

## **Clinical significance of serum IL-6, TNF- $\alpha$ , Hepcidin, and EPO levels in anaemia of chronic disease and iron deficiency anaemia: The laboratory indicators for anaemia.**

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### **Abstract**

**Background:** Pathogenesis of anaemia of chronic disease is multifactorial. It is related with corrupted erythropoietin-dependent erythropoiesis due to C-reactive protein, and pro-inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-alpha. It is also related with decreased red cell survival and impaired iron mobilization. Hepcidin level in iron deficiency anaemia is low than controls but it is high in patients with chronic renal failure. Pro-inflammatory cytokines are associated with the pathogenesis of rheumatoid arthritis and development of anaemia in rheumatoid arthritis.

**Methods:** In this study we aimed to evaluate the relationship between serum hepcidin, interleukin-6, tumor necrosis factor-alpha, erythropoietin levels and whole blood count parameters and clinical properties of patients with iron deficiency anaemia, chronic renal failure and rheumatoid arthritis which were compared with control group, in addition we also aimed to evaluate the differences between patients and controls. Serum interleukin-6, tumor necrosis factor-alpha, hepcidin and erythropoietin levels of 31 patients with iron deficiency anaemia, 15 patients with rheumatoid arthritis, 18 patients with chronic renal failure and 31 healthy controls were measured from peripheral blood samples.

**Results:** We found no differences between groups according to hepcidin and tumor necrosis factor-alpha levels but we found higher interleukin-6 levels and lower erythropoietin levels in patients with anaemia of chronic disease.

**Conclusion:** Ferritin is the most valuable parameter for making the differential diagnose of anemia of chronic disease and iron deficiency anemia, in anemic patients but in some situations that ferritin cannot help for making the differential diagnose, interleukin-6 and erythropoietin may be useful for making the differential diagnose of iron deficiency anemia, and anemia of chronic disease.

**Keywords:** Anemia, Chronic disease, Cytokine.

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### **Introduction**

Anemia of Chronic Disease (ACD) and Iron Deficiency Anemia (IDA) are two most common types of anemia [1]. Typical forms of ACD include microcytic or normocytic normochromic anemias which are associated with chronic infections, chronic non-infectious inflammatory diseases, and malignancies. Multiple factors take part in the pathogenesis of ACD that involve impaired Erythropoietin (EPO)-dependent erythropoiesis in relation to pro-inflammatory cytokines such as IL-6, and TNF- $\alpha$  in addition to decreased erythrocyte life span and poor mobilization of iron [2-4]. IDA constitutes a significant public health issue. As well as the most common

reason, chronic blood loss, the etiology includes nutrition disorders and insufficiencies [1]. As it may also be elevated in a subclinical inflammatory condition, ferritin, an acute phase reactant, may not be helpful in differential diagnosis of ACD from IDA particularly in patients with a chronic disease [5-8]. Hepcidin is a cysteine-rich, cationic, antimicrobial peptide acting as a hemostatic regulator in iron metabolism and a mediator in host defense and inflammation [9]. The gene encoding hepcidin is affected by hypoxia and inflammation [10]. During the course of an inflammation, IL-6 is required for hepcidin induction and, therefore, hepcidin is a key mediator in ACD [11]. Haemolysis, bleeding, decreased erythrocyte life

span as a result of the increased oxidative stress, and reduced EPO secretion due to renal dysfunction are the causes of anemia in Chronic Renal Failure (CRF). CRF is a chronic inflammatory condition in which inflammatory molecules such as CRP and IL-6 may be elevated as renal functions are impaired [6,12,13]. In chronic inflammatory diseases, iron gets blocked in the reticuloendothelial system following the hepcidin release [14]. Hepcidin levels in IDA are lower than healthy individuals, whereas patients with CRF have higher levels of hepcidin [15-17]. On the other hand, Rheumatoid Arthritis (RA) is the most common inflammatory arthritis across the globe and one of its most predominant complications is anemia of inflammation. In particular, pro-inflammatory cytokines TNF- $\alpha$ , IL-6 and IL-1 play an essential role in the pathogenesis of RA and in the development of RA-associated anemia by means of inhibiting iron metabolism and erythropoiesis taking place in the bone marrow [18-20]. In this study, our aim is to determine any influence of serum hepcidin, IL-6, TNF- $\alpha$ , and EPO levels on anemia in both anemia of chronic disease (RA and CRF) patients and in IDA patients and to find out whether their levels differ among these disease groups.

