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Original Article

Relationship of Homocysteine and Related Cofactors Levels to Cardiac Changes in Children with Chronic Renal Failure

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ABSTRACT

Background: Patients with chronic renal failure (CRF) exhibit an abnormally high incidence of cardiovascular diseases usually due to cardiomyopathy and ischemic heart disease. A high frequency of hyperhomocysteinemia has been demonstrated in children with chronic renal failure. Reasons are: decreased renal clearance, accumulation of toxic metabolites, inhibition of homocysteine (Hcy) metabolism and a decrease in serum level of vitamin B_{12} and folic acid which are necessary for Hcy metabolism.

Hyperhomocysteinemia is recognized as an independent risk factor for cardiovascular disease, especially atherosclerosis in adult patients with CRF. However, there is little information about the relationship between plasma homocystiene levels and cardiac diseases in children with CRF.

Objectives: The aim of this study was to determine relation between plasma homocysteine, folic acid, vitamin B_{12} levels and cardiac changes in children with CRF, both conservative and hemodialyzed groups.

Methods: Fifty children were studied in this work and were divided into two groups. Group I comprised 40 patients: twenty patients suffering from end stage renal disease (ESRD) on regular hemodialysis (HD) and 20 patients with CRF under conservative treatment. Group II comprised 10 control healthy children being age- and sex-matched with patients. The two groups were subjected to full history taking, clinical examination, routine investigations for CRF (blood urea, serum creatinine, serum calcium and phosphorus level, CBC). In addition the following markers were estimated once in the conservative group and before and after dialysis, in HD group: serum homocysteine, folic acid and vitamin B_{12} levels by high performance liquid chromatography. Cardiac measurements by echocardiography were done for all groups.

Results: Serum (Hcy) level was significantly elevated in HD and conservative group than in the control group. There was a significant increase in its level in HD compared with the conservative group. There was insignificant variation in Hcy level before and after dialysis. Serum vit. B_{12} and folic acid were significantly lower in HD than in conservative groups. There was a significant decrease of their levels after dialysis than before.

There was a significant negative correlation between Hcy and both folic acid and vit. B_{12} levels, while a highly significant positive correlation between Hcy, blood urea and serum creatinine was found. Also there was a highly significant negative correlation between Hcy and glomerular filtration rate (GFR), and a significant positive correlation between Hcy and systolic and diastolic blood pressure. There is no significant correlation between Hcy and left venricular mass index (LVMI). However, interventricular septum diastolic thickness (IVSDT), left ventricular posterior wall diastolic and systolic thickness (LVPWDT), (LVPWST), left ventricular end diastolic thickness (LVEDD) and relative wall thickness (RWT) were significantly higher in dialyzed than in the conservative group.

Conclusions: These data showed that serum homocystiene was significantly elevated in children with CRF which was more serious in the HD group due to lower level of vit. B_{12} and folic acid which were dialysed vitamins. Hyperhomo-cysteimia, may be one of risk factors for hypertension, and left ventricular hypertrophy in children with CRF.

INTRODUCTION

Chronic renal failure (CRF) is characterized by progressive destruction of renal mass with irreversible sclerosis and loss of nephrons over a period of at least months to many years depending on the underlying etiology. Glomerular filtration rate (GFR) progressively decreased with nephron loss

and the term CRF should be reserved more specifically for patients whose GFR is less than 30 ml/min[1].

Homocysteine (Hcy) is a sulphydryl-containing amino acid byproduct that comes from the demethylation of methionine. The major source of methionine is from animal protein[2]. The kidneys play an important role in the metabolism of Hcy, as demonstrated in both animal and human studies[3].

Hyperhomocysteinemia in chronic renal failure may be seen in various situations including deficient intake or deranged metabolism of vitamins (folic acid, B_6 and B_{12})[4], due to decreased renal clearance of homocysteine, accumulation of toxic metabolites inhibiting homocysteine metabolism and mutation of the gene of the methylene tetra-hydrofolate reductase[5].

Hyperhomocysteinemia (Hcy) is defined by a plasma homocysteine concentration greater than 15 μmol/L which is observed in at least 85% of patients with end stage renal disease (ESRD) undergoing maintenance peritoneal or hemodialysis[6].

Cardiovascular and peripheral vascular diseases are the major cause of morbidity and mortality in the general population today. Hyperhomocysteinemia, a new independent risk factor for atherosclerotic vascular disease, has been described in the last ten years. Moderate hyperhomocysteinemia occurs in 5-7% of the general population and is associated in the third and fourth decade of life with premature coronary disease, stroke, arterial and venous thrombo-embolism. Hyperhomocysteinemia occurs in 85% of patients with end stage renal failure[7]. This is significant because of this population's depending on patent fistulas and prosthetic devices to re-receive dialysis[8].

It is possible that Hcy promotes the growth of vascular smooth muscle cells and inhibits endothelial cell growth, thus, providing an explanation of the pathogenesis of arterio-sclerosis[9].

