Hepcidin and GDF-15 are potential biomarkers of Iron Deficiency Anaemia in Chronic Kidney Disease Patients in South Africa

CURRENT STATUS: UNDER REVIEW

BMC Nephrology  ▶ BMC Series

AISHATU MUHAMMAD NALADO  aishnld@yahoo.co.uk
University of the Witwatersrand Faculty of Health Sciences
Corresponding Author
ORCiD: 0000-0003-3662-8150

Gbenga Olorunfemi
University of the Witwatersrand

Therese Dix-Peek
University of the Witwatersrand

Caroline Dickens
University of the Witwatersrand

Lungile Khambule
University of the Witwatersrand

Tracy Snyman
University of the Witwatersrand

Graham Paget
University of the Witwatersrand

Johnny Mahlangu
University of the Witwatersrand

Raquel Duarte
University of the Witwatersrand

JAYA George
University of the Witwatersrand

Saraladevi Naicker
University of the Witwatersrand

**DOI:** 10.21203/rs.2.19401/v1

**SUBJECT AREAS**  
*Urology & Nephrology*

**KEYWORDS**  
*iron deficiency anaemia, GDF-15, hepcidin, utility, chronic kidney disease*
Abstract

Background

Iron deficiency anaemia is a significant cause of morbidity and mortality among chronic kidney disease (CKD) patients. There is a paucity of information on the role of hepcidin and growth differentiation factor-15 (GDF-15) as potential biomarkers of iron deficiency anaemia among non-dialysis CKD patients. This study aimed to determine the utility of hepcidin and GDF-15 as biomarkers of iron deficiency among non-dialysis CKD patients at an academic hospital in Johannesburg, South Africa.

Method

A cross-sectional study of 312 consecutive consenting non-dialysis CKD patients and 184 controls at Charlotte Maxeke Academic Hospital was conducted from June 2016 to December 2016. Socio-demographic and clinical characteristics were recorded. Plasma hepcidin and GDF-15 were measured using mass spectrometry and ELISA, respectively. Spearman rank correlation, linear and logistic regression and receiver operator curves were utilised to evaluate the predictive and diagnostic/reference values of hepcidin and GDF-15 in absolute and functional iron deficiency anaemia.

Results

The mean age of participants was 49.7 ±15.8 years, and 50.6% of them were females. The predictive value of diagnosing iron deficiency anaemia among CKD patients using GDF-15 and hepcidin was high (AUC=0.723 and 0.714, respectively). There was a weak negative correlation between hepcidin levels and GFR (r=-0.19, p=0.04) in anaemic CKD patients, and between serum GDF-15 and haemoglobin (r=-0.34, p=0.001). Serum ferritin (β=0.005, P-value<0.001), MCV (β=0.0276, P-value=0.029) and gender (β=-0.2188, P-value=0.042) were predictors of log
hepcidin. Urea (β=0.0062, P-value=0.044), MCHC (β = -0.0816, P-value<0.001) and gender (β=-0.0755, P-value=0.001) were predictors of logGDF-15. Both GDF-15 (P=0.028) and hepcidin (P=0.049) were associated with iron deficiency anaemia. Subgroup analysis showed that GDF-15 predicted absolute iron deficiency, while hepcidin predicted functional iron deficiency anaemia

**Conclusion**

GDF-15 and hepcidin are potential predictors of iron deficiency anaemia among CKD patients.

**Introduction**

Anaemia is a common presenting feature among patients with chronic kidney disease (CKD) and is associated with poor quality of life and attendant poor clinical outcomes (1). The pathogenetic mechanisms of anaemia in CKD include reduced erythropoietin production and reticuloendothelial iron blockade secondary to chronic kidney inflammation (2). While largely responsive to erythropoietin replacement, anaemia due to chronic inflammation may be resistant to erythropoietin (EPO) treatment (3). Thus, the management of iron homeostasis should be optimised to enhance the effect of EPO treatment on CKD-associated anaemia (4).

Hepcidin is a peptide hormone that regulates iron balance in the body (5). It is synthesised by hepatocytes and reduces iron absorption and cellular release of iron, through binding to ferroportin (6). Thus, in chronic diseases, elevated levels of hepcidin can decrease absorption of dietary iron and impair the release of iron from its reservoir in the hepatocytes and macrophages (7). Further, increased hepcidin production leads to a decline in plasma iron levels, with an attendant negative
impact on erythropoiesis (2). Studies have also shown that patients with chronic infections and inflammatory disease are predisposed to have increased levels of hepcidin (6, 8). Elevated hepcidin levels may, therefore, play a role in the anaemia of chronic kidney disease (5). The impact of hepcidin on iron metabolism may be increased among CKD patients who have chronic underlying inflammation (5).

Hepcidin inhibits iron release from macrophages as well as intestinal iron absorption. In inflammatory states, hepcidin production is no longer regulated by iron burden (i.e., if the iron level is low, hepcidin synthesis should be downregulated) but is somewhat increased through IL-6 stimulation (9). Moreover, higher levels of hepcidin may occur among CKD patients who are likely to have a poor renal clearance of plasma hepcidin, as their kidney function declines (2). The hepcidin and GDF-15 levels (Growth differentiation factor-15) in CKD patients may not be of greater diagnostic value than conventional parameters, but further studies on these biomarkers are needed. (10). Moreover, detection of the association of GDF-15 expression with serum iron parameters in patients with IDA may be helpful for the diagnostic and pathogenic impact of GDF-15 in anaemia, the role of GDF-15 in IDA is still controversial (11). It is also plausible that increased hepcidin concentrations may cause iron-restricted erythropoiesis in CKD associated anaemia (12). From the previous observations, we postulate that the serum hepcidin level may be a useful biomarker of iron status among CKD patients. Hepcidin inhibitors or hepcidin lowering agents may be effective stand-alone or adjunctive therapy for maintaining normal iron homeostasis, thereby improving anaemia among CKD patients (13), these inhibitors of hepcidin production or action have shown promise to treat anaemia of inflammation in pre-clinical studies, and are now entering human clinical trial (14). However, the utility of hepcidin as a biomarker in the
diagnosis of iron deficiency anaemia (IDA) among non-dialysis CKD patients is unclear, especially in low resource settings such as ours. Growth Differentiation Factor-15 (GDF-15), an anti-inflammatory cytokine, has been suggested as a significant regulator of hepcidin. (15) Oxidative stress and inflammatory conditions can stimulate secretion of GDF-15 by macrophages (16), and GDF-15 has been implicated in the evolution of tumours (17, 18) and atherosclerosis (18). In addition, a strong positive correlation has been reported between hepcidin and GDF-15 in some anaemic patients (19). Emerging evidence shows that in response to anaemia (20), erythroblasts secrete GDF-15, which in turn suppresses hepcidin expression and decreases iron stores (16, 21, 22). It is also plausible that GDF-15 could signal intra-renal injury as circulating GDF-15 levels strongly correlated with intrarenal expression of GDF-15, and GDF-15 was found to be associated with increased risk of CKD progression in two independent cohorts of CKD patients in the Clinical Phenotyping and Resource Biobank (C-PROBE), and the Seattle Kidney Study (SKS) (23, 24).

Little is known about the relationship between GDF-15, hepcidin, and IDA among patients with non-dialysis requiring CKD. The aim of this study was to evaluate serum GDF-15 and hepcidin as surrogates or diagnostic markers of IDA among non-dialysis requiring CKD patients.

Materials and methods

We conducted a comparative cross-sectional study among 312 consecutive patients with CKD attending the renal outpatient clinic of Charlotte Maxeke Johannesburg Academic Hospital, South Africa from 1 June 2016 to 31 December 2016, and 184 healthy controls (which included patients’ relatives and staff members). The
inclusion criterion was all adult stable consenting non-dialysis CKD patients aged 18 years and above. The Human Research Ethics Committee at the University of the Witwatersrand approved this study (150929). All patients gave written informed consent.

Exclusion criteria included active inflammation, active infection, haemoglobinopathies, and blood transfusion within three months preceding the study, immunosuppressive therapy, oral iron treatment, and use of intravenous iron therapy at least two weeks before enrolment, and persons currently on erythropoietin stimulating agents (ESA).

We defined absolute iron deficiency as serum ferritin <100ug/l and transferrin saturation (TSAT <20%), while functional iron deficiency was defined as serum ferritin >100ug/l and TSAT <20%, according to Capellini et al (25). Anaemia was defined as haemoglobin <13g/l in men and <12g/l in women. Patients were considered to have IDA if they presented with absolute iron deficiency, functional iron deficiency and anaemia with low mean corpuscular volume (MCV) (26, 27).

