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Risk Factors of Anemia in Pre-end-Stage Renal Disease

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ABSTRACT
Background: Anemia is a common complication of chronic kidney disease (CKD). Observational studies indicate that low Hb levels in such patients may increase risk for progression of kidney disease and cardiovascular morbidity and mortality. **Objective:** The aim of the work is to study the different risk factors contributing to anemia in pre-end stage renal disease patients (stage 3 and 4 CKD).
Subjects and methods: The study involved 50 patients with history of CKD, not receiving any treatment or dialysis, 29 men and 21 women, 22-72 years old. In addition to 20 healthy persons as a control group. They were randomly selected from the outpatient clinics and inpatient department of Internal Medicine Departments, Al-Azhar Assiut University Hospitals over a period of two years.
Results: There were different risk factors causing anemia among patients group; 1-Anorexia was present in (44%) of patients, 2-Fecal occult blood test was positive in (6%) of patients, 3-The indicators of inflammation including high levels of CRP, ESR (were high in 100%) and serum ferritin was high in (80%) of patients, 4-The indicators of "uremic milieu" including increased level of blood urea and S. creatinine and moderate to severe albuminuria in addition to decreased level of GFR, all were present in (100%) of patients, and 5-erythropoietin deficiency also was present in (54%) of patients. **Conclusion:** Our study concluded that anemia in pre end stage renal disease (ESRD) is multifactorial and the prevalence of different risk factors is inversely correlated with hemoglobin level.
Keywords: Risk Factors, Anemia, Pre-end Stage Renal Disease.

INTRODUCTION
Chronic kidney disease (CKD) is an important, chronic disease that affects humans all over the world. It is characterized by irreversible impairment of excretory, metabolic and endocrine functions of the kidney leads to the development of clinical syndrome of uremia **(1)**. Anemia is a common complication of CKD. The prevalence of anemia varies with the degree of renal impairment in pre-dialysis patients with CKD, but once end-stage kidney failure occurs, all patients are eventually affected **(2)**. Anemia develops once renal function decreases to 50% because of a deficiency in endogenous erythropoietin production by the kidney, decreased red cell survival, blood losses, and increased red blood cell destruction once the patients begin dialysis treatment, particularly hemodialysis **(3)**. Observational studies indicate that low Hb levels in such patients may increase risk for progression of kidney disease and cardiovascular morbidity and mortality **(4)**.

AIM OF THE WORK
The aim of the work is to study the different risk factors contributing to anemia in pre-end- stage renal disease patients (stage 3 and 4 CKD).

SUBJECTS AND METHODS
Subjects: The study involved 50 patients with history of (CKD), not receiving any treatment or dialysis, 29 men and 21 women, 22-72 years old. In addition to 20 healthy persons as a control group. They were randomly selected from the outpatient clinics and inpatient department of Internal Medicine Departments, Al-Azhar Assiut University Hospitals over a period of two years.

Inclusion criteria*: Patients with documented chronic kidney disease of:* serum creatinine $\geq$ 2 mg/dl , GFR < 60 and >15 mL/ minute/1.73 m^2 (i.e. CKD stage III and IV(pre ESRD).
Exclusion criteria**;** f*or patients group*: patients with ESRD or those on dialysis.
For both patients and control groups:
- Recent severe hemorrhagic episode.
- Recent blood transfusion
- patients receiving iron or erythropoietin therapy
- Malignancy or known hematological disorder

Methods:
Ethical consideration and Written informed consent:
An approval of the study was obtained from Al-Azhar University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

After providing informed consent, all subjects were subjected to:
1-Full history taking with stress on nutritional history and anorexia, history of chronic diseases, any drug use, stigmata of CKD.
2- Complete physical examination and assessment of body mass index.
3-Abdominal US.

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4-Laboratory investigations including; CBC, ESR, C-reactive protein, S. albumin and total protein, S. potassium and Kidney function tests, urinary albumin-creatinine ratio in early morning spot urine sample, overt proteinuria by dipstick method and stool examination for occult blood, iron measurements including : S. iron, S. ferritin, total iron binding capacity and transferrin saturation percent, and erythropoietin assay.

5-Estimation of (GFR) using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula.

Statistical analysis

Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as frequency and percentage.

