

Platelet Aggregation Test and Immunoglobulin M as Predictors For Inflammation and Erythropoietin Resistance in Chronic Hemodialysis Patients

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ABSTRACT

Background: Anemia is a constant feature of chronic renal failure patients. There are many causes for this anemia ranging from deficient erythropoietin production, iron deficiency, blood losses, to the effect of uremic toxins such as hyperparathyroid state, some inflammatory cytokines and aluminum toxicity. Inflammation is a common cause of severe anemia and inadequate response to recombinant erythropoietin (EPO) therapy in maintenance hemodialysis (HD) patients.

Objectives: This study was performed to assess recently determined predictors for inflammation rictocetin platelet aggregation test (RIPA) and IgM. We also studied the link between early detection of inflammation and the response to EPO in HD pediatric patients.

Methods: Twenty-eight children on regular HD, their age ranging from 1-16 years with stable general condition were included in this study. They were on EPO treatment and iron supplementation. Thorough clinical examination, routine laboratory work, CBC, CRP, urea, serum creatinine, serum calcium, serum sodium and serum phosphorus were done. Parathormone level, IgM and platelet aggregation test were also done for these patients.

Results: There was a strong correlation between RIPA and Hb changes in HD patients under EPO therapy. The mean level of RIPA was $71.7 \pm 4.1\%$ in the responder group. The activity in the non-responder group was impaired with mean level $49 \pm 6.6\%$. Also there was a strong correlation between RIPA and Hb changes. Increased Hb level is associated with better function of platelets ($r = 0.4, p < 0.01$). There is no significant correlation between IgM level and Hb changes in either group.

Conclusions: A low grade chronic inflammatory state is an important determinant of anemia in clinically stable maintenance HD patients. The reasons for such impaired response to erythropoietin therapy could be some micro-inflammatory effect of HD procedures and an inflammation induced platelet dysfunction of vascular endothelial cells.

INTRODUCTION

Anemia is a constant feature of chronic renal failure patients. There are many causes for this anemia, ranging from deficient erythropoietin production, to iron deficiency and blood losses during the hemodialysis process. At the same time many uremic toxins contribute to bone marrow failure and failure to respond to

erythropoietin. These toxins may be endogenous such as the hyperparathyroid state and some inflammatory cytokines and may be exogenous, such as aluminum toxicity⁽¹⁾.

Recombinant human erythropoietin was licensed for the treatment of renal anemia, and over 90% of patients respond to it⁽²⁾. Unfortunately, some patients may not

respond to it adequately. Inflammation is by far one of the most common factors blunting the response to erythropoietin. Generally, these states result from suppression of endogenous EPO synthesis, resistance of marrow erythroid progenitors, and decreased bioavailability of iron⁽³⁾. Inflammation in dialysis patients may be related to processes associated with renal failure itself, a consequence of treatment of renal failure (dialysis related), or other causes like infections⁽⁴⁾. Prosynthetic modification of protein is one of the causes of inflammation related to renal failure, and this is due to accumulation of pro-inflammatory compounds or products of metabolism. The second cause is oxidative stress due to loss of antioxidants like zinc, selenium and vitamins C & E⁽⁵⁾. Causes related to the dialysis process include exposure of the blood to bio-incompatible dialysis membranes, causing activation of mononuclear cells which has been implicated as a potential cause of inflammation⁽⁶⁾. Also, the quality of water used to prepare the dialysate may contribute to the process of inflammation⁽⁴⁾. Infections which occur more commonly in HD patients, due to impaired humoral and cellular immunity and vascular access, contribute to inflammation⁽⁴⁾.

Many studies were applied addressing the link between the poor response to erythropoietin and inflammation. Acute phase reactant (APR) markers such CRP⁽⁷⁾, serum ferritin, fibrinogen⁽⁸⁾, and hypoalbuminemia⁽⁹⁾, have been found to be associated with low hemoglobin (Hb) levels and hyporesponsiveness to recombinant EPO in HD patients. However, few studies

were applied for early prediction of inflammation in chronic renal failure patients under regular hemodialysis. In vitro ristocetin induced platelet aggregation (RIPA) in whole blood was found to be inversely associated with Hb levels in HD patients treated or not treated with recombinant EPO⁽¹⁰⁾. Borawski et al.⁽¹¹⁾, hypothesized that enhanced whole blood RIPA was a marker of endothelial cell (EC) inflammation leading to exacerbation of anemia in maintenance HD patients.

