Homocysteine and related metabolites in essential hypertension and associated complications; possible role of cysteinylglycine

Mohamed A. Abdel Hafez[1], **Nagwa Kamal Roshdy**[1], **Fatma Taha**[1], **Soliman Nasr**[2]
[1]Medical Biochemistry, and [2]Internal Medicine Departments, Kasr Al-Aini Faculty of Medicine, Cairo University

ABSTRACT

In the present work, the relation between plasma homocysteine (Hcy) and its metabolites (S-adenosyl homocysteine, SAH, and S-adenosyl methionine, SAM) was studied. Plasma folic acid and vitamin B_{12} were evaluated to clarify their role in Hcy-methionine interaconversion in pathogenesis of essential hypertension and related vascular disorders. The study was conducted on 65 patients suffering from essential hypertension either uncomplicated or with renal and non-renal (vascular) complications. Fifteen healthy age and sex matched subjects were included as controls. Plasma Hcy, SAH and SAM were determined by high performance liquid chromatography (HPLC). Plasma folate and vitamin B_{12} were determined by dual count radioimmunoassay. Significant elevation in plasma Hcy and SAH and decrease in SAM/SAH were observed in hypertensive patients versus controls. Significantly higher levels of Hcy and SAH were found in hypertensive patients with renal complications. Also, significantly higher levels of Hcy and SAH were found in hypertensive patients with CHD and CVS. There was significant decreases in plasma folate and vitamin B_{12} in hypertensive patients. Plasma cysteinylglycine was significantly higher in hypertensives. In conclusion, the risk of homocysteine-associated hypertensive complications may partly be due to low folate and vitamin B_{12} which decreased significantly with the severity of hypertension and coincided with the increased homocysteine level. Possible role of cysteinylglycine (Cysgly) particularly for end-stage renal disease was discussed.

RIASSUNTO

Omocisteina e metaboliti correlati nella ipertensione essenziale e nelle sue complicazioni; possibile ruolo della cisteinilglicina

E' stata studiata la relazione tra omocisteina (Hcy) del plasma ed i suoi metaboliti (S-adenosil omocisteina, SAH, and S-adenosil metionina, SAM). L'acido folico e la vitamina B_{12} del plasma sono stati anche valutati per chiarire il loro ruolo nella interconversione Hcy-metionina nella patogenesi della ipertensione essenziale e disordini correlati. Lo studio è stato condotto su 65 ipertesi essenziali, senza o con complicazioni renali od extra-renali. 15 soggetti sani, di comparabile età e sesso, sono stati inclusi come controlli. Hcy, SAH e SAM del plasma sono stati determinati mediante cromatografia liquida ad alte prestazioni (HPLC); il folato e la vitamina B_{12} del plasma mediante metodo radioimmunonologico a doppio conteggio.Negli ipertesi si sono osservati valori significativamente elevati di Hcy e di SAH e diminuzioni di SAM/SAH. Valori significativamente più elevati di Hcy e di SAH si osservavano negli ipertesi con complicazioni renali, nonché negli ipertesi con complicazioni vascolari cardiache e cerebrali. Si riscontrava un significativo abbassamento di folato e vitamina B_{12} del plasma negli ipertesi. La cisteinilglicina del plasma era significativamente più elevate negli ipertesi. In conclusione, il rischio complicazioni ipertensive associate alla omocisteina può parzialmente essere dovuto a bassi livelli di folato and vitamina $B_{12,}$ che diminuivano significativamente con la severità della ipertensione, in coincidenza con aumentati livelli di omocisteina. Viene discusso il possibile ruolo della cisteinilglicina (Cysgly), in partcolare nella malattia renale in stadio finale.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world (1). Primary or essential hypertension is an important modifiable factor for CVD. It is a polygenic disease involving major contributors of various environmental factors, making it difficult to predict which type of therapeutic agents is the most appropriate for the treatment of the given patient (2).

The derangement of endothelial function in hypertension is likely to be caused in part by genetic factors and also by the elevated blood pressure itself (3). During the past few years, an increasing body of evidence has accumulated suggesting the contribution of non-traditional or alternative cardiovascular risk factors including hyperhomocysteinemia and oxidative stress to the development of essential hypertension (4)

Homocysteine (Hcy) is a sulfur-containing amino acid generated during the metabolism of methionine. During the process of methylation, S-adenosylmethionine (SAM) derived from methionine is converted to S-adenosylhomocysteine (SAH), which is additionally hydrolyzed to simultaneously produce Hcy and adenosine by SAH

hydrolase in a variety of mammalian cells. Hcy is remethylated to form methionine by methionine synthase (MS) (vitamin B_{12}- and folate-dependent) or it is trans-sulfurated by cystathionine β-synthase (vitamin B6- dependent) to cystathionine. The later is subsequently converted to cysteine, the precursor of Glutathione (5).

