ORIGINAL RESEARCH

Differentiation of absolute iron deficiency anaemia from reticuloendothelial block anaemia (functional iron deficiency) in chronic kidney disease by the evaluation of serum iron, total iron binding capacity, transferrin saturation, serum ferritin and red cell indices

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ABSTRACT

Aim: The main aim of this study is to know the ferrokinetic alterations like serum iron, TIBC, transferrin saturation and ferritin levels in absolute IDA & functional IDA patients with CKD. **Methodology:** A comparative ferrokinetic study was conducted in iron deficiency anaemia and anaemia of chronic renal disease subjects from Command Hospital Air Force Bangalore. Each subject gave an informed consent and this study was approved by the ethical and research committee of Command Hospital Air Force Bangalore to use human subjects in the research study. The patients voluntarily participated in the study.A total of 150 patients of CKD with age >18 years were included in our study, of which 40 (26.7%) cases were excluded because they did not satisfy inclusion criteria. Out of 110 subjects, 22 (20%) cases had absolute IDA, 60 (54.5%) had functional IDA and the remaining 28 (25.5%) cases had equivocal results. In all subjects, complete haemogram, serum iron, TIBC, transferrin saturation and serum ferritin were estimated. By using serum ferritin and TSAT, we classified patients into two groups absolute IDA and functional IDA & compared traditional indicators of iron status such as Hb, routine hemogram & correlated these two groups with EPO therapy, hypertension, diabetes status, haemodialysis & various stages of CKD in outpatient department patients. **Results:** In the present study, Results show that functional IDA is more common than absolute IDA in CKD patients. Absolute IDA was more common in females. No statistical significance in gender or age distribution was seen in the two subgroups of absolute and functional IDA. The severity of anaemia is significantly higher in patients with absolute IDA (Hb-6.78 g/dL ± 1.025) compared to patients with functional IDA (Hb-7.29g/dL ± 0.887) (p=0.0298). Mean values of MCV (70.5fl ± 5.4 and 84.7fl ± 4.5) (p <0.0001) and MCH (22.7pg ± 4.06 and 27.8pg ± 3.2) (p<0.0001) were significantly low in the absolute IDA group when compared to functional IDA group. There was no significant difference in the mean values of MCHC between the two groups (p=0.06).The differences in values of mean serum iron (30.15 µg/dL ± 9.5 versus 58 µg/dL ± 11.3; p<0.0001) and TIBC (484.8µg/dL ± 69.29 versus 325.23µg/dL ± 61.8; p<0.0001) were statistically significantly between absolute IDA group when compared to functional IDA group. The mean TSAT % was found to be significantly low in the absolute IDA group (6.60% ± 2.2) than compared to functional IDA cases (18.13% ± 6.6)(p<0.0001). The mean ferritin was found to be significantly low in the absolute IDA group (28.29ng/ml ± 12.6) when compared to functional IDA cases (336.6ng/ml ± 57) (p<0.0001). All of the patients with absolute IDA had microcytic hypochromic anaemia on PBS unlike patients with functional IDA who predominantly had normocytic normochromic anaemia. There were no statistically significant differences in the EPO requirement, prevalence of HTN or DM or stage of CKD between the two groups. **Conclusion:** Present study suggests that functional IDA is more common than absolute IDA in CKD patients. Serum iron, TIBC, TSAT, and serum ferritin are practical methods of quantifying iron deficiency in both IDA and CKD groups with sensitivity of serum ferritin and TSAT% together being 74.5%. These markers may help to take direct iron & EPO therapy in CKD patients.

Key words:CKD, TIBC, TSAT, HTN, Anemia

INTRODUCTION

Chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for > 3 months, with implications for health [1].

