

ASSESSMENT OF HOMOCYSTEINE AND ANTIOXIDANT ENZYMES LEVELS IN STROKE

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Article Received on
10 Feb. 2017,

Revised on 02 March. 2017,
Accepted on 22 March 2017

DOI: 10.20959/wjpr20174-8155

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ABSTRACT

Free radicals could play an important role in brain ischemia and reperfusion injury while the generated reactive oxygen species via homocysteine (Hcy) metabolism might also be involved in the induction of lipid peroxidation by malondialdehyde (MDA) formation. The objective of this study has been to investigate the behavior of Hcy, superoxide dismutase SOD, MDA and lipids in the plasma of stroke patients. Forty stroke patients from both genders (26 males and 14 females) were compared with another 40 healthy individuals (25 males and 15 females). Plasma total Homocysteine (tHcy), SOD, MDA and lipids [total Cholesterol (t-Ch)], Triglyceride (TG), Very Low Density Lipoprotein (VLDL), High Density Lipoprotein (HDL) and Low

Density Lipoprotein (LDL)] level were measured. The levels of tHcy, SOD, MDA and lipid (except HDL) had significantly increased ($p \leq 0.05-0.01$) in the serum of stroked patients in comparison with control. These data support that free radical mechanism may play a role in development of stroke and support the consideration of plasma Hcy, SOD, MDA and lipids as a regular and routine screening markers to protect target organ from damage.

KEY WORDS: Stroke, Homocysteine, SOD, MDA, Cholesterol, Triglyceride, LDL, HDL, VLDL.

1.0. INTRODUCTION

In general, stroke might be a major cause of global disability and the second most leading cause of mortality worldwide^[1,2]. Stroke occurs mainly due to elevation in the total plasma homocysteine levels in the elderly and is a common and well-established risk factor for

cardiovascular disease^[3-5]. Homocysteine, ($C_4H_9NO_2S$), a non-protein α -amino acid is the homologue of the amino acid cysteine, differing by an additional methylene bridge ($-CH_2-$). The homocysteine a sulphurous amino acid formed as an intermediary product during the conversion of the essential amino acid methionine to cysteine. It is biosynthesized from methionine by the removal of its terminal C methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of certain B-vitamins. A high level of homocysteine in the blood (hyperhomocysteinemia) incur a person more susceptible to endothelial cell injury, that leads to inflammation in the blood vessels, which in turn may cause atherogenesis, resulting in ischemic injury^[6]. The hyperhomocysteinemia could be classified into three classes depending on the plasma levels i.e. 16-30 $\mu\text{mol/l}$, 31-100 $\mu\text{mol/l}$ and $>100 \mu\text{mol/l}$ that correspond to light, mild and serious form of hyper-homocysteinemia, respectively^[7,8].

Moreover, in adults and mainly in the elderly individuals the cerebrovascular accidents (CVA) are strongly related to cardiovascular risk factors such as hypertension, hypercholesterolemia, diabetes, nicotine-abuse and obesity. During the last few years hyperhomocysteinemia has also been presented as a cardiovascular risk factor. Hyperhomocysteinemia originates from a deviation in the methionine-homocysteine metabolism, and it therefore exerts a possible risk factor for coronary artery disease. The latter occurs when an atherosclerotic plaque blocks blood flow to the coronary arteries, which supply the heart with oxygenated blood. Hyperhomocysteinemia has also been associated with early pregnancy loss and with neural tube defects^[9]. Various causes that can lead to hyperhomocysteinemia, hereditary abnormalities that lead to disturbances of enzymes related to the homocysteine metabolism, vitamin deficiencies and different other factors i.e. lifestyle factors, chronic renal insufficiency, hypothyroidism, pernicious anaemia, systemic lupus erythematosus (SLE), end stage diabetes, cancers and medication^[8, 10-12].

Oxidative stress, the over-generation of reactive oxygen species (ROS) and lipid peroxidation play an important role in the pathogenesis of neuronal damage induced by ischemia-reperfusion^[13]. The presence of high levels of polyunsaturated fatty acids in the membrane lipids of the brain is a source for lipid peroxidation reactions^[14]. Products of lipid peroxidation, such as malondialdehyde (MDA) and 4-hydroxynonenal, were found to be increased in subjects with thrombotic or cardioembolic ischemic stroke than in controls^[15-17].