The following tests were done:

- Independent-samples t-test of significance was used when comparing between two means.
- Chi-square (x^2) test of significance was used in order to compare proportions between two qualitative parameters.
- The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered significant as the following:
- Probability (P-value)
- P-value <0.05 was considered significant.
- P-value <0.001 was considered as highly significant.
- P-value >0.05 was considered insignificant.

RESULTS

1- Our results revealed other chronic diseases and anorexia in the studied patients as shown in Table 1.

Table (1): Past and nutritional history in the studied patients

Parameter	No. (n= 50)	%
Diabetes mellitus	26	52.0
Hypertension	26	52.0
Ischemic heart diseases,	11	22.0
Rheumatoid arthritis	2	4.0
Rheumatic heart disease	1	2.0
Systemic lupus erythematosus	8	16.0
Anorexia	22	44.0

2- The clinical signs in the studied patients included: pallor, facial puffiness, lower limbs edema and other clinical signs as illustrated in table (2).

Table (2): Physical examination of the studied patients

Parameter	No. (n= 50)	%
Pallor:		
Absent	**5**	
Present	**45**	
Facial puffiness:		
Absent	**29**	
Present	**21**	
Lower limbs edema:		
Absent	**31**	
Present	**19**	
Others:		
Basal lung crepitations	**8**	
Cushioned face Malar flush	**3**	
	1	
Mitral regurge	**1**	
No other clinical signs	**37**	

3- Our study revealed (Table 3):

- Highly significant differences between patients and control in Hb, HCT, RBCs count, MCH, MCHC and reticulocyte count. All of these are higher in control than patients.
- Significant differences between patients and control in platelets count; it is higher in the control than patients group.
- No significant differences between patients and control in WBCs count and MCV.
- Highly significant differences between patients and control in complete haemogram (CHg) as follows: the prevalence of anemia is higher in patients group than control.

Table (3): Hematology Panel

	Patients (n= 50)	Control (n= 20)	P-value
HB (g/dl): Mean ± SD	**10.20 ± 1.22**	**14.02 ± 1.40**	**0.001***
Hematocrit value (%) Mean ± SD	**32.26 ± 3.63**	**41.21 ± 4.05**	**0.001***
WBCs (x 10^3): Mean ± SD	**6.06 ± 1.49**	**6.63 ± 1.36**	**0.120**
Platelets (x 10^3): Mean ± SD	**222.86 ± 42.33**	**248.40 ± 36.43**	**0.021***
RBCs (x 10^6): Mean ± SD	**4.22 ± 0.28**	**4.79 ± 0.41**	**0.001***
Mean corpuscular volume (femtoliter): Mean ± SD	**85.74 ± 7.64**	**83.84 ± 5.11**	**0.128**
Mean corpuscular hemoglobin (picogram/cell): Mean ± SD	**28.19 ± 2.20**	**29.55 ± 1.83**	**0.008***
M mean corpuscular hemoglobin concentration, (g/deciliter): Mean ± SD	**32.31 ± 2.25**	**34.69 ± 1.81**	**0.001***
Reticulocyte count (%): Mean ± SD	**0.62 ± 0.19**	**0.84 ± 0.17**	**0.001***
Anemia type according to RBC indices(MCV , MCH &MCHC) Microcytic-Hypochromic Normocytic-Hypochromic Normocytic-Normochromic Normal: **complete blood count.**	**7 (14.0%) 7 (14.0%) 36 (72.0%) 0 (0.0%)**	**2 (10.0%) 1 (5.0%) 0 (0.0%) 17 (85.0%)**	**0.001***

4- There were highly significant differences between patients and control in prevalence of anemia, it is higher in patients than control (Figure 1).