## Materials and Methods

### Patient selection

IDA patient who have applied to the Department of Haematology, RA patients with ACD who have applied to the outpatient Department of Rheumatology and CRF patients with ACD who have applied to the outpatient Department of Nephrology were enrolled to this study. Both patients and healthy controls were included to corresponding patient or healthy control groups only after that have been informed about the study and signed consent form. Admitted patients were anemic according to the WHO criteria, that is, with a haemoglobin value of <12 g/dL in women and <13 g/dL in men. In IDA classification, cut-off value for ferritin level was taken as 13 ng/ml and 30 ng/ml in females and males, respectively, and any lower levels were accepted as IDA. Regarding ACD, cut-off value for ferritin level was taken as 100 ng/ml in both sexes. Inclusion criteria to the study were selected to require the patients: (i) Being at an adult age (over 18 years old), (ii) Diagnosed to have isolated IDA, (iii) Diagnosed to have RA which is not treated with biological agents, but having conventional DMARD treatment and RA-related ACD. (iv) Diagnosed to have CRF which is not treated with iron or EPO, and having CRF-induced ACD. (v) Have agreed to take part in the study through signing the informed consent form of the study. The following are criteria were employed for the exclusion of patients: (i) Not giving consent to participate to the study, (ii) Patients who have an anaemia other than IDA and ACD. A total of 33 ACD patients composed of 18 CRF patients not undergoing dialysis and 15 RA patients, 31 IDA patients and 31 healthy individuals were included to our study. Age, gender, diagnosis, duration of their chronic illnesses, if any, accompanying diseases and medication use of these

patients were questioned. Test results for routine CBC parameters and acute phase reactants, namely, Erythrocyte Sedimentation Rate (ESR), C-Reactive Protein (CRP), fibrinogen, serum iron, transferrin saturation, Total Iron Binding Capacity (TIBC), and serum ferritin levels, DAS-28 activity score of RA patients as an indicator of their disease activity, and Creatinine Clearance (CrCl) and CRF stage of CRF patients were recorded. The study was initiated after obtaining the approval of our local ethics committee.

### Collection and evaluation of samples

Peripheral blood samples were used as study material in the patient and control groups. Blood samples from all cases were collected in the morning after 8-12 hours of fasting, as a minimum. Blood samples were withdrawn into empty tubes to separate serum fraction. In the shortest possible time, sample tubes were delivered to the laboratory, centrifuged at 3000 rpm, at +4°C for 10 min and sera were stored at -75°C until the day of testing. On the test day, the sera were brought to room temperature and their IL-6, TNF- $\alpha$ , EPO, and hepcidin levels were measured.

### Statistical analysis

SPSS for Windows version 21.0 was used for the statistical analysis of the results. The value  $p < 0.05$  was accepted as statistically significant. Measuremental variables are presented as mean SD (Standard Deviation). Normality assumptions were tested by Shapiro Wilk test. Parametric tests (ANOVA, Post Hoc tests) were carried out with normally distributed data, and non-parametric tests (Kruskal Wallis test and Spearman correlation) were carried if with data is not normally distributed. Abnormally distributed data is presented as median and 25-75<sup>th</sup> percentiles. Chi-square test was employed to conduct analyses of cross tables. The four groups; IDA, CRF-ACD, RA-ACD and healthy control groups were compared with each other.

## Results

Demographic and haematological features of our ACD and IDA patients as well as the healthy control group are given in Table 1. Haemoglobin (Hb) and erythrocyte (RBC) values were lower in each of IDA, RA, and CRF groups compared to those in the healthy control group ( $p < 0.0001$ ,  $p < 0.0001$ , respectively). MCV levels were the lowest in the IDA patients ( $p < 0.0001$ ). While predominant anemia type was hypochromic microcytic anemia in IDA patients, CRF and RA patients were featuring as normochromic normocytic anemia. Red cell distribution width (RDW) was measured to be higher in all patient groups compared to those in the healthy control group ( $p < 0.0001$ ). Leukocyte count was higher in the ACD patients regardless they have CRF or RA ( $p = 0.005$ ). Absolute neutrophil count (ANC), however, was higher in the CRF patient of the ACD group ( $p < 0.0001$ ). When compared to the control group, platelet level was highest in the IDA group

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( $p=0.006$ ). Ferritin levels were significantly different among the four groups ( $p<0.0001$ ).

