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# Growth-differentiation factor-15 expression in anemia of chronic disease and iron-deficiency anemia

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**Abstract:**

**BACKGROUND:** Iron-deficiency anemia (IDA) is a common hematological disorder. Anemia of chronic disease (ACD) is mild-to-moderately severe anemia associated with chronic diseases. Growth-differentiation factor-15 (GDF-15) is a member of the transforming growth factor-$\beta$, produced by late-stage erythroid precursors in the bone marrow; it suppresses hepcidin expression during ineffective erythropoiesis. The aim of this study was to measure the level of the GDF-15 level in ACD and IDA and to evaluate its ability to differentiate between ACD and ACD with coexisting iron deficiency (mixed anemia).

**PATIENTS, MATERIALS AND METHODS:** The present study including 87 persons "30 with IDA, 30 with ACD, and 27 controls." The following investigations were done: complete blood count, erythrocyte sedimentation rate, iron profile (serum iron and total iron-binding capacity), C-reactive protein, ferritin, hepcidin, and GDF-15, depending on the results of these investigations, some patients of ACD group appear to be ACD with co-existing iron deficiency. The analysis of data was carried out using the Statistical Packages for the Social Sciences software version 24.

**RESULTS:** The serum hepcidin was significantly lower in IDA, whereas the serum GDF-15 is comparable to that of the control group. The serum hepcidin and serum GDF-15 were significantly higher in (ACD and mixed anemia) group compared to the control group.

**CONCLUSION:** GDF-15 is high in ACD group and comparable to the control in iron-deficiency group, and it is not a useful marker to differentiate between ACD and ACD with coexisting iron deficiency.

**Keywords:**

Anemia of chronic disease, anemia, growth-differentiation factor-15, hepcidin, iron-deficiency anemia

## Introduction

Iron-deficiency anemia (IDA) is the most common type of anemia, in which the iron supply to maintain normal erythropoiesis is inadequate.[1] It starts with the depletion of iron stores, when the serum iron is still normal, then iron-deficient erythropoiesis develop and serum ferritin below 15 μg/L, but the mean corpuscle volume (MCV) and mean corpuscular hemoglobin (MCH) still within normal, and then IDA develops.[2] The most common cause of IDA is blood loss, usually from the gut, menorrhagia, and hemorrhoid. Other causes decrease dietary intake and increase physiological iron needs and malabsorption.[2]

Anemia of chronic disease (ACD) is the anemia that complicates the course of the acute or chronic disease, for example, infection, inflammation, malignancies, and autoimmune disorders.[3] The pathogenesis of ACD is manifested by the dysregulation of iron homeostasis, in addition, to the effect of (tumor necrosis factor-alpha, interleukin-1, and gamma-interferon) that directly represses the differentiation and proliferation of erythroid progenitor cells, and also, they reduce the formation and the biological activity of erythropoietin (EPO).[4]

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The most important the acute-phase protein hepcidin is the main regulator of iron homeostasis, it effectively prevents iron efflux from monocytes and macrophages.[5]

Growth-differentiation factor-15 (GDF-15) is a member of the transforming growth factor-β, bone morphogenetic protein (BMP) super family.[6] The source of circulating GDF-15 is uncertain; probably, it secreted from the liver, although it is also expressed in other tissues such as the kidney, lung and adipose tissue, endothelial cells, placenta, prostate, erythroid progenitors, and others.[7] The GDF15 is one of the major secreted proteins induced by the tumor-suppressor protein p53, suggesting that GDF15 induction is associated with cell-cycle arrest and apoptosis.[8] GDF-15 is required for normal erythroid differentiation. Moreover, the GDF15 is involved in ineffective erythropoiesis, being strongly increased in β-thalassemia and congenital dyserythropoietic anemia. The cytokine blocks hepcidin expression and increases iron absorption, thus leading to iron loading in these anemia.[9]

## Patients, Materials and Methods

This was a cross-sectional study. Samples from 87 participants were included in this study, and they were classified into three groups:

1. Thirty patients with IDA
2. Thirty patients with ACD which then according to the parameters mentioned below and depending on Table 1[10] subdivided to 17 ACD patients and 13 ACD/IDA patients
3. Twenty-seven healthy controls as a control group.