The ROS production was increased in the brain during ischemia that was detectable even in plasma^[16].

Excitotoxic stimulation of superoxide and nitric oxide (NO) production in ischemia-reperfusion leads to formation of highly reactive products, including peroxynitrite and hydroxyl radical, which are capable to damage lipids, proteins and DNA^[17]. In this way, NO and ROS act independently as well as cooperatively to induce neuronal death in acute ischemic stroke^[18]. Hyperhomocysteinemia has been correlated with the occurrence of blood clots, heart attacks and strokes, though it is unclear whether hyperhomocysteinemia is an independent risk factor for these conditions. The aim of this study has been to assess the levels of both homocysteine and antioxidant enzymes in stroke patients to establish a correlation between the stroke cases and these levels.

2.0. MATERIALS AND METHODS

Venous blood was drawn from stroke patients and control subjects after (12-14) hour fasting and allowed to clot in a plain tube at room temperature. The serum was aspirated after centrifugation at (3000 rpm) for 30 minutes, then divided into aliquots in plastic tubes and stored at (-20°C) until the estimation time. Serum samples were collected from 80 individuals, 40 (25 male and 15 female) of these individuals were normal and used as control to compare with another 40 (26 male and 14 female) cases of stroke patients admitted to the Tikrit Teaching Hospital in Tikrit city from March 2013 to August 2013 with ages ranged from (35-60 year).

The total homocysteine was determined by HPLC with UV detection at 254 nm according to method of Araki and Sako^[19]. The SOD was determined by colorimetric method according to method of Fridovich^[20] while the MDA was determined by colorimetric method according to method of Guidet and Shah^[21]. Lipid profiles (cholesterol, triglyceride, HDL, VLDL, LDL) were determined by colorimetric method (Biolabo kit, France). Results were analyzed statistically using Student T-test via statistical Minitab program. The arithmetic means were compared to calculations of the characteristics of the application Duncan's Multiple Range Test by probability level $p \leq 0.05$.

RESULTS

A highly significant increased ($p \leq 0.01$) of tHcy in stroke patients (84.3 ± 6.3 $\mu\text{mol/L}$) were comparable to those of control (10.82 ± 1.4 $\mu\text{mol/L}$). The antioxidant enzymes (SOD and

MDA) showed a significant increased ($p \leq 0.05$) in stroke patients (3.805 ± 0.34 mol/L) and (9.84 ± 0.22 mol/L) in comparison with control (2.103 ± 0.042 mol/L) and (4.21 ± 0.31 mol/L), respectively. The lipid profiles (Cho, TG, HDL, VLDL and LDL) scored significant ($p \leq 0.05$) differences in stroke patients (289.6 ± 6.2 mg/dL, 273 ± 6.2 mg/dL, 35.9 ± 4.3 mg/dL, 45.24 ± 2.26 mg/dL and 226 ± 11.3 mg/dL) in comparison with controls (144.5 ± 6.4 mg/dL, 135.1 ± 8.3 mg/dL, 58.02 ± 1.54 mg/dL, 27.02 ± 1.66 mg/dL and 96.38 ± 5.23 mg/dL), respectively (Table-1).

(Table-1): The arithmetic mean \pm SD of all parameters in both 40 stroke patients and 40 control. Student T-Test was performed and differences ranged between ($p < 0.05-0.01$).

Parameters	Stroke patients (n=40)	Control (n=40)
Homocysteine ($\mu\text{mol/L}$)	84.3 ± 6.3	10.82 ± 1.4
SOD ($\mu\text{mol/L}$)	3.80 ± 0.34	2.10 ± 0.042
MDA (mol/L)	9.84 ± 0.22	4.21 ± 0.31
Cholesterol (mg/dl)	289.6 ± 6.2	144.5 ± 6.4
Triglyceride (mg/dl)	273 ± 6.2	135.1 ± 8.3
HDL (mg/dl)	35.9 ± 4.3	58.0 ± 1.54
VLDL (mg/dl)	45.24 ± 2.26	27.0 ± 1.66
LDL (mg/dl)	226 ± 11.3	96.4 ± 5.23