**Table 1.** Demographic and haematological features of ACD and IDA patients.

	IDA (1) n=31	RA (2) n=15	CRF (3) n=18	Control (4) n=31	p,**	p, 1 vs. 2	p, 1 vs. 3	p, 1 vs. 4	p, 2 vs. 3	p, 2 vs. 4	p, 3 vs. 4
M/F	Mar-28	03-Dec	12-Jun	13/18	<0.0001	NS	NS	<0.0001	NS	<0.0001	NS
Age, mean $\pm$ SD, (year)	42.48 $\pm$ 16.38	62.8 $\pm$ 9.07	66.5 $\pm$ 10.8	45.45 $\pm$ 12.68	<0.0001	0.0001	0.0001	NS	NS	<0.0001	<0.0001
Hb, mean $\pm$ SD, (g/dl)	10.82 $\pm$ 1.66	11.84 $\pm$ 0.58	10.53 $\pm$ 1.56	14.23 $\pm$ 1.3	<0.0001	0.023	NS	<0.0001	NS	NS	NS
Hct%, median	35.1 (32.3-36.5)*	35.3 (34.8-35.8)*	31 (27.5-34.6)*	41.9 (38.5-43.9)*	<0.0001	<0.0001	<0.0001	0.032	NS	NS	0.006
MCV, mean $\pm$ SD, (fl)	76.2 $\pm$ 8.77	86.4 $\pm$ 5.07	85.5 $\pm$ 5.49	85.8 $\pm$ 3.93	<0.0001	<0.0001	<0.0001	<0.0001	NS	NS	NS
MCH, mean $\pm$ SD, (pg)	25.06 $\pm$ 4.20	28.92 $\pm$ 1.76	28.84 $\pm$ 2.40	29.67 $\pm$ 1.78	<0.0001	<0.0001	<0.0001	<0.0001	NS	NS	NS
RBC, mean $\pm$ SD, (10 <sup>12</sup> /L)	4.52 $\pm$ 0.42	4.10 $\pm$ 0.38	3.64 $\pm$ 0.69	4.62 $\pm$ 0.48	<0.0001	NS	<0.0001	NS	0.011	0.047	<0.0001
RDW, median	15.0 (13.5-18.6)*	15.1 (14.1-16.0)*	14.2 (13.3-16.4)*	12.9 (12.5-13.3)*	<0.0001	NS	NS	<0.0001	NS	<0.0001	0.005
WBC, mean $\pm$ SD ( $\mu$ L)	6538.7 $\pm$ 1563	6893.3 $\pm$ 2010.4	8372.2 $\pm$ 2616.8	6487.09 $\pm$ 1567.1	0.005	NS	0.007	NS	NS	NS	0.006
ANC, mean $\pm$ SD ( $\mu$ L)	3896.7 $\pm$ 1404.4	3926.6 $\pm$ 1617.5	6116.6 $\pm$ 2709	4010.3 $\pm$ 1227.4	<0.0001	NS	0.022	NS	0.045	NS	0.031
ALC, mean $\pm$ SD ( $\mu$ L)	1977.4 $\pm$ 438.7	2040 $\pm$ 1099.8	1416.6 $\pm$ 759.4	2034.4 $\pm$ 555	0.014	NS	0.050	NS	NS	NS	0.034
Platelet, median, ( $\mu$ L)	314000 (221000-350000)*	262000 (208000-282000)*	218000 (167000-264250)*	231000 (204000-264000)*	0.006	NS	NS	<0.0001	NS	<0.0001	0.005
Cr, median, (mg/dl)	0.59 (0.55-0.70)*	0.67 (0.55-0.70)*	2.56 (2.21-4.04)*	0.68 (0.59-0.73)*	<0.0001	<0.0001	<0.0001	0.032	NS	NS	0.006
Vitamin B12, median, (pg/ml)	303.1 (238.4-408.1)*	379.7 (295.5-421.5)*	412 (343.6-1703.5)*	-	NS	NS	NS	NS	NS	NS	NS
Folic acid, median, (ng/ml)	7.08 (6.39-9.39)*	9.38 (8.56-12.4)*	7.09 (5.74-8.49)*	-	0.010	0.037	NS	NS	0.030	NS	NS

