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Evaluation of serum Hepcidin level and Iron profile among Sudanese patients with anemia of end stage kidney disease

Abstract

Background: Anemia of chronic disease is anemia found in certain chronic disease state, is typically marked by the disturbance of iron homeostasis or hypoferremia. This condition leads to shortage of iron for hemoglobin synthesis but the iron storage in bone morrow is left undisturbed. Patients with chronic kidney disease are usually anemic because of defective erythropoeisis and inflammation.

Materials and methods: Some of red blood cell profile (Hb, PCV, RBCs count and RBCs indices) were determined by the automated Hematology Analyzer and Cobas e 411 was used to determine the levels of serum iron, ferritin, TIBC, and transferrin saturation percentage. Enzyme – Linked immunoassay (ELISA) was used to determine the level of hepcidin**.**

Results: The results show the mean of the RBCS profile (RBCs count, Hb, PCV) (3.353±0.88cell/l, 10.62±2.4g/dl, 32.59±6.82%) in patients with ACKD Vs (4.048±0.47cell/l, 12.52±1.57g/dl, 37.92±4.79%) in control groups P.value (0.000, 0.000, and 0.000) respectively. Serum hepcidin levels higher in patients with ACKD compared with healthy controls mean (161.55±29.8ng/ml Vs 82.05±13.4ng/ml. P. value (0.000). The mean value of the iron profile, S. iron, S. ferritin and TS % (61.353±29,8ug/dl, 195.3.62±19.4ng/ml, 21.59±12.82%) in patients with ACKD Vs (82.048±0.47ug/dl, 80.52±1.57ng/ml, 28.92±4.79%) in control groups P.value (0.000, 0.000, and 0.000) respectively.

Conclusion: In the present study there is significant association between CKD and RBCS profile (RBCs count, Hb, PCV). The hepcidin levels were significantly higher in patients with ACKD compared with healthy controls. The Statistical significant differences showed in the comparison between the study variables (RBCs profile, Iron profile, hepcidin level) and the end stage of CKD (dialysis dependent), in the RBCs count, Hb, PCV, S. iron, S.ferritin, TIBC. TS %, hepcidin level.

Keywords: hepcidin, anemia of chronic kidney disease

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Abbreviations: ACKD, anemia of chronic kidney disease; CKD, chronic kidney disease; CRP, c-reactive protein; ESKD, end stage kidney disease; ELISA, enzyme linked immuno assay; GFR, glumerate filtration rate; Hb, hemoglobin, TIBC, total iron binding capacity; TS, transferrin saturation; RBCS, red blood cells; SPSS, social packages statistical; S, significant; S. Iron, serum iron; S. Ferritin, serum ferritin; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; NS, non significant

Introduction

Anemia of chronic disease as it is now understood is to at least some degree separate from the anemia seen in chronic renal disease in which anemia results from poor production of erythropoietin, or the anemia caused by some drugs, that have the side effect of inhibiting erythropoiesis. In other words, not all anemia seen in people with chronic disease should be diagnosed as anemia of chronic disease.[1,2] **(Liao et al., 2012) (Roy et al., 2010)**. Anemia of renal failure by insufficient erythropoietin production in response to inflammatory cytokines, increasingly IL-6, the liver produces increased amounts of hepcidin. Hepcidin in turn causes increased internalisation of ferropoietin molecules on cell membranes which prevents release from iron stores. Inflammatory cytokines also appear to affect other important elements of iron metabolism, including decreasing ferropoietin expression, and probably directly blunting erythropoiesis by decreasing the ability of the bone marrow to respond to erythropoietin.

Materials and methods

Study participants

The study was carried out on diagnosed 200 patients with anemia of end stage chronic kidney disease, 100 dialysis dependent and 50 non dialysis. Besides 50 apparently healthy individuals were recruited from the same center as co-patients and included as healthy control group (age 35.90 +_ 12.58). All selected individuals were subjected to assessment of history, thorough clinical examination, and routine laboratory investigations such as complete blood cell count CBC (RBcs profie), Iron profile (Serum. iron, Serum, ferritin, TIBC transferring saturation percentage), and measurement of serum hepcidin by using ELISA. They were selected from Nephrology Unit of Bahri Hospital, the dialysis sessions for these patients were three to two times weekly and the duration range of each sessions was four hours.