The pathogenicity of intracellular SAH accumulation lies in its high-affinity binding to the catalytic region of most SAM-dependent methyltransferases, probably due to structural similarity with SAH enabling it to act as a potent product inhibitor. For this reason, continual hydrolysis of SAH to Hcy and adenosine is essential for SAH homeostasis to assure normal methylation of DNA, RNA, protein, phospholipids, histones and neurotransmitters (6)

High SAH level may compromise the growth and integrity of the endothelial cells through reducing carboxyl methylation of $p21^{ras}$ in vascular endothelial cells (7).

This work was planned to study the correlation between the levels of plasma homocysteine and its related metabolites (SAH and SAM) on one hand, and the severity of essential hypertension on the other. It also aimed at investigating their possible role as risk factors for thrombovascular complications associated with hypertension. To get a clear explanation of the results, it was mandatory to evaluate folic acid and vitamin B_{12} plasma levels. Variable values of cysteinylglycine (Cysgly) was observed during the chromatographic run. This motivated us to assume possible pathophysiological significance.

SUBJECTS AND METHODS

Our patients were diagnosed and classified according to the criteria of the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (8). Patients with conditions thought to influence homocysteine concentrations (such as cancer, thyroid disease, liver cell disease or using anticonvulsant therapy), were excluded. No patients were taking folic acid, vitamin B_6 or B_{12} supplements. Those with renal disease were hypertensive pre-dating renal disorder according to history taking data. Two main groups of patients were studied:

Group I (n=30): patients with uncomplicated essential hypertension; (M/F=18/12), aged (Mean±SD) 51.2±7.4 years. This group was sub-classified into: Subgroup Ia (n=15): patients with moderate hypertension; (M/F=7/8), with a age of 49±7.1 years. Subgroup Ib (n=15): patients with severe hypertension; (M/F=10/5), with age of 52±7.5 years. Group II (n=35): patients with complicated essential hypertension; (M/F=21/14), with age of 51.6±6.8 years.

Because of the wide variation in plasma Hcy in group II, this group was sub-classified into: Group IIa; those with renal function impairment (n=8) and the rest of group II was considered as group IIb having normal renal functions (n=27).

Group IIb was further subdivided into hypertensives complicated with coronary heart disease (CHD) (group IIbi) (n=12) and hypertensives complicated with cerebrovascular stroke (CVS) (group II bii) (n=15).

Group III (n=15): age and sex matched healthy controls, (males/females=10/5), with a mean (±SD) age of 48.8±8.3 years.

All groups were subjected to: careful history taking, full clinical examination with special emphasis on: blood pressure (BP) measurement and fundus examination. Estimation of fasting and 2h-pp blood sugar, serum urea and creatinine, aspartate transaminase (AST) and alanine transaminase (ALT), lipid profile: total cholesterol, triacylglycerols (TAG), high-density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were performed by routine chemical methods. Electrocardiography (ECG), as well as other echocardiography or brain computed tomography (CT) and/or magnetic resonance imaging (MRI) were done when indicated.

Plasma Hcy, SAH and SAM were measured for all patients and controls using reversed-phase high performance liquid chromatography (HPLC). Plasma vitamin B_{12} and plasma folate were measured by dual-count radioimmunoassay.Cysteinylglycine evaluation was done by comparative standard solution inclusion in the chromatographic run for SAM and SAH.

Estimation of Plasma Homocysteine was carried out by a method adopted from that of Ubbink et al (9) on the basis of the chemical description provided by Aracki and Sako (10), which was built on labeling of plasma homocysteine with a thiol-specific fluorogenic reagent, ammonium 7-fluorobenzo-2-oxa-1,3diazole-4sulfonate (SBD-F), followed by HPLC and fluorescence detection. The addition of internal standard mercaptopropionylglycine was done according to Vester and Rasmussen (11)

Chemicals and Reagents were obtained from Sigma ® (St. Louis, MO, USA):

After an overnight fasting, 5 mL of venous blood were withdrawn into an ice-cold vacutainer tube containing EDTA cooled centrifuged at 2000 g for 10 min. Plasma was stored as aliquots at -80^{0}C till assay. 20 μL of the prepared sample were used for chromatographic determination of homocysteine. Standard solution (40 μmol/L) was prepared by dissolving Hcy in normal saline.

