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Biochemical Markers of Iron Status in Hemodialysis Patients

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Introduction: Secondary lack of iron in patients on hemodyalisis is the main cause of inadequate answer on therapy of recombinant human erythropoietin (rHuEPO). Therefore, it is very important to follow the status of iron in these patients. Objectives: The objectives of our study were to define the value of hemoglobin content in reticulocytes as predictor of functional iron deficiency on hemodialyzed treated patients with erythropoietin (rHuEPO) then evaluate the eficiency of using the value of hemoglobin content in reticulocytes in administration of iron HD (Patients on hemodialyzed). Patients and methods: It is a prospective study which included 53 patients treated on chronical hemodialysis and continuing hospital peritoneal dialysis (CAPD), all patients were given additional iron therapy intravenously in order to keep the level of ferritin between 300 µg/l and 500µg/ and transferrin saturation over 20%. The patients were both male and female randomly chosen. The following parameters conected to iron deficiency were compared in this study. The study was taken in the period from august to december 2008 at University Clinical Centar Tuzla Results and discussion:The study included patients from chronical HD programme in therapy with rhEPO, iron intravenously, than patients on CAPD also in therapy with rh EPO and intravenously iron and patients on chronical HD with intravenously iron without rh EPO therapy. There wasn't any significant difference between numbers of male and female patients that were examined and in control group. In this study the following parameters conected to iron deficiency were compared. There wasn't any significant difference in values of seruum ferritin, Ret-he and hemoglobin between the examined and control group. Still, it's clear that members of the examined group had higher values of these parameters comparing to the control group. If we would use criterias like the saturation transferrin and the level of ferritin as referent standard we would have 26/53 (49,1%) patients with iron deficiency in the whole sample. Conclusion: Following chematological and biochemic parameters in examined patients on HD are giving us essential information for planing and leading an adequate erythropoietin therapy. For the maximum effect of rhEPO therapy, an adequate compensation of iron is necessary. Keywords: reticulocytes, iron deficiency, hemodyalized patients.

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1. INTRODUCTION

Renal anaemia is an early sign of chronical kidnies damage and it's an often comlpication of kidniey insufficiency. Three pathophysiology mechanism are responsible for the beginning of anaemia. The insufficiency of erythropoeitin synthesized is the primary factor, another mechanism includes inhibition of hema synthesized. Additional mechanisms are a short life time erythrocytes and bleeding tendency that contributes to the developing of anaemia as an hemolitic factor. Other possible causes of anaemia with patients on hemodialysis are lack of iron, folate, vitamin B12 and damaging blood red cells (1). Anaemia with patients who have chronical disactive kidnies is being diagnosed when the value of hemoglobin falls under 11 g/dl. (hematocrit < 33%).

Anaemia diagnosis includes determing concetration of hemoglobin and hematocrit (level of anaemia) and indicators of blood red picture are: number of erythrocytes, mean volumen of erythrocytes MCV (Mean Corpuskular Volumen erythrocitaes), mean content of hemoglobin on erythrocytes MCH (Mean Corpuskular Hemoglobin), mean concetration of hemoglobin on erythrocytes MCHC(Mean Corpuskular Hemoglobin concentration) for evaluating type of anemia, absolute number of reticulocytes (erythropoietic activity), serum ferritin (reserves of iron), functional iron available for erithropoesis (measured by percentage of hypochromic erythrocytes, transferrin saturation and content on hemoglobin in reticulocytes Ret-He), C-reactive protein (level inflammation) and quality dialyzed thretment Kt/V. In some cases additional researches that need to be done include evaluating of ocult gastrointestinal bleeding, concentration of vitamin B12 in serum and folata in erythrocytes, differentional blood pic-

ture, number of thrombocytes, presence of hemolisys and examination of bony marrow.[2]

2. OBJECTIVES

The objective of our study is to define the value of hemoglobin content in reticulocytes as predictor of functional iron deficiency in patients on hemodyalisis treated with erythropoietin (rHuEPO) then evaluate the eficiency of using value of hemoglobin content in reticulocytes in administration in iron HD (Patients on hemodialysis).