ANAEMIA IN CKD

Anaemia constitutes a common problem in clinical practice and haematological laboratories. Anaemia is neither a diagnosis in itself nor a specific entity but a manifestation of an underlying disease process which is often related to the severity of the disease process.[2]
Anaemia is an almost invariable manifestation of CKD becoming much more common when GFR reaches 60ml/min or less, contributing to the morbidity and mortality of the condition [2].
Normocytic normochromic anaemia is observed as early as stage 3 CKD and is almost universal by stage 4. The primary cause in patients with CKD is insufficient production of erythropoietin (EPO) by the diseased kidneys. Additional factors include iron deficiency (ID), acute and chronic inflammation with impaired iron utilization ("anaemia of chronic disease"), severe hyperparathyroidism with consequent bone marrow fibrosis, and shortened red cell survival in the uremic environment. In addition, co-morbid conditions such as hemoglobinopathy can worsen the anaemia [3-5].
In patients with CKD and anaemia (regardless of age and CKD stage), include the following tests in initial evaluation of the anaemia: Complete blood count (CBC), which should include haemoglobin concentration, red cell indices, white blood cell count and differential, and platelet count, absolute reticulocyte count, serum ferritin level, serum transferrin saturation (TSAT), serum vitamin B12 and folate levels [6, 7].
The most common cause of anaemia worldwide is ID, resulting from prolonged negative iron balance, caused by inadequate dietary iron intake or absorption, increased needs for iron during pregnancy or growth periods, and increased iron losses as a result of menstruation and helminth (intestinal worms) infestation. An estimated 50% of anaemia in women worldwide is due to iron deficiency. Other important causes of anaemia worldwide include infections and other nutritional deficiencies [8-10].
The consequences of ID are numerous as iron plays a central part in the transport of oxygen in the body and is also essential in many enzyme systems like cytochrome oxidase, xanthine oxidase. ID affects neurotransmitter systems in brain causing changes in behaviour such as attention, memory and learning in infants & small children. It also negatively influences the normal defence system against infection [11, 12].
Amongst all anaemia, ID is the most common nutritional deficiency disorder in the world.IDA is the most common microcytic anaemia [13].
Anaemia is an almost invariable manifestation of CKD becoming much more common when GFR reaches 60ml/min or less, contributing to the morbidity and mortality of the condition [14, 15].
In the third National Health and Nutrition Examination Survey (NHANES), the prevalence of anaemia in stage 3 CKD (i.e. GFR of 30-59 ml/min/1.73m^2) was 5.2%, rising to 44.1% in stage 4 and becoming almost universal in stage 5 [16].
The present study is carried out to study the prevalence of absolute IDA and reticuloendothelial block anaemia (functional ID) in CKD patients and to evaluate the correlation of red cell indices with serum iron, serum ferritin, TIBC and serum TSAT [17].

OBJECTIVES OF THE STUDY

1. To study the prevalence of absolute iron deficiency anaemia and reticuloendothelial block anaemia (functional iron deficiency) in CKD patients.
2. To study the correlation of red cell indices with serum iron, serum ferritin, total iron binding capacity and serum transferrin saturation.
3. To guide the clinician in supplementing appropriate dosage of iron or ESA by differentiating absolute IDA from reticuloendothelial block anaemia (functional iron deficiency) of any origin in CKD patients.

METHODOLOGY

A comparative ferrokinetic study was conducted in iron deficiency anaemia and anaemia of chronic renal disease subjects from Command Hospital Air Force Bangalore. Each subject gave an informed consent and this study was approved by the ethical and research committee of Command Hospital Air Force Bangalore to use human subjects in the research study. The patients voluntarily participated in the study.

MATERIALS AND METHODS

SOURCE OF DATA: Clinical records of patients of CKD and blood samples sent for evaluation of IDA in all the chronic kidney disease patients during study period.

STUDY DESIGN: Hospital based cross sectional, correlational study (analytical study).

STUDY AREA: Command Hospital Air Force, Bangalore.

STUDY SUBJECT: Samples from 150 patients of CKD with anaemia with age >18years. The level of haemoglobin for anaemia should be less than 10g/dl.