After obtaining the patient consent and hospital approval to conduct the study, 6 ml of peripheral blood were withdrawn by aseptic technique from the patients and control groups, each sample was divided into two parts: Two ml were added to K3EDTA-coated tubes and mixed gently for complete blood count, blood film, ESR and reticulocyte count, and 4 ml were added to gel and clot activator tubes and then sample let for 2 h to clot and then centrifuged for 15 min to obtain sera.

The serum sample kept in small volumes in Eppendorf tubes in deep freeze (−40) and used to determine the iron profile (serum iron and total iron-binding capacity [TIBC]) and C-reactive protein (CRP), ferritin by chemiluminescence technique, hepcidin by ELISA, and GDF15 also by ELISA.

### Inclusion criteria

#### *For iron-deficiency anemia group*

Patient with no history of chronic disease, with signs and symptoms of IDA, and according to the World Health Organization definition for anemia, the Hb level for females was <12 g/dl, and for males were <13 g/dl both gender was older than 18 years, MCV and MCH below the reference range, hypochromic microcytic on blood film, low serum iron, high-TIBC level, low serum ferritin, and CRP normal.

#### *For anemia of chronic disease group*

Patients with a history of chronic disease, for example, cancer, infection, and rheumatoid arthritis, Hb level for females was <12 g/dl and for males were < 13 g/dl, both gender was older than 18 years, MCV and MCH either normal or mildly decrease, low serum iron, normal or high TIBC level, high or normal serum ferritin, CRP, and ESR increased.

The exclusion criteria for both groups included the patient were not on iron therapy, no recent blood transfusion, and females were not pregnant.

### Statistical analysis

Analysis of data was carried out using the Statistical Packages for the Social Sciences software version 24 (IBM, New York, ver. 25).

## Results

As shown in Table 2, in the IDA group, the serum iron, serum ferritin, serum hepcidin, and transferrin saturation index percentage were significantly lower as compared to that of the control group. While TIBC were significantly higher compared to the control group, and the S. GDF15 and CRP are comparable to the control group results. In (ACD and ACD/IDA) group, the serum iron, TIBC, and transferrin saturation index percentage were significantly lower compared to the control group.

**Table 1: Differentiation between types of anemia**

| Parameters | Iron deficiency without anemia | IDA | ACD | ACD/IDA |
|---|---|---|---|---|
| Hb level | Normal | Low | Low | Low |
| Inflammatory markers | Negative | Negative | Raised | Raised |
| Ferritin | Low | Low | Normal/Increased | Normal |
| Transferrin saturation | Low | Low | Low | Low |
| sTFR/log ferritin ratio | Raised | Raised | Low | Raised |
| serum heocidin | Low | Low | Raised | Normal |
| GDF15 | Normal | Normal | Raised | Raised |

IDA=Iron-deficiency anemia, ACD=Anemia of chronic disease, GDF=Growth-differentiation factor, Hb=Hemoglobin, sTFR=Serum transferrin receptor

The serum ferritin, serum hepcidin, serum GDF15, ESR, and CRP were significantly higher.

As shown in Table 3, there is no significant correlation between serum GDF15 and serum ferritin in the IDA group ($P$ = 0.179, $r$ = 0.252), while there is a positive correlation between serum GDF15 and serum ferritin in both healthy control group and (ACD + ACD/IDA) ($r$ = 0.603, $P$ = 0.001 and $r$ = 0.366, $P$ = 0.046) for each group, respectively.

There is no significant correlation between serum GDF15 and TIBC in IDA patient group ($P$ = 0.223, $r$ = 0.229), while there is a significant inverse correlation between serum GDF15 and TIBC in both healthy control group and (ACD + ACD/IDA) group ($P$ = 0.046, $r$ = −0.388 and $P$ = 0.006, $r$ = −0.487) for each group, respectively.

There is no significant correlation between serum GDF15 and transferrin saturation percentage in IDA patient group, (ACD + ACD/IDA) group and healthy control group ($P$ = 0.513, $r$ = 0.124, $P$ = 0.841, $r$ = −0.038 and $P$ = 0.232, $r$ = 0.238) for each group, respectively.

There is a significant correlation between S. GDF15 and CRP in IDA patient group ($P$ = 0.004, $r$ = 0.510), while there is no significant correlation between the two parameters in the healthy control group ($P$ = 0.161, $r$ = 0.278), and also there is no significant correlation between the two parameters in the (ACD + ACD/IDA) patient group ($P$ = 0.261, $r$ = 0.212).

