INTRODUCTION

Cerebral stroke (CS) is considered as one of the major causes of death and disability worldwide ***(Bushueva et al., 2015).*** Ischemic stroke is the most common type of stroke in Egypt, as in other countries, accounting for 43% to 79% of all stroke types ***(Khedr et al., 2013)***. 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 contribute to predisposition to ischemic stroke ***(Flossmann et al., 2013).***

Endothelium derived nitric oxide (NO) is critical to vascular homeostasis. NO has potent vasodilator and anti-proliferative effects, as well as antithrombotic properties. It stimulates smooth muscle cell relaxation and inhibits platelet aggregation and leukocyte adhesion. Impaired endothelium-dependent vasodilation is a general attribute of atherosclerotic vessels, which to some extent could be due to the reduction in the activity of vascular endothelial nitric oxide synthase (eNOS). This impaired NO-dependent vasomotor reactivity has been implicated in the pathophysiology of stroke ***(Stagliano et al., 1997).***

Previously published studies have indicated that the (eNOS) gene plays a crucial role in the pathogenesis of

many diseases including stroke, essential hypertension and has an important role in development of coronary artery disease ***(Men et al, 2011 and Rahimi et al, 2012).*** The eNOS gene is situated on chromosome 7 (7q35–q36), it consists of 26 exons and codes for an enzyme that produces Nitric Oxide (NO) in the vascular endothelium. ***(Hu et al., 2014; Yang et al, 2014 and Kumar et al, 2016).*** Different genetic variations of eNOS were identified to be associated with development of stroke as (4b/a, 786T>C, A-922G and Glu298Asp) gene polymorphisms ***(Cheng et al., 2008; Guldiken et al., 2009 and Saidi et al., 2010)*** Among investigated genetic variations in eNOS is G894T polymorphism that leads to a change of glutamate to aspartate at site 298 and is said to have increased susceptibility to cleavage of eNOS enzyme which contribute to the development of stroke ***(Kaur et al., 2015).***

Aim of the Work

This study aims to explore the relationship between G894T polymorphism of endothelial nitric oxide synthase gene and acute ischemic stroke.

Chapter (I): Acute Ischemic Stroke

A. Definition:

According to World Health Organization **(WHO)** stroke is a "neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours" ***(WHO Media Center, 2014).*** This can be due to ischemia caused by thrombosis, embolism or hemorrhage. Approximately 87 % of strokes are ischemic in nature; 10% are intra cerebral and 3% are subarachnoid hemorrhage ***(Kaur et al., 2015).***

B. Epidemiology:

Cerebrovascular stroke **(CVS)** is the most frequent cause of permanent disability in adults worldwide, 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).***

C. Causes and Classifications of Acute Ischemic Stroke:

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: Thrombosis, embolism and systemic hypo perfusion ***(Stam, 2005).***

There are various systems for classification of acute ischemic stroke which include:

1. **The Oxford Community Stroke Project classification (OCSP):**

It is a common clinical stroke classification tool, which categorizes stroke into 4 subtypes: total anterior circulation infarct (TACI), partial anterior circulation infarct (PACI), lacunar infarct (LACI) and posterior circulation infarct (POCI). OCSP classification can predict the extent of the stroke, the area of the brain affected, the underlying cause, and the prognosis ***(Asdaghi et al., 2011).***

2. **The Trial of Organization 10172 in Acute Stroke Treatment (TOAST) classification:**

It is based on clinical symptoms and results of further investigations. On this basis, a stroke is classified into 5 categories based on etiology ; thrombosis or embolism due to atherosclerosis of a large artery, embolism of cardiac origin, occlusion of a small blood vessel, other determined cause and undetermined cause (no cause identified***) (Chung et al., 2014).***

3. **Classification according to the type of the occluded vessels:**

Thrombotic stroke also can be divided into two types according to the type of vessel the thrombus is formed on**;** large vessel disease which involves the common and

internal carotids, vertebral arteries, and the Circle of Willis and small vessel disease that 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 ***(Donnan et al., 2008).***

D. Risk Factors of Acute Ischemic Stroke:

1. Non-modifiable Risk Factors:

a. Age:

Atherosclerosis increases with age, subsequently increasing the risk of ischemic stroke. The prevelance of stroke for individuals older than 80 years of age is approximately 27%, compared with 13% for individuals 60 to 79 years of age ***(bastan et al., 2016).***

b. 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).***

c. Ethnicity/race:

Previous studies show that black patients have a higher incidence of stroke than white patients. The mechanisms of this association are poorly understood but might be related with genetic or nutritional factors ***(Catalan Society of Neurology, 2011).***

d. Family history:

Family history of stroke in a first-degree relative increases the risk of suffering from an acute cerebrovascular event, this increased risk may be due to different mechanisms, including inherited predisposition, genetic transmission of susceptibility to stroke, familial-related lifestyle, cultural and environmental factors, or interactions between genes and environmental factors ***(Arboix, 2015).***

