PACIFIC JOURNAL OF MEDICAL SCIENCES
{Formerly: Medical Sciences Bulletin}
ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)
www.pacjmedsci.com. Email: pacjmedsci@gmail.com.

Seminar presentation:

Assessment of Hyperhomocysteinemia among Cardiovascular Patients in Port Moresby General Hospital: A Prospective Study

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INTRODUCTION:

Cardiovascular disease remains the leading cause of mortality in industrialized countries and is becoming increasingly prevalent in developing countries [1]. In the last four decades tremendous gains have been made in understanding the effect of the risk factors for cardiovascular disease. Well-known (or traditional) risk factors such as abnormal cholesterol levels, increased blood pressure, hereditary, advancing age, diabetes mellitus, obesity, lack of physical activity and smoking are helpful in predicting the likelihood of heart attacks or strokes [2, 3]. Modification of some of these risk factors can reduce the risk of having a heart attack or stroke [3]. These traditional risk factors do not fully account for all the cardiovascular disorders reported in hospitals and clinics worldwide [3]. Correlation between Hyperhomocysteinemia and vascular diseases was observed by clinicians and scientists as early as the 1960's [4]. Since then the number of studies have greatly increased and the trend continues to support the Hyperhomocysteinemia as an important risk factor for coronary atherosclerosis, coronary artery disease (CAD), myocardial infarction, stroke, thromboembolism and peripheral vascular disease [4, 5]. It is now widely accepted that an elevated level of Homocysteine (>15umol/L) is an independent risk factor for cardiovascular disease [3, 6]. According to the American Heart Association (AHA) advisory statement [5], the total Homocysteine concentration in blood can be characterized as normal (5 – 15umol/L), intermediate (31 – 100umol/L) or severe (> 100umol/L). In 2002 the Centers for Disease Control and Prevention (CDC) Environmental Health division proposed normal reference ranges for total plasma homocysteine levels according to gender and age groups (Table 1) [7].

In addition, the CDC also published the cut-off points for homocysteine concentration in blood plasma that indicates moderate (16 – 30 umol/L), intermediate (31 – 100umol/L) and severe (> 100umol/L) Hyperhomocysteinemia [7]. Homocysteine is

a non-standard, non-protein sulphur-containing (thiol) amino acid with the molecular formula $C_4H_9NO_2S$ [8, 9, 10]. Homocysteine is produced in the liver as a metabolic intermediate in biosynthesis of cysteine from Methionine [8, 9]. In humans, the metabolism of Homocysteine occurs at the intersection of the Trans-sulfuration and Remethylation pathways [8, 9]. In the Trans-sulfuration pathway Homocysteine condenses with Serine to form Cystathionine, catalyzed by Cystathionine β-synthetase (CBS) that requires the cofactor Pyridoxal-phosphate (B_6-Phosphate) [8, 9]. Hydrolysis of Cystathione to Cysteine and α-Ketobutyrate is catalyzed by γ-Cystathionase [8, 9].

Table 1: Homocysteine Reference Ranges from CDC Environmental Health [7]

	Total Homocysteine (umol/L)	Age	Gender
Normal	4.6 - 8.1	< 30 years	All
Normal	4.5 - 7.9	30 - 59 years	Females
Normal	6.3 - 11.2	30 - 59 years	Males
Normal	5.8 - 11.9	> 60 years	All

The Remethylation pathway involved methylation of Homocysteine to Methionine [8, 9]. The methyl group is obtained from either N-5-Methyltetrahydofolate or Betaine.

N-5-methyl-tetrahydofolate is formed from 5-10-methyltenetrahydofolate by 5-10-methyl-tenetrahydofolate reductase (MTHR).

The reaction that uses N-5-methyl-tetrahydofolate occurs in all tissues and is catalysed by Methionine synthase (MS) that utilizes Vitamin B_{12} as cofactor [8, 9]. The Remethylation reaction involving Betaine occurs in the liver; it is catalyzed by Betaine-Homocysteine methyl-transferase (BHMT) [8, 9].

Some causes of Hyperhomocysteinemia include the following: Vitamin B_6 (Pyridoxine phosphate) and Vitamin B_{12} (Cobalamin) are cofactors and Folic Acid is a co-substrate needed for Homocysteine metabolism [9, 10]. Deficiencies in the B-complex vitamins may cause accumulation of Homocysteine in the body [9, 10, 11];

Genetic defects (mutations) in genes encoding for enzymes involved in the Homocysteine metabolic pathways can

result in Hyperhomocysteinemia. Some of the enzymes are 5,10-Methylene-Tetrahydrofolate (MTHFR); Cystathionine β-synthetase (CBS); Methionine synthase (MS); Betaine-Homocysteine Methyl-Transferase (BHMT) [8, 9, 10, 11, 12]; Renal failure may cause accumulation of Homocysteine in blood because of the less efficient renal clearance of Homocysteine by the kidneys. [4, 8, 11, 20]; certain drugs such as Methotrexate and Cyclosporine A can cause elevation of Homocysteine [11, 18]; Homocysteine levels increase with age [13]. On average Homocysteine levels are higher in males than females [13]; Stress, physical inactivity, smoking, and coffee drinking cause elevation of Homocysteine. [18, 19, 20]. The link between blood Homocysteine level and cardiovascular disease was first suggested by McCully [14], who encountered patients with Homocystinuria. This is an autosomal recessive disorder characterized by abnormalities of the long bones, ocular lens dislocation, mental retardation and venous thromboembolism that can cause aggressive vascular disease [9, 14].