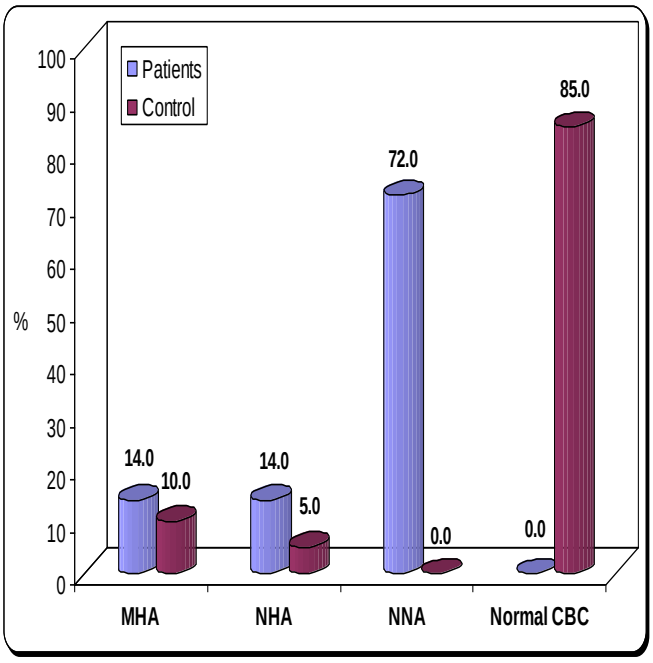

Figure (1): Anemia type according to RBC indices

5- There was highly significant difference between patients and control in: serum albumin and total protein (figure 2); blood urea and serum creatinine (Table 4); in GFR (Table 4); urinary albumin/creatinine ratio (Table 4) and in erythropoietin level (figure 3).

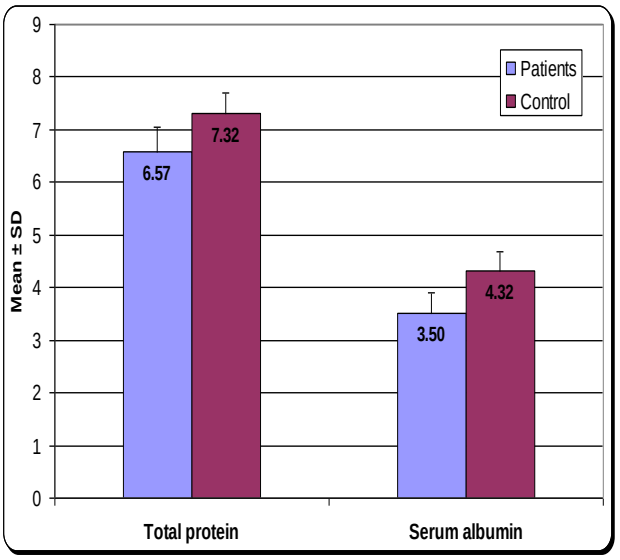

Figure (2): Serum total protein and albumin

Table (4): Traditional kidney function tests, Glomerular Filtration Rate and Urinary albumin/creatinine Ratio of both groups

Renal profile	Patients (n= 50)	Control (n= 20)	P-value
Blood urea: Mean ± SD	105.62 ± 1.1	33.70 ± 4.65	0.001*
S. creatinine: Mean ± SD	2.56 ± 0.58	0.79 ± 0.1	0.001*
GFR: Mean ± SD	26.46 ± 6.91	100.97 ± 9.87	0.001*
Urinary albumin/ creatinine Ratio: Mean ± SD	390.34 ± 9.72	17.72 ± 0.39	0.0001*

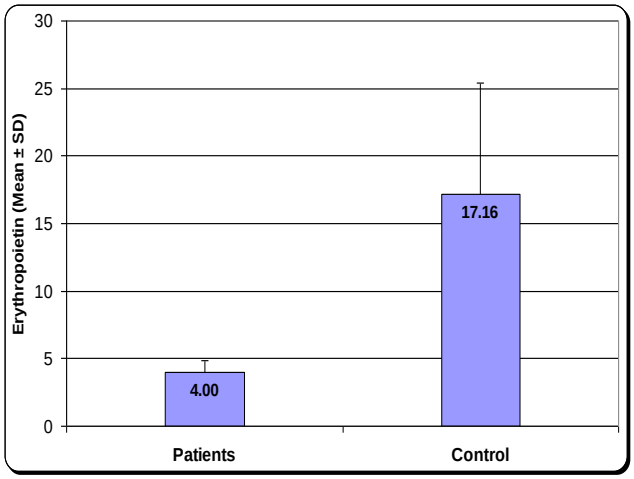

Figure (3) Erythropoietin of Patients and control

6- The prevalence of CKD stages (III and IV) among studied patients is shown in Table 5.

