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Diagnostic performance of peripheral blood film and red blood cell indices as markers of iron deficiency among patients with chronic kidney disease in low resource settings

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Abstract

Introduction: *Iron deficiency anaemia (IDA) is a common finding among patients with chronic kidney disease (CKD) and a major contributor to the high morbidity, mortality and poor quality of life associated with the disease. Assessment of iron deficiency anaemia has become routine in the evaluation of patients with CKD and iron studies such as serum ferritin, total iron binding capacity and transferrin saturation are recommended as standard diagnostic work up. However, in Nigeria and other low and middle income countries (LMICs) where most patients pay out of pocket, only few patients could afford iron studies, in addition to other cost of care. It is therefore imperative to find and establish the utility of other relatively affordable markers of iron deficiency among patients in LMICs. In studies done within the general population, the percentage of hypochromic red blood cells (PHRC), red blood cell RBC indices and reticulocyte haemoglobin concentration (CHr) have been shown to predict iron deficiency. However, the usefulness of these in CKD patients had not been established. Thus, we determined the utility of PHRC and RBC indices among patients with CKD.* ***Methods:*** *this is a cross sectional survey of 157 participants with CKD and 157 apparently healthy controls. The patients with CKD were individuals receiving care at the renal unit of the University College Hospital, Ibadan. Clinical data were collected using standard care report forms and information obtained were demographic details, aetiologies of CKD, dialysis status, symptoms of iron deficiency anaemia, modalities of anaemia treatments and other medications. Blood samples were collected for iron studies, full blood count, red blood cell (RBC) indices, peripheral blood film for PHRC, plasma highly sensitive C-reactive protein, serum electrolytes, urea and creatinine. Data was analyzed using SPSS version 23.* ***Results:*** *a total of 314 participants were enrolled in the study, half of them were patients with CKD while the remaining half were controls without CKD. The mean age for cases and controls were (45.5±14.4 vs 46.1±15.5 years respectively p < 0.72) while females were 57% vs 60.1%, p < 0.06). The prevalence of IDA using iron studies (67 (42.6%) vs 33 (21.0%), p < 0.01), RBC indices (46 (29.3%) vs 25 (15.9%), < 0.002), and PHRC (48 (30.5%) vs 32 (20.4%), p < 0.038). The sensitivity and specificity of RBC indices and PHRC as surrogate markers of iron deficiency anaemia were (73.8% ; 92.0%) and (80.0% ; 92.3%) respectively.* ***Conclusion:*** *this study confirmed that the 3 methods of diagnosis of iron deficiency anaemia vis-a-vis iron studies (Serum ferritin and TSAT), RBC indices and percentage hypochromic RBC, all demonstrated high prevalence of iron deficiency anaemia among patients with CKD. While RBC indices and percentage hypochromic RBC are reliable and highly specific surrogate markers of iron deficiency anaemia among individuals with CKD.*

Introduction

Iron deficiency anaemia (IDA) is a common finding among patients with chronic kidney disease (CKD) and a major contributor of high morbidity, mortality and poor quality of life associated with the disease [1-3]. The prevalence of IDA among CKD is estimated to be between 30 - 70% and increases with the increasing severity of the kidney disease [4-8]. In view of the high burdens of IDA in patients with CKD, early diagnosis and prompt treatments have been shown to improve quality of life and reduce morbidity and mortality associated with CKD [9-10]. The need to assess for IDA is more imperative among patients with CKD, most of whom are on erythropoiesis stimulating agents (ESA) [11]. In view of the benefits of early diagnosis and need for prompt treatments, assessment of IDA has become routine in the evaluation of patients with CKD. The routine method of assessment of IDA include the use of iron studies such as serum ferritin, total iron binding capacity (TIBC) and transferrin saturation (TSAT) and have become standard of care in most clinics [11,12]. The gold standard for the diagnosis of IDA is bone marrow biopsy for stainable iron, however this is not recommended in routine clinical care because of its invasive nature [12]. Other methods of