There is no significant correlation between serum GDF15 (Pg/mL) and serum hepcidin (ng/mL) in IDA patient group, ($P$ = 0.993, $r$ =–0.002), while there is a significant positive correlation between serum GDF15 (Pg/mL) and serum hepcidin (ng/mL) in (ACD + ACD/IDA) patient group with $P$ = 0.008, $r$ = 0.477, and there is a significant positive correlation between serum GDF15 (Pg/mL) and serum hepcidin (ng/mL) in the healthy control group with $P$ = 0.0001, $r$ = 0.678.

## Discussion

In this study, serum hepcidin was significantly decreased in IDA, and this was the same as to results of Giridhar *et al.*,[11] who studied the role of serum hepcidin in the detection of IDA in HIV-infected patients with anemia of inflammation.

In term of hepcidin in (ACD + ACD/IDA), there were a significant increase in hepcidin levels in ACD and

**Table 2: The biochemical parameters of the two patient's groups and the control group**

| | IDA | ACD and ACD/IDA | Healthy controls | *P* |
|---|---|---|---|---|
| Serum iron (ug/dL) | 18.41±9.55 (8.7-49.0) | 25.46±10.91 (5.6-47.0) | 78.11±22.70 (50.0-158.3) | 0.0001* |
| Serum ferritin (ng/ml) | 5.47±5.14 (1.0-27.0) | 584.86±527.29 (52.7-1540.0) | 72.34±56.40 (11.5-231.0) | 0.0001* |
| UIBC (ug/dL) | 413.10±68.01 (285.0-553.1) | 168.89±51.10 (67.2-268.8) | 201.87±61.02 (90.7-306.9) | 0.0001* |
| TIBC (ug/dL) | 431.79±66.16 (302.7-569.3) | 194.32±54.67 (94.0-302.0) | 279.63±58.83 (147.3-378.9) | 0.0001* |
| Transferrin saturation (%) | 4.39±2.52 (2.09-13.65) | 13.54±5.91 (3.36-28.62) | 29.08±9.49 (17.0-46.44) | 0.0001* |
| CRP (mg/L) | 3.36±2.34 (1.8-9.50) | 109.93±92.49 (12.0-325.0) | 3.05±1.50 (2.5-8.23) | 0.0001* |
| Serum hepcidin (ng/mL) | 10.38±18.37 (3.88-105.20) | 154.06±99.99 (32.58-449.19) | 46.58±39.75 (12.0-147.97) | 0.0001* |
| Serum GDF 15 (pg/mL) | 421.84±133.61 (234.83-693.98) | 1086.94±469.22 (490.9-2316.5) | 413.55±96.62 (293.64-647.39) | 0.0001* |

Data were presented as mean±SD (range), *Significant difference among three independent means using the ANOVA test at 0.05 level.
GDF=Growth-differentiation factor, SD=Standard deviation, CRP=C-reactive protein, TIBC=Total iron-binding capacity, UIBC=Unsaturated iron-binding capacity, IDA=Iron-deficiency anemia, ACD=Anemia of chronic disease

**Table 3: Correlation between serum growth-differentiation factor-15 (Pg/mL) and different parameters in iron-deficiency anemia, anemia of chronic disease, and anemia of chronic disease/iron-deficiency anemia patient groups and healthy control groups**

| Correlations | Serum ferritin (ng/ml) | UIBC (ug/dL) | TIBC (ug/dL) | Saturation (%) | CRP (mg/L) | Serum Hepcidin (ng/mL) |
|---|---|---|---|---|---|---|
| 1-IDA | | | | | | |
| S GDF 15 (pg/mL) | | | | | | |
| *R* | 0.252 | −0.241 | −0.229 | 0.124 | 0.510 | −0.002 |
| *P* | 0.179 | 0.200 | 0.223 | 0.513 | 0.004** | 0.993 |
| 2-ACD and ACD/IDA | | | | | | |
| Serum GDF 15 (pg/mL) | | | | | | |
| *R* | 0.366 | −0.450 | −0.487 | −0.038 | 0.212 | 0.477 |
| *P* | 0.046* | 0.013* | 0.006** | 0.841 | 0.261 | 0.008** |
| 3 healthy controls | | | | | | |
| Serum GDF-15 (pg/mL) | | | | | | |
| *R* | 0.604 | −0.382 | −0.388 | 0.238 | 0.278 | 0.678 |
| *P* | 0.001** | 0.049* | 0.046* | 0.232 | 0.161 | 0.0001** |

