ASSOCIATION BETWEEN WHITE BLOOD CELL COUNT AND LEVELS OF SERUM HOMOCYSTEINE IN END-STAGE RENAL FAILURE PATIENTS TREATING WITH HEMODIALYSIS

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Background: In hemodialysis patients, plasma levels of total homocysteine are influenced by nutritional status in patients with chronic kidney disease .To investigate the association between serum homocysteine (Hcy) level as a marker of nutritional status and WBC counts as a marker of inflammation, a cross-sectional study was conducted on patients with end-stage renal disease (ESRD), who were undergoing maintenance hemodialysis treatment. **Methods:** Serum homocysteine (total) and WBC count were measured. Other biochemical analysis including serum predialysis creatinine (Creat), post and predialysis blood urea nitrogen (BUN), albumin (Alb), serum C-reactive protein (CRP) and serum ferritin were measured, also intact serum PTH (iPTH) and plasma HCO3 was measured too. For the efficacy of hemodialysis the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data. The body mass index (BMI) was also calculated. For correlations the partial correlation test was used. **Results:** Total patients were 36(f=15 m=21), consisting of 25non-diabetic HD patients and 11diabetic HD patients. The mean patient's age was 47±17years. In all patients a significant inverse correlation of serum homocysteine with WBC count and a significant positive correlation of serum Homocysteine with BMI and a near significant positive correlation of WBC count with serum CRP were found. **Conclusion:** In hemodialysis patients an inverse correlation between WBC count as a marker of inflammation with serum Hcy level as a marker of nutritional status, further support the hypothesis of the malnutrition-inflammation cachexia syndrome

Keywords: White blood cell, Serum homocysteine

INTRODUCTION

Several reports have demonstrated that moderate or elevated hyperhomocysteinemia is considered an independent risk factor for atherosclerosis, cardiovascular disease, stroke and peripheral vascular disease. in patients with normal renal function [1-3]. In patients with renal impairment, particularly at risk of cardiovascular disease (CVD),[4] markedly elevated plasma homocysteine (Hcy) levels have been found.[5] The mechanism by which homocysteine exerts its effects has not been clearly defined, although it is generally accepted that the accumulation of homocysteine in plasma can damage the endothelium. It has been suggested that homocysteine may induce vascular injury (including endothelial dysfunction, smooth muscle cells proliferation and thiolation of lipoprotein) and affect platelet aggregation and coagulation.[6] Atherosclerosis is a multifactorial disease associated with a variety of risk factors and among them infection and inflammation may contribute to vascular injury and atherogenesis. Inflammation may also promote atherosclerotic plaque rupture and thrombosis[7].

White blood cells (WBC) may serve as an important biomarker for these disease processes. Elevated WBC may also be considered a risk factor for acute myocardial infarction, coronary artery disease and stroke[8]. The white blood cell count (WBC) decreases during hemodialysis and it was investigated as a function of different dialysis membranes[9]. It was shown that hollow-fiber dialyzers activate complement by releasing C3a, C3d, and C5a levels peaking 15 min after beginning dialysis. Total white blood cell (WBC) count dropped simultaneously by 76%, and the decrease in leukocytes was inversely correlated with the levels of C3a and C5a.C3a, C3d, and C5a levels peaking 15 min after beginning dialysis [10]. Indeed there is an increased inflammatory activity in the majority of patients undergoing hemodialysis.

Activation of the immune system during treatment with various dialyzer membranes, is one of the examples of inflammatory status .The result of these activations are release of inflammatory mediators.[9,10] In hemodialysis patients, plasma levels of total homocysteine are influenced by nutritional status in patients with chronic kidney disease.[11,12] Recent studies showed that while an increased level of total plasma homocysteine is a risk factor for poor cardiovascular outcome in the general population, a decreased, rather than an increased, tHcy concentration may predict poor outcome in maintenance hemodialysis patients(MHPs), a phenomenon referred to as reverse epidemiology.[13,14] This seems to be due to the association between a low Hcy and protein-energy malnutrition, which is *per se* a known risk factor for poor clinical outcome in dialysis patients.[15] This

recently described paradoxical association between Hcy and clinical outcome in dialysis patients has now been referred to as a possible component of the reversal of the cardiovascular risks.[16-18] It is believed that both inflammation and protein-energy malnutrition, each independently or together as the "malnutrition-inflammation complex syndrome".[16-18]

Although an *in vitro* association between homocysteine and inflammation has been previously observed.[19]there is quiet little information about the relationship between homocysteine level and WBC counts in maintenance hemodialysis patients. We hypothesized that there may be an association between serum homocysteine level as a marker of nutritional status and WBC count as a marker of inflammation exist. We therefore test the association of WBC count and serum homocysteine in a group of end-stage renal failure patients undergoing regular hemodialysis.