diagnosis of iron deficiency with varying sensitivities and specificities include the use of reticulocyte hemoglobin content (CHr), soluble transferrin receptor (sTfR), red blood cell (RBC) indices and percentage of hypochromic red blood cells (PHRC) [13-15]. CHr and sTfR are readily not available in most of the low and middle income countries (LMICs) despite their high accuracies in diagnosis and ability to diagnose IDA even before clinical manifestations. Where these tests are available, the exorbitant cost puts them out of reach of the majority of the patients with CKD in LMICs [16,17]. The PHRC and RBC indices are tests of IDA that are readily available and affordable among patients with CKD in LMICs, surprisingly the use of these markers as diagnostic tool for IDA among patients with CKD are not commonly being employed. PHRC and RBC indices have been shown to be an accurate markers of IDA among the general population, while only few of these studies were among patients with CKD [18,19]. Serum ferritin and transferrin saturation (TSAT) for diagnosis of IDA among patients with CKD has been recommended by several guidelines. Their sensitivity and specificity have been estimated to be 64.9% and 96.1% respectively [20]. The average cost of iron studies (serum ferritin and TSAT) is $100 in Nigeria. However, most patients with CKD in LMICs including Nigeria pay out of pocket and only a few patients could afford iron studies, in addition to other cost of care [21,22]. It is therefore imperative to confirm and establish the utility of other relatively affordable and accessible markers of iron deficiency among patients with CKD in resource poor settings. This study determined the utility of PHRC and RBC indices among patients with CKD.

Methods

The study was a cross sectional study of 157 participants with CKD and 157 apparently healthy controls that was conducted at the University College Hospital, Ibadan, Nigeria. The patients with CKD were individuals receiving care at the renal unit of the University College Hospital, Ibadan. The methodology has been fully described elsewhere [8] and only a brief summary is provided here. The study was carried out between 1st February 2015 and 31st July 2016, while the ethical approval was obtained from the Joint University of Ibadan and University College Hospital Ethics Committee with the approved protocol number UI/EC/14/0314. The study adhered to the declaration of Helsinki and written informed consent was obtained from all participants. Included in the study were individuals with CKD who were 18 years or older as cases while subjects with other causes of anaemia were excluded, such cases include sickle cell disease, leukemia, multiple myeloma and Human Immunodeficiency Virus (HIV) infection, ongoing blood loss, current febrile illness or those with history suggestive of inflammatory diseases, malignancies. Furthermore, participants with highly sensitive C-reactive protein (hsCRP) > 3ng/l were excluded from the study. The controls were apparently healthy volunteers who were staff and students of the University College Hospital and College of Medicine, University of Ibadan. Clinical data were collected using standard case report forms and information obtained were demographic details, aetiologies of CKD, dialysis status, symptoms of IDA, anaemia treatments and other medications. Ten milliliters of venous blood (5mls each into K2EDTA and lithium heparin specimen bottles) and 10ml of spot urine were collected from each participant. The blood K2EDTA sample was analyzed for full blood count and peripheral blood film while the serum from the heparinized sample was used to process serum iron, ferritin, transferrin, hsCRP, electrolytes, urea and creatinine. The spot urine was used to determine the urinary albumin-creatinine ratio. TIBC and TSAT were calculated from the obtained serum iron, ferritin and transferrin, using iron indices equations [23]. Estimated glomerular filtration rate (eGFR) was estimated from the serum creatinine using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [24]. Peripheral blood smears were made and the microscopy for PHRC was carried out in all participants.

Definition of terms: anaemia was defined as haemoglobin concentration of < 12g/dl in female and < 13.5g/dl in male [14,25]. Functional iron deficiency was defined as serum ferritin > 100ng/ml and TSAT < 20% while absolute iron deficiency was defined as serum ferritin < 100ng/ml and TSAT < 20% [14,25]. An hsCRP > 3mg/L was taken as suggestive of an ongoing infection or inflammatory process [26]. CKD was defined as eGFR < 60ml/min/1.73m^2 and/or albuminuria > 3mg/mmol lasting at least 3 months or the presence of markers of CKD at presentation, these markers include radiological or histological evidence of kidney damage.[27] IDA was defined as PHRC of greater than 8% using peripheral blood film microscopy while RBC indices diagnosis of IDA as Mean Corpuscular Volume (MCV) < 80Fl and Mean Corpuscular Haemoglobin (MCH) < 27pg [28,29].