AIM OF THE WORK

The aim of our study is to find a link between early predictors of inflammation and the response to erythropoietin in HD pediatric patients.

PATIENTS AND METHODS

PATIENTS:

Twenty eight children with CRF, were randomly selected from the Nephrology and Dialysis Unit, Benha University Hospital and Abu El-Rish Children Hospital, Cairo University.

Inclusion criteria included children on regular HD for not less than 4 months and their ages ranging from 1-16 years with a stable general condition. EPO was used in a median dose of 100 u/Kg/wk subcutaneously, and these patients were also receiving iron supplementation 100 mg once a week, with transferrin saturation < 50%. There was no evidence of worsening anemia or absolute iron deficiency (defined on the basis of transferrin saturation < 20% and serum ferritin < 100 ng/ml). All these patients were on adequate hemodialysis as determined by Kt/V and urea reduction rate

(URR). Exclusion criteria included patients less than 1 year and over 16 years. None of these patients suffered from any inflammatory or infectious diseases, or received blood transfusion at least 2 months before the study, or were positive for occult blood in the stool. Also, patients with severe hyperparathyroidism were excluded from the study.

The patients were divided into 2 groups: Group A: included patients who were adequately responding to EPO therapy as shown by elevation of Hb by 1 gm/dL/month, or elevation of the hematocrit level by 1-2% within 1-2 weeks of intake of EPO, 75-150 u/Kg/week, according to the National Kidney Foundation Dialysis Outcome Quality Initiative⁽¹²⁾.

Group B: included patients who were not adequately responding to EPO (showing less Hb or hematocrit elevation).

METHODS

All children in this study were subjected to full history taking and thorough clinical examination. The following laboratory works were done for all patients: CBC (at the start and 6-8 weeks later), renal function tests, s. albumin, ALT, iron indices (s. iron, TIBC, s. ferritin), hepatitis markers (HCV Ab, HBs Ag, HBs Ab), HIV and parathormone level. Also estimation of IgM level, platelet aggregation test (in platelet rich plasma ristocetin "RIPA") and CRP were done for all patients.

Platelet aggregation test: Platelet rich plasma aggregation in response to ristocetin (0.75 mg/ml, Sigma, St. Louis, Mo, USA) was monitored by measuring electric impedance using a 350 VS dual channel aggregometer according to the method of

Wilsoncroft. The extent of aggregation was assessed by measuring the maximal extension of the aggregation curve at 6 min after the addition of the agonist and expressed in ohms. Platelet aggregation in PRP (platelet rich plasma) induced by ristocetin (1.5 mg/ml) was monitored by measuring the maximum percentage of light transmission using a 490 2A dual channel automatic optical aggregometer.

Statistical Analysis

Data were expressed in terms of mean and standard deviation. Analysis of variance, student "t" test, chi square were used to detect statistical significance. Correlation Coefficient "r" was used for correlation between two parameters.

RESULTS

Our study included 28 patients. These patients were divided into 2 groups according to their response to EPO therapy.

Group (A): [Responders] adequately responding to r-Hu EPO therapy and included 17 patients, 9 boys and 8 girls. Their mean age was 10.52 ± 2.18 years.

Group (B): [Poor responders] not adequately responding to EPO therapy and included 11 patients, 6 boys and 5 girls. Their mean age was 11.81 ± 2.99 years.

It is shown in Table (1) that there was a significant difference in Hb level between the two studied groups. As regards serum creatinine, serum ferritin and parathormone level there was no significant difference between the studied groups. Concerning the efficacy of dialysis (determined by Kt/V), both groups were under adequate dialysis.

Table (2) shows that there was no significant difference between both groups

as regards prevalence of hepatitis B & C. One patient in group (A) was suffering from both hepatitis B & C. No patients were suffering from HIV in either group.

It is shown from Table (3) that there was a highly significant difference as regards RIPA between the two groups ($p < 0.01$). Also there was a highly significant difference between the two groups as regards the increase in Hb level after r-Hu-EPO therapy ($p < 0.01$). For IgM level there was no significant difference between both groups.