MATIENTS AND METHODS

This cross-sectional study was conducted on patients with end-stage renal disease (ESRD), who were undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membranes. The study carried out in hemodialysis section of Hajar Medical, Educational &Therapeutic Center of Shahrekord University of Medical Sciences in Shahrekord of Iran. According to the severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism was given oral active vitamin D3 (Rocaltrol), calcium carbonate, and Rena-Gel capsules at various doses. According to the severity of anemia, patients were under i.v. iron therapy with iron sucrose (venofer) at various doses after each dialysis session, all patients were under treatments of 6mg folic acid daily, 500mg L-Carnitine daily, oral Vitamin B-complex tablet daily and also 2000U i.v. Eprex(recombinant human erythropoietin (rHuEPO) after each dialysis session routinely .Exclusion criteria were active or chronic infection and using NSAID or ACE inhibitor drugs or other drugs affect bone marrow.

Serum Homocysteine (total) was measured as follows. Blood samples were drawn after an overnight fast. Each blood samples were centrifuged within 15 min of venepuncture, and were measured by enzyme-linked immunosorbent assay (ELISA) method using DRG of Germany .Serum total Homocysteine (Hcy) have a normal range of 25-125 µmol/L. Also peripheral venous blood samples were collected after an overnight fast for complete blood count (CBC) containing white blood cells (WBCs) with lymphocyte and polymorphonuclear cell (PMNs) differentiation which were measured using Sysmex-KX-21N Cell counter, within 30 minutes from taking the blood samples. Other biochemical analysis including serum predialysis creatinine (Creat), post and predialysis blood urea nitrogen(BUN), albumin(Alb), serum C-reactive protein(CRP) and serum ferritin (by radio immune assay method;RIA) were measured using standard methods. Intact serum PTH (iPTH) was measured by the radioimmunoassay (RIA) method using DSL-8000 of USA (normal range of values is 10-65 pg/ml). Plasma HCO3 was measured by arterial blood gas. For the efficacy of hemodialysis the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data.[20] The body mass index (BMI) was calculated using the standard formula (postdialyzed weight in kilograms/height in square meters;kg/m^2).[21] The duration and the amount of sessions of hemodialysis treatment were calculated from the patients' records. The duration of each hemodialysis session was 4 hours.

Results are expressed as the Mean ± SD and median values. Comparison between the groups was done using Student's t-test. Statistical correlations were assessed using a partial correlation test. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at a *p*-value <0.05.

RESULTS

Total patients were 36(f=15 m=21), consisting of 25(f=11 m=14) non-diabetic HD patients and 11(f=4 m=7) diabetic HD patients. Tables 1, 2 and 3 summarized the patients' data. The mean patient's age was 47(±17) years. The value of serum homocysteine of total HD patients was 5(±2.4) µmol/l (median: 4.5µmol/l). The value of serum Hcy of diabetic and no diabetic-dialysis patients were 5(± 2) and 5(±2 .4) µmol/l respectively. The white blood cell count of total patients was 56 00±2 000cells/mm^3while diabetic and non-diabetic HD patients had a 6300(±2200) and 5300±1800 cells/mm^3respectively. The value of BMI of total patients was 21.8(±4.5) kg/m^2. In this study a weakly significant difference of serum CRP between diabetic and non-diabetics of total HD patients with more values in diabetics was seen (r = 0.065). In all patients a significant inverse correlation of serum homocysteine with WBC count (r = - 0.34, p =0.036 figure 1) (adjusted for age and URR) was seen.

In this group also a significant positive correlation of serum homocysteine with BMI (r = 0.35, p =0.039 fig. 2) (adjusted for duration of dialysis) and a significant positive correlation of WBC count with logarithm of serum CRP (r = 0.33, p =0.048 fig.3) (adjusted for duration of dialysis, plasma HCO3, serum ferritin and iPTH) was existed.