Statistical analyses: data obtained was analyzed using Statistical Package for Social Sciences Version 23 (SPSS 23). Continuous variables were presented as means with standard deviation while categorical variables were presented as proportions. The difference between the means of continuous variables was determined using independent Student t-test while those between categorical variables was tested using Chi-squared statistics. Sensitivity, specificity, positive predictive and negative predictive values were calculated for PHRC and RBC indices as markers of IDA with serum ferritin and TSAT as standard for comparison. Receiver Operating Curve (ROC) and Area Under the Curve (AUC) were analyzed for each of the parameter´s performance for the diagnosis of IDA. In all situations a P-value less than 0.05 was considered statistically significant.

Results

A total of 314 participants were enrolled in the study, 157 were patients with CKD while the remaining half were controls without CKD. The mean age for cases and controls were (45.5±14.4 vs 46.1±15.5 years) while females were 57% vs 60.1%. The mean haemoglobin concentration, packed cell volume, PHRC, MCV, TSAT and reduced eGFR were lower among participants with CKD compared to the control subjects (Table 1). While serum creatinine and urea were significantly higher in patients with CKD compared with the control (Table 1). Among the participants with CKD, majority were in stage III while chronic glomerulonephritis (CGN) 59 (37.6%), hypertension 51 (32.5%), diabetes mellitus 26 (16.5%) and obstructive uropathy 21 (13.4%) were the causes of CKD. Fifty-two of the subjects with CKD (33.1%) have had their kidney disease for over 12 months while majority were pre-dialysis CKD subjects 103 (65.6%) (Table 1). The prevalence of IDA using iron studies (67 (42.6%) vs 33 (21.0%), p < 0.01), RBC indices (46 (29.3%) vs 25 (15.9%), p < 0.002), and PHRC (48 (30.5%) vs 32 (20.4%), p < 0.038) (Table 1). The sensitivity and specificity of RBC indices among CKD cases were 79.5% and 90.3% respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 0.76 and 0.96 respectively (Table 2). The sensitivity and specificity using RBC indices among health controls were 80.9 and 94.1%, while PPV and NPV were 0.71 and 0.95 respectively. The sensitivity and specificity of using PHRC among CKD cases were 72.9% and 86.7% respectively, while PPV and NPV were 0.63 and 0.91 in health controls (Table 2). Among the controls the PHRC had a sensitivity of 75.0% while specificity was 89.5 %, the PPV and NPV were 0.56 and 0.95 respectively (Table 2). Determination of the cut offs for the IDA diagnostic parameters showed that PHRC predicted IDA better with AUC of 0.786 (Figure 1). The range of cut offs and the accompany sensitivity and specificity for PHRC are shown in Table 3. The predictive ability of the RBC indices parameters was modest with the following AUCs; MCH 0.532 (Figure 2), MCHC 0.521 (Figure 3) and MCV 0.528 (Figure 4). The ranges of cut offs and coordinates for MCH, MCHC and MCV are shown in Table 3.

Discussion

The primary objective of this study was to determine the utility of RBC indices and PHRC as

