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# Atherosclerosis at Extracranial Carotid Vessels and Serum Homocysteine

*Mei-Ling Sharon Tai, Kuo Ghee Ong, Tsun Haw Toh, Hafez Hussain, Abdul Rashid Mat Mahidin and Esther Kar Mun Yeow*

## Abstract

In this chapter we will discuss more about the role of homocysteine in atherosclerosis and also association between serum homocysteine with extracranial carotid atherosclerosis. Carotid atherosclerosis comprises an increase in carotid intima-media (CIMT) thickening, plaque formation and carotid stenosis. Atherogenic property of homocysteine was discovered in 1969. Atherosclerosis is initiated by endothelial dysfunction. One of the causes of endothelial abnormality is homocysteine. The development of aggregates of homocysteinyllated lipoproteins with microorganisms obstructs the vasa vasorum in vulnerable plaques. In one study, serum homocysteine in the highest quartile was independently associated with extracranial carotid artery stenosis  $\geq 50\%$ . In another study, raised serum homocysteine was also independently associated with severe extracranial carotid stenosis in both genders. In other studies, serum homocysteine was significantly associated with carotid artery stenosis in internal carotid arteries and external carotid arteries as well as the degree of stenosis. The hypertensive patients who had raised serum homocysteine were reported to have higher risk of developing asymptomatic extracranial carotid artery stenosis.

**Keywords:** homocysteine, carotid, extracranial, atherosclerosis, stenosis

## 1. History

Premature atherosclerosis was first reported by McCully in 1969 [1]. He described it on two infant patients with raised homocysteine with similar arterial changes [1]. These two patients had large- and medium-sized arterial narrowing [1]. The histology was focal fibrosis of intima and media layers, focal proliferation of perivascular connective tissue of small arteries, as well as prominent internal elastic membranes in medium- and small-sized arteries [1].

Since then, numerous studies on homocysteine have been conducted. The level of homocysteine-cysteine mixed disulphide after a methionine load was shown to be slightly higher in the patients with coronary artery disease (CAD) in 1976 [2]. In addition, the fasting level of serum homocysteine was 31% higher in the patients with all vascular diseases than in controls [3]. Raised serum homocysteine was

found to be an independent risk factor for vascular diseases with odds ratios (OR) of 1.5 to 1.8 for every increase of 5  $\mu\text{mol/L}$  in serum homocysteine [4]. In a meta-analysis, raised serum homocysteine was an independent predictor of ischaemic stroke and CAD in the healthy population [5].

## **2. Introduction: molecular aspects of homocysteine**

Homocysteine is a sulphur-containing amino acid which is derived from methionine [6]. Methionine is activated by ATP to S-adenosylmethionine (SAM) [6]. In turn, S-adenosylhomocysteine (SAH) is produced from SAM by transmethylation process [6]. Subsequently, SAH is then hydrolysed into homocysteine [6].

Cystathionine  $\beta$ -synthase (CBS) has the role of catalysing the condensation of homocysteine together with serine into cystathionine by process of transsulfuration [6]. The conversion of cystathionine into cysteine depends on pyridoxal 5'-phosphate [6].

## **3. Introduction to extracranial carotid atherosclerosis**

Cardiovascular diseases due to atherosclerosis include ischaemic stroke, transient ischaemic attack (TIA), CAD and peripheral vascular disease [7]. One of the causes of ischaemic stroke is the atherosclerosis involving the extracranial carotid arteries [8, 9]. Ischaemic stroke occurs secondary to ischemia caused by flow-limiting carotid artery stenosis or by embolism due to plaque rupture [8]. 20–30% of ischaemic strokes in the Western countries are caused by stenosis or occlusion of the extracranial carotid arteries [7].

Atherosclerosis is initiated by endothelial dysfunction [10, 11]. This endothelial abnormality is mainly caused by free radicals, homocysteine, lipoproteins, free radicals and infectious agents [10, 11]. In addition, atherosclerosis develops by activation and proliferation of smooth muscle cells [10, 11]. This leads to thickening of the arterial wall [10, 11]. Moreover, there is infiltration of macrophages which result in fatty streak and plasma-derived extracellular lipid accumulation in the thickened intima layer [10–12].

Beginning in the mid-1980s, subclinical atherosclerosis was assessed by measurement of carotid intima-media thickness with ultrasound carotid Doppler [13]. Later, other parameters such as carotid plaques were used to evaluate for atherosclerosis [14]. These parameters of subclinical atherosclerosis are useful in assessment of cardiovascular diseases, such as CAD and ischaemic stroke [15–18].