Table (4) shows that there was no significant correlation between Hb changes and either CRP or IgM separately. There is a highly statistically significant correlation between RIPA and Hb change in group (A) and a statistically significant correlation in group (B).

Table (5) shows that there was a significant negative correlation between RIPA and serum ferritin. As regards Hb change, there was a significant negative correlation with group (A).

Table 1: Comparison between mean \pm SD of both patient groups regarding age and laboratory investigations.

Parameter	Group A (17 pt)		Group B (11 pt)		P
	Range	X \pm SD	Range	X \pm SD	
Age (years)	6-14	10.52 \pm 2.18	8-16	11.81 \pm 2.99	> 0.05
Hb (gm/dL)	6.1-10.8	8.37 \pm 1.3	5.3-8.1	6.45 \pm 1.2	< 0.05*
S. creatinine (mg/dL)	5.5-3.7	6.3 \pm 1.4	7.1-10.8	8.2 \pm 1.1	> 0.05
Kt/V	0.9-1.4	1.24 \pm 0.17	0.8-1.3	1.14 \pm 0.28	> 0.05
CRP (mg/L)	6-12	6.9 \pm 1.4	6-24	10.2 \pm 2.6	> 0.05
S. ferritin (ng/ml)	868-1871	1368 \pm 480	1071-1721	1573 \pm 522	> 0.05
S. iron (μ g/dL)	107-189	130 \pm 39	139-207	163 \pm 42	> 0.05
Parathormone (pg/ml)	104-165	136.2 \pm 21	115-192	174.4 \pm 18	> 0.05
Platelet c $10^3/mm^3$	196-350	239.3 \pm 32	185-313	226 \pm 35	> 0.05
WBCs $10^3/mm^3$	3.9-8.2	5.5 \pm 1.2	2.9-9.4	5.4 \pm 2.4	> 0.05

Table 2: Comparison between both groups according to prevalence of hepatitis B & C and HIV.

Variable \ Group	Group A (17 pt)		Group B (11 pt)		p
	No.	%	No.	%	
HBV	4	23.52	3	27.27	> 0.05
HCV	7	41.17	7	63.63	> 0.05
HIV	0	0	0	0	

Table 3: Comparison between both groups according to Ig M level, ristocetin platelet aggregation test and Hb change after treatment.

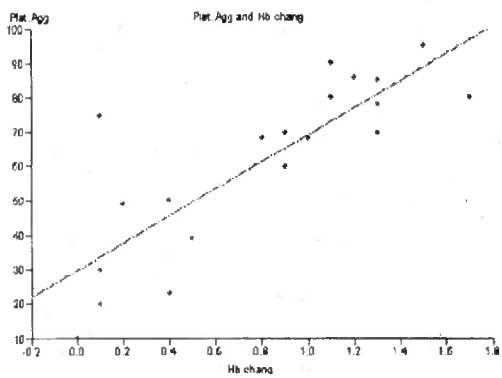
Variable \ Group	Group A	Group B	p
	X ± SD	X ± SD	
IgM (mg/dl)	93.3 ± 38.6	105.3 ± 37.1	> 0.05
RIPA %	71.7 ± 5.9	49 ± 8.16	< 0.01*
Hb change after treatment (gm/dl)	1.3 ± 0.2	0.3 ± 0.15	< 0.01*

Table 4: Correlation between Hb changes after r-HuEPO therapy and CRP, IgM and RIPA in both groups.

Variables \ Hb change	Group A		Group B	
	r	p	r	p
CRP	-0.07	> 0.05	-0.01	> 0.05
IgM	-0.03	> 0.05	-0.06	> 0.05
RIPA	0.4	< 0.01*	0.08	< 0.05*

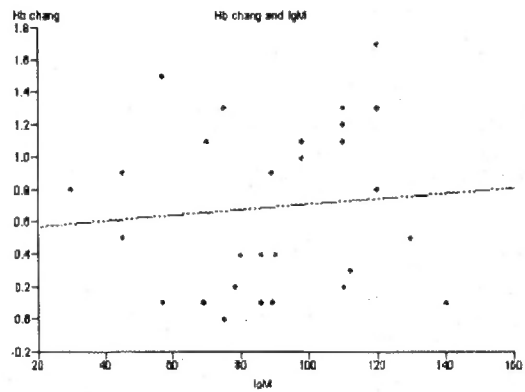
Table 5: Correlation between serum ferritin level and Hb changes and RIPA.