surrogate makers of IDA among patients with CKD. Our study confirmed the well-known fact that IDA is common among patients with CKD using iron studies (42.6%), RBC indices (29.3%) and PHRC (30.5%). The sensitivities and specificities were similar using different IDA diagnostics parameters and in different study groups. In CKD cases RBC indices (79.5%, 90.3%) and PHRC (72.9% and 82.7%), in healthy controls RBC indices (80.9% and 94.1%) and PHRC (75.5% and 89.5%). RBC indices is a measure of IDA that is readily available and affordable in most medium and low income countries (LMICs) unlike other markers of IDA such as serum ferritin, TSAT, Soluble transferrin receptor sTfR. Our study has demonstrated high sensitivity and specificity among subjects with CKD, using RBC indices and PHRC. Expectedly, RBC indices as a marker of IDA had higher sensitivity and specificity in the healthy control subjects compared to those with CKD. The lower prevalence of anaemia among the healthy controls may be responsible for the observed difference. Although, the positive predictive value for RBC indices among patients with CKD was moderately high (76.0%), this was compensated for by high negative predictive values (92.0%) in them. Conditional on a test result returning negative, RBC indices were able to predict to a larger extent those with IDA among controls compared to the CKD group. The utility of PHRC as a diagnostic tool for IDA must be weighed against the background of exclusion of other causes of hypochromia and microcytosis, such as thalassemia, sideroblastic anaemia and chronic inflammatory or infectious disease and malignancies [30,31]. One of the strength of this study is that participants with evidence of ongoing infection or inflammation were excluded using plasma hsCRP as screening tool. Accurately diagnosing IDA requires the use of appropriate cut off for the study population, for this study, a cut off of less than 8% was used for PHRC. However, our study suggests that using a cut off less than 4.5% may be more predictive of IDA among the patients with CKD, this cut off comes with a sensitivity of 70.6% and specificity of 82.4%.

The use of the individual component of RBC indices alone did not accurately diagnose IDA as the coordinates of sensitivities and specificities were low for different cut offs, therefore the values of MCH, MCHC and MCV should be combined to diagnose IDA among patients with CKD. Our study has shown that PHRC is a reliable marker of IDA among patients with CKD. This is similar to the report by Luo *et al*. [32] who found PHRC more predictable of IDA in women with menometrorrhagia. Furthermore, the AUC for PHRC performed better than that of the MCH, MCHC and MCV, which was similar to report by Urrechaga *et al*. [33,34] among patients with CKD. The advances in technology has improved the accuracy of analysis of PHRC, there are autoanalyzers that can automatically generate PHRC along with other RBC indices, but such autoanalyzers are usually not available in LMICs [34,35]. In our study, PHRC was determined using peripheral blood films prepared by an experienced Haematology Technologist and read by equally experienced trained Haematologist. Measures to increase the accuracy of the 2 surrogate makers of IDA is to combine both PHRC and RBC indices, as this has the potential of increasing the accuracy of diagnosis of IDA. Other marker of IDA that is equally accurate but cheaper than serum ferritin and TSAT is reticulocyte haemoglobin concentration (CHr) [36], however, the technology for the assay is not readily available in most of the resource limited settings. Despite the usefulness of PHRC and RBC indices, neither of them can differentiate between absolute and relative IDA and should be used in resource limited setting where accessibility and affordability of other more accurate markers of IDA is limited. Another benefit of using PHRC as marker of IDA is that it has the potential of detecting iron deficiency prior to the development of anaemia. Hence making it a readily accessible screening tool. The use of serum ferritin and TSAT as the gold standard for which the surrogate markers were compared is a limitation of this study, as the ideal gold standard for IDA is the bone marrow biopsy for stainable iron but it is an invasive procedure and prone to higher

risk of complications [37]. Nevertheless, serum ferritin and TSAT are used routinely for the diagnosis of IDA in clinical practice. Although PHRC and RBC indices are readily available in most of the LMICs, often most physicians do not pay attention to them, perhaps because studies on their utility in the local environment have not yet been conducted. Our study has provided evidence that in resource limited setting especially where the preferable and usually expensive tests for the diagnosis of IDA are not affordable or are impracticable, PHRC and RBC indices could serve as accurate surrogate markers of IDA among patients with CKD.

Conclusion

We confirmed a well-known fact using three different diagnostic methods that iron deficiency anaemia is prevalent among sub-Saharan African patients with CKD, while we are also able to document that red blood cell indices and percentage hypochromic red blood cells on peripheral blood film are reliable and accurate surrogate markers of iron deficiency anaemia among individuals with CKD.