3. PATIENTS AND METHODS

It is a prospective study which includes 53 patients on chronical hemodyalisis and continuing hospital peritoneal (CAPD), all patients were given additional iron therapy intravrnously in order to keep the level of ferritin between 300 µg/l and 500µg/ and transferrin saturation over 20%. The patiens were both male and female randomly chosen. In moment of including patients had sabilized hemoglobin (9-11g/dl)- at least two measures in a row.

The normal concentration of iron in organism takes just 30%- 40% capacity transferrin for attaching iron, while the other part of transferrin is free-not saturated with iron.[3]

The Study didn't include patients with malignant disease present. There are no ideal tests for following the iron value in patients who don't have enough iron and are receiving rhEPO therapy. Available tests are measuring only a specific part of methabolic iron which makes a a detailed analysis impossible. Tests that are measuring serum ferritin, transferrin saturation and part in hypochrom erythrocytes are used the most. The main problem with measuring serum ferritn value is not showing presence of functional iron deficiency. Transferrin saturation is presented by a part that is made by dividing serum ferritin with the total capacity conecting iron and it is a better indicator of functional iron deficiency (Fe /TIBC X 100%). The disadvantage of this method is a significant daily biological variation (30%) .

The measuring of hypochrom red cells is a quite new technique, which came out from a new technological advance in automatization of counting cells which makes finding values of hemoglobin in erythrocytes more directly much easier. This procedure uses the flow cytometry method which determines intracellular concentration of hemoglobin in red cells population. Determination of hypochrom red cells is relativly faster and not expensive. The hemoglobin value in reticulocytes is measured by the same methods. The parameters is Ret-he determined on Sysmex XE 2100. In 53 patients from chronical programme dialysis and on CAPD who were on erythropoietin therapy and iron intravenously over three months, usual cheamatological and biochemichal parameters were determined along with concentration of hemoglobin in reticulocytes(Ret-he); red cells, hemoglobin, hematocrit, MCV, MCH. MCHC, thrombocytes reticulocytes, iron, capacity of total iron binding-TIBC, UIBC, ferritin, transferrin saturation, C-RP, transaminases, Ik/V (measure doses of dalysis). All patients were determined by chematological and biochemical parameters monthly. Most patients were given additional iron therapy in order to keep the level of ferritn between 300-500 µg/L. Study was taken in the period from Aaugust to December 2008 year. All patients on chronical dialysis programme received epoietin alfa or beta, three times a week after hemodialysis and intravenously.

4. RESULTS

Statistics are made in software package SPSS 15.0 (Chicago, IL, USA).

The results were analyzed by standard method descriptive statistics. Statistic significance of mean values of the measured parameters were tested by Kolmogorov-Smirnoff's test. For all the calculations we used the significance level of $p<0,05$.

The average values of erithrocytes are compared, MCV, MCH, MCHC and hematocrit under experimental and control group (table 1). Table 1 shows that the control group had significantly higher erithrocytes values, while the experimental group had significantly higher MCH values. There was no significant difference between other analized parameters. Values of examined parameters refering to iron deficit are compared (table 2). There wasn't any significant differences in values of serum iron, reticulocytes of hemoglobin and hemoglobina between experimental and control group. Still, it's obvious that members of the examined group had slightly higher values of presented parameters, than members of control group.

Values od TIBC, UIBC, and transferin were significantly higher in control group (table 3). However, values of tansferin saturation were significantly higher in examined group (table 3). Refrering values of transferin, significant difference was found here significantly higher values of ferritin were found between examiners (table 3).

