Effect of Anemia and Hyperhomocysteinemia on Mortality of Patients on Hemodialysis

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Introduction. Anemia and hyperhomocysteinemia are risk factor of mortality of patients on dialysis. This study was conducted to assess the relationship of hemoglobin and homocysteine levels and mortality of patients on hemodialysis.

Materials and Methods. Fifty patients on hemodialysis and 20 healthy individuals were enrolled in the study. Blood samples were drawn for measurement of hematological parameters, serum iron, serum ferritin, transferrin saturation, and homocysteine levels. The patients were followed up for 1 year to determine the mortality rate and evaluate its association with anemia and hyperhomocysteinemia.

Results. The majority the patients (54%) were not on erythropoietin therapy. Forty-three patients (86%) were anemic (hemoglobin < 11 g/dL). Serum ferritin was high (> 500 ng/mL) in 33 patients (66%). Mortality was 28% in 1 year (33% in anemic patients versus no death among patients with a hemoglobin level greater than 11 g/dL). The relative risk of mortality was increased by 1.58 with every 1 g/dL decrease in hemoglobin level. All of the patients had a high homocysteine level, and a significant difference was observed between the homocysteine levels of the patients on hemodialysis and the control group ($P < .001$). Hyperhomocysteinemia did not affect mortality. In multivariate Cox regression analysis, only hemoglobin level was associated with mortality.

Conclusions. Almost all of our patients on hemodialysis were anemic and this condition was a risk factor of mortality. Iron stores, however, were adequate in more than half of the patients. The reason of anemia could be untreated erythropoietin deficiency. Hyperhomocysteinemia was present in the majority of the patients, but it did not independently affect mortality.

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INTRODUCTION

End-stage renal disease (ESRD) is characterized by nutritional impairment, anemia, hypertension, renal bone disease, neuropathy, nutritional impairment, and reduced life expectancy. Anemia is a most common complication of chronic kidney disease (CKD), including those undergoing maintenance hemodialysis treatment.[1] The central role of anemia in the development of cardiovascular dysfunction is now well established. It leads to progressive deterioration in cardiac function, leading to congestive heart failure. There is a triangular relationship and a vicious circle between congestive heart failure, CKD, and anemia, called cardiorenal

anemia syndrome.[2,3] Anemia in CKD has also been associated with slower deterioration in kidney function in patients in whom anemia has been corrected with erythropoietin. The anemia of CKD is usually normochromic and normocytic, and its cause is the decrease in erythropoietin production, reduced erythrocyte survival, inhibition of bone marrow by uremic toxins, and deficiency of folate, iron, and vitamin B12.[4,5] Anemia of ESRD can be managed relatively successfully by recombinant erythropoietin.[6] Before the erythropoietin injections, assessment of iron status (serum iron, total iron-binding capacity, serum transferrin, and transferrin saturation) of these patients should be done and iron deficiency should be corrected accordingly.

Homocysteine is a sulfur amino acid that has inverse relationship with glomerular filtration rate (GFR). The prevalence of hyperhomocysteinemia is 85% to 100%, when ESRD is developed.[7] Anemia and hemocysteine level are also influenced by nutritional status and both are predictors of mortality in patients on dialysis.[8] This study was conducted to evaluate the effect of anemia and hyperhomocysteinemia on mortality in patients on maintenance hemodialysis.

MATERIALS AND METHODS

This study was conducted at the hemodialysis unit of Shalimar Hospital, Lahore, from June 2008 June 2009. Fifty patients with ESRD who were on regular hemodialysis for more than 3 months were included in the study. Twenty gender-, age-, and socioeconomic status-matched subjects with normal kidney function were included as controls. All of the patients were informed of the study protocol and consented to be enrolled in the study. Demographic data containing age, sex, cause of ESRD, hepatitis B surface antigen, and antihepatitis C virus antibody were collected. Predialysis blood samples of the patients were drawn to measure hematological and biochemical markers (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, blood urea, serum creatinine, serum iron, serum ferritin, and transferrin saturation, as well as serum total homocysteine level. Routine laboratory studies were performed on daily basis in our laboratory. Samples for serum ferritin and homocysteine were stored at -80°C and measured in a batch on Vitros Hormone Analyzer and Biorad automated enzyme-linked immunosorbent assay system.

