Chapter

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 homocysteinylated 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 ≥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 μmol/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 β-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-α 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, homocysteinylated 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 A2, 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 ≥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 μmol/L was associated with asymptomatic carotid artery stenosis in the non-smoker participants aged ≥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 ≥15 μmol/L was a predictor of extracranial carotid stenosis [20] and serum homocysteine level > 14.4 μmol/L was associated with increased extracranial carotid stenosis ≥25% in the elderly people [88], whereas Samson et al. reported that serum homocysteine >10 μmol/L was associated with carotid artery stenosis [89].

Every 1 μmol/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 μmol/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 ≥50%, with OR 1.32 (95% CI: 1.02–1.72) for every rise of 5 μmol/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 μmol/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 ≥8.6 μmol/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.

Acknowledgements

We would like to thank Dr. Lattish Rao Threemurthy and Dr. Parathythasan a/l Rajaandra for their help.

Funding

Supported by University of Malaya UMCares grant RU013-2017C.

Author details

Mei-Ling Sharon Tai[1*], Kuo Ghee Ong[2], Tsun Haw Toh[1], Hafez Hussain[2], Abdul Rashid Mat Mahidin[2] and Esther Kar Mun Yeow[1]

1 Division of Neurology, Department of Medicine, Faculty of Medicine, University Malaya, Kuala Lumpur, Malaysia

2 SOCSO Tun Razak Rehabilitation Centre, Melaka, Malaysia

*Address all correspondence to: sharont1990@gmail.com

References

[1] McCully KS. Vascular pathology of shomocysteinemia: Implications for the pathogenesis of arteriosclerosis. The American Journal of Pathology. 1969;**56**(1):111

[2] Wilcken D, Wilcken B. The pathogenesis of coronary artery disease. A possible role for methionine metabolism. The Journal of Clinical Investigation. 1976;**57**(4):1079-1082

[3] Ueland PM, Refsum H, Brattstrom L. Plasma Homocysteine and Cardiovascular Disease. Atherosclerotic Cardiovascular Disease, Hemostasis, and Endothelial Function. Vol. 183. New York: Marcel Dekker; 1992

[4] Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: Probable benefits of increasing folic acid intakes. Journal of the American Medical Association. 1995;**274**(13):1049-1057

[5] Collaboration HS. Homocysteine and risk of ischemic heart disease and stroke: A meta-analysis. Journal of the American Medical Association. 2002;**288**(16):2015-2022

[6] Durand P, Prost M, Loreau N, Lussier-Cacan S, Blache D. Impaired homocysteine metabolism and atherothrombotic disease. Laboratory Investigation. 2001;**81**(5):645

[7] Edward IB, Barbara AC. The extracranial cerebral vessels. In: Carol MR, Stephanie RW, Charboneau JW, Deborah L, editors. Diagnostic ultrasound. 4th ed ed. Philadelphia: Elsevier; 2010

[8] Griggs RM, Bluth EI. Noninvasive risk assessment for stroke: Special emphasis on carotid atherosclerosis, sex-related differences, and the development of an effective screening strategy. American Journal of Roentgenology. 2011;**196**(2):259-264

[9] Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU. Epidemiology of ischemic stroke subtypes according to TOAST criteria: Incidence, recurrence, and long-term survival in ischemic stroke subtypes: A population-based study. Stroke. 2001;**32**(12):2735-2740

[10] Qureshi AR, Alvestrand A, Divino-Filho JC, Gutierrez A, Heimbürger O, Lindholm B, et al. Inflammation, malnutrition, and cardiac disease as predictors of mortality in hemodialysis patients. Journal of the American Society of Nephrology. 2002;**13**(Suppl 1):S28-S36

[11] Stolić R, Trajković G, Šubarić-Gorgieva G. Ultrasound diagnostics of atherosclerosis in chronic renal insufficiency. Medicinski Pregled. 2006;**59**(5-6):270-272

[12] Nakagawa K, Nakashima Y. Pathologic intimal thickening in human atherosclerosis is formed by extracellular accumulation of plasma-derived lipids and dispersion of intimal smooth muscle cells. Atherosclerosis. 2018;**274**:235-242

[13] Bond MG, Strickland HL, Wilmoth SK, Safrit A, Phillips R, Szostak L. Interventional clinical trials using noninvasive ultrasound end points: The multicenter isradipine/diuretic atherosclerosis study. The MIDAS Research Group. Journal of Cardiovascular Pharmacology. 1990;**15**:S30-S33