Glomerular filtration rate (GFR) was determined by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for eGFR (28).

**Laboratory Measurements**

All collected venous blood samples were immediately centrifuged, separated into aliquots, and stored at -80 degrees Celsius for future assays. Haematological measurements were made on fresh venous blood with EDTA and clotted blood samples. Haemoglobin concentrations (Hb), red blood cell count (RBC), platelet count, creatinine and urea were measured using a haematology analyser (Siemens Diagnostics, Tarrytown, USA). Serum iron was determined by the ferrozine calometric method, total iron-binding capacity (TIBC) by a colorimetric chromazurol
dye-binding method using ADVIA 1800 (Siemen Medical Solutions Diagnostic, USA), and serum ferritin were determined by using a two-site chemiluminescent immunometric assay by IMMULITE®2000 system (Siemens Medical Solutions Diagnostics, USA). Transferrin saturation (TSAT) was calculated as the ratio of serum iron and TIBC and expressed as a percentage.

Hepcidin and isotope labelled internal standards $[^{13}\text{C}_9,^{15}\text{N}_3]$ were purchased from Peptide Institute (Osaka JPN). The serum hepcidin-25 and isotope labelled internal standards $[^{13}\text{C}_9,^{15}\text{N}_3]$ were extracted and separated as previously described by Li et al. (29) Briefly, samples were extracted with 4% HPLC grade trichloroacetic acid (TCA, Merck, Kenilworth, NJ, U.S.A) and separated using reverse-phase liquid chromatography using a 0.5x50mm Halo Peptide- ES column (Sciex, Framingham, USA) with a total run time of 5 min per sample. The analysis was performed on an AB Sciex 5500 QTRAP (Sciex, Framingham, USA), coupled to a micro-Liquid Chromatography (Exigent M3) system with Turbo IonSpray ionisation source operated in positive ion mode Multiple reactive monitoring (MRM) detection was applied using the Sciex 5500 for identification and quantification using the ion transitions (m/z) of 698.0>354.0 and 703.2>354.1 for hepcidin and stable isotope labelled hepcidin $[^{13}\text{C}_9,^{15}\text{N}_3]$ respectively.

Concentrations of serum hepcidin were expressed in nanograms per millilitre (ng/ml). The intra-and inter-assay coefficients of variation (CV) were <6.7% and < 8.8%, respectively. The lower limit of detection was 0.5ng/ml. The median reference level for plasma hepcidin in healthy controls was 5.7 ng/ml. Serum levels of GDF-15 were measured by ELISA (R&D Systems, Minneapolis, MN, USA) with intra- and inter-assay CVs of <2.8 and <6, respectively.
Statistical analysis

Statistical analysis was performed using Stata version 14 (Stata Corp., TX, USA) software. Normally distributed continuous variables were presented as mean (± standard deviation), and non-normally distributed continuous variables were presented as median (interquartile range). Categorical variables were presented as numbers and percentages. The 5th and 95th percentile of the hepcidin and GDF-15 levels among non-anaemic apparently healthy controls were determined as the reference range for the study population. Student’s t-test or Mann-Whitney U tests were used to compare continuous variables among anaemic and non-anaemic groups. Association between anaemic status and categorical variables were assessed using Pearson’s Chi-square test. Spearman’s rank correlation was used to determine the linear relationship between hepcidin or GDF-15 and other linear variables among anaemic and non-anaemic participants. Univariable and multivariable linear regression was conducted with log hepcidin and logGDF-15 as outcome variables respectively. Univariable and multivariable logistic regression was conducted to determine the association between IDA and hepcidin. Confounding variables based on the literature and a univariable p-value of <0.2 were utilised to build the multivariable model using backwards stepwise regression. Age and gender were chosen a priori. A post-estimation receiver operator characteristic curve (ROC) with an area under the curve (AUC) was then utilised to determine the predictive value of hepcidin for IDA among CKD participants. Similar regression analysis was conducted between IDA and GDF-15. The AUC of ROC for hepcidin and GDF-15 were then compared. A non-covariate table of cut-offs with corresponding sensitivity and specificity was generated for hepcidin as a diagnostic marker for IDA among the CKD population. The optimum cut-off point of hepcidin
was then calculated based on the maximal value of the Youden Index (= (sensitivity + specificity) -1). Similarly, an optimum cut-off value for GDF-15 was obtained. The above-mentioned analysis (regression, ROC and cut-offs) was conducted to determine the predictive value of hepcidin and then GDF-15 for functional and absolute IDA. Sub-group analysis was conducted to see if there was a disparity in the performance of hepcidin and GDF-15 in the diagnosis of absolute and functional IDA. For all analyses, a 2-tailed test of the hypothesis was assumed and the level of statistical significance was set at P-value <0.05 (95% confidence interval).

Results

The mean age of the participants was 49.7 (± 15.8) years and there was an almost equal proportion of male (49.4%, n = 245/496) and female (50.6%, n = 251/496) participants. The prevalence of anaemia among CKD cases was about three times that of controls, 33.0% (95%CI: 27.99% – 38.45%) vs 9.78% (95%CI: 6.22% – 15.05%) respectively.

The median levels of serum GDF-15 [1024.5 (429.4–1489.5 pg/ml) vs. 447.25 (188.25–1192.3 pg/ml), P-value < 0.0001] and hepcidin [7.1 (3.9–36.2) vs 3.1(2.1–10.9), P-value < 0.0001], Table 1, were more than doubled among the CKD participants as compared to the controls. Among the CKD participants, the median GDF-15 level was higher among the anaemic as compared to the non-anaemic participants (P-value < 0.0001, Table 1). Similarly, median GDF-15 levels were higher among the anaemic controls as compared to the non-anaemic controls (P = 0.0155). In contrast, there was no difference in the serum levels of hepcidin by anaemia status among the CKD participants (P-value = 0.2790) and the controls (P-
Table 1
Socio-demographic, haematological and biochemical characteristics of participants by iron-deficiency-anaemia status

| Characteristic | CKD (n = 312) | Controls (n = 184) |
|---------------|---------------|-------------------|
|               | Non-anaemic   | Non-anaemic       |
|               | n = 209 (%)   | n = 166 (%)       |
| **Hepcidin**  | **n = 103 (%)** | **n = 18 (%)** |
| (ng/ml)       | (median, IQR) | (median, IQR)    |
| 8.4 (4-45.5)  | 6.8 (3.9-33.6) | 3.1 (2.3-7.9)    |
| 1256.8 (919.1-1618) | 700 (335.1-1327.8) | < 0.0001 |
| 0.2790        | 3.1 (2.1-10.9) |
| Age (mean ± SD) years | 54.5 ± 15.2 | 45.2 ± 17.5 |
| < 40          | 15 (14.6)     | 8 (44.4)         |
| ≥ 40          | 88 (82.3)     | 79 (47.6)        |
| Race          | Blanks        | Whites            |
|               | 95 (92.2)     | 165 (79.0)       |
|               | 8 (7.8)       | 44 (21.1)        |
| Gender        | Male          | Female            |
|               | 41 (39.8)     | 126 (60.3)       |
|               | 62 (60.2)     | 83 (39.7)        |
| Systolic Blood Pressure (median, IQR) (mmHg) | 139 (125-157) | 140 (130-160) |
|               | 132 (120-140) | 132 (130-140) |
| Diastolic Blood Pressure (median, IQR) (mmHg) | 80 (70-91) | 80 (70-90) |
|               | 81 (70-90) | 80 (72-90) |
| Serum Urea (median, IQR) (mmol/L) | 17.1 (9.8-25.7) | 11 (7.1-15.9) |
|               | 4 (3-4.9) | 4.35 (3.6-5.2) |
| Serum Creatinine (median, IQR), μmol/L | 265 (158-520) | 171 (120-255) |
|               | 70.5 (64-78) | 78.5 (69-91) |
| GFR (median, IQR), ml/min/1.73 m² | 27.0 (12.6-27.0) | 43.6 (27.7-66.0) |
|               | < 0.0001 | < 0.0001 |

The reference values (5% – 95% range) among the non-anaemic controls of this study for hepcidin and GDF-15 were 1.5–28.1 ng/ml and 108.2–2833 pg/ml, respectively. There was a significant negative linear relationship between hepcidin and GDF-15 among anaemic CKD patients (r= -0.28, P-value = 0.0037), supplementary Table 1.

Serum ferritin (r = 0.5, P-value < 0.0001), and serum creatinine levels (r = 0.21, P-value = 0.0368) were positively correlated with hepcidin, while GFR (r = −0.19, P-
value = 0.0493) was negatively correlated with hepcidin among the participants with CKD, supplementary table 1. GDF-15 was negatively correlated with serum ferritin and haemoglobin levels, while TSAT level correlated with GDF-15, especially among the anaemic CKD cases (supplementary table 2).