Blood sample collection

Whole blood 5 ml was collected by venepuncture for complete blood cell count. Red blood cell profile, Hb, PCV, RBcs count, MCV, MCH and MCHC), and 2.5 ml was collected into a plain tube. The serum was aliquoted and stored at -70^{0}c for iron profile serum iron,

ferritin, TIBC, and transferrin saturation percentage, hepcidin is measured before hemodialysis for stage 5 patients.

Laboratory analysis

Complete blood cell count (RBcs pofile) Hb, PCV, RBcs count and RBcs indices were measured and calculated by the automated Hematology Analyzer K x-21 (Kobe, Japan). Cobas e 411 (Roche, analyzer series, Germany) was used to determine the levels of serum iron, ferritin, TIBC, and transfeerin saturation percentage. Enzyme Linked immunoassay was used to determine the level of serum hepcidin state fax 4200. (CDRG, Germany. Sandwich- ELISA).

Statistical analysis

Statistical assessment was carried out with SPSS. Data of correlation between serum hepicdin level and IL-6 level variants and qualitative variables were tested by crosstablatien and chi-square test, means of age and duration were compared by anova test.

Results

A total of 250 individuals (100 patients with end stage of dialysis, 100 patients with ACKD divided into 25 stage 1, 25 stage 2, 25 stage 3 and 25 stage 4 and 50 healthy control). 50% male other 50% female with a mean age 33-$^{+}$10.1 years were recruited for the present study. Statistical significant differences were observed in the mean of red blood cell profile (Rbcs count, Hb and PCV) between patients and control P. value (0.000, 0.000, and 0.000), respectively, and not observed in the MCV, MCH and MCHC (Table 1). The mean value of the iron profile (serum. iron, serum. ferritin and TS %) between patients and control showed significant differences P. value (0.000, 0.000, 0.000), respectively, and not observed in TIBC (Table 2). The statistical significant differences observed in hepcidin level between patients and control p. value (0.000) (Table 3). Also statistical significant differences observed in patients with ACKD according to gender (male and female) in the RBCs count, Hb and PCV, p. value (0.004, 0.004, 0.001) and no observed in the red blood cell indices (Table 4). The mean of hepcidin level in patients with ACKD show no significant differences according to gender (Table 5).

Table 1 Mean of red blood cell profile in patients with chronic kidney disease and control

RBCs profile	Case	Control	P value
RBCs count cell/l	3.353±0.88	4.048±0.47	0
HB g/dl	10.62±2.4	12.52±1.57	0
PCV %	32.59±6.82	37.92±4.79	0
MCV fl	93.09±10.12	93.04±4.28	0.974
MCH pg	34.95±33.55	30.59±1.42	0.068
MCHC g/l	316.34±37.58	368.10±397.37	0.07

Table 2 Mean of serum iron profile in patients with chronic kidney disease and control

Iron profile	Case	Control	P value
Iron µg/dl	61.55±29.8	82.05±13.4	0
Ferritin ng/ml	195.322±192.24	80.89±60.94	0
TIBC µg/dl	253.97±77.87	260.32±52.49	0.586
TS %	21.33±12.72	28.17±4.70	0

Table 3 Mean of serum hepcidin level in patients with chronic kidney disease and control

Variable	Case	Control	P value
Hepcidin ng/ml	61.55±29.8	82.05±13.4	0

Table 4 Mean of red blood cell profile in patients with chronic kidney disease according to gender

RBCs profile	Male	Female	P value
RBCs count cell/l	3.770±0.93	3.462±0.67	0.004
HB g/dl	11.38±2.52	10.51±2.05	0.004
PCV %	34.84±7.16	32.10±5.80	0.001
MCV fl	92.85±9.89	93.40±8.36	0.639
MCH pg	36.64±39.66	30.72±3.03	0.079
MCHC g/l	316.01±36.40	340.73±271.75	0.285

Table 5 Mean of serum hepcidin level in patients with chronic kidney disease according to gender

Variable	Male	Female	P value
Hepcidin ng/ml	15.97±14.24	14.24±10.83	0.293

Correlation studies

Correlation coefficients were obtained, and significant correlations were seen between red blood cell profile (RBCs count, Hb and PCV) and duration of disease p value (0.001, 0.005, 0.003) respectively, (Table 6) and also significant correlation between Serum.iron and Serum.ferritin p value 0.000 and 0.010 (Table 7). No statistical significant correlations were observed between the level of hepcidin, age and duration of disease (Table 8). The comparison of the study variables (RBCs profile, Iron profile, hepcidin levels) between the 5 stages of CKD showed statistical significant differences in the RBCs count, Hb, PCV, s.iron, s.ferritin, TIBC. TS %, hepcidin level and no statistical significant differences were seen in the MCV, MCH and MCHC (Table 9).