Increased thromboxane-mediated platelet aggregation is another proposed mechanism of arteriosclerosis[10]. Homocysteine metabolism in cardiovascular cells relies exclusively on folate and vitamin B_{12} dependent remethylation since no trans-sulfuration has to been demonstrated in endothelial cells of human blood vessels. Because of the absence of irreversible breakdown of homocystine to cysteine, homocysteine synthesis may rapidly exceed cell export, resulting in specific cell injury to the point of cell death compared with other organ systems, the cardiovascular system is therefore particularly sensitive to elevated homocysteine levels[9].

AIM OF THE WORK

The aim of this work was to study Hcy, vit. B_{12} and folic acid levels in CRF patients, both dialyzed and conservative groups and their relation to cardiac changes detected by ecchocardiography.

SUBJECTS AND METHODS

The present study was carried out at the Pediatrics Dialysis Unit, Children's Hospital, Zagazig University, during the period from October 2003 to August 2004.

Subjects:

Subjects were classified into two groups:

Group I:

It comprised 40 patients suffering from chronic renal failure. They included 20 patients suffering from end stage renal disease on regular hemodialysis. They included 8 males (40%) and 12 females (60%). Their ages ranged from 3 to 17 years.

The duration of dialysis ranged from six months to five years.

This group (dialyzed patients) was on regular vitamin B_{12} and folic acid supplementation as a routine supplementation of folic acid in a dose of 5 mg/day and vitamin B_{12} in a dose of 125 mcg/day.

Thirteen patients had a history of hypertension and received antihypertensive agents for the duration of the study.

In these patients, hemodialysis was performed with citrate dialysate for 2.5 to 3 hours in each session, three times per week. Dialyzer surface area ranged from 0.3 to 1 m^2.

Inclusion criteria: Stable, maintenance, regular hemodialysis and duration of dialysis at least 6 months.

Exclusion criteria were: - medical instability - known cases of genetic homocystinuria, - known cases of pernicious anaemia before renal failure.

Our work included another 20 patients suffering from chronic renal failure under conservative treatment. They included 9 males (45%) and 11 females (55%). Their age ranged between 3 and 14 years. The duration of illness ranged between one year and 4 years.

Inclusion criteria were as follows: stable clinically and duration of CRF of at least 6 months.

The conservative patients were not taking regular supplementation of vitamin B_{12} and folic acid.

Group II:

It comprised 10 healthy children age- and sex-matched with patients of group I. Their ages ranged between 6 and 15 years (9.9 ± 2.88). They included 4 males (40%) and 6 females (60%).

The following was done to all subjects:

- **Full history taking.**
- **Thorough clinical examination.**
- **Laboratory investigations:**

1. Complete blood count.
2. Blood urea using enzymatic rate method on synchrome CX_5 system, BECKMAN, (USA)[11].
3. Serum creatinine using a modified rate Jaffé method on synchronm CX_5 system from BECKMAN, (USA)[12].
4. Serum calcium and phosphorus using timed endpoint method on synchrone CX_5 system from BECKMAN, (USA)[13].
5. Serum albumin using Opera systems from Bayer, (USA)[14].
6. Estimation of serum Hcy level by HPLC[15].
7. Estimation of vitamin B_{12} and folic acid by HPLC [chromatography, catalog 350 (Alltech-Associated-Inc, 1995)].
8. Echocardiography[16].

Methods:

Sample collection:

Six mls of venous blood were drawn. In patients on hemodialysis, samples were taken before and after dialysis. The plasma and serum were separated and stored at -20°C.

Estimation of serum homocysteine (Hcy) and sample preparation was done as

described by Ubbink et al. (1991)[15].

Chromatography conditions were as follows:

- Flow rate of 1 ml/min. -U.V. detector 270 nm.
- Retension time 2.1 min.
- Mobile phase: The separation of Hcy was carried out using 0.1 mol/L potassium dihydrogen phosphate buffer (pH 2.1) adjusted with orthophosphoric acid containing 4% acetonitrile.

Estimation of vitamin B_{12} and folic acid was done by HPLC according to Alltech-Associate-Inc (1995): Column: LC18, flow rate: 0.1 ml/min., temperature: 30°C U.V. dectector 254 nm, retension time is 7 min. for folic acid and 4 min. for vit. B_{12}.

Eluent: 20 mM KH_2PO_4-K_2-HPO_4 (pH 6.6) and 20 mM KH_2PO_4-0% B (0-5 minutes), 0-100% B (5-20 minutes, linear) and 100% B (20-25 minutes).

Echocardiography: "colour Doppler, 2 D-echo"[16].

Statistical analysis:

Data were entered, checked and analysed using Epi-Info version 6 and SPSS for Windows version 8[17].

RESULTS

Table (1) shows that 16 patients on dialysis were suffering from pallor (80%) as compared with conservative patients, where 8 patients were suffering from pallor (40%).

Eight patients in the dialysis group were suffering from cardiac murmurs (40%) while in the conservative patients; 6 patients were suffering from cardiac murmurs (30%) and 13 patients in the dialysis group were suffering from cardiomegaly (65%) as compared with 9 patients in the conservative group suffering of cardiomegaly (45%).

Thirteen patients on dialysis were suffering from hypertension (65%) as compared with 5 patients (25%) in the conservative group.