DETAILS OF THE PROCEDURE AND METHODOLOGY PROPOSED TO BE USED IN INVESTIGATIONS

150 patients of chronic kidney disease with anaemia reporting to nephrology clinic over a period of 2 yrs were included in the study. Patients were divided into dialysis dependent and non-dialysis dependent. Blood was collected for routine haemogram including haemoglobin, red cell indices and peripheral smear in Ethylene diamine tetraacetic acid vial and for serum ferritin, TSAT and TIBC in a sterile vial after taking a written informed consent. Serum from these blood samples was separated and stored at 4 degree Celsius. Study samples were analysed by automated haematologyanalyser for the following parameters and recorded as

1. Hb% in g/dL.
2. RBC counts in million cells/cumm.
3. MCV in fl.
4. MCHC in %.
5. MCH in pg.
6. RDW-SD in fl.

Serum ferritin was assessed by chemiluminiscence on Beckman Coulter Access 2 Immunoassay system. Serum iron levels and TIBC levels were assessed using semiautomated colorimetric methods on Erba Chem 5 plus semiautomated analyser. Red cell indices was correlated with serum ferritin, serum iron, TIBC, TSAT. Haematological indices was performed on 5 part haematology cell analyser. TSAT was calculated by the formula serum iron ×100/TIBC. Statistical analysis was done on IBM SPSS 20 software (version 20). Sensitivity of serum ferritin and TSAT together in segregating CKD patientsinto two subgroups of absolute and functional IDA was assessed based on patients that could be accurately classified based on these two parameters. GFR was calculated online using the recommended CKD-EPI equation in the National Kidney Foundation application (version 2.3).

INCLUSION CRITERIA

1. All the chronic kidney disease patients with age >18 years.
2. All CKD patients with stage 3, 4, 5 (Non dialysis dependent) and 5 (Haemodialysis dependent/Peritoneal dialysis dependent).
3. The level of anemia should be <10g/dL and eGFR<60ml/min/1.73m^2.

EXCLUSION CRITERIA: Patients fulfilling any one or more of the following criteria were excluded:

1. Blood transfusion in the last 3 months.
2. IV iron in the last 6 months.
3. Oral iron in the last 7days.
4. CKD complicated by bacterial and viral infections.
5. Acute renal failure.
6. GFR > 60ml/min/1.73m^2.
7. CKD with pregnancy.

ESTIMATION OF SERUM IRON AND TIBC

Serum Iron and TIBC were estimated by Iron and TIBC kit in semi auto analyser, (Erba Chem 5plus semiautomated analyser) which uses Ferrozine method.

STATISTICAL ANALYSIS

A cut off of serum ferritin of 100ng/ml in predialysis and peritoneal dialysis patients and 200ng/ml along with TSAT of < 20% was used to differentiate absolute ID from functional iron deficiency (ACD). These patients were divided into two groups. Descriptive data was presented as mean ± SD and range values. Student's "t" test was used to study and compare difference of mean values of data in the two groups data i.e. serum iron, TSAT and serum ferritin levels in the two subgroups of absolute ID andfunctional ID (anaemia of chronic renal disease). Further categorical variables namely DM, HTN, CKD stage, haemodialysis and EPO therapy were compared using Wilcoxon signed-rank test in the two groups. All analyses were conducted using IBM SPSS Statistics 20 software (version 20). For all the tests, a p-value of 0.05 or less was considered for statistical significance.

RESULTS

The results obtained in present study were from a total 150 subjects of which 40 (26.7%) subjects were excluded because of the followings reasons:

1. 14 of them were having Hb> 10%.
2. 8 of them were given PRBCs in the last 3 months
3. 10 of them were given IV iron in the last 6 months.
4. 8 of them had GFR >60ml/min/1.73m^2.

The following were the criteria based on which the patients were divided into functional IDA and absolute IDA.

Absolute iron deficiency was defined as

- TSAT% < 20.
- The serum ferritin concentration <100ng/ml among predialysis and PD patients &<200ng/ml among haemodialysis patients.

Functional Iron deficiency was defined as

- Serum ferritin level is either normal or elevated.
- TSAT%≤20.

GROUP1: Absolute IDA patients (22 cases): males 10, females 12.

GROUP 2: Functional IDA (60 cases): males 34, females 26.

GROUP 3: Equivocal results (28 cases): males 12, females 16.
GROUP 3:I.e. those patients in whom the ferritin and TSAT values gave equivocal results were also excluded from the statistical analysis.