[14] Spence JD. Technology insight: Ultrasound measurement of carotid plaque—Patient management, genetic research, and therapy evaluation. Nature Reviews. Neurology. 2006;**2**(11):611

[15] Aronow W, Ahn C, Schoenfeld M, Gutstein H. Extracranial carotid arterial disease: A prognostic factor for atherothrombotic brain infarction and cerebral transient ischemic attack. New York State Journal of Medicine. 1992;**92**(10):424-425

[16] Blankenhorn D, Selzer RH, Crawford D, Barth JD, Liu C, Liu C, et al. Beneficial effects of colestipol-niacin therapy on the common carotid artery. Two-and four-year reduction of intima-media thickness measured by ultrasound. Circulation. 1993;**88**(1):20-28

[17] Falke P, Stavenow L. Advanced carotid stenosis in TIA and minor stroke as a predictor of coronary heart disease: A 3-year follow-up. International Angiology: A journal of the International Union of Angiology. 1989;**8**(4):175-178

[18] O'leary DH, Polak JF, Kronmal RA, Kittner SJ, Bond MG, Wolfson SK Jr, et al. Distribution and correlates of sonographically detected carotid artery disease in the cardiovascular health study. The CHS Collaborative Research Group. Stroke. 1992;**23**(12):1752-1760

[19] Kakkos SK, Nicolaides AN, Charalambous I, Thomas D, Giannopoulos A, Naylor AR, et al. Predictors and clinical significance of progression or regression of asymptomatic carotid stenosis. Journal of Vascular Surgery. 2014;**59**(4):956-967

[20] Wang J, Shao B, Da Lin XH, Zhang Y, Zhang L, Jiang T, et al. Ideal cardiovascular health metrics associated with reductions in the risk of extracranial carotid artery stenosis: A population-based cohort study. Scientific Reports. 2018;**8**(1):12277

[21] Jia J, Wang A, Wang J, Wu J, Yan X, Zhou Y, et al. Homocysteine and its relationship to asymptomatic carotid stenosis in a Chinese community population. Scientific Reports. 2016;**6**:37361

[22] Stolić R, Trajković G, Perić V, Jovanović A, Šubarić-Gorgieva G. Impact of arteriosclerosis on the functioning of arteriovenous fistula for hemodialysis. Vojnosanitetski Pregled. 2007;**64**(1):13-18

[23] Crouse JR, Goldbourt U, Evans G, Pinsky J, Sharrett AR, Sorlie P, et al. Risk factors and segment-specific carotid arterial enlargement in the atherosclerosis risk in communities (ARIC) cohort. Stroke. 1996;**27**(1):69-75

[24] Kulshreshtha A, Goyal A, Veledar E, McClellan W, Judd S, Eufinger SC, et al. Association between ideal cardiovascular health and carotid intima-media thickness: A twin study. Journal of the American Heart Association. 2014;**3**(1):e000282

[25] Kaspar M, Baumgartner I, Staub D, Drexel H, Thalhammer C. Non-invasive ultrasound-based imaging of atherosclerosis. Vasa. 2019;**48**:126-133

[26] Zhang Y, Guallar E, Malhotra S, Astor BC, Polak JF, Qiao Y, et al. Carotid artery wall thickness and incident cardiovascular events: A comparison between US and MRI in the multi-ethnic study of atherosclerosis (MESA). Radiology. 2018;**289**(3):649-657

[27] Spence JD, Eliasziw M, DiCicco M, Hackam DG, Galil R, Lohmann T. Carotid plaque area: A tool for targeting and evaluating vascular preventive therapy. Stroke. 2002;**33**(12):2916-2922

[28] Barnett PA, Spence JD, Manuck SB, Jennings JR. Psychological stress and the progression of carotid artery disease. Journal of Hypertension. 1997;**15**(1):49-55

[29] McCully KS. Chemical pathology of homocysteine. IV. Excitotoxicity,

oxidative stress, endothelial dysfunction, and inflammation. Annals of Clinical and Laboratory Science. 2009;**39**(3):219-232

[30] Kumar A, Palfrey HA, Pathak R, Kadowitz PJ, Gettys TW, Murthy SN. The metabolism and significance of homocysteine in nutrition and health. Nutrition & Metabolism (London). 2017;**14**(1):78

[31] Faraci FM, Lentz SR. Hyperhomocysteinemia, oxidative stress, and cerebral vascular dysfunction. Stroke. 2004;**35**(2):345-347