Table 6 Correlation of red blood cell profile with duration of disease and age

RBCs profile	Duration R/Pearson	P .value	Age R/Pearson	P value
RBCs count cell/l	-0.239	0.001^{S}	-0.08	0.21
HB g/dl	-0.198	0.005^{S}	-0.05	0.428
PCV %	-0.209	0.003^{S}	-0.043	0.494
MCV fl	0.028	0.698^{NS}	0.03	0.641
MCH pg	-0.056	0.430^{NS}	-0.076	0.234
MCHC g/l	-0.028	0.698^{NS}	-0.067	0.289

Table 7 Correlation of serum iron profile with duration of disease and age

Iron profile	Duration R/Pearson	P. value	Age R/Pearson	P value
Iron µg/dl	-0.285	0.000^{S}	-0.044	0.493
Ferritin ng/ml	0.182	0.010^{S}	-0.111	0.081
TIBC µg/dl	0	1.000^{NS}	-0.008	0.902
TS%	-0.207	0.003^{S}	-0.01	0.877

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Table 8 Correlation of serum hepcidin level with duration of disease and age

Variables	Duration R/Pearson	P. value	Age R/Pearson	P .value
Hepcidin ng/ml	0.15	0.034[S]	0.102	0.106

Table 9 Comparison of the red blood cell profile, iron profile, hepcidin between all CKD stages (5 stages)

Variables	CKD stages (I)	CKD stages (J)	Mean of difference (I -J)	P value
		Stage 1	-0.524	0.005
		Stage 2	-0.036	0.847
Rbcs count Cell/L	Stages	Stage 3	0.62	0.001
		Stage 4	1.06	0
		Stage 5	1.989	0
		Stage 1	-0.196	0.643
		Stage 2	1	0.019
HB g/dl	Stages	Stage 3	2.692	0
		Stage 4	4.229	0
		Stage 5	4.767	0
		Stage 1	-0.804	0.518
		Stage 2	2.116	0.09
PCV %	Stages	Stage 3	6.916	0
		Stage 4	11.627	0
		Stage 5	11.98	0
		Stage 1	-4.012	0.454
		Stage 2	-14.076	0.009
S.Iron ug/dl	Stages	Stage 3	20.048	0
		Stage 4	43.263	0
		Stage 5	43.989	0
		Stage 1	1.16	0.981
		Stage 2	21.844	0.66
Ferritn ng/ml	Stages	Stage 3	5.792	0.907
		Stage 4	170.96	0
		Stage 5	170.96	0
		Stage 1	4.036	0.851
		Stage 2	19.016	0.378
TIBC ug/dl	Stages	Stage 3	34.056	0.115
		Stage 4	45.669	0.008
		Stage 5	47.767	0
		Stage 1	0.452	0.893
		Stage 2	2.528	0.451
TS%	Stages	Stage 3	7.904	0.019
		Stage 4	11.219	0
		Stage 5	13.456	0

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Table Continued...

Variables	CKD stages (I)	CKD stages (J)	Mean of difference (I -J)	P value
		Stage 1	8.908	0.007
		Stage 2	3.092	0.342
Hepcidin ng/ml	Stages	Stage 3	0.8	0.805
		Stage 4	18.751	0
		Stage 5	19	0

