up the innermost layer of blood vessels and may induces vascular damage by promoting

platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation and endoplasmic reticulum stress (**Pagiliani, 2012**). The Homocysteine Studies Collaboration reported that homocysteine levels reduced by 25% (about 3 mmol/L) are associated with 19% reduction in stroke risk (**Souviksen, 2011**).

vii. Hypercoagulability: Several studies suggest that the presence of antiphospholipid antibodies is an independent risk factor for stroke in young women (**Rebbeca et al., 2008**). Inherited thrombophilias, specifically factor V Leiden mutation, are associated with increased risk for venous thrombosis (**Hankey et al., 1999**). However, no study has established a relationship between factor V Leiden mutation and cerebral stroke (**Souviksen, 2011**).

In a study by **Souviksen (2011)**, 10 of 60 patients (17%) had an acute ischemic venous stroke that was attributed to deficiencies in protein C, protein S, or antithrombin III(ATIII). No clear-cut association has been found between protein C or AT III deficiency and arterial strokes, although patients with low protein C levels at the time of acute stroke have poor outcomes. However, a prospective study found free protein S deficiency in 23% of young patients with stroke of uncertain cause, but this finding could be associated with higher levels of C4b (complement C4b; an acute phase reactant that decreases the free protein S levels) (**Souviksen, 2011**).

Table (1): Risk Factors of AIS:

a- Non modifiable risk factors	b- Well-Established Modifiable Risk Factors	c- Potentially Modifiable Risk Factors
i. Age: Elderly, especially >80 years of age	i. Hypertension: Blood pressure >140/90	i. Asymptomatic carotid stenosis: >80% stenosis
ii. Race: Blacks> Hispanics> Whites	ii. Diabetes: Multiple co morbidities	ii.Dyslipidemia: increases the risk of atherosclerosis and thereby ischemic stroke
iii. Sex: Men> women, except in age group (35-44)		iii. Diet: High sodium, low potassium diet
iv. Family History: Monozygotic twins, dominant genetic disorders (CADASIL)		iv. Obesity: Overweight individuals with body mass index>30kg/m ²
		v. Hormone replacement therapy: postmenopausal women or HRT
		vi. Hyperhomocysteinemia: Elderly, young males
		vii. Hypercoagulability: Young women who have antiphospholipid antibodies

(Rebbeca et al., 2008)

2- Pathophysiological mechanisms of acute ischemic stroke (AIS):

Acute ischemic stroke is associated with a rise in systemic markers of inflammation, endothelial activation and oxidative stress. In addition, at the site of brain injury, ischemic cascade, oxidative stress and brain inflammation are thought to

contribute to pathophysiology of cerebral injury in acute stroke (**Beer et al., 2010**).

The initiation and formation of atherosclerotic plaque represents a complex interplay of environmental factors and genetic susceptibility that results in a chronic inflammatory process, that eventually leads to stroke. Exposure of the endothelial surface to a variety of risk factors such as hypertension, hyperglycemia, oxidized low-density lipoprotein, cigarette smoke toxins, infections, and other inflammatory compounds results in endothelial injury (**Yilmaz and Granger, 2008**). Atherosclerosis is being re-defined from its original perception as a disorder of lipid deposition to one of an ongoing inflammatory process (**DeGraba, 2004**).

Lp-PLA2 (Lipoprotein associated phospholipase A2) was primarily recognized as an anti-inflammatory enzyme because of its capability of degrading platelet- activating factor. Intriguingly, this anti-inflammatory activity is gradually opposed by its pro-inflammatory action (**Figure 1**). Lp-PLA2 participates in the oxidative modification of low-density lipoprotein (LDL) by cleaving oxidized phosphatidylcholines, generating lysophosphatidylcholine (lyso-PC) and oxidized non-esterified fatty acids (oxNEFAs), which are two important pro-inflammatory mediators (**Shi et al., 2007 and Cai et al., 2013**).