Chapter

3

Hyperhomocysteinemia and its Implications in Atherosclerosis — The Indian Scenario

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HOMOCYSTEINE — ITS IMPORTANCE IN VASCULAR DISEASE

The relationship between hyperhomocysteinemia and atherosclerosis was suggested by McCully way back in 1969[1]. During an autopsy of two small children who had homocystinuria and had died of myocardial infarction, McCully found extensive atherosclerotic lesions in most of their blood vessels. These two children had very high blood homocysteine levels which seemed to be apparent cause of the atherosclerotic lesions. In the community, a mild to moderate rise in the plasma homocysteine level is often present. McCully hence, hypothesized that raised levels of homocysteine could be a risk factor for atherosclerosis[1]. It took nearly thirty years to prove the same. It is now well accepted that elevated plasma homocysteine is a strong, graded, independent risk factor for stroke, myocardial infarction and other vascular events[2]. In a meta-analysis of 27 trials, comparing persons with homocysteine levels above the 90th percentile to the rest, the risk ratio for coronary artery disease was reported to be 1.7, for cerebrovascular disease 2.5, for peripheral vascular disease 6.8, and for venous thrombosis 2.95. Moreover, it was estimated that a 5 mmol/L increment in homocysteine increased the CAD risk by as much as cholesterol increases of 20 mg/dL.[3] The meta-analysis showed that raised homocysteine was an independent risk factor for vascular disease equal to smoking and hypercholesterolemia. Another important study was the Hordaland Homocysteine study. This was a prospective study of 2127 men and 2639 women age between 65 and 67 years recruited for a cardiovascular screening program. The follow up period for the study was 4.1 years (median). The study showed a strong relationship between plasma homocysteine and all cause mortality. A plasma homocysteine increment of 5 mmol/L was associated with a 49% increase in all cause mortality and 50% increase in cardiovascular mortality (deaths due to CAD and cerebrovascular disease)[4]. Within the cardiovascular mortality group, the strongest association of plasma homocysteine was observed for cerebrovascular disease. Although, recognized to be an independent risk factor, hyperhomocysteinemia is not considered to be a major risk factor for atherosclerosis by the AHA and NCEP ATP III[5,6]. One of the reasons cited for this is the low incidence of hyperhomocysteinemia, just 5-7%, in the American population[6,7].

HYPERHOMOCYSTEINEMIA IN INDIANS

In contrast to the west, Indian studies examining the prevalence of hyperhomocysteinemia in the community have reported a much higher incidence of 52 to 84%[8-10]. The mean homocysteine levels too are quiet high, varying from 19.5 to 23.2 mmols/L[8-10]. In view of these high levels, it is felt that hyperhomocysteinemia can be considered to be an important cardiovascular risk factor in Indians[11].

It is easy to appreciate the reasons for the high incidence of hyperhomocysteinemia in Indians based on the metabolism of homocysteine (Fig. 1).

Homocysteine is an amino acid, which is metabolized either by the remethylation pathway to methionine or the trans-sulfuration pathway to cysteine. The former pathway is dependent on the proper functioning of the enzymes methionine synthetase (MS) and methylene tetrahydrofolate reductase (MTHFR) as well as adequate blood levels of vitamin B_{12} and folic acid. The later pathway is dependent on the enzyme cystathionine beta synthetase (CBS) and adequate blood levels of

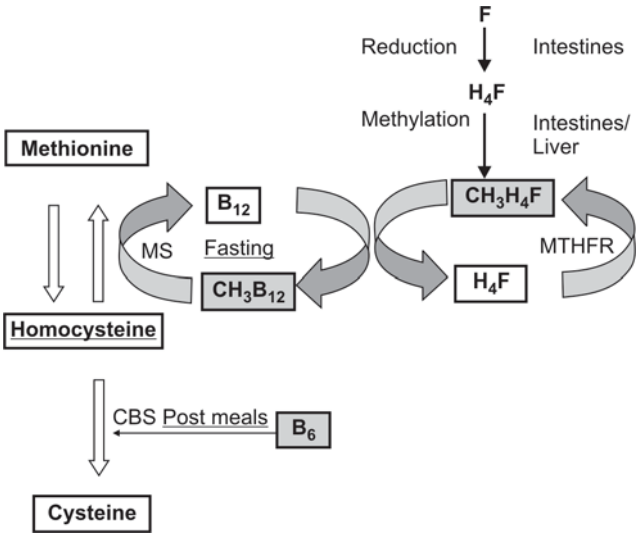

Fig. 1: Metabolism of homocysteine

pyridoxine (vitamin B_6). A genetic defect in the enzymes or a dietary deficiency of the vitamins involved in the metabolism of homocysteine can result in hyperhomocysteinemia.

Amongst Indians, a dietary deficiency of the above mentioned homocysteine lowering B vitamins is often present. The main source of vitamin B_{12} in the diet is non-vegetarian food viz. meat and eggs. Vegetarian food contains practically no vitamin B_{12}. Milk contains small amounts of vitamin B_{12} but most of it is destroyed by boiling. As Indians are often vegetarian, it predisposes them to vitamin B_{12} deficiency. In a recent study conducted in Pune, 441 middle aged men were examined, of which 149 came from rural areas, 142 from slums and 150 from urban middle class. Overall 67% of the men had low vitamin B_{12} concentration and 58% had hyperhomocysteinemia. In the urban middle class, 81% had low B_{12} and 79% had high homocysteine levels. Vegetarians had a 4.4 times higher risk of low vitamin B_{12} than those who ate non - vegetarian food frequently and also a 3 times greater chance of hyperhomocysteinemia. Urban men were significantly more likely to have hyperhomocysteinemia than rural men[12].