What is known about this topic

- *Anaemia is a universal finding among patients with chronic kidney disease and contributes to the increased morbidity and mortality among this group of patients;*
- *Prompt diagnosis and treatment of iron deficiency anaemia among patients with chronic kidney disease retards disease progression and improve survival;*
- *The cost of diagnosis of iron deficiency anaemia, in addition to other cost of care is burdensome to patients with chronic kidney disease in resource limited settings.*

What this study adds

- *Demonstrated the accuracy of cheaper and accessible surrogate markers of iron deficiency anaemia among patient with chronic kidney disease in low resource setting;*
- *Compared the specificity and sensitivity of three markers of iron deficiency anaemia among patients with chronic kidney disease;*
- *Validate the high prevalence of iron deficiency anaemia among patients with chronic kidney disease using three different methods of assessment.*

Competing interests

The authors declare no competing interests.

Authors' contributions

YRR, SOA and BLS were involved in idea conceptualization. YRR, SOA, TSA, AOA KSA and BLS were all involved in the study design. YRR and SOA supervised recruitment and data collection. YRR analyzed the data and wrote the manuscript. YRR, SOA, TSA, AOA KSA and BLS edited and approved the manuscript. All the authors have read and agreed to the final manuscript.

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Tables and figures

Table 1: baseline characteristic of the participants

Table 2: diagnostic accuracy of iron deficiency using red blood cell indices (RBC) and Percentage hypochromic red blood cells (PHRC) in the chronic kidney disease cases and control subjects.

Table 3: range of cut offs for the percentage hypochromic red blood cell, mean corpuscular haemoglobin, mean corpuscular haemoglobin

concentration, and red blood cell indices with their sensitivities and 1-specificity

Figure 1: ROC curve for percentage hypochromic red blood cells

Figure 2: ROC curve for mean corpuscular haemoglobin

Figure 3: ROC curve for mean corpuscular haemoglobin concentration

Figure 4: ROC curve for mean corpuscular volume

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Table 1: baseline characteristic of the participants				
Mean age (year)	**Cases n = 157**	**Controls n = 157**	**Total subjects n = 314**	**p values**
Mean age (years)	45.5 ± 14.4	46.0 ± 15.5	45.75±15.1	0.75
Gender Female Male	 88 (56.1%) 69 (43.9%)	79 (50.3%) 78 (49.7%)	167 (53.2%) 147 (46.8%)	0.31 0.28
Mean serum creatinine (µmol/L)	282.9 (106.1 - 3,625.3)	79.6 (44.2 - 309.4)	190.4 (85.3 - 3,145.2)	0.01
Mean serum urea (mmol/L)	20.4 (10.6 - 49.8)	6.7 (4.3 - 13.3)	16.9 (7.4 - 38.3)	0.01
Mean eGFR (ml/min/1.73m2)	22.7 (3.4 - 59.5)	110.2 (60.3 - 152. 8)	76.8 (37.1 - 124.3)	0.01
Mean haemoglobin concentration (g/dl)	9.3 ± 2.6	11.4 ± 1.7	10.4 ± 2.3	0.01
Mean PCV (%)	27.8 ± 8.7	38.7 ± 5.2	33.3 ±6.5	0.01
Mean corpuscular haemoglobin concentration (pg/dL)	34.6 ±3.9	35.2 ± 4.8	34.9 ± 4.6	0.18
Mean corpuscular haemoglobin (pg)	30.3 ±4.2	32.7± 3.6	31.5 ±3.8	0.41
Mean corpuscular volume (Fl)	87.8 ± 15.8	91.2 ± 12.5	88.4 ± 14.7	0.06
Mean serum iron (µg/dl)	102.8 ± 11.8	106.0 ± 13.3	104.4 ± 12.7	0.28
Mean serum ferritin (ng/mL)	320.5 ± 21.2	289.3 ±18.9	304.9 ±19.7	0.09
Mean TSAT (%)	27.9 ± 6.4	34.8 ± 8.1	31.4 ±7.5	0.04
Mean TIBC (µg/dl)	247.3 ± 13.1	256. 6 ± 15.4	252.0 ± 15.2	0.53
Mean percentage hypochromic RBC	5.6 ± 1,5	4,7 ± 1.2	5.2 ± 1.3	0.36
Prevalence of IDA using PHRC	48 (30.5%)	32 (20.4%)	80 (25.5%)	0.01
Prevalence of IDA using RBC indices	46 (29.3%)	25 (15.9%)	71 (22.6%)	0.01
Prevalence of IDA using serum ferritin and TSAT	67 (42.6%)	33 (21.0%)	100 (31.9%)	0.04
Stages of CKD				
Stage 3	78 (49.7%)			
Stage 4	34 (21.7%)			
Stage 5	45 (28.7%)			
Dialysis status				
Pre-dialysis	103 (65.6%)			
Dialysis	54 (34.4%)			
Duration of CKD Less than 12 months 12 months and above	 105 (66.9%) 52 (33.1%)			
CKD - Chronic Kidney Disease, eGFR - estimated Glomerular Filtration Rate, PCV - Packed Cell Volume, SD - Standard Deviation, TIBC - Total Iron Binding Capacity, TSAT - Transferrin Saturation				