Table (5): Chronic kidney disease stages (III and IV)

Chronic Kidney disease stage	No. (n= 50)	%
Stage III	16	32.0
Stage IV	34	68.0

In the current study, there were (Table 6);

7- Highly significant differences between patients and control in serum iron.

- Significant differences between patients and control in TSAT and serum ferritin.
- No significant differences between patients and control in TIBC.

Table (6): Iron profile of studied groups

	Patients (n= 50)	Control (n= 20)	P-value
S. iron: Mean ± SD	**52.38 ± 2.40**	**76.90 ± 2.16**	**0.001***
TIBC: Mean ± SD	**244.40 ± 58.99**	**260.25 ± 35.33**	**0.187**
TSAT: Mean ± SD	**23.03 ± 8.25**	**30.28 ± 1.35**	**0.009***
S. ferritin: Mean ± SD	**686.69 ± 36.89**	**387.71 ± 86.37**	**0.001***

TIBC: Total iron binding capacity, TSAT: Transferrin saturation

8- The different grades of medical renal diseases in the studied patients as detected by abdominal ultrasonography are shown in Table 7.

Table (7): Abdominal ultra-sonogram of studied patients

Abdominal ultra-sonogram	No. (n= 50)	%
Grade I MRD	6	12.0
Grade II MRD	20	40.0
Grade III MRD	24	48.0

9- There was significant positive correlation between hemoglobin (Hb) level and glomerular filtration rate (GFR) (Figure 4).

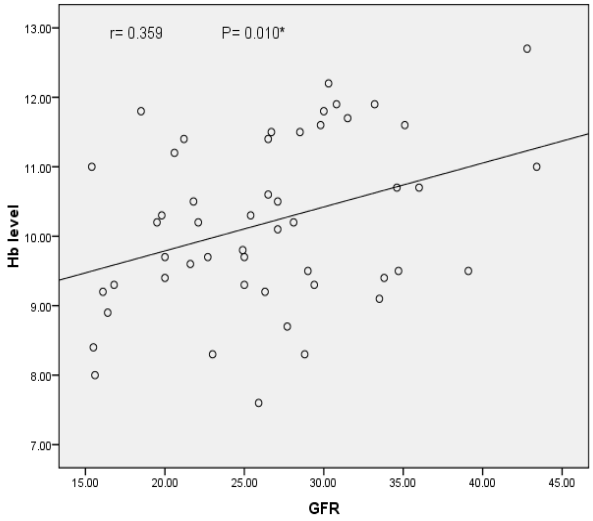

Figure (4): Correlation between hemoglobin (Hb) level and glomerular filtration rate (GFR)

10- There was highly significant positive correlation between erythropoietin level and glomerular filtration rate (GFR) (Figure 5).

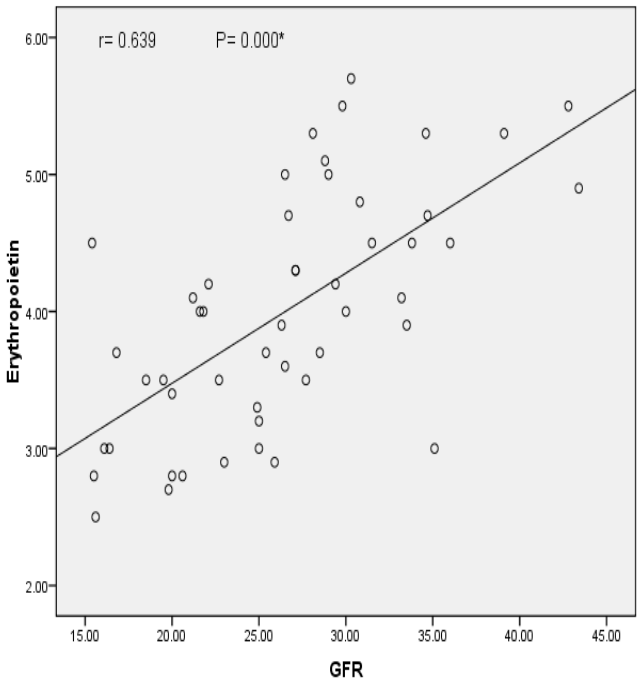

Figure (5): Correlation between erythropoietin level and glomerular filtration rate (GFR)

11-Prevalence of risk factors of anemia in the studied patients are shown in Table 8.