Surprisingly, although majority of Indians are vegetarian, there is also a high incidence of folic acid deficiency reported[13]. The reason for this is that Indians usually cook their food for prolonged periods, which can destroy upto 90% of the folic acid[14]. Pyridoxine deficiency is also reported to be quite common amongst Indians[13,15].

A second important factor that predisposes Indians to hyperhomocysteinemia is a genetic defect in the enzymes that metabolize homocysteine, especially MTHFR. Studies have reported that upto one-third of Indians have a genetic defect which predisposes to decreased activity of MTHFR[16,17]. Two polymorphisms of the MTHFR enzyme commonly exist amongst Indians viz. C677T and A1298C. In a study where the presence of either of the polymorphism was examined, deficiency of MTHFR was reported in 43.5% of the population[18]. Compared to diet, however, the effect of genetic factors in raising the homocysteine level seems to be modest.

Thus, the presence of a dietary deficiency of one or more of the vitamins involved in the metabolism of homocysteine superimposed on a background of MTHFR deficiency seems to be responsible for the very high incidence of hyperhomocysteinemia and the high homocysteine levels noticed in the Indian population.

HYPERHOMOCYSTEINEMIA—IMPLICATIONS IN CARDIOLOGY

India is facing an epidemic of cardiovascular disease. Indians have been reported to have the highest incidence of CAD[19]. The prevalence rate is almost 80-120 per 1000 population[20]. CAD also occurs more prematurely, often affecting people under the age of 40 years[20]. In one study Asian Indians were found to have significantly higher homocysteine levels than Europeans, which was believed to cause twice as many CAD deaths in Asian Indians as compared to Europeans. This study concluded that homocysteine was an independent risk factor in Asian Indians, which probably contributed to the increased CAD risk[21].

Hyperhomocysteinemia is now recognized to be an independent risk factor for atherosclerosis[7]. Homocysteine is an unstable amino acid, which undergoes autooxidation to produce free oxygen radicals[7]. Hyperhomocysteinemia, thus causes increased production of free oxygen radicals and an oxidative stress. This is believed to contribute to atherosclerosis in two ways (Fig. 2).

The free oxygen radicals convert LDLc deposited in the sub-endothelial tissue to oxidized LDLc (oxLDLc). OxLDLc then acts as the key mediator of the inflammatory process in atherosclerosis[22]. OxLDLc causes the release of vascular cell adhesion molecule (VCAM) and monocyte chemoattractant protein (MCP1), which in turn causes monocyte adhesion and penetration respectively. The monocytes then get converted to macrophages, which take up oxLDLc to get converted to foam cells. The foam cells get deposited below the endothelium to form a fatty streak, the first lesion in atherosclerosis. The free oxygen radicals also combine with nitric oxide (NO), inactivating it to peroxynitrite. The resulting endothelial dysfunction, also contributes significantly to atherosclerosis[7].

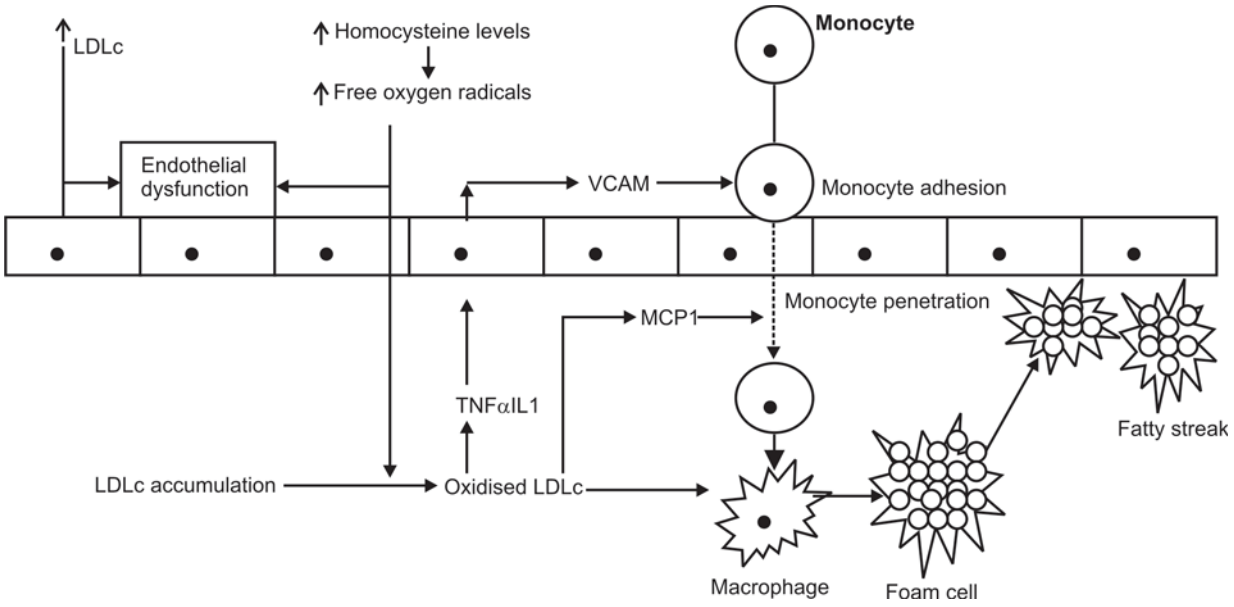

Fig. 2: Hyperhomocysteinemia and etiopathogenesis of atherosclerosis

Hyperhomocysteinemia is associated with not just a greater risk of CAD but also with more severe disease and higher mortality. In patients with CAD diagnosed by electron beam computed tomography (EBT), the coronary calcification over a period of a year progressed by 35% in patients with hyperhomocysteinemia as compared to just 17% in patients with normal homocysteine levels[23]. Homocysteine levels in CAD patients with triple vessel disease have been reported to be significantly higher than patients with single vessel disease[24]. When patients with angiographically confirmed CAD were followed up for a period of 4 years, it was noticed that patients with hyperhomocysteinemia had 6 times greater mortality than patients with low homocysteine levels[25].