Separation and quantification were performed with GBC system, Australia (pump LC 1150, fluorescence detector LC 1255) equipped with phenomenex (USA, UK) phenosphere ODS analytical column (150 mm X 4.6 mm ID, 5 μm particle size). The fluorescence intensities were measured with excitation at 385 nm and emission at 515 nm. Mobile phase consisted of 0.1 mol/L potassium dihydrogen phosphate buffer (pH 2.1) at room temperature, containing 4% acetonirile at a flow rate of 2 ml/min (isocratic mode). The peak of homocysteine was identified according to the retention time of the standard. The concentration was automatically calculated by dividing the ratio between the area of the homocysteine peak and the mercaptopropionylglycine (internal standard) peak.

Estimation of Plasma S-adenosylhomo-cysteine and S-adenosylmethionine was as follows:

Chemicals and Reagents were obtained from Sigma ®

(St. Louis, MO, USA). Fixed volume of standard solutions of SAM and SAH was added to plasma to improve sensitivity of the method. Plasma was cleared off by 0.4 M perchloric acid. 100 μL of the clear supernatant was used for chromatographic determination of the investigated parameters (12). The same system was used but with UV/Vis detector LC 1200 (set at 254 nm) equipped with Hypersil® (UK) ODS analytical column (250 mm X 4.6 mm ID, 5 μm particle size). The mobile phase consisted of 0.1 mol/L sodium acetate, 5 mmol heptanesulfonic acid, adjusted to pH 4.5 with acetic acid and finally containing 4.2% acetonirile with flow rate 1.5 ml/min. The peaks were automatically calculated by relating the peak area of the unknowns with those of standards. The added standard values to samples were subtracted according to the software program of the calibration unit of the system.

Estimation of Plasma Vitamin B12 and Folate: Vitamin B_{12} and folate were determined in plasma (13) using dual count radioassay kit for the simultaneous quantitative determination of vitamin B_{12} (^{57}Co) and folate (^{125}I) provided by the Diagnostic Products Corporation (DPC) (Los Angles, CA, USA).

Statistical Analysis Methods: The data collected were filed on an IBM compatible PC and were processed to get the statistical results that graphically represent some of the important findings of the study (14).

RESULTS

Plasma Hcy was significantly higher in Group II compared to other groups (P<0.001). Plasma SAM/SAH and plasma folate were significantly lower in Group II as compared to both Group Ia and Group Ib (P=0.016, P<0.001 respectively). Plasma Vitamin B_{12} was significantly lower in Group Ib compared to both Group Ia and Group II. (P<0.001). Cysteinylglycine (Cysgly) was significantly higher in group II compared to other groups. (P=0.006) (Table 1).

Plasma Hcy was significantly higher in patients with renal impairment than in control and non renal impairment. Plasma SAH was significantly higher in patients with renal impairment than in control . Plasma SAM/SAH was significantly lower in patients with renal impairment than in control. Plasma folate was significantly lower in group II a than in control and group II b. Plasma vitamin B_{12} was significantly lower in patients with renal impairment than in control and nonrenal subjects. Cysgly was significantly higher in group II a than in control and group II b. (Table 2)

Plasma Hcy was significantly higher in patients with CHD (group IIbi) compared to control and CVS (group IIbii) (Table 3). Plasma Hcy was also significantly higher in group IIBii than in control. Plasma SAH was significantly higher in group IIBii than in control. Plasma folate and vitamin B12 were significantly lower in group IIBi than in control. Plasma folate and plasma vitamin B12 were also significantly lower in group IIBii than in control. Cysgly was significantly higher in group IIbi as compared with control and group IIbii. Plasma Cysgly was also significantly higher in group IIBii than in control. (Table 3)

DISCUSSION

The present work showed a significant increase in plasma Hcy in hypertensive patients compared to controls. Similar results were obtained by Yasmin et al (15). Level of Hcy was significantly higher in patients with complicated hypertension as compared to uncomplicated hypertension. A significant positive correlation between Hcy and blood pressure was found. Tsioufis et al (16) found that Hcy levels are related to 24-hour systolic and diastolic BP values.