Table (8) Prevalence of risk factors of anemia

Risk factors	No.	%
Anorexia	**22**	**44.0**
Positive occult blood in stool	**3**	**6.0**
Increased CRP	**50**	**100.0**
Increased ESR	**50**	**100.0**
Increased Serum Ferritin	**40**	**80.0**
Increased Blood urea	**50**	**100.0**
Increased Serum creatinine	**50**	**100.0**
Decreased GFR	**50**	**100.0**
Erythropoietin deficiency	**27**	**54.0**

12-There was highly significant inverse correlation between number of risk factors and hemoglobin level. The more increase in the number of risk factors of anemia in renal patients, the more is the decrease in hemoglobin level (Figure 6).

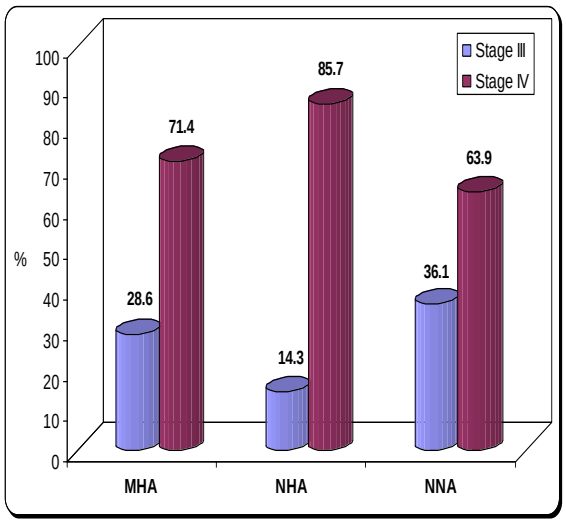

Figure (6): Correlation between number of risk factors of anemia in CKD and Hb level

13-The relation between type of anemia and CKD stage in the studied patients was as follows

- Among patients of microcytic-hypochromic anemia (MHA); 2 patients (28.6%) are stage III CKD while 5 patients (71.4%) are stage IV CKD.
- Among those of normocytic-hypochromic anemia (NHA); 1 patient (14.3%) is stage III CKD while 6 patients (85.7%) are stage IV CKD.
- Among patients of normocytic-normochromic anemia (NNA); 13 patients (36.1%) are stage III CKD while 23 patients (63.9%) are stage IV CKD.

MHA, NHA and NNA all are present in studied patients, but the prevalence of every type is increasing on transition from stage III to Stage IV. (Figure 7)

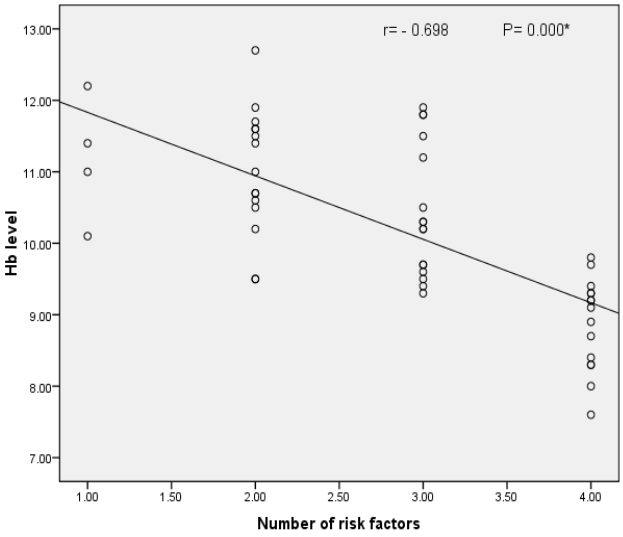

Figure (7): Relation between type of anemia and CKD stage in studied patients

DISCUSSION

Anemia is strongly predictive of complications and death from cardiovascular causes in patients with chronic kidney disease [5]. The morbidity and mortality depend greatly on the underlying etiology of the patient's anemia as well as the stage of the disease, whether early or advanced. In fact, in individuals with advanced stages of chronic kidney disease, the etiology of anemia tends to be multifactorial [6].

By doing abdominal ultrasonography (US) for studied patients to detect the underlying cause and as evidence of chronicity of kidney disease we found that; different grades of medical renal disease (MRD) were present among the studied patients as follows: 6 patients (12%) were grade I MRD, 20 patients (40%) were grade II MRD, and 24 patients (48%) were grade III MRD. These results agree with **Chen *et al.*** [7] who found same changes in US among the studied patients of CKD and were associated with laboratory results reflecting renal damages.