For every ng/ml increase in serum ferritin level among CKD patients, log hepcidin increased by 0.0058 (β = 0.0058, P-value < 0.001). In other words, hepcidin levels increased by $10^{0.0058}$ units for every ng/ml increase in serum ferritin. Similarly, for every g/dl increase in MCV, log hepcidin increased by 0.0276 (β = 0.0276, P-value = 0.029). Similarly, logGDF-15 increased with every unit increase of urea (β = 0.0062, P-value = 0.044) but decreased with every unit increase of MCH (β = -0.0816, P-value < 0.001), Table 2.

| Variable  | Log Hepcidin  | Log GDF-15  |
|-----------|---------------|-------------|
|           | Coefficient   | SE          | P-value | Coefficient | SE | P-value |
| Ferritin  | 0.0058        | 0.00045     | < 0.0001 | 0.000018    | 0.0003 | 0.953 |
| Age       | 0.0010        | 0.0037      | 0.787    | -0.00103    | 0.0027 | 0.704 |
| Race      | Black        | Reference   | Reference | Reference   | Reference | Reference |
| Whites    | 0.0948        | 0.1353      | 0.484    | 0.08541     | 0.1037 | 0.411 |
| Gender    | Male         | Reference   | Reference | Reference   | Reference | Reference |
| Female    | -0.2188       | 0.1071      | 0.042    | 0.2621      | 0.0755 | 0.001 |
| GFR       | -0.000041     | 0.0009      | 0.965    | -0.0014     | 0.0009 | 0.136 |
| MCHC      | -0.0780       | 0.0335      | 0.021    | -0.0816     | 0.0164 | < 0.001 |
| MCV       | 0.0276        | 0.0126      | 0.029    | -           | -      | - |
| Urea      | -            | -           | -        | 0.0126      | 0.0062 | 0.0440 |

The predictive value of GDF-15 and hepcidin for diagnosing IDA among CKD participants was 72% (95%CI: 66% – 78%) and 71% (95%CI: 65% – 78%), respectively. There was no statistically significant difference between the AUC of the ROC curves of GDF-15 and that of hepcidin, (P-value = 0.49, Fig. 1A, Table 3,4). A combination of the two parameters did not improve the diagnostic value of either of the two tests, as the AUC of the ROC of the model with the combination was 73%, 95%CI: 67% – 79%; P-value = 0.29.
### Table 3
Predictors of iron deficiency anaemia with Hepcidin or GF-15 as the primary biomarker among CKD participants

| Variable | **1 Multivariable logistic regression analysis with Hepcidin as the primary factor** | **2 Multivariable logistic regression analysis with GFD15 as the primary factor** |
|----------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
|          | Odds ratio | 95%CI   | P-value | Odds ratio | 95%CI       | P-value |
| Hepcidin | 1.0023     | 1.0000-1.0045 | 0.049 | - | - |
| GDF-15   | -          | -       | -      | 1.0024     | 1.000026-1.00455 | 0.028 |
| Age      | 1.00       | 0.98-1.02 | 0.964  | 1.00       | 0.98-1.02     | 0.947  |
| Gender   |            |         |        |            |             |        |
| Male     | Reference  | Reference | Reference | Reference  | Reference   | Reference |
| Female   | 2.80       | 1.65-4.74 | <0.0001 | 2.73       | 1.60-4.67    | <0.001 |
| eGFR     | 0.99       | 0.98-1.00 | 0.028  | 0.99       | 0.98-1.00    | 0.33   |
| CRP      | 1.01       | 1.00-1.01 | 0.042  | 1.01       | 1.001-1.015  | 0.025  |
| MCV      | 0.94       | 0.90-0.98 | 0.004  | 0.94       | 0.90-0.98    | 0.006  |

**1 Multivariable logistic regression analysis of the model of the relationship between Hepcidin and iron deficiency anaemia.**

**2 Multivariable logistic regression analysis of the model of the relationship between GDF-15 and iron deficiency anaemia.**

The 2 models corrected for gender, age, glomerular filtration rate, C-reactive protein and mean corpuscular volume. OR: Adjusted odds ratio.

### Table 4
Predictors of Absolute iron deficiency anaemia with Hepcidin or GF15 as the primary biomarker among CKD participants

| Variable | **1 Multivariable logistic regression analysis with Hepcidin as the primary factor** | **2 Multivariable logistic regression analysis with GFD15 as the primary factor** |
|----------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
|          | Odds ratio | 95%CI   | P-value | Odds ratio | 95%CI       | P-value |
| Hepcidin | 1.01       | 0.99-1.01 | 0.30   | - | - |
| GDF-15   | -          | -       | -      | 1.001     | 1.00-1.01   | 0.0016 |
| Age      | 1.01       | 0.98-1.03 | 0.54   | 1.006     | 0.98-1.03   | 0.65   |
| Race     |            |         |        |            |             |        |
| White    | Reference  | Reference | Reference | Reference  | Reference   | Reference |
| Black    | 0.23       | 0.78-0.66 | 0.007  | 0.35       | 0.14-0.91   | 0.30   |
| Gender   |            |         |        |            |             |        |
| Male     | Reference  | Reference | Reference | Reference  | Reference   | Reference |
| Female   | 2.86       | 1.38-5.91 | 0.005  | 2.95       | 1.37-6.34   | 0.006  |
| eGFR     | 0.99       | 0.98-1.01 | 0.503  | 0.99       | 0.98-1.01   | 0.318  |
| CRP ()   | 1.01       | 0.99-1.01 | 0.436  | 1.01       | 0.99-1.02   | 0.160  |
| MCV()    | 0.91       | 0.88-0.96 | 0.002  | 0.92       | 0.87-0.97   | 0.002  |
| MCHC()   | 0.73       | 0.52-1.01 | 0.056  | 0.71       | 0.48-1.03   | 0.069  |
| Phosphate() | - | -       | -      | 0.50       | 0.26-0.99   | 0.046  |
| DM       | 1.87       | 0.94-3.71 | 0.008  | 1.77       | 0.86-3.64   | 0.012  |

**1 Multivariable logistic regression analysis of the model of the relationship between Hepcidin and Absolute iron deficiency anaemia.**

**2 Multivariable logistic regression analysis of the model of the relationship between GDF-15 and Absolute iron deficiency anaemia.**

The 2 models corrected for gender, age, glomerular filtration rate, C-reactive protein, MCV, Phosphate, Diabetes Mellitus and mean corpuscular volume. OR: Adjusted odds ratio.
Table 5
A Predictors of Functional iron deficiency anaemia with Hepcidin or GF15 as the primary biomarker among CKD participants

| Variable | 1 Multivariable logistic regression analysis with Hepcidin as the primary factor | 2 Multivariable logistic regression analysis with GFD15 as the primary factor |
|----------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
|          | Odds ratio | 95% CI | P-value | Odds ratio | 95% CI | P-value |
| Hepcidin | 1.01       | 1.01–1.01 | 0.013 | - | - | - |
| GDF-15   | -          | -      | -      | 1.01       | 0.99–1.01 | 0.761 |
| Age      | 0.98       | 0.95–1.01 | 0.123 | 0.99       | 0.96–1.01 | 0.169 |
| Gender   |            |        |        |            |        |        |
| Male     | Reference  | Reference | Reference | Reference | Reference | Reference |
| Female   | 3.23       | 1.47–7.29 | 0.004 | 2.9        | 1.29–6.29 | 0.009 |
| Race     |            |        |        |            |        |        |
| White    | Reference  | Reference | Reference | Reference | Reference | Reference |
| Black    | 15.09      | 1.20–188.96 | 0.035 | 15.88      | 1.16–216.46 | 0.038 |
| eGFR     | 0.99       | 0.98–0.99 | 0.007 | 0.98       | 0.98–0.99 | 0.008 |
| CRP      | 1.01       | 1.00–1.01 | 0.002 | 1.01       | 1.01–1.02 | 0.001 |
| MCHC     | 0.79       | 0.62–0.99 | 0.041 | 0.78       | 0.61–0.99 | 0.044 |
| DM       | 1.35       | 0.55–4.78 | 0.070 | 1.31       | 0.56–4.79 | 0.00 |

1 Multivariable logistic regression of the model of the relationship between Hepcidin and iron deficiency anaemia
2 Multivariable logistic regression of model of the relationship between GDF-15 and iron deficiency anaemia.
The 2 models corrected for gender, age, glomerular filtration rate, C-reactive protein and mean corpuscular volume. OR: Adjusted odds ratio.