It has been reported that the risk conferred by homocysteine, adds to or multiplies the risk conferred by other factors such as smoking, hypertension, diabetes and lipid disorders, all of which are common amongst Indians[26-28]. In a study examining the synergy between hyperhomocysteinemia and hypercholesterolemia, it was reported that the presence of hyperhomocysteinemia in patients with hetrozygous familial hypercholesterolemia increased the risk of CAD by 5.7 times in men[29]. In the AFCAPS / TexCAPS study too, it was noticed that patients who developed an acute coronary syndrome had significantly higher homocysteine levels. Moreover, it was reported that patients with both elevated homocysteine and LDLc levels were at the highest risk[30]. Likewise, it has been reported that when hyperhomocysteinemia is present with smoking or 2 other risk factors, the CAD risk increases by 12 times and when present with increased Lipoprotein(a), the risk increases upto 30 times[19].

Studies examining homocysteine levels in Indian patients with or without CAD have shown mixed results. In the study conducted at Jaslok hospital, Mumbai, homocysteine levels were examined in angiographically confirmed CAD patients (n=65) and compared with age matched controls (n=65). The homocysteine levels were significantly elevated in the CAD patients as compared to controls. CAD patients also had significantly low levels of vitamin B_{12} and folic acid[31]. Likewise, in a study conducted at Kanpur in young CAD patients, the incidence of hyperhomocysteinemia as well as mean homocysteine levels, were shown to be significantly higher in CAD patients as compared to controls[32]. On the other hand, studies have also shown no difference in the homocysteine levels between patients with CAD and control[33-35]. A large multi-centric prospective trial to examine the implications of hyperhomocysteinemia in the Indian CAD patients is required. However, considering the fact that Indians have much higher mean homocysteine levels and a high prevalence of hyperhomocysteinemia which might be contributing to the high risk of CAD and CAD mortality in Indians, it would be prudent to lower the homocysteine levels with homocysteine lowering vitamins.

HYPERHOMOCYSTEINEMIA – IMPLICATIONS IN DIABETOLOGY

India tops the world diabetes list with 31.7 million diabetic patients and this is likely to rise to 79.4 million by the year 2030[36]. Over ninety percent of these have type 2 diabetes mellitus (T2DM)[37]. T2DM patients are frequently prone to atherosclerotic complications which account for more than 80% of all diabetic mortality[38]. About three-fourths of these deaths are due to CAD.

Diabetic patients have 2-4 times higher risk of CAD mortality as compared to the general population[39]. Moreover, CAD has a few peculiarities in diabetic patients. CAD usually occurs earlier, progresses faster and is more diffuse. CAD also carries a higher mortality and morbidity in diabetic patients than the general population[40]. Traditional risk factors often do not fully explain the higher cardiovascular mortality in T2DM patients. The increased risk might be due to the presence of other risk factors. Hyperhomocysteinemia is one such risk factor.

Hyperhomocysteinemia is common in many diabetic patients and may contribute to the accelerated risk of atherosclerosis and cardiovascular disease[41]. The adverse vascular effect of homocysteine in diabetes appears to be related primarily to T2DM[42]. In the west, the incidence of hyperhomocysteinemia in diabetic patients is reported to be approximately 5 times greater than the general population[7,43,44].

The exact cause of hyperhomocysteinemia in diabetic patients is not known. Hyperhomocysteinemia has been shown to be associated with insulin resistance and obesity[45-47]. Decreased glomerular filtration and overt nephropathy, often present in diabetic patients, can contribute to hyperhomocysteinemia. Some drugs like metformin and fenofibrate, commonly used in the management of diabetes, have also been reported to cause hyperhomocysteinemia. It has been suggested that hyperhomocysteinemia caused by these drugs may negate some of their cardiovascular benefits[48]. In the FIELD study, a reduction in the coronary events with fenofibrate was noticed with just 11% of patients[49]. Homocysteine levels in the patients treated with fenofibrate increased by 4 µmols / L, which was believed to have increased the CAD risk by 10-20%. Although more number of patients in the placebo group had received statins which may have masked the treatment benefits of fenofibrate, it was felt that the increased homocysteine levels due to fenofibrate may also have negated its cardiovascular benefit[49].

Both hyperhomocysteinemia and diabetes mellitus are individually known to increase the risk of cardiovascular disease. Hence, when present together, the risk is likely to increase further. Moreover, patients with both T2DM and CAD co-existent, have been reported to have significantly higher homocysteine levels than patients with CAD alone[50] or diabetes alone[51]. In a large population based study, the combined effects of hyperhomocysteinemia and diabetes mellitus were examined. In this study, it was noticed that the presence of hyperhomocysteinemia increased the risk of cardiovascular disease by 1.6 times in diabetic subjects as compared to non-diabetic subjects[52].

Hyperhomocysteinemia has also been reported to be a predictor of CAD severity in T2DM. In a study carried out in T2DM patients with stenotic coronary arteries, the degree of coronary artery stenosis was noticed to be related to the serum homocysteine levels. Patients with triple vessel disease had significantly higher homocysteine levels as compared to patients with two vessels or single vessel disease (16.1, 14.0 and 12.7 µmols / L respectively)[53].