Table (2) shows a highly significant increase in creatinine, BUN and phosphorus in dialyzed patients than in conservative CRF patients and control group ($p < 0.001$ by L.S.D.).

Hemoglobin, albumin, calcium and GFR showed a highly significant decrease in the dialyzed and conservative CRF groups than in the control group and a significant decrease in Hb and albumin in dialyzed patients than in the conservative patients ($p < 0.001$ by L.S.D.).

Table (3) shows a highly significant increase in homocysteine in both conservative and dialyzed patients than in the control group and a highly significant decrease in folic acid and vitamin B_{12} in both dialyzed and conservative patients than in the control group.

Table (4) shows a highly significant increase as regards homocystiene in dialyzed than in conservative group.

Table (5) shows a highly significant decrease of folic acid in dialyzed patients before H.D than in the conservative group.

Table (6) shows a highly significant decrease of vitamin B_{12} in dialyzed than in the conservative group.

Table (7) shows a non-significant difference in homocysteine before and after dialysis. A highly significant decrease of folic acid and vitamin B_{12} was found after dialysis than before dialysis.

Table (8) shows a significant positive correlation between homocysteine, BUN and creatinine and a significant negative

correlation between homocysteine and GFR in the conservative CRF patients.

Table (9) shows a highly significant increase in LVMI in the conservative CRF patients than in the control group ($p < 0.001$). However, LVMI was highly significantly increased in CRF under dialysis patients than conservative and control group ($p < 0.001$).

The prevalence of LV hypertrophy was 80% in CRF under dialysis (16 patients) and 50% in conservative (10 patients).

In the dialyzed and conservative CRF group, there is highly significant difference in IVSDT, LVPWDT and LVEDD than in control group.

In dialyzed patients, IVSDT, LVPWDT, LVEDD, LVPWST and LVESD were highly significantly increased compared with the control group. In dialyzed patients, IVSPT, LVPWDT, LVEDD and LVPWST were significantly higher than in the conservative patients.

In the dialyzed and conservative CRF groups, LVEF and FS were lower than in the control group but this decrease was not statistically significant. In the dialyzed and conservative CRF groups, RWT was significantly higher than in the control group. RWT was significantly higher in dialyzed patients than the conservative patients.

Table (11) shows a significant positive correlation between LVMI and systolic and diastolic blood pressure.

There is a significant negative correlation between LVMI and hemoglobin and albumin.

There is a non-significant negative correlation between LVMI and age.

Table 1: Comparison between the clinical data of patient group.

		Patients (n = 40)	
		Conservative (n = 20)	**CRF on dialysis (n = 20)**
Age	Mean ± SD Range	8.35 ± 2.87 3-14	10.85 ± 4.36 3-17
Weight	Mean ± SD Range	26.32 ± 11.8 14-54	20.99 ± 8.06 13-64
Duration of illness	Mean ± SD Range	3.6 ± 1.9 1-4	4.05 ± 1.3 0.5-5
Sex	Male No (%) Female No (%)	9 (45%) 11 (55%)	8 (40%) 12 (60%)
Pallor	Female No (%)	8 (40%)	16 (80%)
Murmurs	No (%)	6 (80%)	8 (40%)
Cardiomegaly	No (%)	9 (45%)	13 (65%)
Hypertension	No (%)	5 (25%)	13 (65%)
Oedema	No (%)	6 (30%)	2 (10%)

Table 2: Laboratory data in all studied groups.

		Control (n = 10)	Patients (n = 40)		F	p
			Conservative (CRF) (n = 20)	CRF (dialyzed) (n = 20)		
Creatinine (mg/dl)	Mean ± SD (mg/dl)	0.77 ± 0.24	3.85 ± 1.25	8.69 ± 1.63	138	< 0.001
	Range	0.4-1.1	1.5-5.5	5.9-12.2		
BUN (mg/dl)	Mean ± SD (mg/dl)	28 ± 2.44	39.15 ± 8.33	82.8 ± 39.65	20.68	< 0.001
	Range	25-32	25-50	25-218		
GFR (ml/min)	Mean ± SD (ml/min)	107.89 ± 8.4	35.21 ± 14.2	9.25 ± 2.5	103.5	< 0.001
	Range	95.4-115.4	22.4-45.8	4.9-13.4		
Albumin (gm/dl)	Mean ± SD (gm/dl)	4.01 ± 0.54	3.21 ± 0.49	2.96 ± 0.79	27.6	< 0.001
	Range	3.4-6.2	2.5-4.8	2-3.1		
Hemoglobin (gm/dl)	Mean ± SD (gm/dl)	13.98 ± 1.19	10 ± 1.11	8.07 ± 0.96	100.91	< 0.001
	Range	12.5-16	7.6-12	6.4-9.9		
Calcium (mg/dl)	Mean ± SD (gm/dl)	10.2 ± 11.4	7.83 ± 1.67	6.2 ± 3.9	12.47	< 0.001
	Range	9.4-11	10.2-11.4	5.2-6.8		
Phosphorus (mg/dl)	Mean ± SD (mg/dl)	3.91 ± 1.04	4.32 ± 1.45	8.23 ± 1.17	7.21	< 0.001
	Range	3.2-5.4	3.7-5.9	7.3-9.4		

Table 3: Homocysteine, folic acid and vitamin B_{12} levels in all studied groups.