Association with categorical variables namely gender, HTN, DM, EPO therapy, HD & stages of CKD in the two subgroups.
Sensitivity of the ferritin and TSAT values together in predicting the diagnosis of absolute and functional IDA is 22+60/110 = 74.5%

All clinically diagnosed anaemia cases with Hb <10g/dL in CKD patients were taken. Hbestimation and PBS study, red cell indices and biochemical evaluation were done in all subjects.
Out of 110 CKD patients 60 (54.55%) patients were found to have functional IDA, whereas 22 (20%) patients were having absolute IDA (segregated based on the above-mentioned criteria). However, another 28 (25.5%) patients could not be classified into the above-mentioned subgroups based on parameters used in this study alone.

Table 1: Gender Distribution of CKD Cases

		Absolute IDA	Functional IDA	P-value
Male	No	10	34	0.063
	%	45.4	56.7	
Female	No	12	26	
	%	54.6	43.3	
Total	No	22	60	
	%	100	100	

Among 60 functional IDA cases 34 patients (56.7%) were males & 26 patients (43.3%) were females, while in absolute IDA among 22 cases, 10 patients (45.4%) were males & 12 patients (54.6%) were females (p=0.063). However, this difference was not statistically significant (p=0.063) in the two groups.

Table 2: Age Wise Distribution of CKD Cases

Age Range (Years)	Absolute IDA		Functional IDA	
	No	%	No	%
21-40	8	36.4	26	43.3
41-60	9	40.9	24	40
> 60	5	22.7	10	16.7
Total	22	100	60	100

In absolute IDA, 8 (36.4%) cases were in the age group of 21-40 yrs, 9 (40.9%) cases in the age group of 41-60 yrs and 5 (22.7%) cases were in the group of >60 yrs. 26 (43.3%) cases of functional IDA were in the age group of 21-40 yrs, 24 (40%) cases in the age group of 41-60 yrs and 10 (16.7%) cases in the age group of >60 yrs.

Table 3: Morphological Classification of Anaemia in CKD Patients Based on PBS

PBS	Absolute IDA	Functional IDA	Total	p-value
Normocytic Normochromic	0	44 (73.3%)	44 (53.7%)	<0.0001
Microcytic Hypochromic	22 (100%)	16 (26.7%)	38 (46.3%)	
Total	22	60	82	

All of the patients with absolute IDA had microcytic hypochromic anaemia on PBS [22/22(100%)]. Further, majority of patients with functional IDA had normocytic normochromic anaemia [44/60(73.3%)]. Thus microcytic hypochromic blood picture correlates well with absolute ID in CKD patients (p<0.0001).

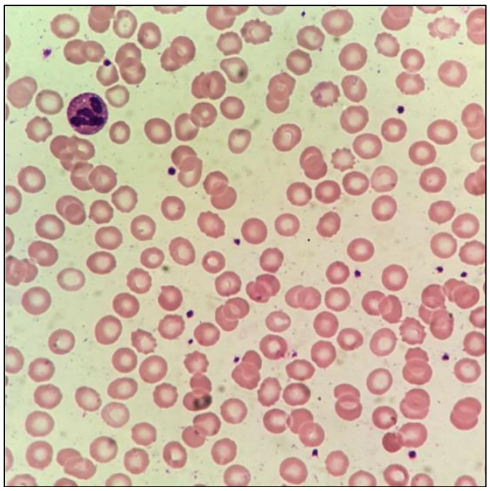

Fig 4: Normocytic normochromic anaemia (Leishman stain 1000x)

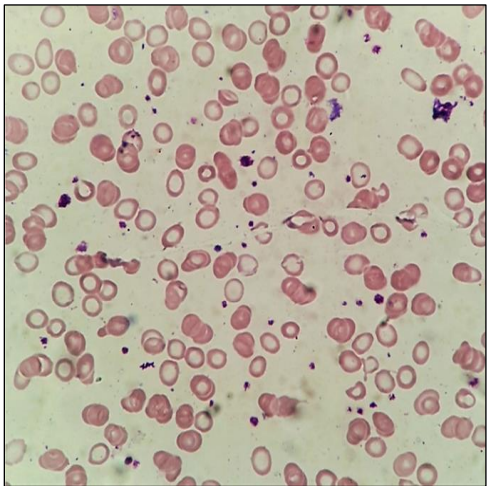

Fig 5: Microcytic hypochromic anaemia (Leishman stain 1000x)

Table 4: Association of Age with Absolute and Functional IDA

Age (Years)	Absolute IDA			Functional IDA			p-value
	No	Mean Age	SD	No	Mean Age	SD	0.574
	22	46.95	13.128	60	44.16	14.16	

The prevalence of absolute IDA is seen in slightly older population (mean age 46.95yrs) compared to functional IDA (mean age 44.16yrs), however this difference was not statistically significant (p=0.574) in the two groups.