Using the non-covariate analysis and Youden’s index, the optimum cut-off value among CKD participants for GDF-15 was 1,030 pg/ml (at a sensitivity of 72.8% and specificity of 61.24%). Similarly, the optimum cut-off for hepcidin was 22.5 ng/ml (at a sensitivity of 38.8% and specificity of 70.8%) (Data not shown).

The predictive value of GDF-15 for diagnosing absolute IDA among CKD participants was 81.9%. (Table 4, Fig. 1). Using the non-covariate analysis and Youden’s index, the optimum cut-off value of GDF-15 for diagnosing absolute IDA among CKD participants was 1129.3 mg/dl (at a sensitivity of 83.64% and specificity of 66.03%). Similarly, the optimum cut-off for hepcidin was 22.5 ng/dl (at a sensitivity of 66.7% and specificity of 70.8%); (Table 6)

Table 6
Cut-off and validity of GDF-15 and Hepcidin in diagnosing Absolute and functional Iron deficiency Anaemia among CKD patients.

| Test Parameter | Predictive value of GDF 15 for AID among CKD patients | Predictive value of Hepcidin for FID among CKD patients |
|----------------|-------------------------------------------------------|-------------------------------------------------------|
| Cut-off        | 1129.3 mg/dl                                          | 22.5 ng/dl                                            |
| AUC            | 81.9%                                                 | 81.4                                                  |
| Sensitivity    | 83.64%                                                | 66.7%                                                 |
| Sensitivity | 83.94% | 90.3% |
|-------------|--------|-------|
| Specificity | 66.03% | 70.8% |
| Likelihood ratio of positive result | 2.462 | 2.28 |
| Likelihood ratio of negative result | 0.2478 | 0.47 |
| Youden’s index | 49.67% | 37.48% |

AID: Absolute iron deficiency anaemia; FID: Functional iron deficiency anaemia; AUC: Area Under Curve.

References
1. Mikhail A, Brown C, Williams JA, Mathrani V, Shrivastava R, Evans J, et al. Renal association clinical practice guideline on Anaemia of Chronic Kidney Disease. BMC Nephrology. 2017;18.
2. Ganz T. Molecular control of iron transport. Journal of the American Society of Nephrology : JASN. 2007;18(2):394–400.
3. Young B, Zaritsky J. Hepcidin for clinicians. Clinical Journal of the American Society of Nephrology : CJASN. 2009;4(8):1384-7.
4. Richardson D. Clinical factors influencing sensitivity and response to epoetin. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2002;17 Suppl 1:53 – 9.
5. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science (New York, NY). 2004;306(5704):2090-3.
6. Arezes J, Nemeth E. Heparin and iron disorders: new biology and clinical approaches. International journal of laboratory hematology. 2015;37 Suppl 1:92 – 8.
7. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. Blood. 2006;108(12):3730-5.
8. Cheng PP, Jiao XY, Wang XH, Lin JH, Cai YM. Heparin expression in anemia of chronic disease and concomitant iron-deficiency anemia. Clinical and experimental medicine. 2011;11(1):33–42.
9. D'angelo G. Role of hepcidin in the pathophysiology and diagnosis of anemia. Blood research. 2013;48(1):10 – 5.
10. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I, et al. Guideline for the laboratory diagnosis of functional iron deficiency. British journal of haematology. 2013;161(5):639 – 48.
11. Abaza HM, Habashy DM, El-Nashar RE. Growth differentiation factor 15 expression in anemia of chronic disease and iron deficiency anemia. The Egyptian Journal of Haematology. 2013;38(1):23.
12. Uehata T, Tomosugi N, Shoji T, Sakaguchi Y, Suzuki A, Kaneko T, et al. Serum hepcidin-25 levels and anemia in non-dialysis chronic kidney disease patients: a cross-sectional study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(3):1076-83.
13. Tsuchiya K, Nitta K. Heparin is a potential regulator of iron status in chronic kidney disease. Therapeutic apheresis and dialysis : official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy. 2013;17(1):1–8.
14. Wang CY, Babitz JL. Heparin regulation in the anemia of inflammation. Current opinion in hematology. 2016;23(3):189.
15. Yalcin MM, Altinova AE, Akturk M, Gulbahar O, Arslan E, Ors Sendogan D, et al. GDF-15 and Heparin Levels in Nonanemic Patients with Impaired Glucose Tolerance. Journal of Diabetes Research. 2016;2016.
16. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(21):11514-9.
17. Unsicker K, Spittau B, Kriegstein K. The multiple facets of the TGF-beta family cytokine growth/differentiation factor-15/macrophage inhibitory cytokine-1. Cytokine & growth factor reviews. 2013;24(4):373 – 84.
18. Eggers KM, Kempf T, Lind L, Sundstrom J, Wallentin L, Wollert KC, et al. Relations of growth-differentiation factor-15 to biomarkers reflecting vascular pathologies in a population-based sample of elderly subjects. Scandinavian journal of clinical and laboratory investigation. 2012;72(1):45–51.
19. Mehmet Muhittin Yalcin AEA, Mujde Akturk, et al. GDF-15 and Heparin Levels in Nonanemic Patients with Impaired Glucose Tolerance. Journal of Diabetes Research. 2016;2016;1–5.
20. Valenti L, Messa P, Pelusi S, Campanostrini N, Girelli D. Heparin levels in chronic hemodialysis patients: a critical evaluation. Clinical chemistry and laboratory medicine. 2014;52(5):613-9.
21. Tanno T, Miller JL. [GDF15 expression and iron overload in ineffective erythropoiesis]. [Rinsho ketsueki]. The Japanese journal of clinical hematology. 2011;52(6):387 – 98.
22. Lakhal S, Balboula H, Trochet R, Townsend AR, Robbins PA, et al. Regulation of growth differentiation factor 15 expression by intracellular iron. Blood. 2009;113(7):1555-63.
23. Wang CY, Babitz JL, Suyto WS, Casu C, Rivello S, Strelau J, Unsicker K, et al. The murine growth differentiation factor 15 is not essential for systemic iron homeostasis in phlebotomized mice. Haematologica. 2013;98(3):444-7.
24. Ho JE, Hwang SJ, Wollert KC, Larson MG, Cheng S, Kempf T, et al. Biomarkers Of Cardiovascular Stress And Incident Chronic Kidney Disease. Clinical chemistry. 2013;59(11):1613-20.
25. Cappellini MD, Comin-Colet J, de Francisco A, Dignass A, Doehner W, Lam CS, et al. Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. American journal of hematology. 2017;92(10):1068-78.
26. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clinical Journal of the American Society of Nephrology : CJASN. 2006;1 Suppl 1:S4-8.
27. Stauffer ME. Prevalence of Anemia in Chronic Kidney Disease in the United States. 2014;9(1).
28. van den Brand JA, van Boekel GA, Willems HL, Kiememeny LA, den Heijer M, Wetzels JF. Introduction of the CKD-EPI equation to estimate glomerular filtration rate in a Caucasian population. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2005;20(4):674–9.
29. Ganz T. The hepcidin-ferroportin system: a key regulator of iron homeostasis. Blood. 2004;103(11):4386-94.
30. Ganz T. Molecular control of iron transport. Journal of the American Society of Nephrology : JASN. 2007;18(2):394–400.
31. Ganz T. Molecular control of iron transport. Journal of the American Society of Nephrology : JASN. 2007;18(2):394–400.
55. Ramirez JM, Schaad O, Durual S, Cossali D, Docquier M, Beris P, et al. Growth differentiation factor 15 production is necessary for normal erythroid differentiation and is increased in refractory anaemia with myelofibrosis. British Journal of Haematology. 2009;144(3):251–62.

56. Peters HP, Laarakkers CM, Swinkels DW, Wetzele JF. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(3):848 – 53.

57. Li HY, Rose MJ, Tran L, Zhang J, Miranda LP, James CA, et al. Development of a method for the sensitive and quantitative determination of hepcidin in human serum using LC-MS/MS. Journal of pharmaceutical and toxicological methods. 2009;59(3):171 – 80.

58. Li XY, Ying J, Li JH, Zhu SL, Li J, Pai P. Growth differentiation factor GDF-15 does not influence iron metabolism in stable chronic haemodialysis patients. Ann Clin Biochem. 2015;52(Pt 3):399-403.

59. Van Wyck DB, Bailie G, Aronoff G. Just the FAQs: frequently asked questions about iron and anaemia in patients with chronic kidney disease. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2002;39(2):426-32.