Using criteria for iron deficiency,

Parameters	Group	Mean	SD	95%CI
Erytrocytes Rbc	experimental	3.38	0.47	0,02
	control	3.89	0.70	
MCV	experimental	96.87	10.14	0,34
	control	94.38	7.74	
MCH	experimental	31.59	2.42	0,02
	control	29.73	2.99	
MCHC	experimental	320.88	8.87	0,09
	control	315.81	12.76	
Hematocrit Ht	experimental	.35	0.12	0,62
	control	.36	0.06	

Table 1. Comparative showing of average values Rbc, MCV, MCH, MCHC i Ht under experimental and control group

Parameters	Group	Mean	SD	95%CI
Iron	experimental	16.84	9.81	0,42
	control	14.70	8.71	
TIBC	experimental	37.95	9.81	0,005
	controa	47.29	13.22	
UIBC	experimental	21.12	11.30	0,002
	controa	32.53	13.84	
Transferin	experimental	1.58	0.42	0,001
	control	2.07	0.60	
Saturation transferin	experimental	44.64	23.90	0,04
	control	32.06	17.37	
Ferrtin	Expe.	715.41	411.95	0,02
	Kontrola	403.03	566.39	
Ret-He	experimental	33.92	3.52	0,16
	control	32.32	4.66	
Hemoglobin	experimental	105.75	15.77	0,06
	control	115.24	18.64	

Table 2. Comparative showing of average values with parameters refering to iron deficit between experimental and control group.

			Ferritin		
			<100	100-500	>500
Group	Experimental	N	3	6	23
		%	9,4%	18,8%	71,9%
	Control	N	12	2	7
		%	57,1%	9,5%	33,3%
Total		N	15	8	30
		%	28,3%	15,1%	56,6%

Table 3. Comparative showing of number of patients with different categories of level of ferritin under experimental and control group.

frequencies of members of examined group considering number of patients with transferrin saturation less than 20 %, and the level of feritin below 100 μg/L (absolute deficit), between 100 i 500 μg/L (relative deficit).

5. DISCUSSION

During the study, patients with chronical hemodialysis who were on **e**rythropoietin (rh EPO) i intravenously therapy or per os iron, values of biochemichal and hematological parameters were determined, that can be helpful in evaluating functional iron deficiency. In time before ESA (agens that stimulates erithropoesys), dialyzed patients often had iron suficit (with ferritin higher than 1000μg/l), because of polytransfusia [4]

Adequate quantity of available iron is increasing erithropoesys and decreasing the need for therapy with agens that are stimulating erithropoesys [5]. Absolute iron deficit is whith low capcities of iron, while functional iron deficit is with wrong mobilization of capacities of bony marrow.[6]

Clear aditude about optimal level of hemoglobin is still not accepted, especialy when talking about wheter it is the same hemoglobin for all patients.

It is proved that lower level of hemoglobin, at the beginning of a dialyzed threatment is significantly increasing risks refering to cardiac complication and death in first year of dialyzed and that early threatment can prevent such condition [8,9]

The average age in all samples was equal (53±9 year with min. 22 and max. 70 years old). There was a significant difference between the experimental (53±11 years) and the control group (53±7), while the number of examined members on chronical hemodialyzed programme and number of examined members of control group on continous (CAPD), was significantly different (p=0,02).

Average values of erithrocytes, MCV, MCH, MCHC i hematocrit under experimental and control group are compared. The control group had significantly higher values of erithrocytes (p=0,02) while the examined group had significantly higher values MCH (p=0,02), which was expected and the reason why they are getting rh EPO .

Values of reticulocytes, fraction immature reticulocytes, reticulocytes low, mean high fluorescentie, thrombocytes i CRP between experimental and control group**.** There wasn't any significant difference, except in higher values of reticulocytes in examined group, p-value (0,02) indicates on statisticly significant difference. These are a reasonable higher number of reticulocytes as response to rhEPO therapy.

In the study (1997)[10] was found that values of hemoglobin in reticulocytes are precisely accurate, and suitable for iron deficiancy diagnosys with hemodialyzed patients who have values less than 26 pg, shows presence of iron deficiancy. Sensitivty of 100% and spcificity of 80%, were significantly more accurate than the ferritin and transferin saturation proved. The same study found that ferritin has no level that is accurate for iron deficiancy diagnosys , and at the same time it's proved that transferin saturation of 21% has a sensitivity of 81%, but specificity of 63%. When only transferrin saturation and serum ferritin are taken, they have small a sensitivity in diagnosys of iron status with patients in hemodialysis. When these values divergate, they became unreliable in leading iron therapy and generaly indicate on functional iron deficit[11]. However, increasing concentration of ferritin in serum is not allways combined with increasing of iron content on depou, but sometimes in pathological conditions is a result of stronger relasing of ferritn from tissue or stronger sintesys of apoferritin mostly combined with strong sintesys of proetina acute phase. By increasing the concentration of ferritin in blood plasma is happening in period of acute virus hepatytis, and also in period of chronical inflammatory process and other pathological conditions[12].