[32] Stanger O, Weger M. Interactions of homocysteine, nitric oxide, folate and radicals in the progressively damaged endothelium. Clinical Chemistry and Laboratory Medicine. 2003;**41**(11):1444-1454

[33] Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: The vitamin intervention for stroke prevention (VISP) randomized controlled trial. Journal of the American Medical Association. 2004;**291**(5):565-575

[34] Dayal S, Arning E, Bottiglieri T, Böger RH, Sigmund CD, Faraci FM, et al. Cerebral vascular dysfunction mediated by superoxide in hyperhomocysteinemic mice. Stroke. 2004;**35**(8):1957-1962

[35] Herrmann W, Obeid R. Homocysteine: A biomarker in neurodegenerative diseases. Clinical Chemistry and Laboratory Medicine. 2011;**49**(3):435-441

[36] Marković AR, Hrnčić D, Macut D, Stanojlović O, Djuric D. Anticonvulsive effect of folic acid in homocysteine thiolactone-induced seizures. Cellular and Molecular Neurobiology. 2011;**31**(8):1221

[37] Petras M, Tatarkova Z, Kovalska M, Mokra D, Dobrota D, Lehotsky J, et al. Hyperhomocysteinemia as a risk factor for the neuronal system disorders. Journal of Physiology and Pharmacology. 2014;**65**(1):15-23

[38] Dudman NP, Temple SE, Guo XW, Fu W, Perry MA. Homocysteine enhances neutrophil-endothelial interactions in both cultured human cells and rats in vivo. Circulation Research. 1999;**84**(4):409-416

[39] McCully KS. Homocysteine metabolism, atherosclerosis, and diseases of aging. Comprehensive Physiology. 2015;**6**(1):471-505

[40] McCully KS. Chemical pathology of homocysteine. II. Carcinogenesis and homocysteine thiolactone metabolism. Annals of Clinical and Laboratory Science. 1994;**24**(1):27-59

[41] Wentworth P, Nieva J, Takeuchi C, Galve R, Wentworth AD, Dilley RB, et al. Evidence for ozone formation in human atherosclerotic arteries. Science. 2003;**302**(5647):1053-1056

[42] McCully KS. Homocysteine and the pathogenesis of atherosclerosis. Expert Review of Clinical Pharmacology. 2015;**8**(2):211-219

[43] Ravnskov U, McCully KS. Vulnerable plaque formation from obstruction of vasa vasorum by homocysteinylated and oxidized lipoprotein aggregates complexed with microbial remnants and LDL autoantibodies. Annals of Clinical and Laboratory Science. 2009;**39**(1):3-16

[44] Stühlinger MC, Tsao PS, Her J-H, Kimoto M, Balint RF, Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: Role of asymmetric dimethylarginine. Circulation. 2001;**104**(21):2569-2575

[45] Fryer RH, Wilson BD, Gubler DB, Fitzgerald LA, Rodgers GM.

Homocysteine, a risk factor for premature vascular disease and thrombosis, induces tissue factor activity in endothelial cells. Arteriosclerosis and Thrombosis: A Journal of Vascular Biology. 1993;**13**(9):1327-1333

[46] Graeber JE, Slott JH, Ulane RE, Schulman JD, Stuart MJ. Effect of homocysteine and homocystine on platelet and vascular arachidonic acid metabolism. Pediatric Research. 1982;**16**:490

[47] Hajjar KA, Mauri L, Jacovina AT, Zhong F, Mirza UA, Padovan JC, et al. Tissue plasminogen activator binding to the annexin II tail domain direct modulation by homocysteine. The Journal of Biological Chemistry. 1998;**273**(16):9987-9993

[48] Lentz S, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. The Journal of Clinical Investigation. 1991;**88**(6):1906-1914

[49] Spence JD. Homocysteine-lowering therapy: A role in stroke prevention? The Lancet Neurology. 2007;**6**(9):830-838

[50] Balcerzyk A, Niemiec P, Kopyta I, Emich-Widera E, Pilarska E, Pienczk-Ręcławowicz K, et al. Methylenetetrahydrofolate reductase gene A1298C polymorphism in pediatric stroke—Case–control and family-based study. Journal of Stroke and Cerebrovascular Diseases. 2015;**24**(1):61-65

[51] Hozyasz KK, Mostowska A, Szaflarska-Poplawska A, Lianeri M, Jagodzinski PP. Polymorphic variants of genes involved in homocysteine metabolism in celiac disease. Molecular Biology Reports. 2012;**39**(3):3123-3130