The patients were followed up for 1 year to determine the effect of anemia and hyperhomocysteinemia on survival and risk factors of mortality. Normal values for laboratory parameters were considered according to the Kidney/Dialysis Outcome Quality Initiative guidelines.[9] The data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Ill, USA). Quantitative variables, including blood urea, serum creatinine, blood hemoglobin, and hematocrit, were expressed as mean ± standard deviation. The Pearson correlation coefficient was used to study the relationship between hemoglobin and homocysteine levels. The *t* test was applied to test the significance of difference in quantitative variable between groups. A Cox proportional hazard regression Model was used to determine the significant factors for predicting mortality in patients on hemodialysis. A *P* value less than .05 was considered significant.

RESULTS

A total of 70 participants were included in the study (50 patients on maintenance hemodialysis for more than 3 months and 20 controls). Causes of ESRD were diabetes mellitus in 30 (60%), hypertension in 10 (20%), nephrolithiasis/obstructive nephropathy in 4 (8%), chronic glomerulonephritis in 3 (6%), and unknown in 3 patients (6%). Twenty-nine patients (58%) were women. The mean ages of the patient and control groups were 46.9 ± 15.5 years and 39.7 ± 13.0 years, respectively (range, 14 to 76 years). The mean duration of dialysis was 13.82 ± 10.53 months (range, 3 to 60 months). Antihepatitis C virus antibody and hepatitis B surface antigen were negative in 28 (56%) and 48 (96%) patients, respectively. Hematological and biochemical data of the patients and controls are shown in Table 1. The majority the patients (54%) were not on erythropoietin therapy. The average dose of erythropoietin being used by the patients was 2652 IU/wk.

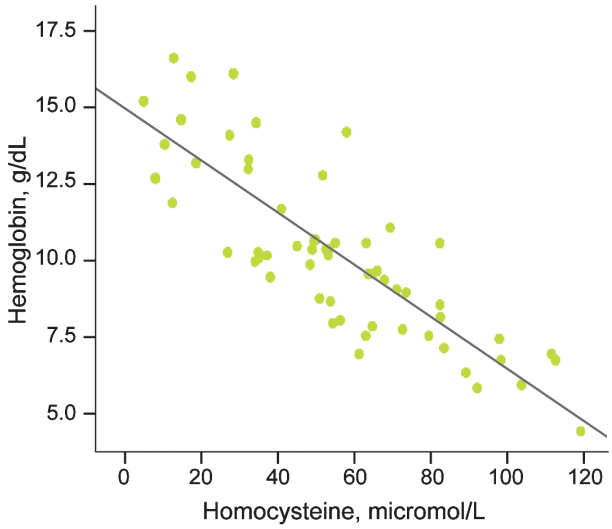
Forty-three patients (86%) were anemic (hemoglobin < 11 g/dL). Serum ferritin (mean, 976.75 ± 1386.01 ng/mL) was high (> 500 ng/mL) in 33 patients (66%). Mortality was 28% in 1 year (33% in anemic patients versus no death among patients with a hemoglobin level greater than 11 g/dL). The relative risk of mortality was

Table 1. Laboratory Parameters of Patients on Hemodialysis and Control Group

Parameters	Hemodialysis Patients	Controls	*P*
Hemoglobin, g/dL	9.58 ± 2.30	13.90 ± 1.80	< .001
Hematocrit, %	28.77 ± 6.77	29.43 ± 3.36	.68
MCV, fL	84.58 ± 5.96	85.07 ± 6.45	.76
MCH, pg	28.15 ± 2.09	34.33 ± 17.68	.02
MCHC, g/dL	33.29 ± 1.17	33.34 ± 0.94	.88
Serum ferritin, ng/mL	976.75 ± 1386.01	57.30 ± 47.58	.004
Serum iron, µmol/L	53.33 ± 44.18	133.90 ± 31.70	< .001
Total iron-binding capacity, µmol/L)	204.24 ± 65.96	208.50 ± 84.54	.82
Transferrin saturation, %	28.81 ± 25.83	73.87 ± 34.59	< .001
Serum hemocysteine, µmol/L	28.58 ± 8.10	8.04 ± 1.91	< .001