3.0. DISCUSSION

The biosynthesis of homocysteine from methionine involves multiple steps where cystathionine β -synthase catalyses the condensation of homocysteine and serine to give cystathionine^[22]. This reaction uses pyridoxine (vitamin B₆) as a co-factor. Cystathionine γ -lyase then converts this double amino acid to cysteine, ammonia, and α -ketobutyrate^[23]. Homocysteine, could mostly develop from eating meat and high levels of it are linked to early development heart disorders and exerts risk of various heart disease. It is found to be associated with low levels of vitamins B₆, B₁₂, and folate, as well as renal disease. Recent researches have shown, however, that getting homocysteine levels down with vitamins doesn't reduce the chance of undergoing heart disease. The mechanism, is not clear enough yet as the general practitioners (GP) cannot be certain if the chance of having heart and blood vessel disease goes up with higher homocysteine level^[24]. However, there appears to be a correlation between high levels of homocysteine and arterial damage that can, in general, lead to atherosclerosis and blood clots.

It has also been noted that damage to cerebral tissue increases the production of oxygen radicals which follows by increase of oxidative stress, leading to possible subsequent changes

in the degree of elimination of thiols, including homocysteine^[25]. Serum homocysteine increases after acute phase of ischemic stroke as a reason of folate and vitamins B6 or B12 deficiency which is particularly common in elderly people. Impairment of renal function also leads to increased concentrations of homocysteine^[26]. Consequently, several physiological and biochemical changes are accompanied with the elevation of tHcy in human during health complaint.

There is growing evidence that high Hcy levels contribute to the pathogenesis of ischemic stroke^[27]. For a few years passed, numerous studies and reviews have identified a strong and independent association between elevated Hcy and vascular disease, including stroke^[28-31]. The Hcy is postulated to cause ischemic stroke via various mechanisms. However neither its mechanism of action nor its role in the acute phase of stroke are enlightened, yet nor any rapid diagnostic test has been established so far.

Histologically, homocysteine levels are believed to cause atherogenesis and thrombogenesis via endothelial damage, vascular smooth muscle proliferation, and coagulation abnormalities^[32]. It produces changes in structure and function of cerebral blood vessels. Oxidative stress appears to play an important role in mediating such changes^[31]. High homocysteine levels induced by acute methionine loading produced impaired auto-regulatory responses in older humans^[33]. High homocysteine levels induced oxidative stress may occur as a result of decreased expression and/or activities of key antioxidant enzymes as well as increased enzymatic generation of superoxide anion^[31]. Superoxide anion and hydroxyl radical generated during oxidation of Hcy initiate lipid peroxidation, an effect that occurs both at the endothelial cell surface as well as within lipoprotein particles in plasma^[34]. These findings were concomitant with similar results^[35-37]. However, other studies disagreed with the present results regarding lipid fraction, as all lipid fractions (except HDL-C) levels were significantly increased^[38] while was related with hypertension as coexisted with hyperlipidemia. Other studies suggested that serum lipids remain stable following acute ischemic stroke and is consistent with the absence of acute phase response or nutritional deficiency^[39].

There is no universal recommendation for checking homocysteine levels. The test is still relatively expensive while isn't widely available^[24]. In the present study blood sera were used to check the plasma total Homocysteine (tHcy), SOD, MDA and lipids [total cholesterol (t-Ch)], triglyceride (TG), very low density Lipoprotein (VLDL), high and low density lipoprotein

(HDL, LDL), respectively were significantly increased in tHcy in stroke patients with significant increased in antioxidant enzymes (SOD and MDA) and lipid profile (except HDL) in comparison with control. This might indicate a positive correlation between tHcy and the antioxidant enzymes. Accordingly, These tests could well be used as a diagnostic tool to a direct and an early check of the health status of the patients who might display any indication of stroke. Such a technique would be a timesaver and help an early diagnose the expected blood ischemia which could be used by GPs as short cut test and routine health check up at hospitals.

4.0. CONCLUSION

It is concluded that free radical mechanism may play a role in development of stroke and support the consideration of plasma Hcy, SOD, MDA and lipids as a regular and routine screening markers to protect target organ from damage.

5.0. REFERENCES

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