		Control (n = 10)	Patients (n = 40)		F	p
			Conservative (n = 20)	Before dialysis (n = 20)		
Homocysteine (µmol/L)	Mean ± SD (µmol/L)	9.83 ± 5.87	58.95 ± 20.63	128.38 ± 62.35	27.18	< 0.001
	Range	2.4-20.2	25.9-92.9	44.8-140.2		
Folic acid (ng/dl)	Mean ± SD (ng/dl)	12.81 ± 4.49	6.55 ± 2.88	2.44 ± 1.05	47.1	< 0.001
	Range	7.2-20.1	2.5-12.1	1.1-4.9		
Vitamin B_{12} (ng/dl)	Mean ± SD (ng/dl)	350.56 ± 58.46	102.63 ± 11.16	97.6 ± 14.93	24.56	< 0.001
	Range	160.2-510	85-123.3	23.4-120		

Table 4: LSD test of different studied groups as regards homocysteine.

	Control	Conservative
Conservative	< 0.001	
CRF (under dialysis)	< 0.001	< 0.001

Table 5: LSD test of different studied groups as regards folic acid.

	Control	Conservative
Conservative (CRF)	< 0.001	
CRF (before dialysis)	< 0.001	< 0.001

Table 6: LSD test of different studied groups as regards vitamin B_{12}.

	Control	Conservative
Conservative	< 0.001	
CRF (before dialysis)	< 0.001	< 0.05

Table 7: Homocysteine, folic acid and vitamin B_{12} levels before and after dialysis.

		Patients (n = 20)		F	p
		Before dialysis	After dialysis		
Homocysteine (µmol/L)	Mean ± SD (µmol/L)	128.38 ± 26.35	126.2 ± 32.96	2.02	> 0.05 NS
	Range	44.8-140.2	50.8-145.5		
Folic acid (ng/dl)	Mean ± SD (ng/dl)	2.44 ± 1.05	1.21 ± 0.51	5.65	< 0.001 HS
	Range	1.1-4.9	0.4-2.3		
Vitamin B_{12} (ng/dL)	Mean ± SD (ng/dl)	97.6 ± 14.93	37.21 ± 17.32	12.84	< 0.001 HS
	Range	23.4-120	10.7-65.8		

Table 8: Correlation between homocysteine and renal function parameters in conservative CRF patients.

	r	p
BUN	0.419	0.039 (S)
Creatinine	0.41	0.043 (S)
GFR	-5.584	< 0.001 (HS)

Table 9: Cardiac measurements in all studied groups.

		Control	Conservative	CRF (on dialysis)	F	p
IVSDT (cm)	Mean ± SD	0.77 ± 0.18	0.79 ± 0.17	1.01 ± 0.29	5.977	0.005 (< 0.001)
	Range	0.55-1.02	0.56-1.2	0.62-1.58		
LVPWDT (cm)	Mean ± SD	0.77 ± 0.19	0.8 ± 0.15	0.92 ± 0.16	3.687	0.033 (< 0.05)
	Range	0.5-1.09	0.5-1.09	0.75-1.32		
LVEDD (cm)	Mean ± SD	3.77 ± 0.5	3.88 ± 0.42	4.21 ± 0.27	5.428	0.008 (< 0.05)
	Range	3.2-4.64	3.2-4.38	3.75-4.64		
IVSST (cm)	Mean ± SD	0.83 ± 0.17	0.87 ± 0.3	0.98 ± 0.25	1.25	0.296 (< 0.05)
	Range	0.6-1.2	0.16-1.65	0.58-1.65		
LVPWST (cm)	Mean ± SD	1.22 ± 0.1	1.2 ± 0.12	1.41 ± 0.11	17.27	< 0.001
	Range	1-1.32	1-1.45	1.22-1.6		
LVESD (cm)	Mean ± SD	3.77 ± 0.37	3.79 ± 0.28	4.27 ± 0.37	12.324	< 0.001
	Range	3.2-4.15	3.2-4.11	3.21-4.71		
LVEF (%)	Mean ± SD	0.68 ± 0.18	0.66 ± 0.13	0.65 ± 0.14	0.058	0.944
	Range	0.42-0.94	0.4-0.91	0.39-0.9		
FS (%)	Mean ± SD	51.3 ± 19.37	44.25 ± 11.87	41.9 ± 8.11	1.918	0.158
	Range	27-92	25-77	23-64		
LVMI (gm/m^2)	Mean ± SD	82.13 ± 23.48	89.54 ± 21.25	134.8 ± 42.42	13.631	< 0.001
	Range	54.2-120.5	43.6-125	82.2-222.3		
RWT	Mean ± SD	0.4 ± 0.21	0.47 ± 0.22	0.56 ± 0.56	11.23	< 0.001
	Range	0.34-43	0.41-0.49	0.48-61		
LVH Prevelane (%)		0%	50%	80%		