The frequency of ipsilateral strokes was higher in the patients with progressive asymptomatic carotid stenosis than those without asymptomatic carotid stenosis [19]. A rate of 5.3% of developing ipsilateral strokes was observed in the patients with moderate asymptomatic carotid stenosis [19].

The presence of extracranial carotid artery stenosis was found to be negatively associated with ideal baseline cardiovascular health in several studies [20, 21]. An assessment of carotid intima-media thickness (CIMT) is a good indicator of coronary atherosclerosis [22, 23]. In addition, CIMT is an independent predictor of cardiovascular mortality [22, 23]. Moreover, reduced frequency of subclinical atherosclerosis is associated with ideal cardiovascular health profile [24]. Several large population studies showed that there was an association between increased CIMT with future cardiovascular events [25]. In the Multi-Ethnic Study of Atherosclerosis (MESA), Zhang et al. reported

that measurement of CIMT with magnetic resonance imaging (MRI) was more consistently associated with incident cardiovascular diseases (especially stroke) than ultrasound carotid [26].

The presence of carotid plaque helps in the identification of the patients with coronary atherosclerosis [14]. The baseline plaque area is believed to be more than 3.4 times more powerful than the Framingham risk Equation [27]. The patients with plaque scores in the highest quartile had 3.4 times higher risk of stroke, myocardial infarction and overall mortality in the last 5 years than those in the lowest quartile [27]. Measurement of plaque area is a sensitive parameter to assess atherosclerosis [28]. In a recent study by Kaspar et al., ultrasound-based carotid plaque analysis techniques are more promising for future research studies on generalised atherosclerosis [25].

#### **4. Pathophysiology**

Raised serum homocysteine results in endothelial dysfunction as manifested by changes in endothelial cell structure and function [29, 30]. The hypothesised mechanisms were pro-inflammatory effects (expression of tumour necrosis factor- $\alpha$  and inducible nitric oxide (NO) synthase), oxidative stress and impaired endothelium-mediated platelet inhibition [31–33]. In addition, raised serum homocysteine leads to a decrease in nitric oxide bioavailability and inflammation [30].

The autoxidation of homocysteine produces oxidative stress [32]. Raised serum homocysteine-related pathologies such as atherosclerosis and thrombosis are believed to be due to oxidative stress [34–37]. Hydroxyl free radicals due to raised serum homocysteine level remove electrons from other molecules including DNA, proteins, lipids and carbohydrates in all the cellular components [34–37]. In addition, the hydroxyl free radicals stimulate lipid oxidation and accumulate intracellular cholesterol [33]. Raised serum homocysteine level increases the adhesion between the endothelial cells and neutrophils, resulting in release of extracellular hydrogen peroxide which damages the endothelial cell [38].

Homocysteine is important in vascular function and atherosclerosis [39]. Ozone activates thioretinaco to produce thioretinaco ozonide which is the active site for oxidative phosphorylation [40]. In addition, ozone has been discovered to be present in human atherosclerotic plaques, thus emphasising the important role of ozone and cholesterol ozonolysis in atherosclerosis [41]. Aggregates of microorganisms, homocysteinyllated and oxidised low-density lipoproteins (LDL) and lipoprotein autoantibodies in regions of high pressure lead to obstruction of the vasa vasorum [39, 42, 43]. This in turn results in ischaemia and rupture into arterial intima to form the vulnerable plaque [39, 42, 43].

Endothelial cell hyperplasia and fibrin deposition in the walls of arterioles may worsen the degree of obstruction of the vasa vasorum by lipoprotein aggregates [1]. Homocysteine activates the proliferation of endothelial cells by inhibiting the nitric oxide production by platelets and endothelial cells [37, 44]. Subsequently, production of glutathione peroxidase is suppressed, and this results in a rise of amount of arachidonic acid from platelets to produce more reactive oxygen species [37].

Homocysteine initiates the coagulation process by tissue factor pathway [45]. Homocysteine activates platelet production of the thromboxane A<sub>2</sub>, a vasoconstrictor and pro-aggregant [46]. Moreover, homocysteine causes thrombosis by inhibiting tissue plasminogen activator binding domain of annexin II [47]. Homocysteine suppresses the activation of protein C and thrombomodulin surface expression [48] as well as increases the adhesion of platelets [49].