Variables \ Ferritin	Group A		Group B	
	r	p	r	p
RIPA	-0.4	< 0.05*	-0.1	< 0.05*
Hb change	-0.2	< 0.05*	0.01	> 0.05



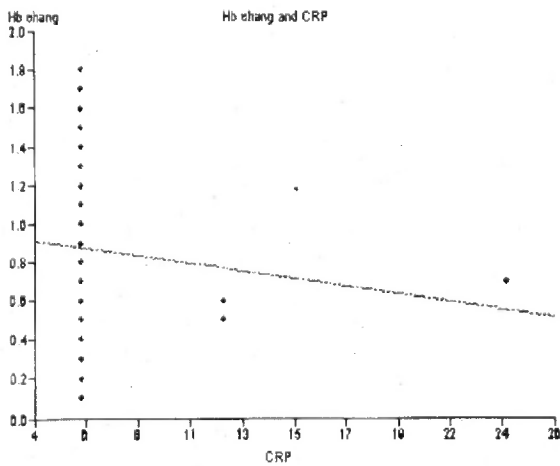
Correlation between Hb changes and Platelet aggregation test

Fig. 1



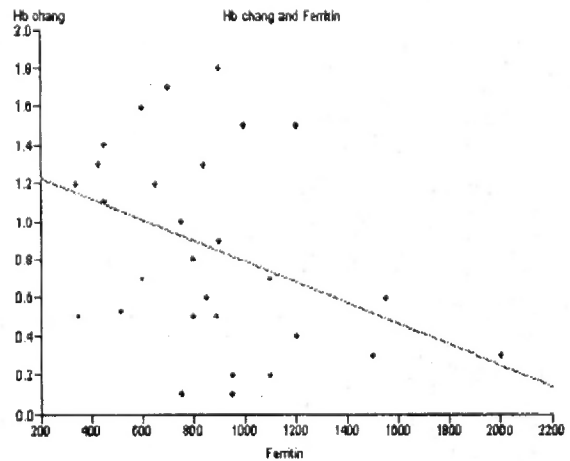
Correlation between IgM and Hb changes

Fig. 2



Correlation between CRP and Hb changes

Fig. 3



Correlation between ferritin and Hb changes

Fig. 4

DISCUSSION

Our study was done on 28 patients, and they were divided into two groups according to their response to EPO. Seventeen patients were good responders (group A) and eleven were poor responders (group B). According to the definition of NKF-DOQI guidelines, good response is defined by elevation of Hb by 0.8-1.2 gm/dL/month, or elevation of hematocrit level by 3% within 2-4 weeks of intake of EPO by a dose of 75-150 u/kg/week. Both groups were similar in all aspects as regards regular hemodialysis, three sessions weekly, under adequate dose of EPO together with iron therapy. None of the patients received blood transfusion in the previous two months before the study. The patients were apparently healthy, not complaining from chronic or blood disease. Also there was no apparent focus of infection. The distribution of age and sex in either group revealed no significant difference. Regarding the prevalence of HBV and HCV infection, there was no statistically significant difference between the two groups.

It is known that inadequate dialysis is associated with impaired response to EPO⁽¹³⁾. Our studied patients were under adequate efficient dialysis with a mean of Kt/V of 1.24 ± 0.14 and 1.14 ± 0.28 in group A & B respectively, and there was no significant difference between both groups as regards adequacy of dialysis.

Iron deficiency in cases of ESRD and under regular HD may blunt the response to EPO therapy⁽¹⁴⁾. The level of serum iron was 130 ± 39 $\mu\text{g/dL}$ for group (A) and 163 ± 42 $\mu\text{g/dL}$ for group (B) with no significant statistical difference.

Regarding serum ferritin, its mean level was 1368 ± 480 ng/ml and 1573 ± 522 ng/ml for groups A & B respectively, with no significant difference. Ferritin is the mobile iron store in the body, and at the same time its level is increased in inflammation⁽¹⁴⁾.