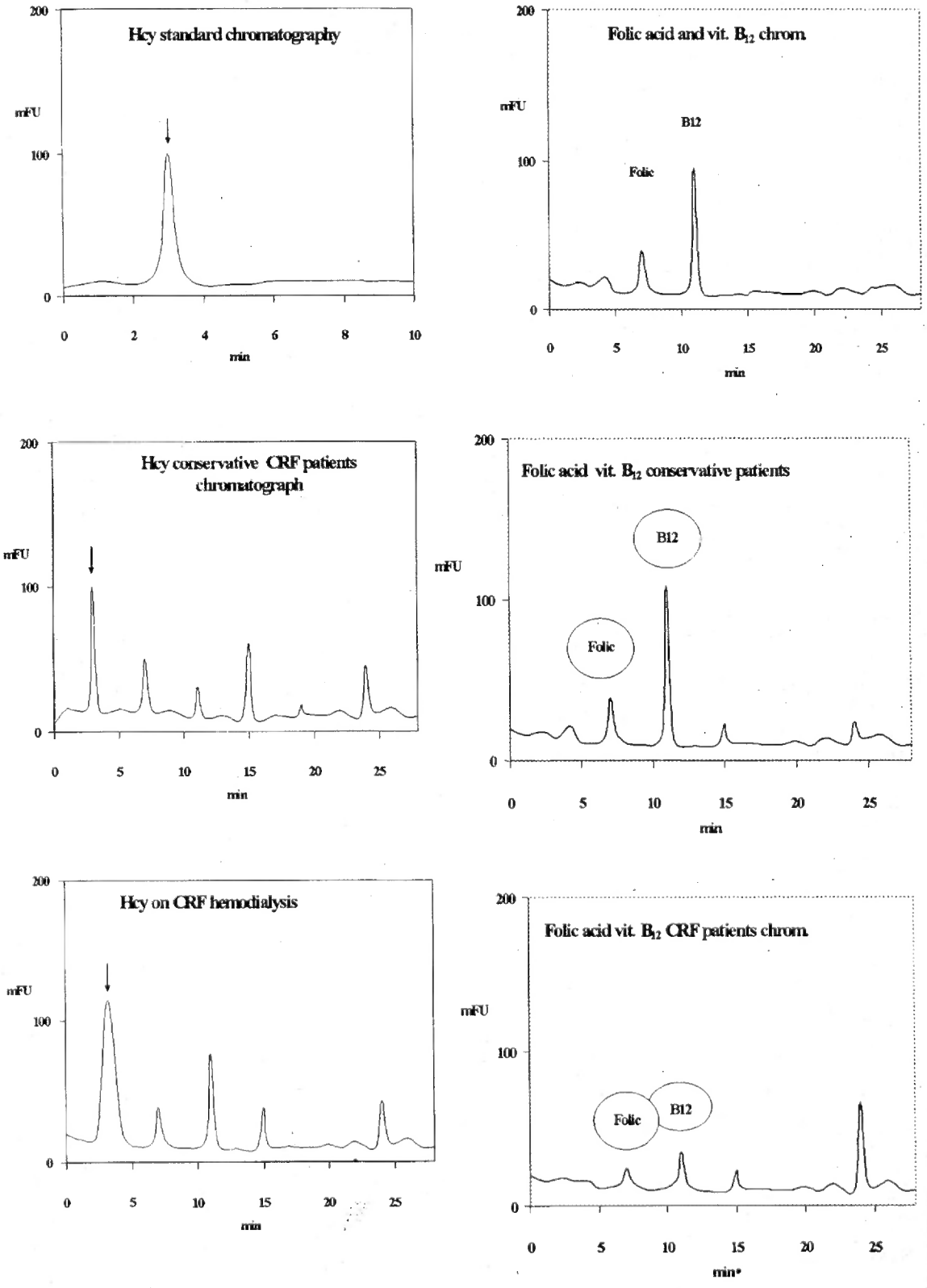
CRF = Chronic Renal Failure under hemodialysis
IVSDT = Interventricular Septum Diastolic Thickness
IVSST = V Septum Systolic Thickness
LVESD = LVE Systolic Diameter
LVPWDT: Left Ventricular Posterior Wall Diastolic Thickness
FS% = Fraction Shortening
RWT: Relative Wall Thickness
LVMI = LV Mass Index
LVPWST = LVPW Systolic Thickness
LVEF% = LV Ejection Fraction

Table 10: Correlation between homocysteine and other parameters in all studied groups.

	r	p
Age	0.214	> 0.05 (NS)
Systolic blood pressure	0.315	< 0.05 (S)
Diastolic blood pressure	0.312	< 0.05 (S)
BUN	0.52	< 0.001 (HS)
Creatinine	0.394	< 0.001 (HS)
Vitamin B_{12}	-0.355	< 0.05 (S)
Folic acid	-0.364	< 0.05 (S)
Hemoglobin	-0.32	< 0.05 (S)
Albumin	-0.32	< 0.05 (S)
LVMI	0.124	> 0.05 (NS)

Table 11: Correlation between LVMI and other parameters in patients groups.