60. Kempf T, Wollert KC. Growth-differentiation factor-15 in heart failure. Heart failure clinics. 2009;5(4):537 – 47.
Discussion

To our knowledge, this is the first study to evaluate the clinical utility of hepcidin and GDF-15 as markers of IDA, among persons with non-dialysis CKD. This study demonstrates that in a cohort of predialysis CKD patients, serum hepcidin and GDF-15 predicted IDA among patients with CKD, with a predictive value of 71.4% and 72.3%, respectively, and GDF-15 predicted absolute IDA, while hepcidin predicted functional IDA (83.6% vs 66.7%). In addition, we showed a negative correlation between hepcidin and eGFR among CKD patients. There was no significant correlation between eGFR and GDF-15 among anaemic patients with CKD. Our observation of a negative correlation of hepcidin with GFR agrees with findings by others (12, 30). Ashby et al, after correcting for ferritin levels, concluded hepcidin inversely correlated with eGFR (31). In contrast with our study, Zaritsky et al (32) and Lee et al (33) found that eGFR had a positive correlation with hepcidin in adult CKD patients, despite using similar assay methods (mass spectrometry) as with our
study; however, hepcidin levels in their paediatric CKD patients did not correlate with eGFR. Sung Woo Lee et al’s (33) findings are also at variance with our findings; they found eGFR to positively correlate with hepcidin levels in patients with advanced CKD (stage 3b-5). This suggests that the relationship between hepcidin and renal function is still unclear. Thus, different etiopathogenic pathways may play varying roles among the CKD stages. Furthermore, the mechanism of action of hepcidin in CKD may differ from other low molecular weight proteins such as $\beta_2$ microglobulins or cystatin-c. Hepcidin pathways may be largely dependent on ferritin metabolism, with renal function playing a minimal role. The smaller sample size of previous studies may affect their conclusions. Our study recruited a relatively larger sample size. The sensitivity of the methods used may also play a role in the observed differences between our findings and others as we used mass spectrometry, in contrast to other studies where ELISA was used (30, 34). Our data supports published studies reporting higher levels of hepcidin in CKD and among haemodialysis patients (31, 35). The increase in serum hepcidin levels in CKD patients compared to controls might be due to increased inflammation and decreased renal clearance of hepcidin attributable to renal impairment in CKD patients (36-38).

We found that serum ferritin the primary storage molecule for cellular iron positively correlated with hepcidin in our CKD patients, as previously documented among patients with CKD and those on dialysis (31, 35, 39-41). This finding is also consistent with studies in non-CKD populations (42-44). In the setting of CKD, the direct relationship of hepcidin with ferritin may represent a protective effect of hepcidin against systemic iron overload (32). However, there was no correlation between hepcidin and inflammatory markers such as hsCRP. The possible
explanation may be that patients with active infections and inflammation were excluded from our study. The reference range (5%-95%) of serum hepcidin levels among healthy controls in this study was 1.5-28.1ng/ml. This value was lower than the reference range reported among healthy male and female adults [median and 5-95% reference range, 112ng/ml (29-254ng/ml) for men and 65ng/ml (17-286ng/ml) for women] (39). A possible explanation may be differences in assay used and racial differences. Thus, there is a need to establish different reference ranges for diverse populations.

We determined a cut-off value for serum hepcidin of <22.5ng/ml, (AUC= 0.71) at a sensitivity of 38.8%, and specificity of 70.8%, to predict IDA, which supports the literature that hepcidin levels are below the reference range in iron-deficient states (45). In 261 premenopausal female blood donors, serum hepcidin <8ng/ml (with a 95% confidence range of serum hepcidin levels from 8.2-199.7ng/ml) had a sensitivity of 41.5% and specificity of 97.6% for diagnosing IDA, while serum hepcidin <18ng/ml had a sensitivity of 79.2% and specificity of 85.6% (46). A study by Vyas et al. (47) determined hepcidin cut-off points to diagnose iron deficiency of <34.55, with AUC 0.845, the sensitivity of 98.33%, specificity 52.94%. Choi et al reported that hepcidin levels <6.895 ng/ml had a sensitivity of 79.2% and specificity of 82.8% for the diagnosis of iron deficiency in children (48). Sanad et al found urinary hepcidin at a cut-off value of <0.94 nmol/mmol to predict IDA with a sensitivity of 88% and specificity of 88% (49). These findings differ from the study by Jonker et al(50), who reported hepcidin to be a poor predictor of bone marrow iron stores (sensitivity of 66.7% and specificity 48.5%). However, their study population included children, and intra-individual variability in serum hepcidin could account for differences in our findings. This is the first study that showed in a sub-
group analysis that hepcidin was predictive of functional IDA in pre-dialysis CKD. Although levels of hepcidin are elevated in CKD, other factors may affect hepcidin levels. Further research is needed to determine whether this biomarker has advantages over conventional markers (ferritin, TSAT) (10).

We found GDF-15 levels to be significantly higher in CKD patients with IDA as compared to CKD patients without IDA, consistent with findings by Yilmaz et al (51). Although Yang Li et al (52) found an increase in GDF-15 levels in their dialysis patients, they were unable to demonstrate a correlation between GDF-15 and iron indices, a finding that was prominent in our study. There are possible mechanisms that may explain the findings of increased GDF-15 in patients with IDA. First, GDF-15 may be an important mediator in a negative feedback loop whereby it increases to suppress high hepcidin levels in CKD patients with iron deficiency. Second, GDF-15 expression could be upregulated in response to chronic inflammatory processes, which often occur in patients with IDA and CKD (53, 54). Another explanation is that iron depletion could independently cause GDF-15 induction in the erythroid precursor cells as a result of iron sequestration in macrophages (55). All these are speculative, however, as the exact mechanism, underlying IDA and GDF-15 levels require further investigation.

Another important finding in this study was GDF-15 predicting IDA, at a cut-off value <1,030 pg/ml with a predictive value of 72% (AUC = 0.723), the sensitivity of 72.8%, and specificity of 61.24%. Lakhal et al (22) also observed a significant increase in GDF-15 in persons with iron deficiency, which was similar to our findings. In contrast, Tanno et al (56) found among blood donors that there was no association between iron deficiency due to blood loss and serum GDF-15 levels. Our study, therefore, suggests that GDF-15 can be a useful diagnostic tool in patients
with IDA, and may relate to the fact that erythroid cells from patients with IDA may secrete a GDF-15-specific stimulator that is induced during iron deficiency (55). However, inflammation-mediated changes seen in iron homoeostasis may not be likely to induce the increased GDF-15 levels in patients with IDA, as serum ferritin levels did not correlate with GDF-15 levels in this study. Mast et al (57) also found the absence of correlation of GDF-15 with ferritin, which was similar to our finding. GDF-15 in this study was found to be predictive of absolute IDA in sub-group analysis, this finding is in contrast with findings of Yilmaz et al (51) where GDF-15 was predictive of functional IDA among haemodialysis and CKD patients. Differences in these findings could be due to differences in the study population, as their population included a dialysis population, and our study population was pre-dialysis. Another explanation could be due to different cut-off values to define functional IDA; their cut-off value was >800, while ours was >100. Further research is required to explore the puzzle of GDF-15 in IDA in CKD patients.

We found a negative correlation between participants’ age and GDF-15 levels, which is at variance with reports by other researchers (58-61). The difference in these findings could be attributed to differences in the study population as our study was carried out in a pre-dialysis population, and their studies involved dialysis patients, and a normal healthy population. The association of GDF-15 with age requires further prospective, multicentre studies.

Another important finding in this study is the negative correlation of GDF-15 with haemoglobin in both CKD patients and controls. This finding is consistent with reports by others (52, 58, 62), but is in disagreement with findings in non-CKD populations, where GDF-15 was positively correlated with haemoglobin (63-65). The difference in findings between our study and others could be explained by
differences in the study population.

Our study has limitations. First, the cross-sectional study design makes it difficult to infer causality between hepcidin, GDF-15 levels, and the risk of anaemia. The cross-sectional study design also precluded us from conducting follow up serial measurements of both hepcidin and GDF-15. The intra-patient variability and diurnal variations in hepcidin assays may interfere with serum hepcidin measurement.

The strengths of our study include the large sample size of pre-dialysis CKD patients, and our use of mass spectrometry, the gold standard for hepcidin assay. In addition, our study defined iron deficiency based on absolute and functional IDA, whereas prior studies focused mostly on functional IDA, and mainly recruited dialysis patients. Finally, to our knowledge, this is the first study to predict the performance of GDF-15 as a diagnostic tool for IDA in non-dialysis CKD patients.