Plasma SAM was unaltered in hypertension vs healthy controls, whereas SAH was significantly raised in the

Table 1
Clinical and laboratory data (mean±SD) in the studied

Parameter	Group Ia n=15	Group Ib n=15	Group II n=35	Ctrl n=15	P value
MBP	118.4±6.02	140.2±11.78	130.8±15.6	87.9±6.44	<0.001**
Age	49.6±7.16	52.9±7.53	51.7±6.83	48.8±8.27	0.372
Duration	3.67±3.13	8.60±4.22	3.69±2.96	0±0	<0.001**
Creatinine	74±6.51	76.8±7.78	129.5±114.92	71.6±7.95	0.022*
HCY	12±1.36	19.4±4.47	27.34±15.6	10.93±1.71	<0.001**
SAH	76±26	87±36	69±23	50±17	0.002**
SAM	72±26	72±28	52±25	51±24	0.012*
SAM/SAH	0.98±0.23	0.86±0.23	0.75±0.25	1.13±0.35	<0.001**
Folate	18.9±1.44	12.9±1.9	12±1.3	24.8±1.6	<0.001**
B_{12}	314.05±37.03	148.1±43.4	202.7±62.14	451.1±50.7	<0.001**
Cysgly	2.36±0.40	2.58±0.83	5.62±5.74	2.34±0.48	0.006**

* Significant. ** Highly significant
MBP= mean blood pressure (mmHg), y= years, HCY= Plasma homocysteine (μmol/L), SAH= S-adenosylhomocysteine (nmol/L), SAM= S-adenosylmethionine (nmol/L), Folate = plasma folic acid (nmol/L), B_{12}=vitamin B_{12} (pmol/L), Cysgly= Cysteinylglycine (μmol/L)

Table 2
Homocysteine and related metabolites in patients with renal disease (Group II a), normal renal function (Group II b) and controls

Parameter	Group II A (n=8)	Group II B (n=27)	Control (n=15)	P_1	P_2
HCY	50.4±11.4	20.5±8.4	10.9±1.7	<0.001**	<0.001**
SAH	83±30	65±20	51±20	0.07	0.002**
SAM	57±20	51±20	52±30	0.5	0.6
SAM/SAH	0.67±0.2	0.77±0.3	1.13±0.4	0.2	0.016*
Folate	10.93±1.4	12.33±1.07	24.8±1.6	0.03*	<0.001**
B_{12}	139.56±17.53	221.45±58.08	451.1±50.7	<0.001**	<0.001**
Cysgly	11.8±9.2	3.7±2.1	2.3±0.48	<0.001**	<0.001**

P1: group IIa vs group II b P2 : group II a vs control,
*Significant ** Highly significant

Table 3
Homocysteine and related metabolites in patients with CHD, CVS and controls

Parameter	CHD (II Bi) (n=12)	CVS (II Bii) (n=15)	Control (n=15)	P_1	P_2	P_3
HCY	24.3±9.8	17.5±6	10.9±1.71	0.04*	<0.001**	<0.001**
SAH	63±24	67±20	50±17	0.63	0.13	0.02*
SAM	46±24	54±24	52±25	0.40	0.5	0.76
SAM/SAH	0.75±0.28	0.8±0.26	1.1±0.35	0.64	0.04*	0.057
Folate	12.08±1.05	12.5±1.08	24.8±1.6	0.34	<0.001**	<0.001**
B_{12}	215.89±72.7	225.89±45.4	451.1±50.7	0.66	<0.001**	<0.001**
Cysgly	4.7±2.8	3.04±0.85	2.3±0.48	0.04*	0.004**	<0.01*

P 1: group IIbi vs group IIBii, P 2 :group IIbi vs control, P3: group IIBii vs control
* Significant, ** Highly significant

former versus the latter. Meanwhile, both plasma folate and vitamin B_{12} were significantly decreased in hypertensives . There was no significant correlation between Hcy on one hand and SAH or SAM on the other in hypertensives. Hcy correlated positively(r=o.399,p<o.o5) with SAH only in group II (complicated hypertension). SAM was found principally in the erythrocytes (17). Both Hcy and SAH were estimated in plasma and the concentration of Hcy exceeds that of SAH by almost three orders of magnitude. This difference, most likely, reflects the more facile transport of Hcy across the plasma membrane as compared to SAH (18).

The levels of plasma folate and vitamin B_{12} were decreased significantly in hypertensive patients compared to controls. These levels further decreased significantly with the severity of hypertension and coincided with the increased Hcy. A significant negative correlation was found between Hcy and folate in hypertensive patients (r =-0.57, P <0.001). Also, a significant negative correlation was found between Hcy and vitamin B_{12} in hypertensive patients (r = -0.595, P <0.001). Correlation became more evidently strong with disease severity.