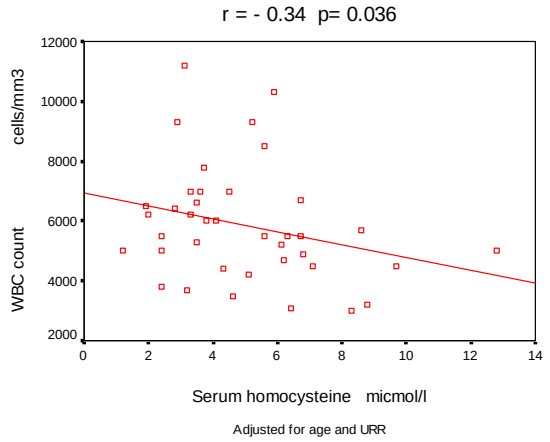

Figure 1: Significant inverse correlation of serum homocysteine with WBC count.

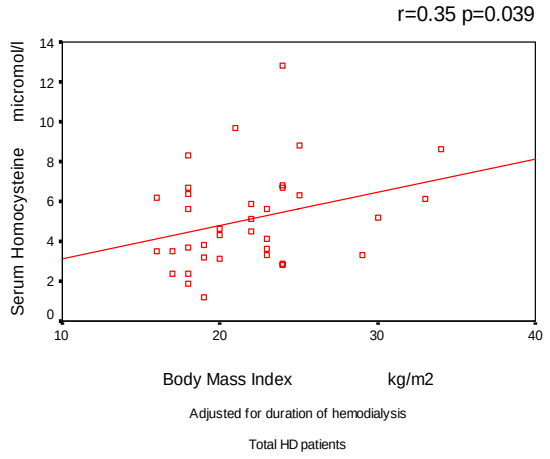

Figure 2: Significant positive correlation of serum Homocysteine with BMI.

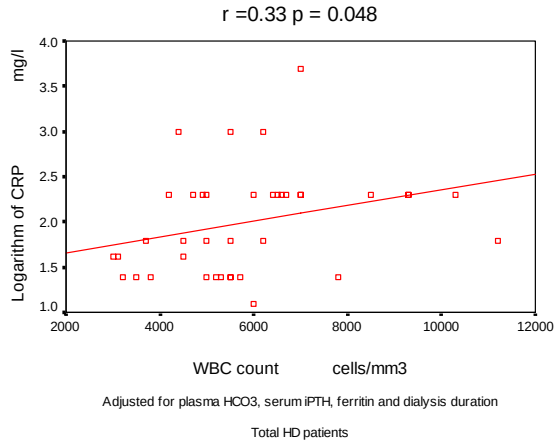

Figure 3: Significant positive correlation of WBC count with serum CRP

Table-1: Mean ±SD, Minimum Maximum and median values of age, duration, doses and also URR of total hemodialysis patients.

Total patients N=36	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age years	18	80	47±17	43
DH* months	2	156	32±36	19
Dialysis sessions	54	216	123±54	156
URR %	39	76	58.5±9	57.5
WBC count s Cells/mm^3	1000	11200	5500±2000	5500
Hb g/dl	5	13	9±2	9
Homocysteine µmol/L	1.2	12.8	5±2.4	4.5
iPTH Pg/ml	16	1980	444 ±	293
Alb g/dl	2.4	4.8	3.8±0.5	3.95
CRP mg/l	3	40	8.7±6.7	8
PMN %	40	85	65±11	67
Lymphocyte %	11	47	27±9	25.5
HCO3 mEq/L	14	25	2.6 ±	20
Ferritin ng/dl	35	1250	± 292 503	420

*Duration of hemodialysis

Table-2: Mean ±SD, Minimum, Maximum and median values of age, duration, doses and also URR of non-diabetic hemodialysis patients.

Non-diabetic patients N=25	**Minimum**	**Maximum**	**Mean±SD**	**Median**
Age years	16	80	44±17	41
DH* months	2	156	40±40.8	22
Dialysis sessions	36	1584	370±452	156
URR %	60	76	61±7.5	60
WBC counts cells/mm^3	1000	9300	5300±1800	5500
Hb g/dl	5	12	8.5±2	9
Hemocysteine µmol/L	1.2	9.7	5±2	5.6
iPTH Pg/ml	22		469 ±	340
Alb g/dl	2.4	4.7	3.8±0.50	4
CRP mg/l	2	20	7.4±3.8	6
PMN %	40	85	65±11	65
Lymphocyte %	1.1	4.7	27.5±9.7	24
HCO3 mEq/L	14	25	2.8 ±	20

Ferritin ng/dl	170		280 ±	470

*Duration of hemodialysis

Table-3: Mean ±SD , Minimum ,Maximum and median values of age, duration ,doses and also URR of diabetic patients.