In our research, values of following parameters refering to iron deficit are compared. There wasn't any significant difference in values of iron serum, Rethe and hemoglobin between experimantal and control group.

However, it is clear that members of the examined group had slightly higher values of these parameters than the control group. Comparing the values of TIBC, UIBC, and transferrin between experimental and conrtol group are statisticly significantly different.

Transferrin saturation values were significantly higher in examined group (44,64 ± 23,9), than control group (32,06±17,37). When talking about values of ferritin, statisticly singinificant difference was found (p=0,02) – significantly higher values of ferritin with examined members (715,41±411,95) than in control group.

Level of serum ferritin is frequently increasing, independent from iron status, with factors such as inflammatory process**,** disease liver. Some studies are showing that with dialysed patients there is inflammation with a frequency of 30-50%[13]. In the last phase of chronical kidney insufficiency, many patients have a biochemical reaction **„**chronical inflammation reaction**"**, that cause increasing of circulating level of reactants acute phase of inflammation such as CRP and amiloid A and secretoric production inflammator cells cytokinins (13) .

Using cirteria for iron deficit, frequencies of examined group refering to number of patients with transferrin saturation less than 20%, and with level of ferritin below 100 μg (absolute deficit), between 100 i 500μg (relative deficit), and over 500μg, are analized. Frequencie**s** o**f** patients who have a level of hemoglobin in reticulocytes less than 28 pg, are also analized. Iron deficiency is either absolute, when all body reserves are unavailable, or functional, when body has insuffcient or even overloaded quantity of iron that can't be activated fast enough for needs of bony marrow**.**

Number of all patients with transferrin saturation below 20% in complete sample was 13/53 (24,5%). Significantly a higher number of control group (X^2=4,26; df=1; p=0,04) had lower transferrin saturation than the examined group.

In the complete sample there were

15/53 (28,3%) members examined with ferritin <100μg, and 8/53 (15,1%) members with ferritin between 100 i 500 μg.

There was significantly a higher number of examined members od control group (X^2=14,27; df=2; p=0,001) who have levels of ferritin below 100μg. Comparative showing of number of patients considering level of contens of hemoglobin in reticulocytes, transferrin saturation and level of ferritin, is made.

If we would use as referent standard criteria of transferrin saturation and level of ferritin, we would have 26/53 (49,1%) patients with iron deficiency in the complete sample.

Concentration of hemoglobin in reticulocytes is tested, using Receiver Operating Characteristics (ROC) analisys.

Surface below ROC (AUROC) curve for reticulocytes Hb was 0,73 (%95 CI=0,59-0,84) and was statisticly significant (p=0,001).

Using information provided by ROC curve, target value reticulocytes Hb with the best dignostics performances was 31,1 pg. This value had sesitivity of 50%, spcificity of 96%, positive predictive value of 93% and negative predictive value of 68%.

Slika 10 : Receiver Operating Characteristics (ROC) curve of diagnostic accuracy retikulocitnog hemoglobina compared to usual criteria for deficit iron serum with patients on dialyzed

6. CONCLUSION

Following chematological and biochemic parameters in examined patients on HD is giving us essential information for planing and leading an adequate erythropoietin therapy. Determinaiton of hemoglobin content in retcoulocyt has atributes that can provide an ideal test of iron status in HD patients spcified for iron deficiency diagnosys. For maximal efect of rhEPO therapy, adequate compensation of iron is necessary. With chronical kidney patients, transfusion of erithrocytes should be avoided[14]. For treatment of renal anemia an optimal dialysis is crucial [15] .

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