Remethylation of Hcy to methionine is folate and vitamin B_{12}-dependent (19). Reductions of the latter agents in hypertensives nullify their role in keeping SAM unaltered, but at the same time cannot exclude their role in elevation of SAH. Rise of plasma SAH, therefore, could partially be due to reduced folate and vitamin B_{12} levels, and diminished trans-sulfuration pathway as well. Absence of significant correlation between plasma Hcy and both SAH and SAM supports this view.

Zhan et al (20) reported that the insignificant difference in Hcy level between hypertensive patients and controls in Chinese population might be due to higher levels of folic acid and vitamin B_{12} which encountered in their patients, the authors did not comment, in their report, on the nutritional regimens or supplementation of these vitamins .Our findings regarding Hcy, although in controversy with that study, yet they are in the same direction of the importance of folate and vitamin B_{12} in Hcy homeostasis .

Christensen et al (21) reported that the risk of vascular disease due to hyperhomocysteinemia might be partly attributable to low folate level. Vitamin B_{12} and folate even within their normal reference range. Woo et al (22) demonstrated that folic acid supplementation for one year declined in Hcy levels and improved flow-mediated dilatation.

Several pathophysiologic mechanisms linking hyperhomocysteinemia to vascular disease were considered. The most commonly reported one is through pro-oxidant/antioxidant imbalance produced by Hcy. Hcy auto-

oxidation in the presence of transition metals generating reactive oxygen species such as thiol free radicals with subsequent formation of superoxide anion, hydrogen peroxide and hydroxyl radicals (23). These directly oxidize low-density lipoprotein (LDL) (24). In addition, peroxynitrite is generated in the reaction between superoxide anion and nitric oxide (25).

Hcy toxicity could also be due to its conversion to homocysteine-thiolactone; an intramolecular thioester of Hcy. It is synthesized by methionyl-tRNA synthetase in an error-editing reaction that prevents translational incorporation of Hcy into proteins. Hcy generates free radicals during its auto-oxidation to thiolactone (26) that inhibits the expression of antioxidant enzymes, such as glutathione peroxidase (27). Hcy thiolactone is known to be toxic to endothelial cells; it was found to induce gross changes in endothelial cell morphology and cell death (28). Vascular damage may also result from an impairment of the tertiary structure of connective tissue protein, because of the reduced degree of cross-linkage between collagen and fibrin monomers (29).

Hcy thiolactone reacts with proteins by a mechanism involving homocysteinylation of protein lysine residues (30). Homocysteinylation of the vascular wall proteins by Hcy thiolactone may impair the basal membrane of the vascular wall and may contribute to LDL sequestration (31). Hcy stimulates the proliferation of vascular smooth muscle cells by activating nuclear factor-kappa beta (NF-?B) which may contribute to the mitogenic effect of Hcy by activating cyclin D1 expression (32).

Hyperhomocysteinemia in CHD and CVS: Hcy level was significantly higher in hypertensive patients with CHD and CVS compared to controls. No significant correlations were detected between Hcy and each of total cholesterol, LDL-c, HDL-c. This implies that Hcy concentration and the traditional risk factors have independent risks for developing cardiovascular diseases.

High levels of Hcy augment the adrenergic activity in patients with essential hypertension increasing the risk of atherosclerotic CHD (33). In hypertensive patients with CHD and CVS, SAH level was significantly higher and SAM/SAH ratio was significantly lower compared to controls, supporting a role for SAH in cardiovascular disease risk. Kerins et al (34) presented convincing evidence that plasma SAH appeared to be a much more sensitive indicator of the difference between patients with cardiovascular disease and control subjects than was Hcy. SAH is a strong noncompetitive inhibitor of the catechol-O-methyltransferase (COMT) that methylates catecholamines. Elevation of blood or tissue levels of catecholamines ensues and consequently, over-stimulation of the cardiovascular system's functions. High levels of endogenous catecholamines in vascular cells incur chronic cumulative damage, and hypertension follows (35) .

In this work, a significant positive correlation was found between Hcy and age(r=o.449,p<0.001) this correlation remained strong after supplementation with folate and vitamins (vitamin B_{12} and B_6) (36). Saw et al (37) suggested that higher Hcy levels in elderly may be related to the decline in renal functions with age.