Diabetic patients n=11	Minimum	Maximum	Mean±SD	Median
Age years	27	75	53±15.8	55
DH* months	6	24	14.5±6	12
Dialysis sessions	54	216	123±54	108
URR %	39	75	53.5±9.8	54
WBC counts cells/mm^3	3500	11200	6300±2200	62
Hb g/dl	5	13	10±2	9
Hemocysteine µmol/L	1.2	12.8	5± 2.4	4.5
iPTH Pg/ml	16	860	268 ±	42
Alb g/dl	2.3	4.8	3.8±0.50	3.9
CRP mg/l	3	40	8.7±6.6	8
PMN %	40	85	65±11	67.5
Lymphocyte %	11	47	27±19	25.5
HCO3 mEq/L	18	25	1.8 ± 20	20
Ferritin ng/dl	35	1000	295 ±	296

*Duration of hemodialysis

These investigations indicated that oxidative stress had a determinant role in the stimulatory effect of homocysteine most likely via activation of the redox-sensitive transcription factor NF-κB. Homocysteine-induced B lymphocyte proliferation is mediated by oxygen radicals such as O2, OH. and H2O2, generated by thiol (-SH) auto-oxidation [22]. Although less reactive than homocysteine, cysteine shares some of the chemical properties derived from the presence of the sulfhydryl group. Cysteine has a general cytotoxicity *in vitro* and promotes the detachment of human arterial endothelial cells in culture. It also exhibits auto-oxidation properties in the presence of metal ions, resulting in the generation of free radicals and hydrogen peroxide that promote the activation of the cellular immune system by enhanced induction of NF-kB and MCP-1. Finally cysteine can support superoxide-mediated modification of low density lipoproteins (LDL), thus facilitating the formation of foam cells [22]. In a study conducted by Kalantar-Zadeh et al. to examine the association between homocysteine level and markers of malnutrition-inflammation complex syndrome and 12-months prospective hospitalization and mortality in 367 MHD patients, aged 54.5 +/- 14.7 years, found a weak to moderate but statistically significant correlations of hyperhomocysteinemia with some laboratory markers of nutrition (serum albumin, prealbumin, creatinine, and urea nitrogen) but no significant correlation with serum C-reactive protein were existed[13]. They found that the hospitalization rates were significantly higher in patients with lower tHcy levels and mortality rate in the lowest tHcy quartile was significantly higher compared with other three quartiles. They concluded that tHcy may be a more exclusive nutritional marker in MHD patients with no association with inflammatory measures[13]. In a study conducted by Suliman et al. on a cohort of 250 patients with chronic kidney disease (CKD) which starting renal replacement therapy to assess the overall mortality in relation to basal tHcy level followed the patients during a 4-year period they found that ninety-three patients (37%) with signs of inflammation (CRP > or = 1 mg/dl) had significantly lower levels of tHcy and serum Alb than 157 noninflamed patients. Serum tHcy levels correlated positively with serum Alb levels and negatively with CRP levels and other inflammation markers. They showed that the presence of both inflammation and malnutrition was associated with lower homocysteine levels than when malnutrition was present without inflammation .They also showed that that serum Alb and CRP levels were independently associated with tHcy levels after adjustment for other variables. Serum tHcy level was significantly greater in survivors than nonsurvivors, and greater tHcy level was associated with better survival, more over they concluded that plasma tHcy level was lower in patients with inflammation. And inflammation may contribute to the reverse association between tHcy level and mortality in patients with CKD starting renal replacement therapy [11]. We also could show the positive correlation of serum homocysteine with BMI further support the association of Hcy with nutritional status in MHPs. More recently in a study conducted by Sakuta et al. to find whether WBC count is associated with homocysteine designed a cross-section all study on middle-aged Japanese men. They showed that a univariate regression analysis WBC count was associated positively with total homocysteine .In a multivariate analysis which included cigarette smoking, physical activity, ethanol consumption, vegetable intake and body mass index, the association between WBC count and total homocysteine remained significant. They concluded that association may partially explain the reported association between elevated WBC count and cardiovascular disease.[24] To our knowledge this is the first report concerning the inverse correlation of serum homocysteine with WBC count .In the malnutrition-inflammation cachexia(MIC) syndrome point of view in hemodialysis patients , we

could show an inverse correlation between WBC count as a marker of inflammation with serum Hcy levels as a marker of nutrition, however further studies are required to understand whether rising serum Hcy levels are the trigger for the WBC activation thus promoting or contributing to the pro-inflammatory and pro-atherosclerotic responses.

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