increased by 1.58 with every 1 g/dL decrease in hemoglobin level. Those who died after 1 year had lower hemoglobin levels than patients who remained alive (8.17 ± 1.85 g/dL versus 12.2 ± 2.24 g/dL; *P* = .006). In univariate Cox regression model, mortality was significantly increased by decreased hemoglobin level (hazard ratio, 3.04; 95% confidence interval, 1.32 to 5.82; *P* = .03). In multivariate Cox regression analysis, only hemoglobin level was associated with mortality (Table 2).

All of the patients had a high homocysteine level, and a significant difference was observed between the homocysteine levels of the patients on hemodialysis and the control group (28.58 ± 8.10 µmol/L versus 2.0 ± 1.29 µmol/L, respectively; *P* < .001). Hyperhomocysteinemia did not affect mortality. However, homocysteine levels were high in anemic patients as compared to nonanemic patients (67.33 ± 2.16 µmol/L versus 41.73 ± 17.14 µmol/L; *P* < .001; Figure).



Relationship between serum homocysteine levels and hemoglobin in patients on hemodialysis (*P* < .001).

DISCUSSION

In this study, most of the patients (86%) were anemic (hemoglobin < 11 g/dL). A similar low level of hemoglobin has also been observed in another local study,[10] in which 44 patients (86.4%) were having low hemoglobin levels and the mean hemoglobin level was 7.97 ± 1.4 g/dL. Table 3 highlights the comparison of different mean hemoglobin levels and percentages of anemic patients in developed countries and Pakistan.[11] The reasons for anemia in these patients are that uremic toxins inhibit the erythropoiesis in kidney failure

Table 2. Maximum Likelihood Estimates of Outcome Predictors (Mortality) in Patients on Hemodialysis

Factor	Regression Coefficient	Exp(B)	95% Confidence Interval	*P*
Hemoglobin	-0.79	0.45	0.24 to 0.84	.01
Age	0.006	1.01	0.97 to 1.05	.75
Sex	-0.04	0.96	0.21 to 4.32	.95
Blood urea	0.004	1.00	0.97 to 1.03	.80
Serum creatinine	-0.27	0.76	0.50 to 1.16	.20
Homocysteine	-0.04	0.96	0.91 to 1.02	.17
Ferritin	< 0.001	1.00	0.99 to 1.00	.70
Iron	< 0.001	0.99	0.98 to 1.02	.96
Total iron-binding capacity	< 0.001	0.99	0.99 to 1.01	.89

Table 3. Hemoglobin Levels and Anemia in Patients on Dialysis Therapy, by Country[12]

Country	Total Number of Patients	Mean Hemoglobin, g/dL	Hemoglobin < 11 g/dL, %
Sweden	466	12.0	23
United States	1690	11.7	27
Spain	513	11.7	31
Canada	479	11.6	29
Belgium	442	11.6	29
Australia	423	11.5	36
Germany	459	11.4	35
Italy	447	11.3	38
United Kingdom	436	11.2	40
France	341	11.1	45
Japan	1210	10.1	77
Pakistan*	50	9.8	86

*Results of the present study.

through bone marrow suppression and reduced life span of erythrocytes.[12] For the correction of anemia, erythropoietin use is recommended in these patients. However, anemia remains in patients who cannot afford erythropoietin treatment due to financial burden of this chronic disease. Dialysis cost is US $ 350 to US $ 400 per month for a three-time per week dialysis program, while the per capita income is US $ 1100.[13] Due to financial gap in income and expenditure, patients cannot afford expensive injections of erythropoietin, because it increases financial stress of extra US $ 200 per month. Even the dose of erythropoietin used by these patients on maintenance hemodialysis is less as compared to the international guidelines (10 000 U/wk 20 000 U/wk). The average dose of erythropoietin in our study was 2652 U/wk. In this study, only 46% of the patients were getting erythropoietin as compared to 90% of patients on maintenance hemodialysis in the United States.[14] Therefore, insufficient dose and disparity in access to erythropoietin is the main reason for anemia in our patients. Anemia is an important predictor of mortality in patients on dialysis.[15,16] In our patients, death was associated with a low hemoglobin level; all deaths occurred in the patients with a hemoglobin lower than 11 g/dL. Similar results were observed by Portoles and coworkers.[17]