Hyperhomocysteinemia has also been reported to increase the mortality in T2DM. In one study, the effect of hyperhomocysteinemia on fatal and non-fatal CAD events in T2DM patients was examined. It was noticed that diabetic patients with hyperhomocysteinemia, had a two times greater risk of CAD deaths as compared to diabetic patients whose homocysteine levels were less than 15 µmols / L (26.1% vs. 13.5%). Also the risk of CAD events was 36.2% and 26.2% respectively. This study concluded that in T2DM patients, hyperhomocysteinemia was a strong and independent risk factor for CAD events[54]. In the Hoorn study, the effect of hyperhomocysteinemia on the five year mortality in diabetic patients was compared to non-diabetic patients. For each 5 µmols / L rise in homocysteine levels, the risk of 5 year mortality rose by 17% in non-diabetic and 60% in diabetic subjects. Thus, hyperhomocysteinemia appeared to be a stronger (1.9 fold) risk factor for mortality in type 2 diabetic patients than in non-diabetic patients[43]. Among diabetics, silent CAD is quite frequent and tends to be a strong predictor of future coronary events and mortality. In diabetic patients, an independent association has been noticed between elevated homocysteine levels and silent CAD[55,56].

Very few studies conducted in India have examined the incidence of hyperhomocysteinemia in diabetic patients. In one study conducted in Pune, hyperhomocysteinemia was recorded in 76% of diabetic patients as

compared to 81% in the community[9]. Likewise, in a study conducted at Dr Talwalkar Clinic, Mumbai, the incidence of hyperhomocysteinemia in the diabetic patients was 68% as against 70% in the community[57]. A study conducted at Jaslok hospital, Mumbai, recorded significantly higher homocysteine levels in diabetic patients as compared to controls[30]. Thus, the incidence of hyperhomocysteinemia as well as mean homocysteine level is quite high in Indian diabetic patients.

In the west, although the incidence of hyperhomocysteinemia in diabetic patients has been reported to be much higher than the community, it is likely that hyperhomocysteinemia develops after the onset of diabetes and then interacts with it to worsen the CAD prognosis. On the other hand, in Indian diabetic patients, as the incidence of hyperhomocysteinemia in the community itself is high, hyperhomocysteinemia is probably already present when the patient develops diabetes. Hence, hyperhomocysteinemia is likely to interact with diabetes from the very beginning and cause greater deleterious effects.

T2DM patients frequently have both hyperhomocysteinemia and dyslipidemia coexistent[58]. In one small pilot study conducted at Dr Talwalkar clinic, Mumbai, the benefits of adding homocysteine lowering vitamins to atorvastatin on the progression of atherosclerosis in diabetic patients was examined. High risk diabetic patients with increased carotid artery intima media thickness (IMT) were randomized to receive either atorvastatin 10 mg or atorvastatin10 mg along with three homocysteine lowering vitamins, viz. methylcobalamin 500 mcg, folic acid 5 mg and pyridoxine 10 mg.The treatment was continued for a period of one year. In patients who received atorvastatin alone, the mean IMT progressed from 0.88 to 0.92 mm; whereas in those who received homocysteine lowering vitamins along with atorvastatin, there was no progression of IMT[59]. The difference between the two groups was however not statistical significant.

A large scale, long term, multi-centric, longitudinal study in Indian T2DM patients to examine the implications of hyperhomocysteinemia and the benefits of homocysteine lowering is warranted. However, such a study may take a long time to be completed. In the mean time, as Indian diabetic patients have a very high incidence of hyperhomocysteinemia which can interact with diabetes to further increase the cardiovascular risk, due consideration should be given to its management. It may be prudent to estimate homocysteine levels in all diabetic patients and if the levels are found to be more than 12 µmols/L, homocysteine lowering vitamins, which are safe and inexpensive, should be administered.

HYPERHOMOCYSTEINEMIA – IMPLICATIONS IN NEUROLOGY

Hyperhomocysteinemia has been shown to be associated with a number of neurological conditions like stroke, silent brain infarct, dementia, movement disorders, etc.

Hyperhomocysteinemia and Stroke- The Ruby Hall Study

At the Ruby Hall Clinic, Pune, serum homocysteine, B_{12} and folate were estimated in consecutive cases of ischemic stroke, arterial or venous infarction. The exclusion criteria included embolic stroke unless it was due to CAD, renal disease, hypothyroidism or those on vitamin supplements or injections of B_{12}/folate. As seen in Table 1, 80.74% of all arterial stroke had raised homocysteine and 75% of venous infarcts had the same. A raised homocysteine was the commonest risk factor for stroke in this population. The mean homocysteine in vegetarians with stroke was 37.7 + 11.9 µmols/L and the mean homocysteine level in all non vegetarians from rare to more than once a week was 25.5 + 13.6 µmols/L in this population[8].

Table 1: Homocysteine levels in Ruby Hall Study (2002)
Elevated Homocysteine in Ischemic stroke (163 cases)

	Cases	*HCY > 16 (%)*
Arterial occlusive disease	135	109 (80.74%)
Arterial occlusive disease (Age < 40 years)	27	25 (92.5%)
Venous sinus thrombosis	28	21 (75.6%)
Control	101	52 (51.5%)

P value significant for controls vs. all 3 groups

Since the publication of the above study, further data from 461 cases of ischemic stroke have been collected. Table 2 shows the present figures. The subsequent cases are not continuous stroke patients but include all those in whom the estimates were done depending chiefly on the treating physician and financial status. The striking features are that in 461 cases of stroke, homocysteine was elevated in 370 (80.26%). In the raised homocysteine group, B_{12} was low in 213 (57.5%) and folic acid was low in 63 (17.0%). B_{12} was borderline in an additional 9.5%. A borderline B_{12} level with elevated homocysteine (or methylmalonic acid) implies metabolic deficiency of vitamin B_{12}.