[52] Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, et al. The natural history of homocystinuria due to cystathionine β-synthase deficiency. American Journal of Human Genetics. 1985;**37**(1):1

[53] Stanger O, Herrmann W, Pietrzik K, Fowler B, Geisel J, Dierkes J, et al. DACH-LIGA homocystein (German, Austrian and Swiss homocysteine society): Consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: Guidelines and recommendations. Clinical Chemistry and Laboratory Medicine. 2003;**41**(11):1392-1403

[54] Frosst P, Blom H, Milos R, Goyette P, Sheppard CA, Matthews R, et al. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nature Genetics. 1995;**10**(1):111

[55] Rozen R. Genetic predisposition to hyperhomocysteinemia: Deficiency of methylenetetrahydrofolate reductase (MTHFR). Thrombosis and Haemostasis. 1997;**78**(1):523-526

[56] Pezzini A, Del Zotto E, Padovani A. Homocysteine and cerebral ischemia: Pathogenic and therapeutical implications. Current Medicinal Chemistry. 2007;**14**(3):249-263

[57] Suwelack B, Gerhardt U, Witta J, Rahn KH, Hohage H. Effect of homocysteine on carotid intima-media thickness after renal transplantation. Clinical Transplantation. 2000;**14**(6):555-560

[58] Schnyder G, Flammer Y, Roffi M, Pin R, Hess OM. Plasma homocysteine levels and late outcome after coronary angioplasty. Journal of the American College of Cardiology. 2002;**40**(10):1769-1776

[59] Selhub J. The many facets of hyperhomocysteinemia: Studies from the Framingham cohorts. The Journal of Nutrition. 2006;**136**(6):1726S-1730S

[60] Apeland T, Mansoor MA, Strandjord RE. Antiepileptic drugs as independent predictors of plasma total homocysteine levels. Epilepsy Research. 2001;**47**(1-2):27-35

[61] Basu TK, Makhani N, Sedgwick G. Niacin (nicotinic acid) in non-physiological doses causes hyperhomocysteineaemia in Sprague–Dawley rats. The British Journal of Nutrition. 2002;**87**(2):115-119

[62] Foucher C, Brugere L, Ansquer J-C. Fenofibrate, homocysteine and renal function. Current Vascular Pharmacology. 2010;**8**(5):589-603

[63] Müller T, Woitalla D, Fowler B, Kuhn W. 3-OMD and homocysteine plasma levels in parkinsonian patients. Journal of Neural Transmission. 2002;**109**(2):175-179

[64] Huang E-J, Kuo W-W, Chen Y-J, Chen T-H, Chang M-H, Lu M-C, et al. Homocysteine and other biochemical parameters in type 2 diabetes mellitus with different diabetic duration or diabetic retinopathy. Clinica Chimica Acta. 2006;**366**(1-2):293-298

[65] Masuda Y, Kubo A, Kokaze A, Yoshida M, Fukuhara N, Takashima Y. Factors associated with serum total homocysteine level in type 2 diabetes. Environmental Health and Preventive Medicine. 2008;**13**(3):148

[66] Niittynen L, Nurminen M-L, Korpela R, Vapaatalo H. Role of arginine, taurine 4 and homocysteine in cardiovascular diseases. Annals of Medicine. 1999;**31**(5):318-326

[67] D'agostino RB, Wolf PA, Belanger AJ, Kannel WB. Stroke risk profile: Adjustment for antihypertensive medication. The Framingham study. Stroke. 1994;**25**(1):40-43

[68] Wolf PA, D'Agostino RB, Belanger AJ, Kannel WB. Probability of stroke: A risk profile from the Framingham study. Stroke. 1991;**22**(3):312-318

[69] Fine-Edelstein J, Wolf P, O'leary D, Poehlman H, Belanger A, Kase C, et al. Precursors of extracranial carotid atherosclerosis in the Framingham study. Neurology. 1994;**44**(6):1046-1050

[70] Zhu Z-Q, Chen L-S, Wang H, Liu F-M, Luan Y, Wu L-L, et al. Carotid stiffness and atherosclerotic risk: Non-invasive quantification with ultrafast ultrasound pulse wave velocity. European Radiology. 2018;**29**(3):1507-1517

[71] Landmesser U, Hornig B, Drexler H. Endothelial function: A critical determinant in atherosclerosis? Circulation. 2004;**109**(21 suppl 1):27-33