In conclusion, our preliminary data indicate that GDF-15 and hepcidin predict IDA in pre-dialysis CKD patients and could be promising tools in the diagnosis of IDA in CKD, and they could predict absolute and functional IDA, among pre-dialysis CKD. These assays are expensive, not readily available, and require technical know-how, which may preclude their use in resource-constrained environments. More extensive randomized prospective studies are necessary to confirm our findings and to help determine a reliable cut-off value of serum hepcidin and GDF-15 in the diagnosis of IDA.

Declarations

Acknowledgements

We acknowledge the patients for participating in the research. We thank the International Society of Nephrology (ISN) for supporting Dr Nalado through the ISN
Declaration of conflicting interest

None of the authors have any conflict of interest to declare.

Funding

This research was partly funded by Trust Educational Funds from Bayero University Kano, and Supervisors research grants.

References

1. Mikhail A, Brown C, Williams JA, Mathrani V, Shrivastava R, Evans J, et al. Renal association clinical practice guideline on Anaemia of Chronic Kidney Disease. BMC Nephrology. 2017;18.

2. Ganz T. Molecular control of iron transport. Journal of the American Society of Nephrology : JASN. 2007;18(2):394-400.

3. Young B, Zaritsky J. Hepcidin for clinicians. Clinical Journal of the American Society of Nephrology : CJASN. 2009;4(8):1384-7.

4. Richardson D. Clinical factors influencing sensitivity and response to epoetin. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2002;17 Suppl 1:53-9.

5. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science (New York, NY). 2004;306(5704):2090-3.

6. Arezes J, Nemeth E. Hepcidin and iron disorders: new biology and clinical
approaches. International journal of laboratory hematology. 2015;37 Suppl 1:92-8.

7. Pak M, Lopez MA, Gabayan V, Ganz T, Rivera S. Suppression of hepcidin during anemia requires erythropoietic activity. Blood. 2006;108(12):3730-5.

8. Cheng PP, Jiao XY, Wang XH, Lin JH, Cai YM. Hepcidin expression in anemia of chronic disease and concomitant iron-deficiency anemia. Clinical and experimental medicine. 2011;11(1):33-42.

9. D'angelo G. Role of hepcidin in the pathophysiology and diagnosis of anemia. Blood research. 2013;48(1):10-5.

10. Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I, et al. Guideline for the laboratory diagnosis of functional iron deficiency. British journal of haematology. 2013;161(5):639-48.

11. Abaza HM, Habashy DM, El-Nashar RE. Growth differentiation factor 15 expression in anemia of chronic disease and iron deficiency anemia. The Egyptian Journal of Haematology. 2013;38(1):23.

12. Uehata T, Tomosugi N, Shoji T, Sakaguchi Y, Suzuki A, Kaneko T, et al. Serum hepcidin-25 levels and anemia in non-dialysis chronic kidney disease patients: a cross-sectional study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(3):1076-83.

13. Tsuchiya K, Nitta K. Hepcidin is a potential regulator of iron status in chronic kidney disease. Therapeutic apheresis and dialysis : official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy. 2013;17(1):1-8.

14. Wang C-Y, Babitt JL. Hepcidin regulation in the anemia of inflammation.
Current opinion in hematology. 2016;23(3):189.

15. Yalcin MM, Altinova AE, Akturk M, Gulbahar O, Arslan E, Ors Sendogan D, et al. GDF-15 and Hepcidin Levels in Nonanemic Patients with Impaired Glucose Tolerance. Journal of Diabetes Research. 2016;2016.

16. Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(21):11514-9.

17. Unsicker K, Spittau B, Kriegstein K. The multiple facets of the TGF-beta family cytokine growth/differentiation factor-15/macrophage inhibitory cytokine-1. Cytokine & growth factor reviews. 2013;24(4):373-84.

18. Eggers KM, Kempf T, Lind L, Sundstrom J, Wallentin L, Wollert KC, et al. Relations of growth-differentiation factor-15 to biomarkers reflecting vascular pathologies in a population-based sample of elderly subjects. Scandinavian journal of clinical and laboratory investigation. 2012;72(1):45-51.

19. Mehmet Muhittin Yalcin AEA, Mujde Akturk, et al. GDF-15 and Hepcidin Levels in Nonanemic Patients with Impaired Glucose Tolerance. Journal of Diabetes Research. 2016;2016:1-5.

20. Valenti L, Messa P, Pelusi S, Campostrini N, Girelli D. Hepcidin levels in chronic hemodialysis patients: a critical evaluation. Clinical chemistry and laboratory medicine. 2014;52(5):613-9.

21. Tanno T, Miller JL. [GDF15 expression and iron overload in ineffective erythropoiesis]. [Rinsho ketsueki] The Japanese journal of clinical hematology. 2011;52(6):387-98.

22. Lakhal S, Talbot NP, Crosby A, Stoepker C, Townsend AR, Robbins PA, et al.
Regulation of growth differentiation factor 15 expression by intracellular iron. Blood. 2009;113(7):1555-63.

23. Casanovas G, Spasić MV, Casu C, Rivella S, Strelau J, Unsicker K, et al. The murine growth differentiation factor 15 is not essential for systemic iron homeostasis in phlebotomized mice. Haematologica. 2013;98(3):444-7.

24. Ho JE, Hwang SJ, Wollert KC, Larson MG, Cheng S, Kempf T, et al. Biomarkers Of Cardiovascular Stress And Incident Chronic Kidney Disease. Clinical chemistry. 2013;59(11):1613-20.

25. Cappellini MD, Comin-Colet J, de Francisco A, Dignass A, Doehner W, Lam CS, et al. Iron deficiency across chronic inflammatory conditions: International expert opinion on definition, diagnosis, and management. American journal of hematology. 2017;92(10):1068-78.

26. Wish JB. Assessing iron status: beyond serum ferritin and transferrin saturation. Clinical Journal of the American Society of Nephrology : CJASN. 2006;1 Suppl 1:S4-8.

27. Stauffer ME. Prevalence of Anemia in Chronic Kidney Disease in the United States. 2014;9(1).

28. van den Brand JA, van Boekel GA, Willems HL, Kiemeney LA, den Heijer M, Wetzels JF. Introduction of the CKD-EPI equation to estimate glomerular filtration rate in a Caucasian population. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(10):3176-81.

29. Li H, Rose MJ, Tran L, Zhang J, Miranda LP, James CA, et al. Development of a method for the sensitive and quantitative determination of hepcidin in human serum using LC-MS/MS. Journal of pharmacological and toxicological methods.
27.

30. Peters HP, Laarakkers CM, Swinkels DW, Wetzels JF. Serum hepcidin-25 levels in patients with chronic kidney disease are independent of glomerular filtration rate. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2010;25(3):848-53.

31. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, et al.
Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney international. 2009;75(9):976-81.

32. Zaritsky J, Young B, Wang HJ, Westerman M, Olbina G, Nemeth E, et al.
Hepcidin—A Potential Novel Biomarker for Iron Status in Chronic Kidney Disease. Clinical Journal of the American Society of Nephrology : CJASN. 2009;4(6):1051-6.

33. Lee SW, Kim JM, Lim HJ, Hwang Y-H, Kim SW, Chung W, et al. Serum hepcidin may be a novel uremic toxin, which might be related to erythropoietin resistance. Scientific Reports. 2017;7(1):4260.

34. van der Putten K, Jie KE, van den Broek D, Kraaijenhagen RJ, Laarakkers C, Swinkels DW, et al. Hepcidin-25 is a marker of the response rather than resistance to exogenous erythropoietin in chronic kidney disease/chronic heart failure patients. European journal of heart failure. 2010;12(9):943-50.

35. Tomosugi N, Kawabata H, Wakatabe R, Higuchi M, Yamaya H, Umehara H, et al.
Detection of serum hepcidin in renal failure and inflammation by using ProteinChip System. Blood. 2006;108(4):1381-7.

36. Kulaksiz H, Gehrke SG, Janetzko A, Rost D, Bruckner T, Kallinowski B, et al. Pro-hepcidin: expression and cell specific localisation in the liver and its regulation
in hereditary haemochromatosis, chronic renal insufficiency, and renal anaemia. Gut. 2004;53(5):735-43.

37. van der Weerd NC. Hepcidin-25 in Chronic Hemodialysis Patients Is Related to Residual Kidney Function and Not to Treatment with Erythropoiesis Stimulating Agents. 2012;7(7).

38. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. The Journal of clinical investigation. 2002;110(7):1037-44.

39. Ganz T, Olbina G, Girelli D, Nemeth E, Westerman M. Immunoassay for human serum hepcidin. Blood. 2008;112(10):4292-7.

40. Wetzels DWSaJFM. Hepcidin: a new tool in the management of anaemia in patients with chronic kidney disease? Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2008;23:2450–3.