The Ruby Hall Study is by far the largest Indian study in stroke. However, several other neurologists in Pune, Mumbai, Guwahati and Hyderabad have reported similar findings. In a study conducted in Guwahati

Table 2: Homocysteine B12 and Folate studies in 461cases of stroke (RHC 2006)

		Raised HCY 15.1+		*HCY Normal*		
Serum Folate (mg/ml)	*Cases*	*B12 low 200 or less (pg/ml)*	*B12 Borderline 201-300 pg/ml*	*B12 N 301+ pg/ml*	*B12 low*	*B12- N*
0-3	63	39	8	8	4	4
3.1-6.8	114	50	7	40	15	2
6.9-14	142	71	10	39	0	22
14.1+	142	53	19	26	2	42
Total	461	213	44	113	21	70

461 Cases HCY> 15 in 370 (80.26%)
In this group B12 low 46.1% Borderlines 9.54%.

recently, hyperhomocysteinemia was reported in 59.1% of 110 ischemic stroke[60]. Likewise, hyperhomocysteinemia was reported in 83% of the 58 patients with ischemic stroke studied in Lucknow[17].

Homocysteine levels, Silent Brain Infarcts and White Matter Lesions in the Elderly

Besides clinical stroke, the MRI enables one to see brain infarcts which are sub-clinical, and the presence of white matter hyperintensity on CT scan represents sub-cortical ischaemia in the elderly due to small vessel disease. The Rotterdam scan study examined elderly people between the ages of 60 and 99. These patients were stratified based on their serum homocysteine into quartiles and the changes seen in the lowest quartile were taken as standard. Compared to the lowest quartile, each higher quartile showed an increase in changes. The rate of the changes in the top quartile was 2.5 times that in the lowest quartile for silent brain infarcts, 2.3 times for severe white matter changes and 3 times more if both silent brain infarcts and white matter changes were included[61]. Thus, sub-clinical sub-cortical ischemia usually due to small vessel disease is also associated with significantly raised homocysteine levels.

Homocysteine and Dementia

The topic of homocysteine and dementia was opened up by a report of the association from the Framingham study, in which 1092 elderly persons without dementia were followed up for 8 years. The serum was available for estimation of serum homocysteine at the onset and 8 years before the start of the study. In follow up 111 persons developed dementia. It was found that those in the highest quintile (top 1/5) of serum homocysteine had an increased risk of developing dementia. The highest quintile, compared to all other quintiles at the end of 8 years, had a 1.9 times greater chance of developing dementia and this risk was not only for vascular dementia but also for Alzheimer's dementia.[62] Thus, high homocysteine levels, preceded the onset of dementia, by years. The Rotterdam Scan study has also reported the results of the follow up of their cases, 1015 persons followed 3697 patient years (approx 3.6 years each). Of these, 30 developed dementia and the ones with the silent brain infarcts (SBI) had a dementia risk of 2.26 times compared to no SBI[61]. The SBI +ve group, even if they did not reach dementia, had a greater decline in global cognitive function. They thus established that raised homocysteine is linked to SBI and SBI is a risk factor for dementia. It was also noted that the ones with SBI who developed dementia were those who had further SBI.

In a study conducted in Japan, it was pointed out that Alzheimer's disease patients also had increased SBI. Of the 143 cases of Alzheimer's disease, 47 (32.9%) had silent brain infarct. The homocysteine levels in the ones with SBI were significantly higher than in those without[63]. After adjustment for gender and age, homocysteine levels correlated with SBI, while hypertension did not.

Hyperhomocysteinemia - Other effects in CNS

Besides stroke and dementia, hyperhomocysteinemia is known to cause abnormal movement and dystonia. There are several reports of patients who had progressive increase of movement disorders while various treatments were being tried. Subsequently the detection of hyperhomocysteinemia led to treatment and significant recovery. A recent study has noted that high homocysteine is associated with an increased risk of L-Dopa dyskinesia in Parkinson's disease[64].

Vitamin B_{12} and folate deficiency produce many other CNS manifestations like neuropathy, myelopathy,

cognitive defect, and neural tube defect. It is still unclear if homocysteine is involved at least in part of these manifestations.

Hyperhomocysteinemia – Treatment

The internationally accepted treatment for hyperhomocysteinemia involves the use of three homocysteine lowering vitamins viz. folic acid, vitamin B_{12} and pyridoxine. Folic acid and B_{12} act predominantly under fasting conditions and pyridoxine acts after meals. In patients with hyperhomocysteinemia, folic acid alone was shown to reduce homocysteine levels by 22% and vitamin B_{12} by 11%[65]. However, when both were administered together, they acted synergistically to cause a reduction in the homocysteine levels by 38.5%[65]. Pyridoxine probably does not add to the effect of folate and B_{12} in the fasting state. Pyridoxine has been shown to cause a reduction in the post methionine loading homocysteine levels by 22%[66].

Evidence that high serum homocysteine is a risk factor for CAD, stroke and vascular dementia is now very convincing. The important question is whether lowering homocysteine will reduce risk for subsequent stroke or myocardial event. The first study in this regard was the Swiss heart study, which followed up 553 patients after successful angioplasty. One group received homocysteine lowering vitamins and one did not. There was a significant reduction in the need for re-vascularization in the supplemented group than those without[67]. Mortality and second non-fatal MI was also less in the supplemented group but this did not reach statistical significance.

There are other studies which have indirectly shown the benefits of homocysteine lowering vitamins on cardiovascular disease. In a study examining the effect of homocysteine lowering vitamins on the endothelial function in patients with CAD, administration of the vitamins for a period of six months was shown to improve the endothelial function and increase the coronary blood flow by 96% as compared to a further reduction in coronary blood flow by 16% in patients who received the placebo[68]. Benefits of homocysteine lowering vitamins have also been noticed on the carotid artery intima media thickness (IMT). In patients with hyperhomocysteinemia and increased IMT, homocysteine lowering vitamins reduced the IMT from 1.50 to 1.42 mm, whereas in those receiving placebo, the IMT increased from 1.47 to 1.54 mm[69].