Table 2: diagnostic accuracy of iron deficiency using red blood cell indices (RBC) and percentage hypochromic red blood cells (PHRC) in the chronic kidney disease cases and control subjects

Diagnostic variable	Iron depleted	Iron replete	Total N = 314	Sensitivity	Specificity	Positive predictive value	Negative predictive value
IDA diagnosis by RBC indices in CKD group							
True positive	35 (79.5%)	11 (9.7%)	46 (29.3%)	79.5%	90.3%	0.76	0.92
False positive	9 (20.5%)	102 (90.3%)	111 (70.7%)				
Total	44 (28.0%)	113 (72.0%)	157 (100.0%)				
IDA diagnosis by RBC indices in Control group							
True positive	28 (72.9%)	7 (5.1%)	25 (15.9%)	80.9%	94.1%	0.71	0.97
False positive	4 (19.1%)	129 (94.9%)	132 (84.1%)				
Total	21 (13.4%)	136 (86.6%)	157 (100.0%)				
IDA diagnosis by PHRC indices in CKD group							
True positive	27 (72.9%)	16 (13.3%)	43 (27.4%)	72.9%	86.7%	0.63	0.91
False positive	10 (27.1%)	104 (86.7%)	114 (72.6%)				
Total	37 (23.6%)	120 (76.4%)	157 (100.0%)				
IDA diagnosis by PHRC indices in Control group							
True positive	18 (75.0%)	14 (10.5%)	32 (20.4%)	75.0%	89.5%	0.56	0.95
False positive	6 (25.0%)	119 (89.5%)	125 (79.6%)				

CKD - Chronic Kidney Disease, IDA- Iron Deficiency Anaemia, PHRC - Percentage Hypochromic Red Blood Cell, RBC - Red Blood Cell

Table 3: range of cut offs for the percentage hypochromic red blood cell, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and red blood cell indices with their sensitivities and 1-specificity		
Parameter values	**Sensitivity**	**1-Sensitivity**
Percentage hypochromic red blood cell (%)		
2.5	0.721	0.324
3.5	0.706	0.263
4.5	0.706	0.186
5.5	0.662	0.142
6.5	0.647	0.117
7.5	0.618	0.105
Mean Corpuscular Haemoglobin (pg)		
24.1	0.706	0.644
24.3	0.691	0.628
24.5	0.676	0.619
24.8	0.647	0.595
25.1	0.529	0.453
25.4	0.500	0.441
Mean Corpuscular Haemoglobin Concentration (g/dL)		
28.9	0.838	0.785
29.1	0.632	0.559
29.3	0.603	0.555
29.5	0.588	0.547
29.7	0.559	0.538
29.8	0.529	0.526
Mean Corpuscular Volume (Fl)		
84.5	0.588	0.555
84.6	0.588	0.534
84.7	0.574	0.530
84.9	0.574	0.510
85.3	0.559	0.486
85.8	0.559	0.474