Iron is an essential ingredient used in erythropoiesis, and its adequate amount is required for new erythrocytes synthesis. Measurement of serum ferritin, serum iron, and transferrin saturation is the primary tool for assessing iron status in patients with CKD.[18] Reduced serum iron level is due to poor dietary intake, poor appetite, and increased iron losses (up to 5 mg/d to 6 mg/d) in patients on hemodialysis. In patients with CKD, iron deficiency anemia is divided into absolute (transferrin saturation < 20%, serum ferritin < 200 ng/mL) and functional iron deficiency anemia (transferrin saturation percentage < 20%, serum ferritin > 500 ng/mL). In this study, transferrin saturation was normal (> 20%) in 26 patients (52%) and it was high (> 500 ng/mL) in 33 (66%). Similar observation was made by Rambod and associates.[19] Intravenous iron preparations are affordable for our patients, and we can eliminate iron deficiency according to the international guidelines.[20] This study, however, showed that although iron level and stores were adequate in most of the patients, the majority of the patients were still anemic. Serum ferritin concentration is a commonly used marker of iron status in maintenance dialysis patients.[21] It was shown that a low serum ferritin concentration is a reliable indicator of iron deficiency among patients with ESRD. However, a high serum ferritin may show adequate iron stores among patients on dialysis and is increased as an acute-phase reactant (inflammatory marker).[22] The high ferritin level in our patients might be due to the fact that double-lumen catheters were being used for a long duration in these patients. These catheters are a source of infection and are the reason for resistance of erythropoietin-stimulating agents. These findings indicate that serum ferritin is almost equally important as an inflammatory marker. Hence, moderately high levels of serum ferritin should not be assumed to indicate high iron stores, and patients with ferritin levels higher than 500 ng/mL should not be automatically labeled as iron overload.[23] There is a need to study other inflammatory markers in these patients for correlation of serum ferritin with them.

Plasma homocysteine is an important risk factor of atherosclerosis in patients on dialysis. Several studies have reported prevalent severe hyperhomocysteinemia in patients on hemodialysis.[24,25] Hyperhomocysteinemia is a risk factor of mortality in these patients.[26-28] However, hyperhomocysteinemia had no significant effect on mortality of our patients on hemodialysis. Homocysteine level was 26.20 ± 10.1 µmol/L in patients who died and 28.55 ± 8.84 µmol/L

in the survived patients. Overall, homocysteine was high in all of the patients, a phenomenon reported by other investigators, as well.[29] Foley and colleagues reported that mild to moderate elevations in plasma total homocysteine levels were observed in the majority (>85%) of patients with ESRD on maintenance dialysis. In the present study, homocysteine was high in anemic patients (67.33 ± 2.16 µmol/L) as compared to nonanemic patients (41.73 ± 17.14 µmol/L). Therefore, the impact of hyperhomocysteinemia on mortality might be masked by anemia. To pinpoint the role of hyperhomocysteinemia in mortality, first hemoglobin level should be improved in these patients. It is possible that the high levels of serum homocysteine in some conditions may increase the micro-inflammatory state of uremia in patients on hemodialysis and play a role in intensification of anemia.[30] In addition, patients on hemodialysis show a positive correlation of serum homocysteine with serum creatinine and urea levels, but it has a negative correlation with serum iron and transferrin saturation.

CONCLUSIONS

Almost all of our patients on hemodialysis were anemic and this condition was a risk factor of mortality. Iron stores, however, were adequate in more than half of the patients. Major reasons of anemia could be erythropoietin deficiency and inadequate dialysis. Hyperhomocystinemia was present in the majority of the patients, but, while homocystine had a reverse correlation with hemoglobin level, it did not independently affect mortality.

CONFLICT OF INTEREST

None declared.

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