Table 5: Association of Hb % with Absolute and Functional IDA

Hb %	Absolute IDA			Functional IDA			p-value
	No	Mean	SD	No	Mean	SD	0.0298
	22	6.78	1.025	60	7.29	0.887	

The severity of anemia is slightly higher in patients with absolute IDA (i.e. mean Hb-6.78 g/dL) compared to patients with functional IDA (i.e. mean Hb-7.29 g/dL) and this difference was statistically significant (p=0.0298).

Table 6: Association of Serum Iron with Absolute and Functional IDA

Serum Iron (µg/dL)	Absolute IDA		Functional IDA		p-value
	Mean	SD	Mean	SD	<0.0001
	30.15	9.5	58.00	11.3	

The mean value of serum iron is found to be significantly low in the absolute IDA group (30.15µg/dL) than compared to functional IDA group (58.00µg/dL) which is statistically significant (p< 0.0001).

Table 7: Association of TIBC with Absolute and Functional IDA

TIBC(µg/dL)	Absolute IDA		Functional IDA		p-value
	Mean	SD	Mean	SD	<0.0001
	484.8	69.29	325.23	61.88	

The mean value of TIBC in absolute IDA cases (484.8µg/dL) was high when compared to functional IDA cases (325.23 µg/dL) and was statistically significant (p< 0.0001).

Table 8: Association of TSAT % with Absolute and Functional ID

TSAT(%)	Absolute IDA		Functional IDA		p-value
	Mean	SD	Mean	SD	< 0.0001
	6.5	2.2	18.13	5.8	

The mean TSAT % was found to be very low in the absolute IDA cases (6.5%) than compared to functional IDA cases (18.13%) and was statistically significant (p< 0.0001).

Table 9: Requirement of EPO Therapy in Patients of Absolute and Functional IDA

		Absolute IDA	Functional IDA	Total	p-value
EPO Not Given	No (%)	9 (40.9)	30 (50)	52 (47.3)	0.288
EPO Given	No (%)	13 (59.1)	30 (50)	58 (52.7)	
Total	No (%)	22 (100)	60 (100)	110 (100)	

Out of 22 patients who had absolute IDA, 13 (59.1%) required EPO therapy as compared to 30 of 60 (50%) patients with functional IDA. This difference was not statistically significant (p=0.288).

Table 10: Association of Hypertension with Absolute and Functional IDA

		AbsoluteIDA	FunctionalIDA	p-value
Non-Hypertensive	No	12	29	0.218
	%	54.5	48.3	
Hypertensive	No	10	31	
	%	45.5	51.7	
Total	No	22	60	
	%	100	100	

Prevalence of hypertension was found more in patients with functional IDA (i.e. 51.7%) when compared to absolute IDA (i.e. 45.5%) and this difference was not statistically significant (p=0.218).

Table 11: Association of Diabetes Mellitus with Absolute and Functional IDA

		Absolute IDA	Functional IDA	p-value
Non-Diabetes Mellitus	No	10	32	0.146
	%	45.5	53.3	
Diabetes Mellitus	No	12	28	
	%	54.5	46.7	
Total	No	22	60	
	%	100	100	

The prevalence of DM was found to be higher (i.e. 54.5%) in patients with absolute IDA as compared to functional IDA (46.7%) but was not statistically significant (p=0.146).

Table 12: Distribution of Patients Who Were Haemodialysed in the Groups of Absolute and Functional IDA

		Absolute IDA	Functional IDA	p-value
Non-Haemodialysis	No	4	18	0.110
	%	18.2	30	
Haemodialysis	No	18	42	
	%	81.8	70	

Total	No	22	60	
	%	100	100	

Absolute IDA (81.8%) was more prevalent in haemodialysis patients as compared to functional IDA (70%). However this difference was not statistically significant (p=0.110).