Recently published double blind trials have documented mixed results about the value of vitamin supplementation in preventing new vascular events. The VISP trial (Vitamin Intervention for Stroke Prevention) was published in 2004. It showed in a double blind controlled trial that giving vitamins failed to prevent stroke[70]. In 2005, however, members who took part in the VISP trial reanalyzed their data. They felt that the results could be erroneous partly because folate supplementation of cereals had been disregarded, and that those who had high B_{12} levels to start with may have already received vitamins, and those whose serum B_{12} was very low to start with (< 250 µmols / L) may have malabsorption. They excluded these cases from the analysis as also cases with low GFR. The exclusions were actually decided before they re-analyzed the data. On re-analyses they found that the high dose B_{12} supplement group had 21% less risk of death, stroke, or coronary heart disease[71]. Moreover, if the initial serum B_{12} was above the median and the patient received high dose B_{12}, this group did the best and conversely the group whose serum B_{12} was below median to start with and received low dose supplements had the poorest outcome. It was concluded that in the era of folate supplementation, B_{12} administration is beneficial and crucial.

The HOPE 2 study randomized 5522 persons age 55 and older to receive vitamin supplements or placebo. Their conclusion was that vitamins failed to reduce the risk of new cardiovascular events. This was true if all cardiovascular events MI, stroke and sudden deaths were included. However, in their own analysis, homocysteine lowering vitamins reduced the risk of all stroke by 25% and non fatal stroke by 28%[72]. Furthermore their results may have been vitiated by the fact that 72% of the placebo and treatment group were from US and Canada where folic acid supplementation of cereals had become mandatory by 1998, so the placebo group also had folate supplements. Moreover, in the HOPE 2 study, the mean homocysteine before treatment in both groups was 12.2 µmols / L and after 5 years treatment in the control group it had risen to 12.9 and in the treatment group homocysteine had fallen to 9.7, leaving a 3.2 µmols / L difference in the two groups.

In India the conditions are quite different than in the US and other countries where the HOPE 2 study was done. In the HOPE 2 study, before treatment the mean Hcy level was 12.2 µmols / L. In the Ruby Hall study, the pure vegetarian control group had homocysteine levels of 27.7 µmols / L and the occasional non vegetarians had homocysteine levels of 21.0 µmols / L. The mean B_{12} level in the HOPE 2 study patients was 426-436 µmols / L. In the Ruby Hall Study vegetarian controls had mean B_{12} levels of 190 µmols / L and 56% of the pure lacto-vegetarian and 55% of occasional non

vegetarians had B_{12} deficiency. At Ruby Hall the homocysteine falls in stroke patients after treatment reached 30 µmols / L(39.7-9.8 µmols / L over 2-8 months). Thus the HOPE 2 study results probably do not apply to the Indian population especially as the placebo group in HOPE 2 was receiving folate supplements in cereals in the majority.

Perhaps the most striking results of vitamin supplementation come from the study of stroke epidemiology in US and Canada from 1990-2002. In the US, from 1990-98, stroke mortality was falling steadily at 0.3% per year. After 1998, when folate substitution in cereals was introduced, the mortality fell to 2.9% per year, a 10 times change[73]. Similarly in Canada the stroke mortality which was falling by 1% per year before 1998 improved to 5.4% per year after the introduction of folate substitution[73]. Most striking is that in England and Wales, where folate supplements were not used, there was no change in stroke mortality in this period, though education of public regarding risk factors and treatment was otherwise similar. Most important, this change occurred across the entire population of US and Canada with similar falls in African American, and Hispanics. This is important because stroke risk of course falls if hypertension is corrected or smoking is stopped, or diabetes is controlled but that happens only to those who are co-operative and take the necessary treatment. Folate substitution in cereals was a methodology, which reduced risk of everyone in the country. If food fortification was tried in India, one would also have to fortify with vitamin B_{12}, not just folate, and find a universally used food which could be fortified.

Until such time, doctors should remember raised homocysteine is a very common and important cardiovascular risk factor in our country, commoner than diabetes, smoking and even hypertension and carrying the same risk roughly as each of the 3 above. Hyperhomocysteinemia is also a risk factor for arterial and venous occlusion. Moreover, it is the *easiest* of the risk factors to modify.