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level. GDF=Growth-differentiation factor, SD=Standard deviation, CRP=C-reactive protein, TIBC=Total iron-binding capacity, UIBC=Unsaturated iron-binding capacity, IDA=Iron-deficiency anemia, ACD=Anemia of chronic disease

normal level in the mixed type ACD/IDA, and this result is in agreement with the study of Hanudel *et al*. in which the hepcidin levels were increased in anemia of chronic kidney disease in a murine model,[12] and also in agreement with Pasricha *et al*. in which the hepcidin levels were increased in anemia of inflammation and decreased in IDA.[13]

In term of serum GDF-15, the results were significantly high in ACD, and mixed type anemia (ACD/IDA) compared to IDA and to control group; a comparable result was mentioned by the study of Abaza *et al*.,[9] Theurl *et al*.,[14] and also to the study of Abbas *et al*.[15]

Since GDF15 is not expressed specifically in erythroblasts, elevations of this cytokine in patients with cancers and inflammatory disease should not be attributed to erythroblast expression. This point may be particularly important when considering the high levels of GDF15 reported in patients with ACD.[8]

There is no significant correlation between serum GDF15 and serum ferritin in IDA. In the IDA with the absence of inflammatory status, the GDF15 is produced because it is necessary for normal erythropoiesis.[16] Hence, it is still within normal level to maintain erythropoiesis.

There is a positive correlation between serum GDF15 and serum ferritin in both healthy control group and (ACD + ACD/IDA), these results were comparable to Theurl *et al*.[14] but not to Abaza *et al*.[9] study that deny a significant correlation between these variables in ACD group. The correlation between GDF15 and ferritin in these groups could be explained by the correlation of GDF15 with the severity of inflammatory process mentioned by Breit *et al*. and regarded GDF15 as a useful biomarker for the detection of active inflammation.[17]

In term of correlation between GDF15 and CRP in the IDA group, there was a strong positive correlation between these two variables; this means that GDF15 and CRP increase with mild subclinical inflammatory process as mentioned by Posthouwer *et al*.'s study.[18]

In (ACD and ACD/IDA) and healthy control group, there was no significant correlation between GDF15 and CRP; this result was in agreement with Abaza *et al*.'s study.[9]

In IDA group, there was no significant correlation between serum hepcidin levels and GDF15 levels, the same correlation was mentioned by Theurl *et al*.,[14] The hepcidin level is dawn regulated in IDA, while GDF15 is still within the normal range to maintain normal erythropoiesis.[19]

In (ACD and ACD/IDA) group and the healthy control group, there was a significant direct correlation between GDF15 and hepcidin, and this result was in agreement with Hong *et al*. in which both hepcidin and GDF15 levels were increased and showed a positive correlation in anemic Type II diabetes mellitus patients.[20]

GDF15 has been shown to suppress hepcidin in patients with hepatocellular iron overload and anemia, in particular β-thalassemia or congenital dyserythropoietic anemia and to a lesser extent, in other types of iron-loading anemia.[21]

In contrast to iron-loading anemia (such as thalassemia major in which there is low hepcidin level and increase iron absorption), ACD is associated with high hepcidin level and reduced duodenal iron absorption.[22]

This means that the increased level of GDF15 in ACD cannot suppress hepcidin level as both parameters were increased in ACD, and this has many explanations:

1. Other ACD-related factors that may overcome the regulatory effect of GDF15 on hepcidin expression during inflammation[14]
2. Erythroid cells in ACD, ACD/IDA may secrete GDF15-specific stimulator that is not induced during IDA[16]
3. Increase EPO production because of hypoxia causing an increase in GDF-15 concentration[21]
4. GDF-15 may not regulate hepcidin expression during inflammation, and hepcidin expression is instead controlled by iron availability and the associated down-stream signals, such as BMP-6[23]
5. Level of GDF-15 is low in comparison with β-thalassemia major, which means it is too low to effectively suppress inflammation and iron triggered hepcidin expression.[14]

## Conclusion

GDF15 is high in ACD group and comparable to the control in iron-deficiency group, and it is not a useful marker to differentiate between ACD and ACD with coexisting iron deficiency.

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### Conflicts of interest
There are no conflicts of interest.

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