Table 13: Association of CKD Stage with Absolute and Functional IDA

		Absolute IDA	Functional IDA	Total	p-value
CKD Stage 4	No	8	27	35	0.256
	%	36.4	47.4		
CKD Stage 5	No	14	30	44	
	%	63.6	52.6		
Total	No	22	57	79	
	%	100	100	100	

Since there were only 3 cases of stage 3 CKD, this subgroup was excluded from the statistical analysis. With respect to stage 4 and stage 5 CKD there was no statistically significant difference in the prevalence of functional IDA and absolute IDA (p=0.256).

Table 14: Correlation of Red Cell Indices with Absolute and Functional IDA

Groups	MCV (fl){Mean ± SD}	MCH (pg){Mean ± SD}	MCHC (%){Mean ± SD}
Absolute IDA	70.5 ± 5.4	22.7 ± 4.06	29.6 ± 3
FunctionalIDA	84.7 ± 4.5	27.8 ± 3.2	31.7 ± 3.5
p-value	<0.0001	<0.0001	0.06

Mean values of MCV (p<0.0001) and MCH (p= <0.0001) were significantly low in the absolute IDA group when compared to functional IDA group. There was no significant difference in the mean values of MCHC between the two groups.

Table 15: Equivocal Cases

Parameters	Males	Females	Total
Ferritin ≥ 100ng/ml TSAT ≥ 20%	8	14	22
Ferritin < 100ng/ml TSAT ≥ 20%	5	1	6
Ferritin >1500ng/ml	1	1	2
Type of anaemia	Normocytic normochromic		28

Equivocal results were obtained in 28 (25.5%) patients. Out of 28 patients, 22 had ferritin >100ng/ml and 6 of them had ferritin <100ng/ml. TSAT was elevated to more than 20% in all the 28 cases.

Table 16: Comparison of Absolute and Functional IDA with Regard to Haemoglobin, Serum Iron, TIBC,Serum Ferritin and TSAT%

Groups	Hb% (Mean±SD)	Serum Iron µg/dL (Mean±SD)	TIBC µg/dL (Mean±SD)	Serum Ferritin ng/ml (Mean±SD)	Transferrin SAT% (Mean±SD)
Absolute IDA	6.78 ±1.025	30.15 ± 9.5	484.8±69.29	28.29 ±12.6	6.50 ± 2.2
Functional IDA	7.29 ± 0.887	58.00 ± 11.3	325.23±61.9	336.6 ± 57	18.13 ± 5.8
p-value	0.0298 (S)	<0.0001 (S)	<0.0001 (S)	<0.0001 (S)	<0.0001 (S)

p<0.05=Significant (S), p>0.05=Non-Significant (NS). Whereas difference in means of haemoglobin, serum iron, TIBC, serum ferritin and TSAT% were found to be statistically significant in absolute IDA group when compared to functional IDA group.

DISCUSSION

Anaemia represents a common clinical problem in subjects with CKD, and is associated with increased morbidity and mortality, especially in patients undergoing chronic HD.[81] The K/DOQI anemia work groups have delineated use of serum ferritin and the TSAT as the primary tools for assessing iron

management in patients with anaemia and CKD, including ESRD. The main aim of this study is to know the ferrokinetic alterations like serum iron, TIBC, TSAT and ferritin levels in absolute IDA & functional IDA patients with CKD. By using serum ferritin and TSAT we have classified patients into two groups absolute IDA and functional IDA & compared traditional indicators of iron status such as Hb, routine haemogram& correlated these two groups with EPO therapy, HTN, DM, HD & various stages of CKD in outpatient department patients [18, 19].

The present study included 150 subjects of which 40 (26.7%) cases were excluded because they did not satisfy inclusion criteria. Out of 110 subjects, 22 (20%) cases had absolute IDA, 60 (54.5%) cases had functional IDA and the remaining 28 (25.5%) cases had equivocal results. Thus functional ID is commoner than absolute ID in CKD patients. Similar results have been observed in earlier studies. In another study by Talib *et al.*,the overall prevalence of IDA in CKD was 42.63% (Table 18). Thus, overall we had fewer cases of absolute IDA (20%) in CKD as compared to other studies[20, 21].