Hypertensive renal disease: Hcy level was significantly higher patients with renal impairment as compared to non-renal patients. Li et al (338) proved that hyperhomocysteinemia may be an important pathogenic factor in the target organ damage, such as glomerular damage, associated with hypertension. Elevation of plasma Hcy might be due to impaired metabolism of Hcy by the kidneys or inefficient clearing off through renal tubules (39). It was reported that 70 % of Hcy is cleared of by the kidneys (40). Renal reabsorption of Hcy in the tubular cells only occurs for the non-protein bound disulfides. The redox status of the tubular cells allows a reduction of the disulfides, which makes homocysteine available for conversion via the transsulfuration or remethylation pathway (41)

Which of hypertension or hyperhomoctsteinemia predisposes to the other? Brattsrom and Wilcken (42) claim that elevation of blood pressure is responsible for nephrosclerosis with consequent deterioration of renal functions. This is highly relevant to the plasma clearance of Hcy rather than plasma creatinine that may still be normal. One may conclude ,according to this claim, that Hcy might be marker of renal malfunction in presence of hypertension regardless of plasma creatinine level.

High level of Hcy induces reversal of the SAH hydrolase (SAHH) reaction that shifts the reaction towards SAH formation, thus trapping adenosine and reduces free adenosine that has important vascular functions. Adenosine dilates coronary and cerebral arteries and increases blood flow in microcirculation. It inhibits platelet aggregation, and decreases proliferation or growth of smooth muscle or glomerular mesangial cells (43). It also dilates renal medullary vessels, increasing renal blood flow (44). These actions of adenosine protect cardiovascular and other systems from ischemic or atherosclerotic injuries. Decrease in adenosine production with a reduction of methylation of many cellular elements as a consequent of hyperhomocysteinemia may activate or promote the sclerotic process in the glomeruli, and thus aggravating impairment of renal functions (45).

Perna et al (46) stated that Hcy is a uremic toxin involved in protein molecular damage due to inhibition of methylation reactions. Methylation has been shown to be inhibited in uremic patients and, at least in the erythrocyte, is caused by Hcy and subsequent SAH accumulation (46). In uremia, inhibition of L-isoaspartyl protein-*O*-methyltransferase, an enzyme involved in the repair of protein damage leading to the accumulation of altered L-isoaspartyl residues in erythrocyte membrane proteins. In uremic patients, plasma and intracellular Hcy levels are significantly elevated (35 to 40 μmol/L) in parallel with rise of SAH concentrations. Hyperhomocysteinemia has been related to the increased CVD risk in uremia (47). Both hyperhomocysteinemia and the impaired transmethylation potential (the low SAM/SAH ratio) can be corrected partially by folate administration in uremia (48). Plasma folate and vitamin B_{12} were significantly lower in patients with renal impairment than in control and non-renal hypertensives.

Cysteinylglycine: Our results showed a significantly higher cysteinylglycine (Cysgly) level in complicated hypertensive patients compared to both non-complicated hypertensive patients and healthy controls (P<0.001). This was particularly in patients having renal impairment. Cysteinylglycine is released from glutathione by the effect of gamma glutamyltranspeptidase (GGT) when glutathione carries amino acids into the cells. The raised cys-gly level in patients with renal impairment could be due to its impaired clearance together with other sulfur compounds (49). It could also be due to altered amino acid transport across cell membranes, the so-called γ-glutamyl cycle that requires GGT. GGT particularly active in renal tubules (50) .Alteration of connective tissue and/or cell membrane proteins as a result of homocysteinaylation reactions, occurring in end-stage renal diseases, may participate in enhancement of γ-glutamyl cycle.

We may suggest that plasma cysteinylglycine rather than homocysteine is a marker of renal disease. Homocysteine level is changed according to folate status in spite of existence of nephrosclerosis and therefore it is unfit as a marker (51) .