In our study; DM and HTN were present equally in (52%) of patients followed by IHD, SLE, RA and RHD. This result was in agreement with **Yali *et al.*** [8] who reported that diabetes and hypertension are the most common causes of CKD followed by other conditions that can damage the kidneys and cause CKD. Hypertension is a well-established cause, a common complication, and an important risk factor for progression of renal disease. Controlling hypertension is the most important intervention to slow the progression of renal disease [9].

Diabetes is a common cause of chronic renal disease. Effective control of blood glucose and blood pressure reduces the renal complications of diabetes. Meticulous control of blood glucose was conclusively shown to reduce the development of microalbuminuria by 35% in type 1 diabetes (diabetes control and complications trial) and in type 2 diabetes (United Kingdom prospective diabetes study) [10].

Moĭseienko and Al'ianova [11] revealed that anorexia was one of the most common gastroenterological symptoms in CKD. In the current study anorexia was found only in (44%) of patients and this can be explained by inclusion of CKD patients and those also on hemodialysis in the previous study.

In our study; pallor, facial puffiness and lower limbs edema were present in the studied patients with percentages of 90%, 42% and 38% respectively, while other clinical signs that were present in the patients including basal lung crepitation, mitral regurge, and malar flush and cushioned face were occurring in the percentages of 16%, 6%, 2% and 2% respectively. These results disagree with **Levin and Stevens** [12] who reported that; facial puffiness followed by lower limbs edema are the most common clinical presentation in CKD patients in percentage of 55% and 43% respectively.

In the current study; occult blood in stool was present in 3 patients equal to a percentage of (6%) while it was absent in 47 patients (94%). These results disagree with **Bini *et al.*** [13] who reported positive fecal occult blood test in 32.8% of 497 subjects with stage 2/3 CKD and 42.6% of 197 patients with stage 4/5 CKD ($P < 0.001$), compared to patients with none/stage 1 CKD. This disagreement can be explained by the large number of patients in addition to stage 2 and 5 CKD patients that were included in the last study.

Our present study revealed highly significant differences between patients and control groups in hemoglobin (Hb) level, P value = 0.001, Hb level was higher in control group. These results agree with **Martínez *et al.*** [14] study, which included 267 patients of stages 3, 4 and 5 CKD, not on dialysis, most of them were in CKD stages 3 and 4, the mean hemoglobin levels in these patients before starting ESA treatment was 10.2±0.9 g/dL.

The current study revealed highly significant differences between patients and control in the prevalence of anemia, it was higher in patients than control. The all 50 patients (100%) had anemia while only 3 subjects (15%) of control had anemia and 17 subjects (85%) had normal CBC. Among patients group 36 patients (72%) had normocytic-normochromic anemia (NNA), 7 patients (14%) had normocytic-hypochromic anemia (NHA) and 7 patients (14%) had microcytic-hypochromic anemia (MHA), while in the control group only 2 individuals (10%) had MHA and 1 individual (5%) had NHA. These results agree with **Talwar *et al.*** [15] who found 100% of advanced CKD patients not receiving treatment had anemia, the prevalence of NNA, NHA, MHA occurred in a percentage of 72% , 9% , 19% respectively, while **Afshar *et al.*** [16] reported that; among 46 predialysis patients 75% had anemia and 25% had normal CBC. By studying the morphological pattern of anemia they found NNA and MHA were occurring in a percentage of 80% and 15% frequently and 5% had macrocytic anemia.

Our study revealed highly significant lower mean GFR in the studied patients compared to the control group, this result was in agreement with **Stevens *et al.*** [17] who reported that; patients with advanced stages CKD had lower mean GFR compared to control due to marked reduction in the functioning units of the kidney.