	r	p
Age	0.26	> 0.05 (NS)
Systolic blood pressure	0.216	< 0.05 (S)
Diastolic blood pressure	0.214	< 0.05 (S)
Hemoglobin	-0.37	< 0.05 (S)
Albumin	-0.33	< 0.05 (S)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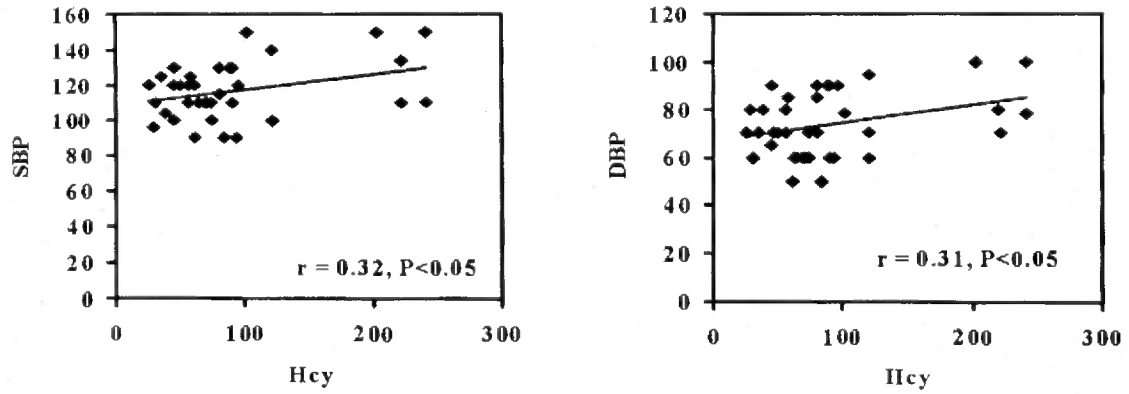
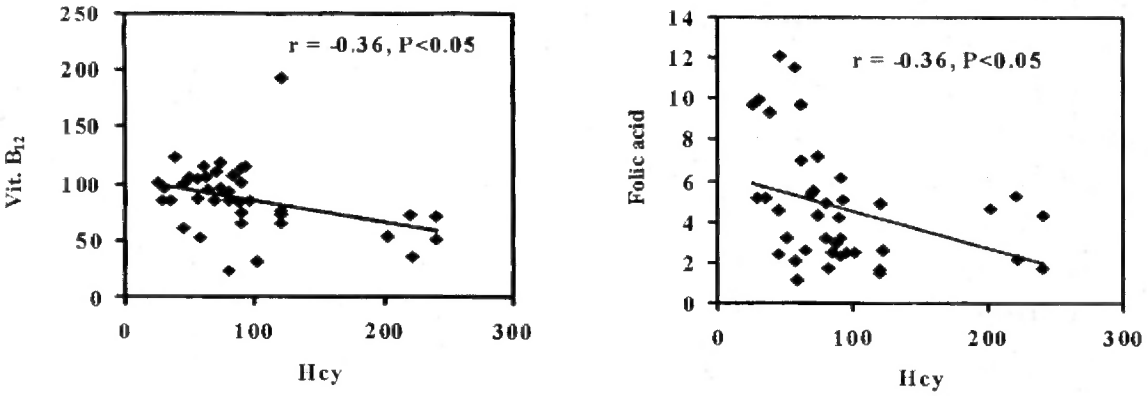
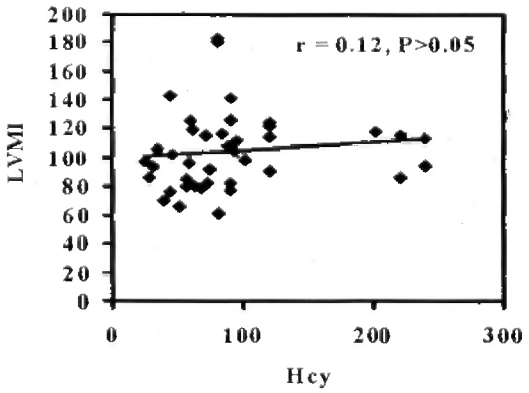


Significant positive correlation between Hcy, systolic and diastolic blood pressure.



Significant negative correlation between Hcy, vit. B_{12} and folic acid.



Non-significant correlation between Hcy and LVMI.

Fig. 2

DISCUSSION

Cardiovascular diseases are a major cause of morbidity and mortality in ESRD patients and they cannot be explained entirely by the prevalence of traditional risk factors for atherosclerosis. Moderately raised homocysteine (Hcy) concentrations have been associated with an increased risk of atherothrombotic events[18].

A high plasma homocysteine concentration has been found in patients with ESRD[19]. Hcy is a sulfhydryl containing amino acid formed by the demethylation of an essential amino acid methionine. A cellular export mechanism and two metabolic pathways - remethylation and trans-sulfuration - afford degradation in order to maintain low intracellular concentration of Hcy. By trans-sulfuration, Hcy is processed to cystathionine and remethylation results in methionine. Remethylation of Hcy to methionine catalyzed by methionine synthase depends on folate as MTHF and vitamin B_{12} as methyl cobalamine[20].

The study of homocysteine, folic acid and vitamin B_{12} in relation to cardiac changes in children with chronic renal failure has been the aim of our work.