One of the factors that depresses the response to EPO is severe hyperparathyroidism⁽¹⁵⁾. The effect of parathormone is due to a direct toxic effect on red blood cell production and survival, and an indirect effect via the induction of marrow fibrosis and interference with erythropoiesis leading to anemia. As regards parathormone level, it was 136 ± 21 ng/ml in group (A), while in group (B) it was 174.4 ± 18 ng/ml, with no statistically significant difference. This level is high in both groups, and this finding cannot be avoided in cases of renal failure, and this factor was the same in both groups. But still this level was not very high enough to cause severe hyperparathyroidism.

On studying the inflammatory markers, it was found that there is no significant difference in the level of CRP between the two groups. Sitter et al.⁽¹⁶⁾, stated that CRP is a useful marker for severity of inflammation in chronic HD patients and this is not in agreement with our results. On the other hand, Kimmel et al.⁽¹⁷⁾, stated that positive acute phase reactants as CRP or ferritin are markers for which serum levels are elevated during acute episode of inflammation.

As regards the level of IgM, our study revealed normal levels in both groups. Our study did not reveal a significant importance for IgM as a marker for inflammation. This is not in agreement with the results of Borawski et al.⁽¹¹⁾, who stated that IgM level in HD patients is increased and it is a

marker of chronic HD induced microinflammatory state. The normal level for IgM in our study can be explained by the fact that conduction of this study was in pediatric uremic patients with lower immunity than adults, where the other comparative studies were applied.

As regards ristocetin induced platelet aggregation (RIPA), its activity was adequate in the responder group (A), with a mean level of activity $71.7 \pm 4.1\%$.

In group (B) the activity was impaired with a mean activity of $49 \pm 6.6\%$. Comparing both levels, there was a highly statistically significant difference between the two groups ($p < 0.01$). Our cut off value was 66% with sensitivity and specificity 85% and 63.6% respectively. Positive and negative predictive values were 77% and 70% respectively. The RIPA test when performed in platelet rich plasma, estimates functional activity of circulating vWF, which is mostly an endothelial cell (EC) derived multimeric glycoprotein involved in platelet adhesion and aggregation and in blood coagulation. This suggests that whole blood RIPA is an important marker for EC injury in HD patients⁽¹⁸⁾.

Our study revealed that there was a strong correlation between RIPA and Hb changes, so that an increase in Hb level was associated with better function of platelets ($p < 0.01$). This is in agreement with the study done by Borawski et al.⁽¹¹⁾, who stated that RIPA was found to be inversely associated with Hb level in HD patients treated with recombinant EPO.

In order to exclude altered function of platelets due to low platelet count, it should be mentioned that thrombocytopenic

patients and those having bleeding tendencies were excluded from the study. The mean platelet count of our patients was adequate in both groups with no significant difference.

When correlating CRP with Hb changes, there was no significant correlation in either group. This is in agreement with the studies done by Gunnell et al.⁽⁷⁾ and Browaski et al.⁽¹¹⁾.

Also there was no significant correlation between IgM level and Hb changes in either group, but this is not in agreement with the result done by Browaski et al.⁽¹¹⁾.

From our results, it was found that there is a significant negative correlation between ferritin level and Hb changes ($p < 0.05$). This is in agreement with the study of Browaski et al.⁽¹¹⁾, who stated that increased serum ferritin level may represent both abundant iron stores and the presence of inflammation. Also NKF-DOQI consider serum ferritin as a marker of inflammation, so it was likely to find a significant correlation between low Hb level and high ferritin level. Moreover a negative correlation was found between ferritin level and RIPA.

Concluding, a low grade chronic inflammatory state is an important determinant of anemia in clinically stable maintenance HD patients. The microinflammatory effects of HD procedures and dysfunction of vascular endothelial cells could be the distinct reasons for impaired erythropoiesis and poor response to EPO therapy. We recommend early recognition and proper management of anemia in chronic HD patients following NKF-DOQI guidelines. Complete aseptic technique

should be followed during intervention with these patients to minimize occurrence of silent infection with subsequent blunting to EPO. RIPA can be used as an early

predictor of inflammation in chronic HD patients. Also other studies are needed to determine other factors impairing response to EPO as anti-erythropoietin antibodies.

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