In the current study (by dipstick test) we found that 70% of the studied patients had overt proteinuria in different grades, while 30% had no proteinuria but they had moderate albuminuria assessed by increased

urinary albumin : creatinine ratio. These results agree with study of **Davidson and Smiley**[18] which include 70 CKD patients,19 diabetic and 51 non diabetic. 54 patients (77%) had proteinuria with dipstick test, 12 patients (17%) had trace positive for protein and 42 patients (60%) had proteinuria more than or equal 1+, also albumin : creatinine ratios were measured in urine samples and they conclude that; in contrast to the recommendations of the American Diabetes Association and the National Kidney Foundation, dipstick positive proteinuria of more than or equal to (1+) can substitute for an albumin: creatinine ratio

In the present study, we found highly significant differences in serum iron, it was lower in the studied patients compared to control, the mean serum iron ± SD was (52.38 ± 12.40 microgram/dl) and (76.90 ± 22.16 microgram/dl) in patients and control respectively. These results are in agreement with **Arogundade *et al.*** [19] who found in the study including 60 predialysis CKD patients that; anemia is very common in the predialysis CKD population and the prevalence of iron deficiency is high, the mean serum iron ± SD was (50.22 ± 11.52 microgram /dl) before starting treatment with intravenous and oral iron. We also found significant differences in TSAT% between patients and control, it was lower among the studied patients, but there was no significant differences between both in TIBC. These results agree with **Chung *et al.*** [20] who reported low TSAT% in non-dialysis stage 3-5 CKD patients but also no significant decrease in TIBC among the same patients group. Also we found significant differences between patients and control in serum ferritin, its mean was higher in the studied patients. This result in agreement with **Meynard *et al.*** [21] who found elevated serum ferritin level in predialysis CKD patients due to infection and inflammation.

Erythropoietin (EPO) in normal adult ranged between 4.1 – 19.5 mlu/ml and decreases as the CKD progresses. EPO measurement is not routinely done for diagnosis of anemia in CKD [22]**.** Our present study revealed highly significant differences in serum erythropoietin (EPO) level between patients and control. This result agree with **Gouva *et al.*** [23]**,** who reported EPO deficiency in chronic kidney disease, which is a functional response to a decreased glomerular filtration rate and the specialized peritubular cells that produce EPO are partially or completely depleted or injured as renal disease progresses. The specialized peritubular cells that produce EPO are partially or completely depleted or injured as renal disease progresses, so that EPO production is inappropriately low relative to the degree of anemia. EPO is produced when its gene is transcribed, in a process that depends on the binding of a molecule called hypoxia-inducible factor 1 alpha to the hypoxia-responsive element on the erythropoietin gene [23].

In our study, we found significant positive correlation between hemoglobin (Hb) level and glomerular filtration rate (GFR). This result agree with **Zarzecki *et al.*** [24] who found significant positive correlation between Hb level and GFR in different stages of chronic renal failure. Also **Afshar *et al.*** [16], reported same result in the study were including 46 predialysis patients. We also found highly significant positive correlation between erythropoietin level and GFR. This result agree with **Gouva *et al.*** [23], who reported highly significant positive correlation between erythropoietin level and GFR in CKD patients not receiving renal replacement therapy.

By studying the prevalence of different risk factors causing anemia among the patients group we found that; anorexia was present in 22 patients (44%) while occult blood in stool was positive in 3 patients (6%). Increased levels of CRP, ESR and serum ferritin as indicators of inflammation were present in 50 patients (100%), 50 patients (100%) and 40 patients (80%) respectively. Also the indicators of "uremic milieu" including increased level of blood urea and serum creatinine in addition to decreased level of GFR, all were present in the 50 patients (100%). Erythropoietin deficiency also was present in 27 patients (54%).

The present study revealed highly significant inverse correlation between number of risk factors and hemoglobin level. The more prevalence of risk factors of anemia in renal patients, the more is the decrease in hemoglobin level. This result is in agreement with **Magwood *et al.*** [25], who reported more reduction in Hb level among CKD patients as the prevalence of different causes of anemia is increasing.

CONCLUSION AND RECOMMENDATIONS

1- Our study concluded that anemia in pre ESRD is multifactorial and the prevalence of different risk factors is inversely correlated with hemoglobin level. The normocytic-normochromic anemia (NNA) is the most common morphological pattern in renal patients followed by normocytic-hypochromic and microcytic-hypochromic types.

2-Anemia may develop early in CKD patients and before the onset of uremic symptoms, so once renal impairment is diagnosed and GFR < 60 ml/min the patients should be screened for anemia by regular CBC and iron profile may be needed.

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