The mean age of our study population was 45.6 years (range 21-60 years). Majority of patients in absolute IDA & functional IDA groups were in the range 21-40yrs and 41-60 yrs respectively. In a study done by Mohammed Idris *et al.*,[86] there were two peaks in IDA i.e. 21-30 yrs and 41-50 yrs, suggesting that majority of patients with IDA were aged between 20-50yrs [22].

In our study population, overall gender distribution of anaemia in CKD comprised of 56 (50.9%) males and 54 (49.1%) females with male to female ratio of 1.03:1. Among 60 cases of functional IDA, 34 patients (56.7%) were males & 26 patients (43.3%) were females, while in absolute IDA among 22 cases, 10 patients (45.5%) were males & 12 patients (54.5%) were females (p=0.063). Thus, we had more female patients with absolute IDA similar to other authors [23, 24].

In the study by Talib *et al.*,in males the prevalence of IDA was 44.4% which was less than female patients (55.6%). Absolute ID was uncommon, but its prevalence depended highly on the ferritin cut-off used to define ID. Using a ferritin level <100 ng/ml and TSAT≤20%, Fishbane*et al.*,[84] found about 25% of the men with estimated GFR 60 ml/min/1.73 m^2 had absolute ID, and more than 60% of women i.e. more than 5 times more than men. Unlike Fishbane*et al.*,who had significantly more females with absolute IDA, in our study we had fewer females with IDA when compared to males due to total number of females in our study group being less as a whole. With respect to gender distribution, we found no significant differences in the prevalence of absolute IDA and functional IDA in the two subgroups. In a similar study by Idris *et al.*,they found more number of females with IDA in CKD [25, 26].

The total amount of transferrin present in plasma is expressed in terms of its capacity to bind and is known as TIBC. The mean values of serum iron and TIBC in absolute IDA cases & functional IDA cases were 30.15 µg/dL ± 9.5 & 484.8µg/dL ± 69.29 and 58 µg/dL ± 11.3 & 325.23µg/dL ± 61.9 respectively. These results correlates with diagnostic definition of absolute IDA having lower values of serum iron and elevated values of TIBC (p<0.0001). In absolute IDA patients, there is increase in iron carrying protein transferrin and the amount of iron which is available to bind is reduced causing decrease in transferrin saturation.[42] Transferrin is a negative acute phase reactant and is decreased in patients of CKD. If it falls below 15%, bone marrow uptake suffers and manifests with iron deficiency [27].

The severity of anaemia was found slightly higher in patients with absolute IDA (mean Hb-6.78g/dL ± 1.025) compared to patients with functional IDA (mean Hb - 7.29g/dL ± 0.887) and the difference was statistically significant (p=0.0298). The results of other studies are similar to our study.[66] Wians *et al.*,reported mean Hb concentrations of 8.8g/dL ± 2.0 in absolute IDA group and Hb was higher in ACD group where values of 9.5g/dL ± 1.7 were seen [28].

All of the patients with absolute IDA had microcytic hypochromic anaemia on PBS [22/22(100%)]. Further, majority of patients with functional IDA had normocytic normochromic anaemia [44/60(73.3%)]. Thus microcytic hypochromic blood picture correlates well with absolute ID in CKD patients (p<0.0001). The anaemia of CKD has been reported as normocytic normochromic type in majority of previous studies too. The presence of microcytosis reflects ID and aluminium excess where there is reduced serum iron concentration and TSAT is < 20% and increased RDW, increased TIBC and serum ferritin levels(>100ng/ml). The anaemia of CKD is generally normocytic normochromic. The presence of macrocytosis reflects vitamin B12 or folate deficiency and or erythropoietin therapy, shifting immature and large reticulocytes into the circulation [29].

We found the sensitivity of the ferritin and TSAT values together in predicting the diagnosis of absolute and functional IDA in CKD patients to be 74.5%. Other authors have found serum ferritin levels <200ng/ml to be 100% specific for the diagnosis but only 41% sensitive. Further, TSAT of less than 20% was 88% sensitive, but only 63% specific in the same study [30].