Discussion

Anemia is commonly seen in all stages of kidney disease but much more pronounced in patients with end-stage kidney disease.[3,4] In this study there is significant association between chronic renal disease and RBCs count, Hb and PCV, hepcidin levels were significantly higher in patients with ACKD compared with healthy controls. comparable results were also reported in other studies.[5–7] It also been indicated that hepcidin levels were approximately two-to three –fold higher in patients with ACKD than in the controls.[8] Hepcidin levels are expected to be elevated in patients with ACKD due to limited hepcidin excretion in urine, tissue iron overload and inflammation.[9–13] Among our study group patients, we found decreased levels of serum iron, TIBC and TS%, However, serum ferritin levels were found to be elevated in this group. Findings consistent to our have been seen in a study on patients with CKD.[14–18] The situation in which the serum iron is low and the serum ferritin is high is frequently seen among ACKD patients, High ferritin levels may be observed in this disease because of functional iron deficiency or reticulo-endothelial blockade. Our study show in the first time the comparison between the study variables (Rbcs profile, Iron profile, hepcidin level) in the all stages of CKD (5) stages, showed statistical significant differences in the RBCs count, Hb, PCV, s.iron, s.ferritin, TIBC. TS %, hepcidin level and IL-6 level and no statistical significant differences seen in the MCV, MCH and MCHC, we found that the severity of CKD can increase the severity of anemia, influencing in the iron status and increase the levels of hepcidin. Evaluate whether the study variables was influenced by the gender affected with ACKD (male and female), significant relation was observed in between male and female in the RBCs profile (RBCS count, Hb and PCV), and was influenced by the age and duration of disease, we found positive correlation between the duration of disease and RBCS profile (RBCS count, Hb and PCV), hepcidin and with serum iron and serum ferritin, concluding that the gender and duration of disease can influence but the levels of hepcidin cannot influenced by the gender and age (Figure 1).[19–23]

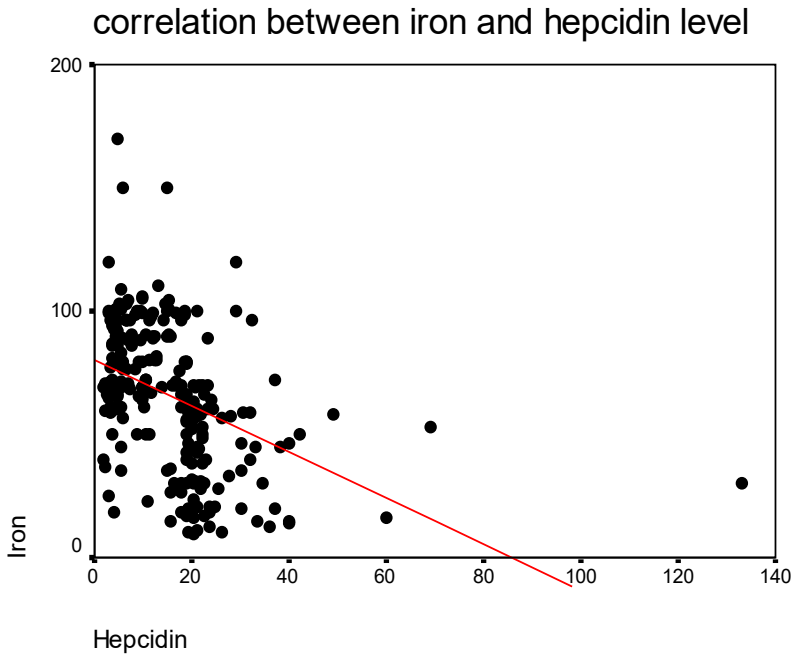

Figure 1 Medium negative correlation of serum iron with hepcidin.

Conclusion

In this study there is significant association between CKD and RBCs count Hb and PCV). Hepcidin level was significantly higher in patients with ACKD compared with healthy controls. It known that hepcidin synthesis is induced by inflammation, and the significant relation was observed between male and female in the RBCS count, Hb and PCV, and was influenced by the age and duration of disease, we found positive strong correlation between the duration of disease and RBCS count, Hb and PCV, hepcidin and with serum iron and serum ferritin, concluding that the gender and duration of disease can influence but the levels of hepcidin cannot influenced by the gender

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and age. Our study show in the first time the comparison between the study variables, RBCs profile, Iron profile and hepcidin level in the all stages of CKD, showed statistical significant differences in the RBCs count, Hb, PCV, s. iron, s. ferritin, TIBC. TS %, hepcidin level and IL-6 level and no statistical significant differences seen in the MCV, MCH and MCHC, we found that the severity of CKD can increase the severity of anemia, influencing in the iron status and increase the levels of hepcidin.

Acknowledgments

None.

Conflicts of interest

None.

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