[72] Elsharawy MA, Alkhadra AH, Ibrahim MFA, Selim F, Hassan K, Elsaid AS, et al. Impact of atherosclerosis risk factors on the clinical presentation of arterial occlusive disease in Arabic patients. The International Journal of Angiology: Official Publication of the International College of Angiology, Inc. 2008;**17**(4):203

[73] Słomka T, Drelich-Zbroja A, Jarząbek M, Szczerbo-Trojanowska M. Intima–media complex thickness and carotid atherosclerotic plaque formation in Lublin's population in the context of selected comorbidities. Journal of Ultrasonography. 2018;**18**(73):133-139

[74] Walker MD, Marler JR, Goldstein M, Grady PA, Toole JF, Baker WH, et al. Endarterectomy for asymptomatic carotid artery stenosis. Journal of the American Medical Association. 1995;**273**(18):1421-1428

[75] Collaborators NASCET. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. The New England Journal of Medicine. 1991;**325**(7):445-453

[76] Turtzo LC, McCullough LD. Sex differences in stroke. Cerebrovascular Diseases. 2008;**26**(5):462-474

[77] Hellings WE, Pasterkamp G, Verhoeven BA, De Kleijn DP, De Vries J-PP, Seldenrijk KA, et al. Gender-associated differences in plaque phenotype of patients undergoing carotid endarterectomy. Journal of Vascular Surgery. 2007;**45**(2):289-296

[78] Wild RA, Reis SE. Estrogens, progestins, selective estrogen receptor modulators, and the arterial tree. American Journal of Obstetrics and Gynecology. 2001;**184**(5):1031-1039

[79] Pines A, Bornstein NM, Shapira I. Menopause and ischaemic stroke: Basic, clinical and epidemiological considerations. The role of hormone replacement. Human Reproduction Update. 2002;**8**(2):161-168

[80] Anand SS, Yusuf S, Vuksan V, Devanesen S, Teo KK, Montague PA, et al. Differences in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: The study of health assessment and risk in ethnic groups (SHARE). The Lancet. 2000;**356**(9226):279-284

[81] Kim SJ, Song P, Park JH, Lee YT, Kim WS, Park YG, et al. Biomarkers of asymptomatic carotid stenosis in patients undergoing coronary artery bypass grafting. Stroke. 2011;**42**(3):734-739

[82] Marcucci R, Sofi F, Fedi S, Lari B, Sestini I, Cellai A, et al. Thrombophilic risk factors in patients with severe carotid atherosclerosis. Journal of Thrombosis and Haemostasis. 2005;**3**(3):502-507

[83] Rahman A, Quraishi FA, Miah MNA, Hakim M, Saha UK, Akteruzzaman M, et al. Relationship between homocysteine and carotid artery stenosis in ischemic stroke. Bangladesh Journal of Neuroscience. 2012;**28**(1):1-9

[84] Wu W, Guan Y, Xu K, Fu X-J, Lei X-F, Lei L-J, et al. Plasma homocysteine levels predict the risk of acute cerebral infarction in patients with carotid artery lesions. Molecular Neurobiology. 2016;**53**(4):2510-2517

[85] Zhang J, Liu Y, Wang A, Wang D, Jiang R, Jia J, et al. Association between H-type hypertension and asymptomatic extracranial artery stenosis. Scientific Reports. 2018;**8**(1):1328

[86] Laxdal E, Eide G, Amundsen S, Dregelid E, Pedersen G, Jonung T, et al. Homocysteine levels, haemostatic risk factors and restenosis after carotid thrombendarterectomy. European Journal of Vascular and Endovascular Surgery. 2004;**28**(3):323-328

[87] Mousavi SA, Ghasemi M, Hoseini T. Association between plasma homocysteine concentrations and extracranial carotid stenosis. Annals of Saudi Medicine. 2006;**26**(2):120

[88] Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PWF, Belanger AJ, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. The New England Journal of Medicine. 1995;**332**(5):286-291

[89] Samson RH, Yungst Z, Showalter DP. Homocysteine, a risk factor for carotid atherosclerosis, is not a risk factor for early recurrent carotid stenosis following carotid endarterectomy. Vascular

and Endovascular Surgery. 2004;**38**(4):345-348

[90] Jeong S-K, Seo J-Y, Cho YI. Homocysteine and internal carotid artery occlusion in ischemic stroke. Journal of Atherosclerosis and Thrombosis. 2010;**17**(9):963-969