TSAT is the amount of iron present in transferrin, expressed on percent basis. The mean serum ferritin and TSAT % were found to be significantly low in the absolute IDA group (28.29ng/ml ± 12.6) & (6.5% ± 2.2) respectively, when compared to functional IDA cases (336.6ng/ml ± 57) & (18.13% ± 5.8) and these differences were statistically significant (p<0.0001). According to Fishbane*et al.*,the only tests with marginal significance were those for serum ferritin and transferrin saturation in classifying CKD patients. In their study, the serum ferritin level was 120. 1 ± 115.8ng/ml. (Table 21) in patients with ID, compared

with 182.4 ± 121.1ng/mL in patients with adequate Iron (P = 0.09). This is also in accordance with studies of Sharma.D.C [31],

Stage 3 CKD patients complain of anaemia where ID is the common cause. This occurs due to decreased production of EPO by diseased kidney.[94] According to a study by Stauffer *et al.*,prevalence of anaemia increased with stage of CKD, from 8.4% at stage 1 to 53.4% at stage 5. In the present study too, prevalence of IDA has increased with stage of CKD, which is similar to previous studies. With respect to stage 4 and stage 5 CKD there was no statistically significant difference in the prevalence of functional IDA and absolute IDA (p=0.256). Further three cases of stage 3 CKD had functional IDA but none of stage 3 CKD patients had absolute IDA.

The prevalence of DM was found to be higher (i.e. 54.5%) in patients with absolute IDA as compared to functional IDA (46.7%) but was not statistically significant (p=0.146) [33].

The limitations of this study were non availability of a gold standard to help calculate the specificity of ferritin and TSAT values and to help classify them into absolute and functional IDA. These may include bone marrow iron staining, serum hepcidin measurements, percentage of hypochromic red blood cells (% HRBC), soluble serum TfR receptors, and reticulocyte haemoglobin content (CHr). As per recent studies, hepcidin, a peptide secreted by the liver, has been identified as controlling the level of plasma iron by regulating the intestinal absorption of dietary iron, as well as the release of iron from macrophages and the transfer of iron stored in the hepatocytes. Increase in hepcidin level in the course of inflammatory disease may be a significant mediator of the accompanying anaemia. We did not have hepcidin correlation in our patients [34].

The % HRBC is a reliable and sensitive indicator for functional iron deficiencyand the measurement of HRBC requires automated cell counter which was not available in our centre thus limiting its use. Measurement of soluble serum transferrin receptor is not routinely used in clinical practice as it is expensive and is recommended to be carried out in patients in whom the quantitation of hypochromic red cells is not available or when the determination of serum ferritin, serum iron, transferrin and TSAT does not lead to an accurate classification of type of anaemia. We did not have this test facility in our centre. Examination of a bone marrow aspirate stained with Prussian blue to determine the presence or absence of iron is regarded generally as the “gold standard” for the assessment of storage iron, especially in hospitalized patients. This being an invasive procedure was not done in our study. CHr measures Hb entering reticulocytes during terminal differentiation and hence reflects the effectiveness of erythropoiesis. It is highly accurate with lowest coefficient of variability, but is not a widely available assay [35].

Further in 28 equivocal cases we did not have follow up of serum B12 and folate levels. Clinically, functional ID is confirmed by the erythropoietic response to a course of parenteral iron and is excluded by the failure of erythroid response to intravenous iron administration.[55] In this study we did not study the response of intravenous iron administration to classify patients as having functional IDA [36, 37]. Despite certain limitations, this study provided us with useful insight into the epidemiology of IDA in CKD. The utility of serum ferritin and TSAT with cut offs as defined by K/DOQI anemia work groups has been shown to be 74.5% in this study.

CONCLUSION

The limitations of this study were non availability of a gold standard to help calculate the specificity of ferritin and TSAT values and to help classify them into absolute and functional IDA. These include bone marrow iron staining, serum hepcidin measurements, percentage of hypochromic red blood cells (% HRBC), soluble serum TfR receptors, and reticulocyte haemoglobin content (CHr). In this study we did not study the response of intravenous iron administration to classify patients as having functional IDA. Correlation with serum folate and vitamin B_{12} levels were not available either in equivocal cases.

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