The basal levels of Hcy were estimated in two groups. Patient groups included twenty patients on conservative treatment, twenty patients on regular hemodialysis (group I) and ten healthy children as a control group (group II).

This study shows that homocysteine levels were high in dialyzed patients and conservative patients as compared with the control group.

In agreement with this result, Robinson et al. (1996)[21,22] found a significantly higher basal Hcy level in dialysis patients than in the control group.

This was also in agreement with Bostom et al. (1997)[23] who found that Hcy concentrations were two to three times higher in patients with ESRD than in the general population.

Basal Hcy level in patients with ESRD on regular hemodialysis was markedly elevated with a mean of 36.3% µmol/L as compared to the control group with a mean of 6.8 µmol/L[24].

Lilien et al. (1999)[25] indicated that homocysteinemia is a feature of ESRD in both children and adults. They found that the level is higher in those on dialysis than those on conservative treatment and that homocysteinemia persists even after renal transplantation. On other hand, Hong et al. (1998)[26] found no difference between the level of Hcy in dialysis patients and those on conservative treatment.

This study shows an increased level of homocysteine with a decrease in renal function. In our results, we found a significant negative correlation between homocysteine and GFR in conservative chronic renal failure and a positive correlation between Hcy, BUN and creatinine, and on comparing basal Hcy level in conservative patients and dialyzed patients, we found that the basal Hcy was higher in dialyzed patients than the conservative patients with a significant difference. This denotes that the level of Hcy increases with the progression of renal disease.

In agreement with this result, Foley et al. (1998)[27] found that the relationship between Hcy level and GFR are consistent

with the presumption that the normal kidneys play a prominent role in plasma Hcy handling. Hcy levels increase as renal function declines and progresses to ESRD, with the vast majority (> 85%) of dialysis patients ultimately experiencing mild to moderate hyperhomocysteinemia.

This relation was also found by Arnodottire et al. (1996); Wollesen et al. (1999) and Boston et al. (2000)[28-30]. A striking consistent inverse relationship exists between Hcy levels and renal function over a broad range of renal function from severe renal insufficiency to level of GFR far above the uremic range. This relationship is a powerful indirect evidence that elevated Hcy levels in renal disease are intimately linked to kidney function.

A significant increase was found in Hcy level in patients with CRF on conservative treatment as compared to healthy controls, which indicates that hyperhomocysteinemia is present from the early stages of chronic renal failure[31].

Homocysteine is metabolized by either the trans-sulfation pathway which requires vitamin B_6 as a cofactor or the trans-methylation pathway requiring folate and vitamin B_{12} as a cofactor[32].

In this study, the basal levels of serum folic acid and vitamin B_{12} were measured in all studied groups. Both vitamins were found to be lower in dialysis patients than the conservative patients and controls. These differences were found to be statistically significant. According to these results, we can say that vitamin supplementation in both the dialysis group and conservative group is not sufficient.

In this study, we compared between serum level of vitamin B_{12} and folic acid after and before dialysis. We found a significantly lower level of vitamin B_{12} and folic acid after dialysis than before it. This is explained by the fact that vitamin B_{12} and folic acid are water soluble vitamins. So, there is loss during the hemodialysis process.

In agreement with this result, Brulez et al. (1999)[20] found that all patients on maintenance hemodialysis are supplemented with multi-vitamin tablets as there will be loss of water soluble vitamins during dialysis.

Folate status is a major determinant of plasma homocysteine in patients with CRF. There is a strong inverse relationship between the plasma homocysteine concentration and plasma folate levels both in normal individuals and patients with CRF[33,34].

The plasma vitamin B_{12} level can also affect the plasma homocysteine level and hyperhomocysteinemia can persist in patients despite normal to supranormal levels of vitamin B_{12}. So, both routine folate and vitamin B_{12} supplementation have been recommended in patients with CRF[34].

Gupta and Robinson (1997)[19] found that the occurrence of hyperhomocysteinemia in patients with ESRD despite high plasma vitamin concentration (folate, B_6 & B_{12}) could be due to altered metabolism or inhibition of intracellular vitamin activity.

Hong et al. (1998)[26] do not agree with this finding. They found that plasma folic acid concentrations were 3-4 times higher in the dialysis than the predialysis group, but these levels of folic acid are not enough to

reduce hyperhomocysteinemia in ESRD. On the other hand, Loeher et al. (1998)[24] found that folic acid and vitamin B_{12} in plasma were similar in patients with ESRD on regular hemodialysis and controls.

This finding can be explained by the fact that homocysteine is remethylated to methionine by the help of the enzyme methionine synthase that requires folic acid as a methyl donor and a metabolite of vitamin B_{12} (methyl-cobalamine) as a cofactor. Hence, the presence of both folic acid and vitamin B_{12} would be more helpful in the reduction of serum homocysteine than folic acid alone[35].

In this study, we compared between the level of homocysteine before and after dialysis, and found no significant difference in Hcy levels before and after dialysis.