Patients with Homocystinuria usually have Hyperhomocysteinemia [4, 9, 14]. Children with Homocystinuria usually do not thrive because of the complications of arteriosclerosis [4, 9, 14]. Untreated patients who are homozygous for Homocystinuria may have Homocysteine concentration in the blood about five times above the normal level for unaffected people [16]. They may also have about 50% chance of developing vascular events by age 30 years [16]. Earlier studies [4, 17] indicated that Homocysteine is an independent risk factor for vascular disease, similar to that of smoking or hyperlipidemia and that the risk was more pronounced in smokers and in those with hypertension. In a recent review by Wald et al [15] meta-analyses of cohort studies show significant positive associations between serum Homocysteine concentrations and Ischemic heart disease events and stroke. According to the authors [15, 16] a 3.0umol/L decrease in serum Homocysteine (achievable by utilizing 0.8mg/day of Folic acid) lowers the risk of myocardial infarction by 15% and stroke by 24%. According to Mann and Green [16] a 5.0umol/L increase in total Homocysteine level in blood can significantly increase the risks of having coronary heart disease and cerebrovascular disease.

Several recent studies [18, 19, 20] have linked Hyperhomocysteinemia to birth defects, Down Syndrome, Diabetes Mellitus, Alzheimer's, Osteoporosis, Renal Failure and some cancers. Despite several well structured research and clinical studies on Hyperhomocysteinemia, the exact mechanism(s) by which it can cause

vascular injuries remains unclear. There are however a number of hypotheses that have been proposed. Some of these hypotheses include the following: The direct toxic effect of Homocysteine that damages the cells lining the intima of arteries [21, 22, 23]; Oxidation and modification of low-density lipoproteins by Homocysteine [24, 25, 26]; Interference of Homocysteine with the blood clotting factors [27, 28, 29]. There are several ongoing research studies to elucidate the mechanism(s) of action of Hyperhomocysteinemia in Atherosclerosis and cardiovascular diseases.

Despite counter arguments by some authors [30] there are evidence indicating strong correlation between Hyperhomocysteinemia and cardiovascular diseases.

Proposed prospective research project:

Hyperhomocysteinemia is a risk factor for cardiovascular diseases [4, 5, 9, 16, 17]. All confirmed cardiovascular cases in Papua New Guinea (PNG) are reported to the Sir Buri Kidu Heart Institute in the Port Moresby General Hospital (PMGH), which is the major specialist and referral hospital in PNG. Most of the cardiovascular cases have been successfully managed and have been related to traditional risk factors.

There are, however no published data indicating the involvement of Homocysteine in the aetiology of cardiovascular disease in PNG. The need to investigate the possible role of Homocysteine in cardiovascular diseases in PNG is strongly supported by the Chief consultant cardiologist and other specialists in PMGH.

The aim of the study will be to assess the Homocysteine level in the blood of patients with cardiovascular diseases admitted to the PMGH. The data obtained will be used to evaluate the relationship (if any) of Homocysteine to cardiovascular diseases among patients in PMGH. The Sir Buri Kidu Heart Institute in PMGH will serve as the sampling site for this collaborative cross-sectional study.

Signed informed consent will be obtained from the relatives of patients that are enrolled in the study.

Fasting blood samples will be collected from patients with heat attack and stroke admitted in the institute.

Assay of plasma Homocysteine levels will be carried out using the HPLC procedures and protocol designed by Immundiagnostiks [32, 33]. Blood samples will also be collected from healthy age-matched controls. Parameters such as age, gender, health status, current and past history of illnesses such as renal failures and those affecting the CVS especially diabetes mellitus will be recorded. Data obtained will be statistically analysed and interpreted using approved standards and cut-off points.

Ethical clearance and permission for this project will be obtained from the relevant authorities. It is hoped that this project will set the stage for more detailed study to investigate the causes of Homocystinemia in PNG. Hopefully this could also lead to studies of nutritional factors affecting Homocysteine levels namely the B vitamins (vitamin B_6, B_{12} and folic acid) and to develop genetic screening of patients and individuals who may be at risk of having cardiovascular disease [2, 6, 10, 11].

ACKNOWLEDGMENTS:

The author thanks Gairo Gerega, A/Prof VJ Temple, Prof Sir Isi Kevau and Dr. Lawrence Teiptoror for their assistance and support.

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