2. Well-Established Modifiable Risk Factors:

a. Hypertension:

Hypertension accelerates the development of atherosclerosis; which leading to an increased number of atherothrombotic events and hence increase risk of stroke ***(Sacco et al., 2006).*** High blood pressure increase the risk for stroke for 4-fold ***(McManus and Liebeskind, 2016).***

b. Diabetes mellitus:

About 40% of all ischemic strokes can be due to the effects of diabetes especially if associated 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 ***(Feigin et al., 2016).***

c. Smoking:

Smoking increases the risk of thrombus formation and accelerates the development of atherosclerosis. Smoking also increases blood viscosity, platelet aggregation, increases in low density lipoproteins (LDL-C) cholesterol and decreases high density lipoprotein (HDL-C) cholesterol, which causes direct damage to endothelium and increase in blood pressure ***(Arboix, 2015).***

3. Potentially Modifiable Risk Factors:

a. 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).***

b. Dyslipidemia:

Dyslipidemia is a major risk factor for coronary heart disease (CHD), 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).***

c. Obesity:

Obesity is a risk factor for ischemic stroke in men and women. Men who have body mass index (BMI) greater than 30 kg/m^2, have a (1.95) relative risk for ischemic stroke ***(Kurth et al., 2002).***

Summary of risk factors of AIS shown in **(Table 1).**

Table (1): Shows Summary of Risk Factors of AIS:

1.Non modifiable Risk Factors	2. Well-Established Modifiable Risk Factors	3. Potentially Modifiable Risk Factors
a. Age: Elderly, especially >80 years of age.	**a. Hypertension:** Blood pressure >140/90.	**a. Asymptomatic carotid stenosis :**> 80% stenosis.
b. Sex: Men> women.	**b. Diabetes:** Multiple co morbidities.	**b. Dyslipidemia:** increases the risk of atherosclerosis and thereby ischemic stroke.
c. Ethnicity/race Blacks> Hispanics> Whites.	**c. smoking***:* smoker >nonsmoker.	**c. Obesity:** Overweight individuals with body mass index>30kg/m².
d. Family History: Monozygotic twins, dominant genetic disorders.		

E. Diagnosis of Acute Ischemic Stroke:

1. History:

Full history including present history, family history, past history and history of risk factors and related diseases, such as hypertension, diabetes or heart disease, as well as prior stroke or transient ischemic attack are taken in consideration ***(Nor et al., 2005)***.

2. Clinical presentation:

Symptoms of a stroke are variable according to the area of the brain involved. One of the common symptom of stroke is sudden weakness and numbness on one side of the body ***(Donnan et al., 2008).***

Neurological symptoms of acute ischemic stroke is different according to cerebral circulation and the artery being occluded:(a) Anterior Cerebral Artery: Confusion, personality change and incontinence, (b) Middle Cerebral Artery: Contrateral motor loss in lower face, contralateral motor or sensory loss (arm more than leg), contralateral visual field loss and language deficit (dominant hemisphere), (c) Posterior Cerebral Artery: Contalateral sensory loss, ipsilateral visual field deficit and cortical blindness ***(Janica et al., 2007).***

3. Physical and neurological examination:

Physical and neurological examination of the patient help in confirming the diagnosis .An examination usually include vital signs assessment such as pulse and blood pressure. The examination will include also the ability to perform certain basic functions if the patient is alert ***(Nor et al., 2005).***

The national institute of health stroke scale (NIHSS) is a rapid and reliable tool for measurement stroke severity ***(Joseph et al., 2010).*** The NIHSS provide accurate information to patients, set Goals for therapy and plan for discharge. It measures many aspects of brain functions as level of consciousness, vision, movement, sensation, speech, and language ***(Joseph et al., 2010).*** A certain number of points are given for each impairment discovered during a focused neurological examination. A maximal score of 42 represents the most severe and devastating stroke **(Table 2)** ***(Kwah and Diong, 2014).***

The level of stroke severity as measured by the NIHSS stroke scale scoring system include:

Score 0 = no stroke

Score 1-4 = minor stroke

Score 5-15 = moderate stroke

Score 15-20 = moderate/severe stroke

Score 21-42 = severe stroke ***(Joseph et al., 2010).***

Table (2): National Institutes of Health Stroke Scale:

Category	Results	Scoring
1.Level of consciousness (LOC)	Alert, kneely responsive. Not alert (arousable- minor stimulation) Not alert (arousable- painful stimulation) Unresponsive	0 1 2 3
2.Visual gaze	No visual loss Partial hemianopia Complete hemianopia Bilateral hemianopia	0 1 2 3
3.Facial palsy	Normal Minor paralysis Partial paralysis Complete paralysis	0 1 2 3
4.Limb ataxia	Ataxia-two limbs No ataxia Ataxia-one limb	0 1 2
5.Sensory	No sensory loss Mild sensory loss Severe sensory loss	0 1 2
6.Articulation	Normal Mild to moderate dysartheria Severe dysartheria	0 1 2

(Janice et al., 2007).