The reason for the high levels of homocysteine in dialysis patients and the absence of a relationship between its level and the effectiveness of dialysis can be probably explained by two factors. The first is that Hcy is highly protein bound and therefore does not dialyze well. The second cause is that the kidney is the main organ of catabolism. So, Hcy is not being removed normally by conversion to methionine and cystathionine in patients with poor renal catabolic function[36].

Bostom et al. (1997)[23] found that the routine hemodialysis treatments acutely decrease Hcy levels by approximately 30 to 40% but levels quickly increase to their elevated pretreatment values. There are ongoing attempts to manipulate the dialysis modality to improve Hcy clearance.

High-flux dialysis membranes, which have the capacity to clear large plasma molecules, including potential uremic inhibitory substances have no significant long-term Hcy lowering effect[37].

Floridi et al. (1998)[38] found that increasing the frequency of dialysis may lead to significantly lower fasting Hcy levels. One potential explanation for this is that Hcy diffuses directly into the dialysate, and more frequent dialysis allows a lower steady-state level to be reached. Alternatively, uremic inhibitory factors may be removed, facilitating normal Hcy metabolism.

The 2nd part of this study was to assess cardiac changes that occur in children with chronic renal failure and its relation to homocysteine level.

Morbidity and mortality from cardiac disease in patients with CRF are usually due to cardiomyopathy and ischemic heart disease. Cardiomyopathy may manifest as concentric LV hypertrophy, LV dilatation or systolic dysfunction[39].

Independent risk factors for LV hypertrophy are increased age, systolic hyper-tension, anaemia, hyperparathyroidism, coronary artery disease, inter-dialytic weight gain and prolonged dialysis therapy[40].

In this study, the plasma homocysteine levels increase in patients with CRF both on conservative treatment and patients on dialysis and there are cardiac changes detected by echocardiography in CRF patients as compared with controls.

The plasma homocysteine levels increase in patients with CRF and may play a role in their cardiovascular disease[31,41,42].

Most previous clinical and experimental studies of homocysteine have focused on

its effect on arterial vessels, specifically when they are atherosclerotic although it is known that coronary artery disease is one of the risk factors for LV hypertrophy[43].

In this study, left ventricular mass index (LVMI) was higher in CRF under dialysis than in both conservative and control groups, LVMI was higher in conservative than control groups and LV hypertrophy prevalence was 80% in dialyzed patients and 50% in conservative patients. This agrees with findings by Hakan et al. (2004)[18]. There was an increase in LVMI in CRF in both conservative and hemo-dialysis patients and hyperhomocysteinemia compared with controls.

LVH and increased LV performance in children with CRF may be an adaptive process in response to increased blood pressure and higher metabolic demand seen in these patients. However, when the cardiovascular system is stressed by exercise, this compensation may be insufficient in children undergoing chronic dialysis. Thus, diminished contractile reserve in these children might reflect the early development of maladaptive stage of LVH with risk of ultimate worsening of cardiac function and development of congestive heart failure over time[44,45].

Hakan et al. (2004)[20] found that there was a positive correlation observed between LVMI and blood pressure (SBP and DBP). In addition, serum hemoglobin levels were low in CRF group. However, in the CRF group, there was no correlation between LVMI and the serum albumin level which is accepted as a marker of malnutrition. So hypertension and anemia are considered to be the most important cause of LV hypertrophy.

In the echocardiographic study, we found 80% of dialyzed patients had left ventricular hyprtrophy of whom concentric hypertrophy represents 20% of cases, eccentric hypertrophy represents 45% of cases and asymmetric septal hypertrophy is seen in 15% of cases. Asymmetric septal hypertrophy is present if the ratio of interventricular septum (IVS) to left ventricular posterior wall (LVPW) is ≥ 1.3. These data correlated with what was reported by Morris et al. (1993)[46]. They suggested that asymmetric septal hypertrophy of uremic children was due to sympathetic overactivity and to increase in circulating catecholamines.

Hyperhomocysteinemia begins when GFR decreases to 70 ml/min and is associated with atherosclerotic occlusive arterial accidents in predialysis CRF patients[43].

In this study, there was no significant correlation between LVMI and homocysteine. These findings are similar to the result of Blacher et al. (1999)[42].

In this study, there was no direct correlation between homocysteine and cardiac hypertrophy. However, we can hypothesize that hyperhomocysteinemia may influence cardiac geometry due to changes in vascular impedance. Hyperhomocysteinemia may contribute to hypertension by affecting vascular impedance. We found a positive correlation between LVMI and SBP and DBP.

In addition, it is known that atherosclerosis begins in childhood and it has been recommended that prevention begins at this time[47].

RECOMMENDATIONS

- All patients with CRF, whether on replacement therapy or conservative treatment should receive adequate doses of folic acid and vitamin B_{12} regularly to overcome the defects in Hcy metabolism.
- Invention of a new type of dialyzer membrane for clearance of excess Hcy in patients with ESRD.
- Further studies to assess normal variations of serum Hcy among Egyptian children.
- Further studies are needed to evaluate vitamin doses which are sufficient to decrease Hcy level in patients with ESRD in a trial to decrease the expenses of treatment.
- Further studies should focus on whether the decrease in Hcy level by vitamin supplementation is accompanied by a decrease in the risk of cardiac changes in children with CRF.

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