REFERENCES

1. Hackam DG and An and SS: Emerging risk factors for atherosclerotic vascular disease. JAMA 2003 290:932-940.
2. Waeber B and Brunner HR: The multifactorial nature of hypertension: the greatest challenge for its treatment?. J Hypertens 2001:19(suppl.):S9-S16.
3. Plante GE: Vascular response to stress in health and disease. Metabolism 2002 51:25-30.
4. Virdis A, Ghiadoni L, Salvetti G, Versari D, Taddei S and Salvett A: Hyperhomocyst(e)inemia: is this a novel risk factor in hypertension. J Nephrol 2002 :279:671-678.
5. Rodwell VW:Catabolism of the carbon skeleton of amino acids. 1999 In Harper's Biochemistry. 25th edition, pp 323-346. Appleton and Lange, Norwalk, Connecticut/San Mateo, California, USA.
6. James S, Melnyk S, Pogribna M, Pogribny I and Caudill M: Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. J Nutr 2002 :132:2361S-2366S
7. Wang X: A theory for the mechanism of homocysteine-induced vascular pathogenesis. Med Hypotheses 1999: 53:386-394
8. Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure : Arch Intern Med 1997:157:2413-2446.
9. Ubbink JB, Vermaak H and Bissbort S:Rapid high performance liquid chromatographic assay for total homocysteine levels in human serum. J Chromatogr :1991: 565:441-446.
10. Araki A and Sako Y:Determination of free and total homocysteine in human plasma by high performance liquid chromatography with fluorescence detection. J Chromatogr: 1987: 422:43-52.
11. Vester B and Ramussen K: High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. Eur J Clin Chem Clin Biochem 1991: 29:549-554
12. Bottiglieri T:Isocratic high performance liquid chromatographic analysis of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in animal tissues: the effect of exposure to nitrous oxide. Biomed Chromatogr 1990: 4; 239-241
13. Becker P.: Vitamin B6, vitamin B12 and folate nutritional status in men with hyperhomocysteinemia. Am J Clin Nutr. 1993: 57: 47- 53.
14. Armitaga P. and Berry G.: Statistical methods in medical research, 3rd edition. 1994: Blackwell, scientific publication, London, UK, pp 1-50.
15. Yasmin I, Falzone R and Brown MJ.: Determinants of arterial stiffness in offspring of families with essential hypertension. Am J Hypertens 2004: 17:292-298.
16. Tsioufis C, Antoniadis D, Stefanadis C, Tzioumis K, Pitsavos C, Kallikazaros I, Psarros T, Lalos S, Michaelides A and Toutouzas P: Relationships between new risk factors and circadian blood pressure variation in untreated subjects with essential hypertension. Am J Hypertens 2002: 15:600-604.
17. Wise CK, Cooney CA, Ali SF, Poirier LA: Measuring S-adenosylmethionine in whole blood, red blood cells and cultured cells using a fast preparation method and high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl. 1997 696:145-152.
18. Kamatani N, Willis EH and Carson DA.:Selection and characterization of a murine lymphoid cell line partially deficient in S-adenosylhomocysteine hydrolase. Biochim Biophys Acta 1983: 762:205-214
19. Finkelstein. JD.:Pathways and regulation of homocysteine metabolism in mammals. Semin Thromb Hemost 2000: 26:219-225
20. Zhan S, Gao Y, Yin X, Huang Y, Hu Y and Li L: A case-control study on the relationship between abnormal homocysteine metabolism and essential hypertension. Zhonghua Liu Xing Bing Xue Za Zhi. 2000: 21:194-197(Abstract in English)
21. Christensen B, Landaas S and Stensvold S:Whole blood folate, homocysteine in serum and risk of first acute myocardial infarction. Atherosclerosis 1999: 147:317-326.
22. Woo KS, Chook P, Lolin YI, Sanderson JE, Metreweli C and Celermajer DS:Folic acid improves arterial function in adults with hyperhomocysteinemia. J Am Coll Cardiol 1999: 34:2002-2006.
23. Munday R:Toxicity of thiols and disulphides: Involvement of free-radical species. Free Radic Biol Med 1989: 7:659-673.
24. Wood JL and Graham A: Structural requirements for oxidation of low-density lipoprotein by thiols. FEBS Lett 1995: 366:75-80.
25. Pryor WA & Squadrito GL: The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am J Physiol. 1995: 268:699-722
26. Stamler JS, Osborne JA, Jaraki O, Rabbani LE, Mullins M, Singel D, Loscalzo J. :Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. J Clin Invest. 1993:91(1):308-318.
27. Outinen et al; 1998Outinen PA, Sood SK, Liaw PCY, Sarge PD, Maeda N, Hirsh J, Ribau J, Rodor TJ, Weitz JI and Austin RC: Characterization of the stress-induced effects of homocysteine.Biochem J 1998: 332: 213- 221.
28. Jakubowski H: Homocysteine is a protein amino acid in humans. J Biol Chem 2002: 277, 30425-30428.
29. Lubec B, Fang-Kircher S, Lubec T, Blom HJ and Boers GHJ:Evidence for McKusick's hypothesis of deficient collagen cross-linking in patients with homocystinuria. Biochim Biophys Acta 1996: 1315:159-162.
30. Jakubowski H: Homocysteine thiolactone: Metabolic origin and protein homocysteinylation in humans. J Nutr; 130 (Suppl) 2000:377S-381S.