## **5. Factors affecting serum homocysteine level**

Genetic polymorphisms of the metabolic genes, such as methylenetetrahydrofolate reductase (MTHFR), cystathionine-beta-synthase (CBS), DNA methyltransferase (DNMT) and nicotinamide N-methyl-transferase (NNMT), results in increased level of homocysteine [50, 51]. This leads to an increased risk of ischaemic stroke [50, 51]. CBS deficiency is the most common cause of homocysteinemia due to genetic cause [52]. In the mutation in the gene coding for the enzyme MTHFR, cytosine is replaced by thymidine (C → T) at the base position 677 of the gene [53]. The carriers have nearly 70% reduction in the enzymatic activity [53]. Therefore, the carriers have 20% increase of serum homocysteine concentrations [54]. Deficiencies in CBS and MTHFR result in very high serum homocysteine levels [55].

Nutritional and metabolic abnormalities can also result in elevated serum homocysteine [42]. Metabolism of homocysteine involves remethylation to methionine requiring folate and vitamin B12-derived methylcobalamin [56]. Furthermore, in the process of transsulfuration to cystathionine, vitamin B6-derived pyridoxal 5'-phosphate is needed [56]. Nutritional deficiencies in the vitamin B cofactors inhibit the metabolism of homocysteine metabolism, and this causes an elevated level of serum homocysteine [56].

Serum homocysteine level was significantly higher in the patients with people with impaired renal function [57]. In various studies, gender was significantly correlated with serum homocysteine [53, 58]. However, in some other studies, there was no variation in gender [59].

Parkinsonism and antiepileptic medications have been reported to lead to raised serum homocysteine [60–63]. Paradoxically, lipid-lowering medications have also been reported to cause raised serum homocysteine [60, 63]. The patients with diabetes mellitus (DM) have higher homocysteine than nondiabetics irrespective of gender and ethnic group [64, 65]. Malignancy also leads to higher concentration of homocysteine [66].

## **6. Factors affecting extracranial carotid atherosclerosis**

Atherosclerosis involves inflammation, intimal injury, proliferation of smooth muscle cells and lipid metabolism [67, 68]. In the Framingham study, fasting cholesterol level, systolic blood pressure (SBP), age and status of smoking were significantly associated with the degree of extracranial carotid stenosis in both genders [69]. In the study by Zhu et al., age was correlated positively with CIMT [70]. Hyperlipidaemia, hypertension, DM and smoking were thought to be associated with endothelial dysfunction [71]. In another study, there was positive correlation between incidence of smoking and hypertension with the severity of presentation of extracranial carotid artery stenosis [72].

In a recent study, CIMT and carotid plaques were associated with hypertension, DM and hyperlipidaemia [73]. Male gender has an increased risk of ischaemic stroke in comparison to female gender for all degrees of carotid stenosis [74, 75]. After the age of 85, female gender had a higher risk of stroke [76]. Carotid plaques present in female gender contain reduced level of pro-inflammatory cytokine and more smooth muscle cell content [77]. Female gonadal hormones provide protective effect by causing favourable lipid profile change and by increasing neuronal viability and cerebral blood flow [78, 79]. Oestrogen protects premenopausal women against atherosclerosis [78, 79].

Various ethnic groups have different associations with vascular risk factors [80]. Therefore, these ethnic groups have varying prothrombotic factors and degrees of plaque rupture [80]. Particularly, South Asians have increased serum homocysteine

levels in comparison to Chinese and European patients [80]. Kim et al. reported that serum homocysteine is a predictor of asymptomatic carotid stenosis in the patients undergoing coronary artery bypass surgery (CABG) [81].

## **7. Homocysteine and extracranial carotid artery stenosis**

Kim et al. reported that serum homocysteine in the highest quartile was independently associated with extracranial carotid artery stenosis  $\geq 50\%$  [81]. In another study, raised serum homocysteine was also independently associated with severe extracranial carotid stenosis in both genders [82]. In other studies, serum homocysteine was significantly associated with carotid artery stenosis in internal carotid arteries and external carotid arteries as well as the degree of stenosis [83, 84]. The hypertensive patients who had raised serum homocysteine were reported to have higher risk of developing asymptomatic extracranial carotid artery stenosis [85]. However, other studies showed conflicting results [86–87].

In a community-based study, serum homocysteine  $>19.3 \mu\text{mol/L}$  was associated with asymptomatic carotid artery stenosis in the non-smoker participants aged  $\geq 40$  without transient ischemic attack and coronary artery disease [21]. In addition, raised serum homocysteine was associated with asymptomatic carotid artery stenosis in the diabetic patients [21]. Wang et al. reported that serum homocysteine level of  $\geq 15 \mu\text{mol/L}$  was a predictor of extracranial carotid stenosis [20] and serum homocysteine level  $> 14.4 \mu\text{mol/L}$  was associated with increased extracranial carotid stenosis  $\geq 25\%$  in the elderly people [88], whereas Samson et al. reported that serum homocysteine  $>10 \mu\text{mol/L}$  was associated with carotid artery stenosis [89].