REFERENCES

1. McCully K. Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am J Pathol 1969;56:111-28.
2. Spence J, et al. Vitamin Intervention for Stroke Prevention Trial-An Efficacy Analysis. Stroke 2005;36:2404-9.
3. Boushey C, et al. A Quantitative Assessment of Plasma Homocysteine as a Risk Factor for Vascular Disease. Probable Benefits of Increasing Folic Acid Intakes. JAMA 1995;274:1049-57.
4. Vollset S, et al. Plasma total Homocysteine and cardiovascular and non cardiovascular mortality. The Hordaland Homocysteine study. Am J Clin Nutrition 2001;74:130-6.
5. Third Report of NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) - Final Report. National Institute of Health. Sept 2002.
6. Malinow M, et al. Homocysteine, Diet and Cardiovascular Diseases – A Statement for Healthcare Professionals From the Nutrition Committee, American Heart Association. Circulation 1999;99:178-82.
7. Welch G, Loscalzo J. Homocysteine and Atherothrombosis. NEJM 1998;338:1042-50.
8. Wadia R, et al. Hyperhomocysteinemia and Vitamin B12 Deficiency in Ischaemic Strokes in India. Ann Ind Acad Neurol 2004;7:387-92.
9. Refsum H, et al. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. Am J Clin Nutr 2001;74:233-41.
10. Misra A, et al. Hyperhomocysteinemia and low intakes of folic acid and vitamin B12 in urban North India. Eur J Nutr 2002;41:68-77.
11. Yagnik C, et al. Vitamin B12 deficiency and Hyperhomocysteinaemia in Rural and Urban Indians JAPI 2006;54:775-81.
12. Indian Study Group on Homocysteine – Consensus meeting on Hyperhomocysteinemia and Atherosclerosis, Goa, 24th June, 2006.
13. Lakshmi A, et al. Plasma Homocysteine level in relation to folate and vitamin B6 status in apparently normal men. Asia Pacific J Clin Nutr 2001;10:194-5.
14. SparkNotes: Water Soluble Vitamins: Folic Acid. www.sparknotes.com / health / vitamins / watersoluble
15. Gheye S, et al. Fibrinogen and Homocysteine levels in Coronary Artery Disease. Indian Heart J 1999;51:499-502.
16. Mukherjee M, et al. A Low Prevalence of the C677T mutation in the methylenetetrahydrofolate Reductase Gene in Asian Indians. Clin Genet 2002;61:155-9.
17. Kalita J, et al. Methykebetetrahydrofolate reductase gene polymorphism in Indian stroke patients. Neurology India 2006;54:260-3.
18. Kumar J, et al. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian Population. J Hum Geneta 2005;50:655-63.
19. Enas E, et al. Dyslipidemia among Indo-Asians Strategies for Identification and Management. Br J Diabetes Vasc Dis 2005;5:81-90.
20. Yeolekar M, et al. Hyperhomocysteinemia and Vascular Disease: Role and Implications. JAPI 2002;50:5-8.
21. Chambers J, et al. Plasma Homocysteine Concentrations and Risk of Coronary Heart Disease in UK Indian Asian and European. Lancet 2000;355:523-7.
22. Kopprasch S, et al. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. Diabetes 2002;51:3102-6.
23. Rasouli M, et al. Plasma Homocysteine Predicts Progression of Atherosclerosis. Atherosclerosis 2005;181:159-65.

24. Kobori Y, et al. Influence of Serum Homocysteine Level on Coronary Atherosclerosis. J Cardiol 2004;43:223-9.
25. Nygard O, et al. Plasma Homocysteine Levels and Mortality in Patients with Coronary Artery Disease. NEJM, 1997;337:230-6.
26. Subramanian R. Hyperhomocysteinemia: Current Evidences And Recommendations. Inches Companion IV 2005;57-64.
27. Reddy N, et al. Prevalence of risk factors for Coronary Atherosclerosis in a Cross-sectional Population of Andhra Pradesh. Indian Heart J 2002;54:697-701.
28. Ramchandran A, et al. Clustering of Cardiovascular risk factors in Urban Asian Indians. Diabetes Care 1998;21:967-71.
29. Pisciotta L, et al. Serum Homocysteine, Methylenetetrahydrofolate Reductase Gene Polymorphism and Cardiovascular Disease in Heterozygous Familial Hypercholesterolemia, Atherosclerosis 2005;179:333-8.
30. Ridker P, et al. Plasma Homocysteine Concentration, Statin Therapy and the Risk of First Acute Coronary Events. Circulation 2002;105:1776-9.
31. Personal Communication : Dr.G.S.Sainani, Work done at Jaslok Hospital, Mumbai.
32. Puri A, et al. Homocysteine and Lipid Levels in Young Patients with Coronary Artery Disease. JAPI 2003;681-5.
33. Chacko K, et al. Plasma Homocysteine levels in patients with Coronary Heart Disease. Indian Heart J 1998;50:295-9.
34. Deepa R, et al. Absence of Association between Serum Homocysteine Levels and Coronary Heart Disease in South Indian Males. Indian Heart J 2001;53:44-7.
35. Sastry B, et al. A Case-Control Study of Plasma Homocysteine Levels in South Indians with or without Coronary Artery Disease. Indian Heart J 2001;53:749-53.
36. Wild, et al. Global Prevalence of Diabetes Mellitus. Diabetes Care 2004;27:1047-53.
37. Raheja B, et al. The DiabCare Asia–India Study Groups: Diabetes Care in India – Current Status. JAPI 2001;49:717-22.
38. Rao S, McGuire D. Epidemiology of Diabetes Mellitus and Cardio Vascular Disease. In: Marso SP, Stern DW (Eds). Diabetes and Cardio Vascular Disease. Lippincot, Williams and Wilkins, Philadelphia 2004;153-78.
39. Pyorala K, et al. Diabetes and Atherosclerosis: An epidemiological view Diabetes Metab Rev 1987;3:463-524.
40. Aronson D. Pharmacologic modulation of autonomic tone: Implications for the Diabetic Patient. Diabetologia 1997;40:476-81.
41. Hu F, Manson D. Management of Diabetes: Diet and Lifestyle Modifications. In: Pickup JC, Williams G (Eds): Textbook of Diabetes. Blackwell Publishing Company, Malden, Massachusetts. 3rd Ed 2003;36-1:36-13.
42. Feener E, Dzau V. Pathogenesis of Cardiovascular Disease in Diabetes. In: Kahn C, et al (Eds): Joslin's Diabetes Mellitus. Lippincott, Williams and Wilkins Philadelphia, 14th Edn 2005;867-84.
43. Hoogeveen E, et al. Hyperhomocysteinemia increases Risk of Death especially in Type 2 Diabetes: 5 year follow up of the Hoorn Study. Circulation 2000;101:1506-11.
44. Buysschaert M, et al. Hyperhomocysteinemia and Type 2 Diabetes. Diabetes Care 2000;23:1816-22.
45. Bjorck J, et al. Associations between Serum Insulin and Homocysteine in a Swedish population - A Potential Link between the Metabolic Syndrome and Hyperhomocysteinemia: The Skaraborg project. Metabolism 2006;55:1007-13.
46. Meigs J, et al. Fasting Plasma Homocysteine levels in the Insulin Resistance Syndrome: The Framingham Offspring study. Diabetes Care 2001;24:1403-10.
47. Narin F, et al. The Association of Plasma Homocysteine Levels with Serum leptin and Apolipoprotein B levels in Childhood Obesity. Ann Saudi Med 2005;25:209-14.
48. Desouza C, et al. Drugs Effecting Homocysteine metabolism : Impact on Cardiovascular Risk. Drugs 2002;62:605-16.
49. The Field Study Investigators, Effects of Long-term Fenofibrate Therapy on Cardiovascular Events in 9795 people with Type 2 Diabetes Mellitus (the FIELD Study) Randomized Controlled Trial. The Lancet 2005;366:1849-61.
50. Rudy A, et al. Homocysteine Concentrations and Vascular Complications in patients with Type 2 Diabetes. Diabetes Metab. 2005;31:112-7.
51. Ndrepepa G, et al. Circulating Homocysteine Levels in Patients with type 2 Diabetes Mellitus. Nutr Metab Cardiovasc Dis. 4th October 2006.
52. Hoogeveen E, et al. Hyperhomocysteinemia is Associated With an Increased Risk of Cardiovascular Disease, Especially in Non-Insulin-Dependent Diabetes Mellitus. A Population-Based Study. Arterioscler Thromb Vasc Biol 1998;18:133-8.
53. Okada E, et al. Hyperhomocysteinemia is a Risk Factor for Coronary Arteriosclerosis in Japanese Patients with Type 2 Diabetes. Diabetes Care 1999;22:484-90.
54. Soinio M, et al. Elevated Plasma Homocysteine Level Is an Independent Predictor of Coronary Heart Disease Events in Patients with Type 2 Diabetes Mellitus. Ann Intern Med 2004;140:94-100.
55. Tarkun I, et al. Homocysteine Concentrations in Type 2 Diabetic Patients with silent Myocardial ischemia : A Predictive marker. J Diabetes Complications 2004;18:165-8.
56. Gazzaruso C, et al. Silent Coronary Artery Disease in Type 2 Diabetes Mellitus: the role of Lipoprotein(a), Homocysteine and apo(a) Polymorphism. Cardiovascular Diabetology 2002;1:5.
57. Keshvani A, Talwalkar P. Homocysteine Levels in patients with T2DM and Vascular Complications. Paper presented at RSSDI Mahacon, 15th March 2005 – Thane.
58. Keshvani A, Athavale U, Talwalkar P. Co-existence of Dyslipidemia and Hyperhomocysteinemia in High Risk Type 2 Diabetic Patients. Paper to be presented at IDF Capetown 7th December 2006.
59. Keshvani A, Athavale U, Talwalkar P. Comparative Effect of Atorvastatin and Atorvastatin with Homocysteine Lowering Vitamins on Carotid Intima Media Thickness in patients with Type-2 Diabetes Mellitus. Paper to be presented at IDF Capetown 7th December 2006.
60. Das R, Borah N C. Homocysteine and Ischaemic stroke a case control study. Annals of Indian Academy of Neurology 2006; 9(Supplement 1):39.