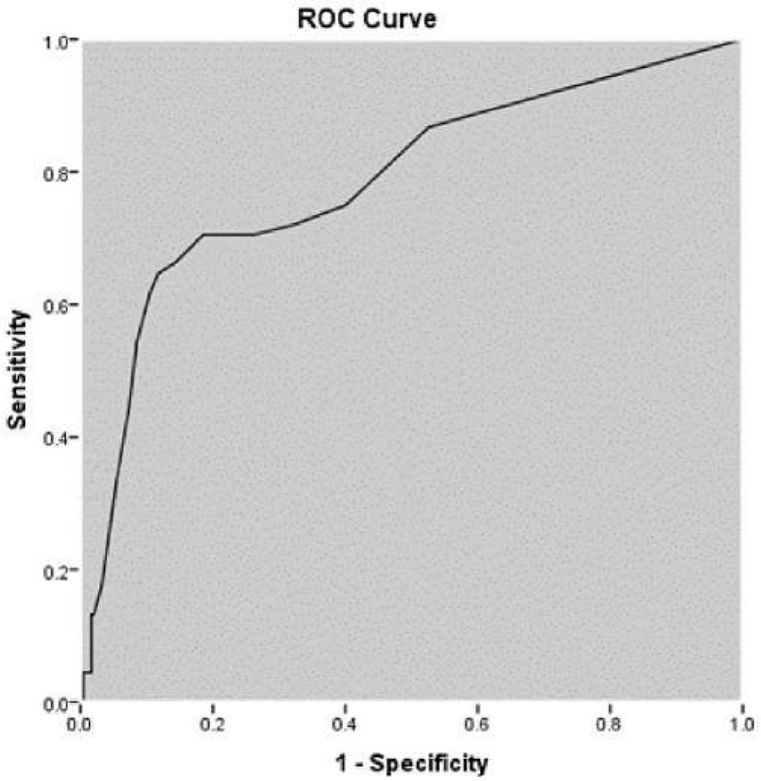

Figure 1: ROC curve for percentage hypochromic red blood cells

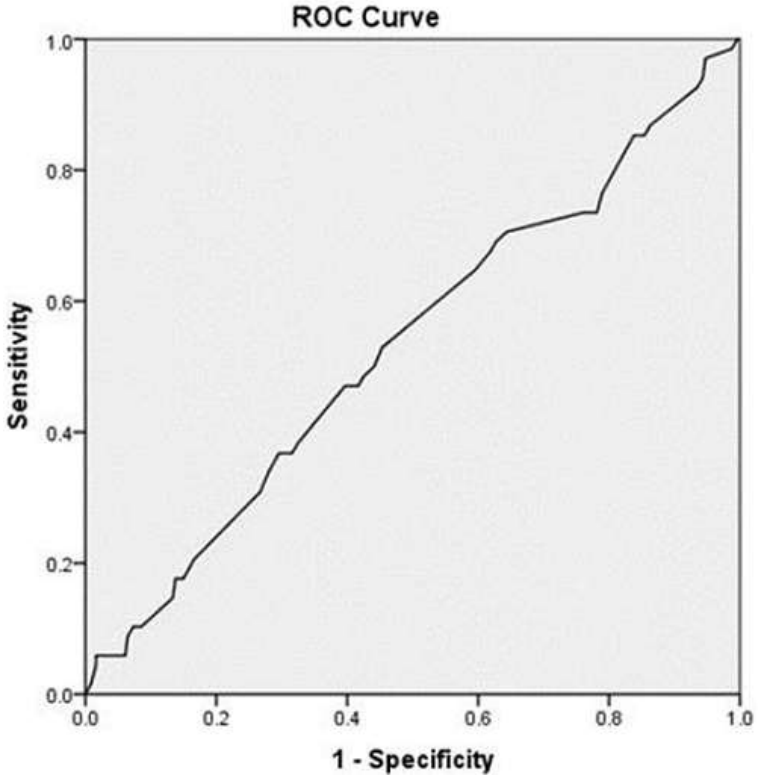

Figure 2: ROC curve for mean corpuscular haemoglobin