4. Investigation:

a- Imaging of the Brain:

The diagnosis of stroke is mainly clinical diagnosis, with assistance of imaging technique like CT scans (mostly without contrast enhancements) or MRI scans, Doppler

ultrasound, and arteriography ***(Kidwell et al., 2004).*** Imaging techniques can also help in determining the subtypes and causes of stroke ***(Kidwell et al., 2004).***

i) Computed axial tomography (CT scan, CAT scan):

CT scan can quickly rule out hemorrhage or a brain tumor causing stroke-like symptoms. It may even show areas of the brain that are in danger of dying. CT is one of the non-invasive, painless imaging test that uses x-rays to produce a three-dimensional image of the interior of the patient's head **(Figure: 1)** ***(Chalela et al., 2007).***

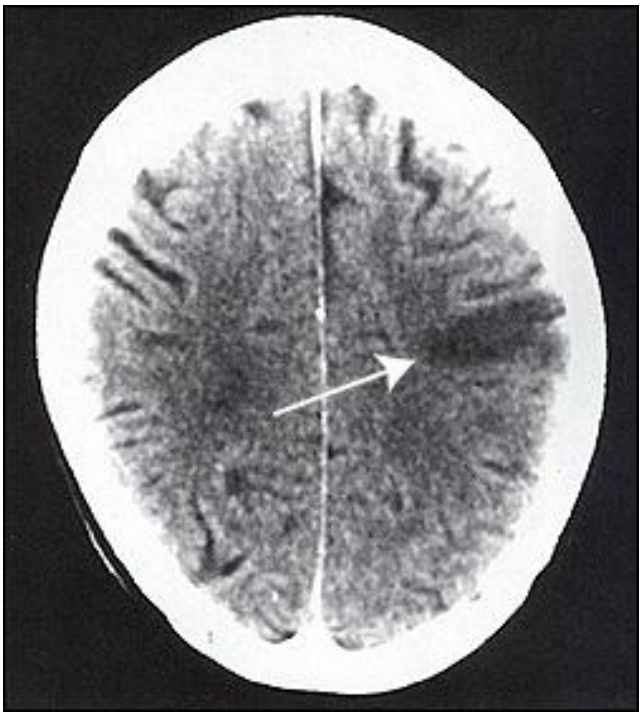

Figure (1): CT scan of a patient with left middle cerebral artery stroke. The arrow indicates the location of the stroke ***(Chalela et al., 2007).***

ii) Magnetic Resonance Imaging (MRI scan, MR scan):

MRI scan shows the brain and spinal cord in more details than a CT scan. MRI can be used in diagnosis of ischemic stroke, hemorrhagic stroke, and other problems involving the brain, brain stem, and spinal cord ***(Chalela et al., 2007).*** This is a non-invasive, painless test that uses magnetic fields to produce a three-dimensional image of the interior of the patient's head **(Figure 2)** ***(Chalela et al., 2007).***

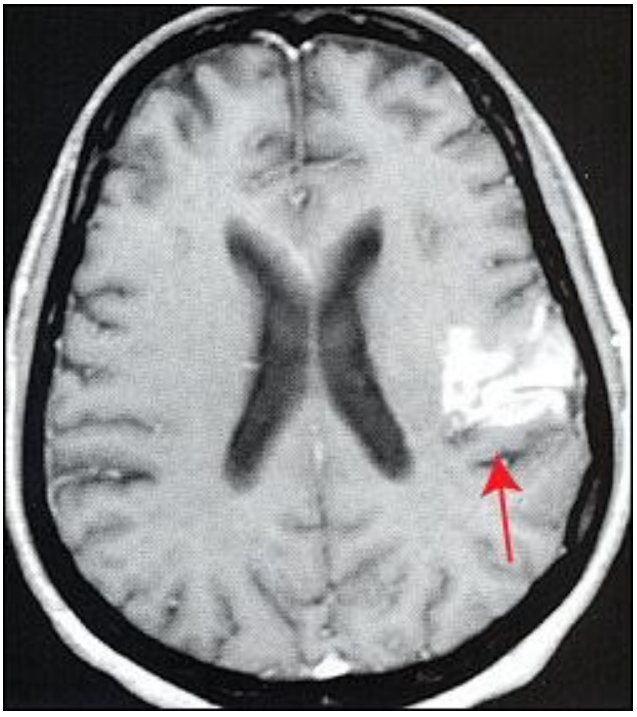

Figure (2): MRI of a patient with stroke of the left hemisphere of the brain. The arrow indicates the area that was affected ***(Chalela et al., 2007).***