Every  $1 \mu\text{mol/L}$  increase of total homocysteine level was associated with 1.12 times the risk for developing internal carotid artery (ICA) occlusion after adjustment for stroke subtypes and risk factors [90]. In the study conducted by Wang et al., every  $1 \mu\text{mol/L}$  increase of total homocysteine level was associated with 1.096 times the risk of developing extracranial carotid stenosis [20]. In addition, Mueller et al. identified serum homocysteine as independent predictor of ICA stenosis  $\geq 50\%$ , with OR 1.32 (95% CI: 1.02–1.72) for every rise of  $5 \mu\text{mol/L}$  [91].

In a previous study, elevated serum homocysteine level is associated with a higher prevalence of 40–100% extracranial carotid arterial disease (ECAD) in older patients [92]. In this study, high serum homocysteine levels were seen in 45% of the older male patients with 40–100% ECAD, whereas only in 20% of the older men with 0–39% ECAD [92]. In addition, elevated serum homocysteine levels were found in 40% of the older female patients with 40–100% ECAD versus 18% of the older women with 0–39% ECAD [92].

Elevated serum homocysteine levels were also associated with a higher prevalence of coronary artery disease (CAD) and peripheral artery disease in older patients [93, 94]. In another study, the significant independent predictors of new cerebral infarction in older patients were serum homocysteine, age, smoking, diabetes mellitus, hypertension and previous cerebral infarcts [95].

Moreover, in a previous study, the significant independent predictors of new-onset CAD in older patients were serum homocysteine, age, smoking, diabetes mellitus, hypertension and hyperlipidaemia [96].

## **8. Homocysteine and carotid plaque**

Increased serum homocysteine level was associated with 1.344 higher risk of developing carotid plaque [97]. Plaque area was reported to be increased in the

patients with raised serum homocysteine level [98, 99]. Furthermore, the presence of complicated atheromatous plaque was significantly associated with serum homocysteine level [98].

The patients with serum homocysteine level  $> 15 \mu\text{mol/L}$  had increased risk of presence of carotid plaque and plaque in bilateral common carotid artery (CCA) [100]. An increase in serum homocysteine was independently associated with plaque morphology and larger plaque area [101].

The patients with serum homocysteine level of  $\geq 8.6 \mu\text{mol/L}$  had higher risk of developing echolucent plaques [101]. In another study, the patients with raised serum homocysteine level had 1.28 times risk of developing advanced carotid plaques after adjustment for age and gender [102]. Advanced carotid plaques were defined as ulcerated plaque and plaques with incomplete fibrous cap [102]. These advanced carotid plaques resulted in a higher ischaemic stroke risk [102]. In the study by Zhang et al., raised serum homocysteine acted synergistically with hypertension; therefore there was a greater risk of having plaque in bilateral CCA [100]. Alvarez et al. reported that in the patients with carotid stenosis of more than 70% and were receiving surgical management, high homocysteine level was present in the patients with extracranial cerebrovascular diseases [103].

## **9. Homocysteine and carotid intima-media thickness**

An increase in homocysteine level was significantly associated with an increase in CIMT carotid intima-media thickness [104]. In a study on the patients with primary hypertension, serum homocysteine level was independently associated with CIMT [105]. A significant positive correlation between homocysteine and intima-media thickness was reported [106]. In another study conducted among the patients with Parkinson's disease receiving treatment, there was positive correlation with statistical significance between CIMT and serum homocysteine level [107]. The patients with raised serum homocysteine as well as hypertension had higher risk of increased CIMT [100].

## **10. Association of serum homocysteine with atherosclerosis**

According to Wu et al., there was correlation between serum homocysteine level with carotid intima-media thickness and total number of plaques and unstable plaques [84]. He also reported that serum homocysteine level was correlated with stenosis of ICAs and external carotid arteries (ECA) [84]. In a study on middle-aged asymptomatic women, serum homocysteine was significantly associated with atherosclerosis change after adjustment for age, LDL, diastolic blood pressure and body mass index [108].

In conclusion, raised serum homocysteine should be diagnosed early as this can lead to increased CIMT, carotid plaque and extracranial carotid stenosis. Raised serum homocysteine level can be managed with folic acid and vitamin supplementation.

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