61. Vermeer S, et al. Homocysteine, silent brain infarcts and white matter lesions. The Rotterdam Scan study Annals Neurol 2002; 51:385-9.
62. Seshadri S, et al. Plasma Homocysteine as a risk factor for dementia and Alzheimer's disease. New Eng J Med 2002; 346: 476-83.
63. Matsui T, Nemolo M, Muruyama M. Plasma Homocysteine and risk of co existing silent Brain infarcts in Alzheimer's disease Neuro deger Dise 2005;2:299-304.
64. Zoccolella S, et al. Hyperhomocysteinaemia in movement disorders: Current evidence and hypothesis. Curr Vasc Pharmacol 2006; 4:237-43.
65. Sato Y, et al. Hyperhomocysteinemia in Japanese patients with convalescent stage ischemic stroke: Effect of combined therapy with folic acid and mecobalamine, J Neurol Sci 2002;202:65-8.
66. Bostom A, et al. Treatment of hyperhomocysteinemia in renal transplant recipients. A randomized, placebo-controlled trial. Ann Intern Med 1997;127:1089-92.
67. Schnyder G, et al. Effect of homocysteine-lowering therapy with folic acid, vitamin b(12), and vitamin B96) on clinical outcome after percutaneous coronary intervention : the Swiss Heart study: a randomized controlled trial. JAMA 2002;288:973-9.
68. Willems FF, et al. Coronary endothelial function in hyper-homocysteinemia: improvement after treatment with folic acid and cobalamin in patients with coronary artery disease. J Am Coll Cardiol 2002;40:766-72.
69. Till U, et al. Decrease of carotid intima-media thickness in patients at risk to cerebral ischemia after supplementation with folic acid, Vitamin B6 and B12. Atherosclerosis 2005;181:131-5.
70. Toole J, 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. JAMA 2004;291:565-75.
71. Spence JD, et al. Vitamin intervention for stroke prevention trial an efficacy analysis. Stroke 2005;36:2404-9.
72. The heart outcomes prevention evaluation (HOPE) 2 investigators, Homocysteine lowering with folic acid and B vitamins in vascular disease. NEJM 2006;354:1566-77.
73. Yang Q, et al. Improvement in stroke mortality in Canada and the United States, 1990 to 2002. Circulation 2006;113:1335-43.