41. Coyne DW. Hepcidin: clinical utility as a diagnostic tool and therapeutic target. Kidney international. 2011;80(3):240-4.

42. Ingo Mecklenburg DR, Elizaveta Fasler-Kan, et al. Serum hepcidin concentrations correlate with ferritin in patients with inflammatory bowel disease. European Crohn's and Colitis Organisation Published by Elsevier BV. 2014;10:1392-7.

43. Haghpanah S, Esmaeilzadeh M, Honar N, Hassani F, Dehbozorgian J, Rezaei N, et al. Relationship Between Serum Hepcidin and Ferritin Levels in Patients With Thalassemia Major and Intermedia in Southern Iran. Iranian Red Crescent
Medical Journal. 2015;17(7).

44. Flávio Augusto Naoum BPE, Milton Arthur Ruiz, et al. Assessment of Labile Plasma Iron and Hepcidin in Patients Who Undergo Hematopoietic Stem Cell Transplantation. Blood. 2014;124:4029.

45. Goyal J, McCleskey B, Adamski J. Peering into the future: hepcidin testing. American Journal of Hematology. 2013;88(11):976-8.

46. Pasricha SR, McQuilten Z, Westerman M, Keller A, Nemeth E, Ganz T, et al. Serum hepcidin as a diagnostic test of iron deficiency in premenopausal female blood donors. Haematologica. 2011;96(8):1099-105.

47. Vyas S, Kapoor A, Nema S, Suman S. Quantification of serum hepcidin as a potential biomarker in diagnosis of iron deficiency anaemia. International Journal of Research in Medical Sciences. 2017;5(7):2926-30.

48. Choi HS, Song SH, Lee JH, Kim HJ, Yang HR. Serum hepcidin levels and iron parameters in children with iron deficiency. The Korean Journal of Hematology. 2012;47(4):286-92.

49. Sanad M, Gharib AF. Urinary hepcidin level as an early predictor of iron deficiency in children: A case control study. Italian Journal of Pediatrics. 2011;37:37.

50. Jonker FA, Calis JC, Phiri K, Kraaijenhagen RJ, Brabin BJ, Faragher B, et al. Low hepcidin levels in severely anemic malawian children with high incidence of infectious diseases and bone marrow iron deficiency. PLoS ONE. 2013;8(12):e78964.

51. Yilmaz H, Cakmak M, Darcin T, Inan O, Bilgic MA, Bavbek N, et al. Can Serum Gdf-15 be Associated with Functional Iron Deficiency in Hemodialysis Patients? Indian Journal of Hematology & Blood Transfusion. 2016;32(2):221-7.
52. Li XY, Ying J, Li JH, Zhu SL, Li J, Pai P. Growth differentiation factor GDF-15 does not influence iron metabolism in stable chronic haemodialysis patients. Ann Clin Biochem. 2015;52(Pt 3):399-403.

53. Van Wyck DB, Bailie G, Aronoff G. Just the FAQs: frequently asked questions about iron and anemia in patients with chronic kidney disease. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2002;39(2):426-32.

54. Kempf T, Wollert KC. Growth-differentiation factor-15 in heart failure. Heart failure clinics. 2009;5(4):537-47.

55. Ramirez JM, Schaad O, Durual S, Cossali D, Docquier M, Beris P, et al. Growth differentiation factor 15 production is necessary for normal erythroid differentiation and is increased in refractory anaemia with ring-sideroblasts. British journal of haematology. 2009;144(2):251-62.

56. Tanno T, Rabel A, Lee YT, Yau YY, Leitman SF, Miller JL. Expression of growth differentiation factor 15 is not elevated in individuals with iron deficiency secondary to volunteer blood donation. Transfusion. 2010;50(7):1532-5.

57. Mast AE, Foster TM, Pinder HL, Beczkiewicz CA, Bellissimo DB, Murphy AT, et al. Behavioral, biochemical, and genetic analysis of iron metabolism in high-intensity blood donors. Transfusion. 2008;48(10):2197-204.

58. Xiang-Yang Li JY, Jiu-Hong Li, et al. Growth differentiation factor GDF-15 does not influence iron metabolism in stable chronic haemodialysis patients. Annals of Clinical Biochemistry 2014;52(3):399-403.

59. Kempf T, Horn-Wichmann R, Brabant G, Peter T, Allhoff T, Klein G, et al. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed
by a new immunoradiometric sandwich assay. Clinical chemistry.
2007;53(2):284-91.

60. Wang F, Guo Y, Yu H, Zheng L, Mi L, Gao W. Growth differentiation factor 15 in different stages of heart failure: potential screening implications. Biomarkers: biochemical indicators of exposure, response, and susceptibility to chemicals. 2010;15(8):671-6.

61. Breit SN, Carrero JJ, Tsai VW, Yagoutifam N, Luo W, Kuffner T, et al. Macrophage inhibitory cytokine-1 (MIC-1/GDF15) and mortality in end-stage renal disease. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(1):70-5.

62. Theurl I, Finkenstedt A, Schroll A, Nairz M, Sonnweber T, Bellmann-Weiler R, et al. Growth differentiation factor 15 in anaemia of chronic disease, iron deficiency anaemia and mixed type anaemia. British journal of haematology. 2010;148(3):449-55.

63. Mei S WH, Fu R, et al. Hepcidin and GDF15 in anemia of multiple myeloma. Int J Hematol. 2014;100:266-73.

64. Omaima M Abbas MAH, Ashraf Y. El Fert, et al. Growth differentiation factor 15 as a marker of ineffective erythropoesis in patients with chronic C virus infection. Menoufia Medical Journal. 2015;30:133-8.

65. De Haan JJ, Haitjema S, den Ruijter HM, Pasterkamp G, de Borst GJ, Teraa M, et al. Growth Differentiation Factor 15 Is Associated With Major Amputation and Mortality in Patients With Peripheral Artery Disease. Journal of the American Heart Association. 2017;6(9).
### Tables

**Table 1: Socio-demographic, haematological and biochemical characteristics of participants by iron-deficiency-anaemia status**

| Characteristics                  | CKD (n=312) | Controls (n=184) |
|----------------------------------|-------------|-----------------|
|                                 | IDA n=103 (%) | Non-anaemic n=209 (%) | P-value | IDA n=18 (%) | Non-anaemic n=166 (%) |
| Hepcidin (ng/ml) (median, IQR)   | 8.4 (4-45.5) | 6.8 (3.9 -33.6) | 0.2790 | 3.1 (2.3 - 7.9) | 3.1 (2.1 - 10.5) |
| GDF-15 (pg/ml) (median, IQR)     | 1256.8 (919.1-1618) | 700 (335.1-1327.8) | <0.0001 | 1175.9 (708.9-1267.1) | 397.95 (18.1101) |
| Age (mean ±SD) years             | 54.5 ± 15.2 | 54.3 ± 14.2 | 0.9340 | 45.2± 17.5 | 41.3 ±13. |
| <40                              | 15 (14.6) | 37 (17.7) | 0.484 | 8 (44.4) | 79 (47.6) |
| ≥40                              | 88 (82.3) | 172 (85.4) | 10 (55.6) | 87 (52.4) |
| Race                             |             |                 |          |             |                 |
| Blacks                           | 95 (92.2) | 165 (79.0) | 0.003 | 12 (66.7) | 133 (80.1) |
| Whites                           | 8 (7.8) | 44 (21.1) | 6 (33.3) | 33 (19.9) |
| Gender                           |             |                 |          |             |                 |
| Male                             | 41 (39.8) | 126 (60.3) | 0.001 | 7 (38.9) | 71 (42.8) |
| Female                           | 62 (60.2) | 83 (39.7) | 11 (61.1) | 95 (57.2) |
| Systolic Blood Pressure (median, IQR) (mmHg) | 139 (125 - 157) | 140 (130 - 160) | 0.1994 | 132 (120 - 140) | 132 (130 - 140) |
| Diastolic Blood Pressure (median, IQR) (mmHg) | 80 (70 - 91) | 80 (70 - 90) | 0.6234 | 81 (70-90) | 80 (72 - 90) |
| Serum Urea (median, IQR) (mmol/L) | 17.1 (9.8 - 25.7) | 11 (7.1 - 15.9) | 0.0001 | 4 (3-4.9) | 4.35 (3.6 - 5.2) |
| Serum Creatinine (median, IQR), mmol/L | 265 (158-520) | 171 (120 - 255) | <0.0001 | 70.5 (64 - 78) | 78.5 (69 - 75) |
| GFR (median, IQR), mls/min/1.73m² | 27.0(12.6 - 27.0) | 43.6 (27.7 - 66.0) | <0.0001 | 129.8(96.3 - 139.5) | 114.4(96.8 - 133.0) |
Table 2: Multiple linear predictors of log Hepcidin and logGDF-15 among CKD patients