[91] Mueller T, Furtmueller B, Aigelsdorfer J, Luft C, Poelz W, HaltmayerM.Totalserumhomocysteine-a predictor of extracranial carotid artery stenosis in male patients with symptomatic peripheral arterial disease. Vascular Medicine. 2001;**6**(3):163-167

[92] Aronow WS, Ahn C, Schoenfeld MR. Association between plasma homocysteine and extracranial carotid arterial disease in older persons. The American Journal of Cardiology. 1997;**79**(10):1432-1433

[93] Aronow WS, Ahn C. Association between plasma homocysteine and coronary artery disease in older persons. The American Journal of Cardiology. 1997;**80**:1216-1218

[94] Aronow WS, Ahn C. Association between plasma homocysteine and peripheral arterial disease in older persons. Coronary Artery Disease. 1998;**9**:49-50

[95] Aronow WS, Ahn C, Gutstein H. Increased plasma homocysteine is an independent predictor of new atherothrombotic infarction in older persons. The American Journal of Cardiology. 2000;**86**:585-586

[96] Aronow WS, Ahn C. Increased plasma homocysteine is an independent predictor of new coronary events in older persons. The American Journal of Cardiology. 2000;**86**:346-347

[97] Sasaki T, Watanabe M, Nagai Y, Hoshi T, Takasawa M, Nukata M, et al. Association of plasma homocysteine concentration with atherosclerotic carotid plaques and lacunar infarction. Stroke. 2002;**33**(6):1493-1496

[98] Bakoyiannis C, Karaolanis G, Moris D, Palla V, Skrapari I, Bastounis E, et al. Homocysteine as a risk factor of restenosis after carotid endarterectomy. International Angiology: A Journal of the International Union of Angiology. 2015;**34**(2):166-171

[99] Spence JD. Carotid plaque burden is associated with higher levels of total homocysteine. Stroke and Vascular Neurology. 2017;**2**(1):40

[100] Zhang Z, Fang X, Hua Y, Liu B, Ji X, Tang Z, et al. Combined effect of hyperhomocysteinemia and hypertension on the presence of early carotid artery atherosclerosis. Journal of Stroke and Cerebrovascular Diseases. 2016;**25**(5):1254-1262

[101] Alsulaimani S, Gardener H, Elkind MS, Cheung K, Sacco RL, Rundek T. Elevated homocysteine and carotid plaque area and densitometry in the northern Manhattan study. Stroke. 2013;**44**(2):457-461

[102] Yang X, Zhou Y, Liu C, Gao X, Wang A, Guo Y, et al. Homocysteine and carotid plaque stability: A cross-sectional study in Chinese adults. PLoS One. 2014;**9**(4):e94935

[103] Alvarez B, Yugueros X, Fernández E, Luccini F, Gené A, Matas M. Relationship between plasma homocysteine and the morphological and immunohistochemical study of carotid plaques in patients with carotid stenosis over 70%. Annals of Vascular Surgery. 2012;**26**(4):500-505

[104] Hayta E, Hizmetli S, Atalar MH, Cinar Z. Association of plasma homocysteine level and carotid intima-media thickness in rheumatoid arthritis patients receiving methotrexate.

Archives of Rheumatology. 2015;**30**(3):214-220

[105] Catena C, Colussi G, Url-Michitsch M, Nait F, Sechi LA. Subclinical carotid artery disease and plasma homocysteine levels in patients with hypertension. Journal of the American Society of Hypertension. 2015;**9**(3):167-175

[106] Memişoğullari R, Erdoğmuş B, Alp HH, Bilgin C, Arbak PM, Yavuz Ö. Serum homocysteine levels in highway toll collectors and the relationship with intima-media thickness of the carotid artery. Turkish Journal of Medical Sciences. 2008;**38**(2):133-137

[107] Yasemin K, Fahriye FÖ, Ali BK, Sema NA, Tuba Ö, Esra YD, et al. The effect of medical therapy on plasma homocysteine levels and carotid intima-media thickness in Parkinson's disease. Journal of Neurological Research and Therapy. 2016;**1**(3):10-19

[108] Mungun-Ulzii K, Erdenekhuu N, Altantsetseg P, Zulgerel D, Huang S-L. Asymptomatic Mongolian middle-aged women with high homocysteine blood level and atherosclerotic disease. Heart and Vessels. 2010;**25**(1):7-13