31. Olszewski AJ and McCully KS:Homocysteine metabolism and the oxidative modification of proteins and lipids. Free Radic Biol Med 1993: 14:683-693.
32. Guttridge DC, Albanese C and Reuther:NF-?B controls cell growth and differentiation through transcripyional regulation of cyclin D1. Moll Cell Biol 1999:19:5785-5799.
33. Wocial B, Berent H, Kostrubiec M, Kuczynska K, Kuch-Wocial A and Nieweglowska N: Homocysteine, adrenergic activity and left ventricular mass in patients with essential hypertension. Blood Press 2002: 11:201-205.
34. Kerins DM, Koury M.J, Capdevila A, Rana S. and Wagner, C :Plasma S-adenosylhomocysteine is a more sensitive indicator of cardiovascular disease than plasma homocysteine. Am J Clin Nutr 2001:74:723-729
35. Zhu BT: On the mechanism of homocysteine pathophysiology and pathogenesis: a unifying hypothesis. Histol Histopathol 2002: 17:1283-1291.
36. Selhub J, Jacques PF, Wilson PWF, Rush D and Rosenberg IH: Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993: 270:2693-2698.
37. Saw SM, Yuan JM, Ong CN, Arakawa K, Lee HP, Coetzee GA and Yu MC: Genetic, dietary and other lifestyle determinants of plasma homocysteine concentrations in middle-aged and older Chinese men and women in Singapore. Am J Clin Nutr 2001: 73:232-239
38. Li N, Chen YF and Zou AP:Implications of hyperhomocysteinemia in glomerular sclerosis in hypertension. J Hypertens 2002: 39:443-448.
39. Hankey GJ and Eikelboom JW:Homocysteine and vascular disease. Lancet 1999: 354:407-413
40. Guttormsen AB,Ueland PM ,Svarstad E, Refsum H.: Kinetics basis of hyperhomocysteinemia in patients with chronic renal failure . Kin Int 1997: 52:495-502.
41. Arnadottir M and Hultberg B:Homocysteine in renal disease, In: " Homocysteine in Health and Disease" (Carmel R and Jacobsen DW eds), 2001 Cambridge University Press, Cambridge, UK, pp 321-330
42. Brattstrom L and Wilchen D:Homocysteine and cardiovascular disease: cause or effect ? Am J Clin Nutr 2000: 27:315-323.
43. Dubey RK, Jackson EK and Rupprecht HD:Factors controlling growth and matrix production in vascular smooth muscle and glomerular mesangial cells. Curr Opin Nephrol Hypertens 1997: 6:88-105
44. Zou AP, Nithipatikom K and Li PL: Role of renal medullary adenosine in the control of blood flow and sodium excretion. Am J Physiol 1999: 276:790-797.
45. Chen YF, Li PL and Zou AP:Effect of hyperhomocysteinemia on adenosine metabolism and renal function in rats.FASEB J 2001: 15:A448 (Abstract in English)
46. Perna AF, Ingrosso D, Lombardi C and Cesare CM: Homocysteine in uremia. Am J Kid Dis 2003: 41:S123-S126.
47. Perna AF, Ingrosso D, Galletti P, Zappia V, Capasso GB and De Santo NG: Enzymatic methyl esterification of erythrocyte membrane proteins is impaired in chronic renal failure. Evidence for high levels of the natural inhibitor S-adenosylhomocysteine. J Clin Invest 1993: 91:2497-2503.
48. Mallamaci F, Zoccali C and Tripepi G:Hyperhomocysteinemia predicts cardiovascular outcomes in hemodialysis patients. Kidney Int 2002: 61:609-614.
49. Perna AF, D'Aniello A, Lowenson JD, Clarke S, De Santo NG and Ingrosso D:D-aspartate content of erythrocyte membrane proteins is decreased in uremia: Implications for the repair of damaged proteins. J Am Soc Nephrol 1997: 8:95-104,
50. Suliman ME, Filho JC, Barany P, Anderstam B, Lindholm B, Bergstrom J.: Effects of methionine loading on plasma and erythrocyte sulphur amino acids and sulph-hydryls before and after co-factor supplementation in haemodialysis patients. Nephrol Dial Transplant. 2001:16:102-10.
51. Coomes MW.: Amino acid metabolism. In "Textbook of Biochemistry, with clinical correlations",2002 Devlin TM, ed, Chap. 18, 5th edition,Wiley-Liss,New York, USA, p 819.