| Variable  | Log Hepcidin | Log GDF-15 |
|-----------|--------------|------------|
|           | Coefficient  | SE         | P-value  | Coefficient  | SE         | P-value |
| Ferritin  | 0.0058       | 0.00045    | <0.0001  | 0.000018     | 0.0003     | 0       |
| Age       | 0.0010       | 0.0037     | 0.787    | -0.00103     | 0.0027     | 0       |
| Race      |              |            |          |              |            |         |
| Blacks    | Reference    | Reference  | Reference| Reference    | Reference  | R       |
| Whites    | 0.0948       | 0.1353     | 0.484    | 0.08541      | 0.1037     | 0       |
| Gender    |              |            |          |              |            |         |
| Male      | Reference    | Reference  | Reference| Reference    | Reference  | R       |
| Female    | -0.2188      | 0.1071     | 0.042    | 0.2621       | 0.0755     | 0       |
| GFR       | -0.000041    | 0.0009     | 0.965    | -0.0014      | 0.0009     | 0       |
| MCHC      | -0.0780      | 0.0335     | 0.021    | -0.0816      | 0.0164     | <       |
| MCV       | 0.0276       | 0.0126     | 0.029    | -            | -          | -       |
| Urea      | -            | -          | -        | 0.0126       | 0.0062     | 0       |

Table 3: Predictors of iron deficiency anaemia with Hepcidin or GF-15 as the primary biomarker among CKD participants
Table 4: Predictors of Absolute iron deficiency anaemia with Hepcidin or GF15 as the primary biomarker among CKD participants

| Variable | 1Multivariable logistic regression analysis with Hepcidin as the primary factor | 2Multivariable logistic regression analysis with GDF-15 as the primary factor |
|----------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
|          | Odds ratio | 95%CI | P-value | Odds ratio | 95%CI |
| Hepcidin | 1.0023     | 1.0000-1.0045 | 0.049 | - | - |
| GDF-15   | - | - | - | 1.00024 | 1.000026-1.00455 |
| Age      | 1.00 | 0.98-1.02 | 0.964 | 1.00 | 0.98-1.02 |
| Gender   | Reference | Reference | Reference | Reference | Reference |
| Male     | Reference | Reference | Reference | Reference | Reference |
| Female   | 2.80 | 1.65-4.74 | <0.0001 | 2.73 | 1.60-4.67 |
| eGFR     | 0.99 | 0.98-1.00 | 0.028 | 0.99 | 0.98-1.00 |
| CRP      | 1.01 | 1.00-1.01 | 0.042 | 1.01 | 1.001-1.015 |
| MCV      | 0.94 | 0.90-0.98 | 0.004 | 0.94 | 0.90-0.98 |

1Multivariable logistic regression of the model of the relationship between Hepcidin and iron deficiency anaemia.
2Multivariable logistic regression of model of the relationship between GDF-15 and iron deficiency anaemia.
The 2 models corrected for gender, age, glomerular filtration rate, C-reactive protein and mean corpuscular volume ratio.
| Variable | \(^{1}\)Multivariable logistic regression analysis with Hepcidin as the primary factor | \(^{2}\)Multivariable logistic regression with GDF15 as the primary factor |
|----------|-------------------------------------------------|-------------------------------------------------|
|          | Odds ratio | 95%CI       | P-value | Odds ratio | 95%CI       |
| Hepcidin | 1.01       | 0.99-1.01   | 0.30    | -          | -          |
| GDF-15   | -          | -           | -       | 1.001      | 1.001-1.01 |
| Age      | 1.01       | 0.98-1.03   | 0.54    | 1.006      | 0.98-1.03  |
| Race     | Reference  | Reference   | Reference| Reference  | Reference |
| White    | Reference  | Reference   | Reference| Reference  | Reference |
| Black    | 0.23       | 0.78-0.66   | 0.007   | 0.35       | 0.14-0.91  |
| Gender   | Reference  | Reference   | Reference| Reference  | Reference |
| Male     | 2.86       | 1.38-5.91   | 0.005   | 2.95       | 1.37-6.34  |
| Female   | 2.86       | 1.38-5.91   | 0.005   | 2.95       | 1.37-6.34  |
| eGFR     | 0.99       | 0.98-1.01   | 0.503   | 0.99       | 0.98-1.01  |
| CRP ()   | 1.01       | 0.99-1.01   | 0.436   | 1.01       | 0.99-1.02  |
| MCV()    | 0.91       | 0.88-0.96   | 0.002   | 0.92       | 0.87-0.97  |
| MCHC ()  | 0.73       | 0.52-1.01   | 0.056   | 0.71       | 0.48-1.03  |
| Phosphate | -          | -           | -       | 0.50       | 0.26-0.99  |
| DM       | 1.87       | 0.94-3.71   | 0.008   | 1.77       | 0.86-3.64  |

\(^{1}\)Multivariable logistic regression of the model of the relationship between Hepcidin and Absolute iron deficiency anaemia.

\(^{2}\)Multivariable logistic regression of model of the relationship between GDF-15 and Absolute iron deficiency anaemia. The 2 models corrected for gender, age, glomerular filtration rate, C-reactive protein, MCV, Phosphate, Diabetes Mellitus, and mean corpuscular volume.

OR: Adjusted odds ratio.

Table 5: A Predictors of Functional iron deficiency anaemia with Hepcidin or GF15 as the primary biomarker among CKD participants
| Variable | 1 Multivariable logistic regression analysis with Hepcidin as the primary factor | 2 Multivariable logistic regression with GDF15 as the primary factor |
|----------|--------------------------------------------------------------------------------|---------------------------------------------------------------|
|          | Odds ratio | 95% CI | P-value | Odds ratio | 95% CI |
| Hepcidin | 1.01       | 1.01-1.01 | 0.013 | -         | -     |
| GDF-15   | -          | -       | -       | 1.01       | 0.99-1.0 |
| Age      | 0.98       | 0.95-1.01 | 0.123 | 0.99       | 0.96-1.0 |
| Gender   | Reference  | Reference | Reference | Reference | Reference |
| Male     | Reference  | Reference | Reference | Reference | Reference |
| Female   | 3.23       | 1.47-7.29 | 0.004 | 2.9        | 1.29-6.2 |
| Race     | Reference  | Reference | Reference | Reference | Reference |
| White    | Reference  | Reference | Reference | Reference | Reference |
| Black    | 15.09      | 1.20-188.96 | 0.035 | 15.88      | 1.16-216. |
| eGFR     | 0.99       | 0.98-0.99 | 0.007 | 0.98       | 0.98-0.9 |
| CRP      | 1.01       | 1.00-1.01 | 0.002 | 1.01       | 1.01-1.0 |
| MCHC     | 0.79       | 0.62-0.99 | 0.041 | 0.78       | 0.61-0.9 |
| DM       | 1.35       | 0.55-4.78 | 0.070 | 1.31       | 0.56-4.7 |

1 Multivariable logistic regression of the model of the relationship between Hepcidin and iron deficiency anaemia.

2 Multivariable logistic regression of model of the relationship between GDF-15 and iron deficiency anaemia.

The 2 models corrected for gender, age, glomerular filtration rate, C-reactive protein and mean corpuscular volume.

Table 6: Cut-off and validity of GDF-15 and Hepcidin in diagnosing Absolute and functional Iron deficiency Anaemia among CKD patients.
| Test Parameter                                | Predictive value of GDF 15 for AID among CKD patients | Predictive value of Hepcidin for FID among CKD patients |
|-----------------------------------------------|------------------------------------------------------|-------------------------------------------------------|
| Value                                         | Value                                                | Value                                                 |
| Cut-off                                      | 1129.3 mg/dl                                         | 22.5 ng/dl                                            |
| AUC                                          | 81.9%                                                | 81.4                                                 |
| Sensitivity                                  | 83.64%                                               | 66.7%                                                 |
| Specificity                                  | 66.03%                                               | 70.8%                                                 |
| Likelihood ratio of positive result           | 2.462                                                | 2.28                                                 |
| Likelihood ratio of negative result           | 0.2478                                               | 0.47                                                  |
| Youden’s index                               | 49.67%                                               | 37.48%                                                |

AID; Absolute iron deficiency anaemia FID; Functional iron deficiency anaemia AUC; Area Under Curve.

**Figures**
Figure 1

Receiver Operator Characteristics curves (ROC) of: (A). Comparison of the predict...

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Supplementary tables 4BMC.docx