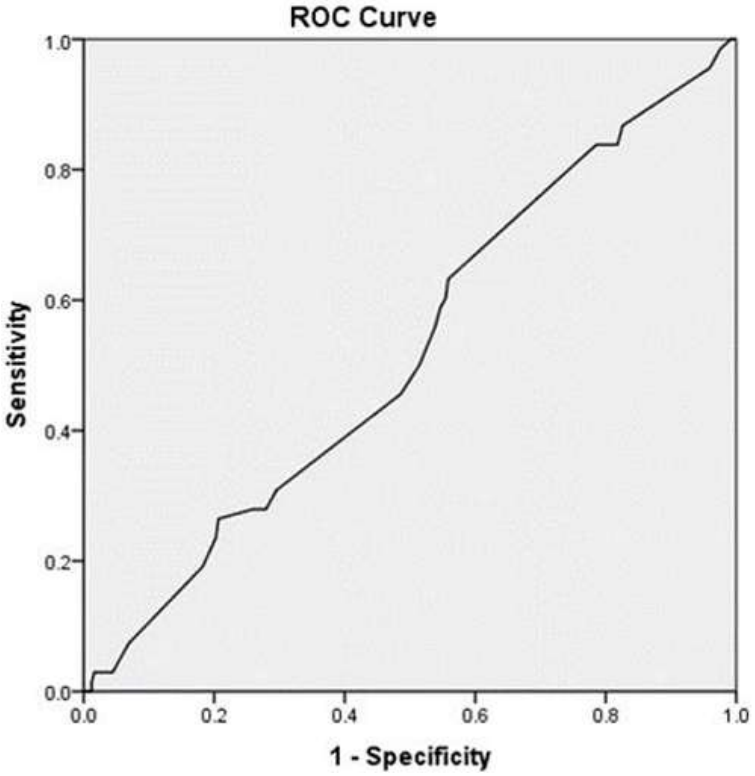

Figure 3: ROC curve for mean corpuscular haemoglobin concentration

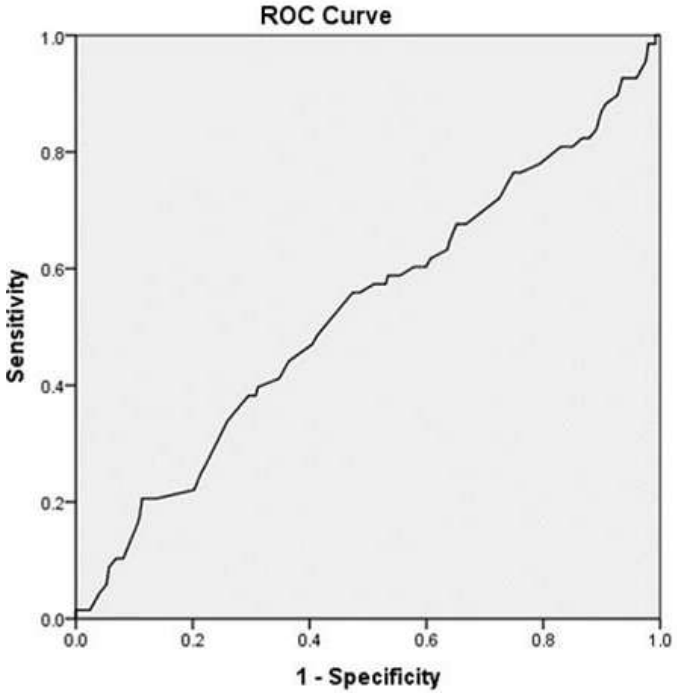

Figure 4: ROC curve for mean corpuscular volume