\* (Q1-Q3), \*\* Only significant statistics among the groups are given. NS: Non-Significant; IDA: Iron Deficiency Anaemia; RA: Rheumatoid Arthritis; CRF: Chronic Renal Failure; M: Male; F: Female; Hb: Haemoglobin; MCV: Mean Cell Volume; MCH: Mean Corpuscular Haemoglobin; RBC: Red Blood Cell; RDW: Red Cell Distribution Width; WBC: White Blood Cell; ANC: Absolute Neutrophil Counts; ALC: Absolute Lymphocyte Counts; Cr: Creatinine;

**Table 2.** Parameters of iron metabolism, ferritin, acute phase reactants, and serum IL-6, TNF- $\alpha$ , EPO, and hepcidin levels in ACD and IDA patients.

	IDA (1) n=31	RA (2) n=15	CRF (3) n=18	Control n=31	(4) p,**	p, 1 vs. 2	p, 1 vs. 3	p, 1 vs. 4	p, 2 vs. 3	p, 2 vs. 4	p, 3 vs. 4
Serum iron, median, ( $\mu$ g/dl)	33 (25-38.5)*	44 (15.75-64)*	49 (44.75-63.5)*	-	0.038	NS	<0.0001	NS	NS	NS	NS
Transferrin saturation, median, (%)	8 (6.75-14)*	16 (13-29.5)*	11.71 (6-30.6)*	-	NS	NS	NS	NS	NS	NS	NS
TIBC, median, ( $\mu$ g/dl)	397 (370.5-427.5)*	345 (300.2-367)*	262 (122-399)*	-	0.006	NS	<0.0001	NS	NS	NS	NS
Ferritin, median, (ng/ml)	7.89 (5.44-12.02)*	110.8 (103.4-124.3)*	131 (108.56-272.0)*	29.87 (26.03-40.41)*	<0.0001	<0.0001	<0.0001	0.032	NS	NS	0.006

ESR, median (mm/h)	13 (8-28.25)*	28 (19-43)*	39.5 (31.75-55)*	2 (4-12)*	<0.0001	NS	<0.0001	NS	NS	NS	NS	0.009
CRP, median (mg/dl)	0.345 (0.344-0.385)*	0.345 (0.344-3.96)*	0.465 (0.344-13.9)*	-	NS	NS	NS	NS	NS	NS	NS	NS
Albumin, mean $\pm$ SD (g/dl)	4.51 $\pm$ 0.30	4.18 $\pm$ 0.26	4.02 $\pm$ 0.47	4.37 $\pm$ 0.28	0.008	NS	0.006	NS	NS	NS	NS	NS
IL-6, median (pg/ml)	1.38 (0.9-2.58)*	3.18 (1.88-11.9)*	4.82 (2.61-13.27)*	1.38 (0.86-2.82)*	<0.0001	0.011	<0.0001	NS	NS	NS	0.017	<0.0001
TNF- $\alpha$ , mean $\pm$ SD, (pg/ml)	5.55 $\pm$ 2.92	5.18 $\pm$ 1.62	4.69 $\pm$ 0.79	5.00 $\pm$ 1.17	NS	NS	NS	NS	NS	NS	NS	NS
EPO, median (mIU/ml)	13.72 (7.46-38.7)*	13.2 (8.96-21.46)*	15.93 (6.64-31.72)*	7.82 (5.46-11.24)*	0.006	NS	NS	0.008	NS	NS	NS	NS
Hepcidin, median, (ng/ml)	211.82 (172.92-747.78)*	141.54 (119.19-231.53)*	184.31 (137.56-259.11)*	206.9 (140.25-775.37)*	NS	NS	NS	NS	NS	NS	NS	NS

\* (Q1-Q3), \*\* Only significant statistics among the groups are given, whereas non-significant ones are not presented. IDA: Iron Deficiency Anaemia; RA: Rheumatoid Arthritis; CRF: Chronic Renal Failure; TIBC: Total Iron Binding Capacity; ESR: Erythrocyte Sedimentation Rate; CRP: C-Reactive Protein; IL-6: Interleukin-6; TNF- $\alpha$ : Tumor Necrosis Factor-alpha; EPO: Erythropoietin.

While ferritin levels of CRF patients with ACD were the highest (median 131 ng/ml), the levels were detected to be lowest in IDA patients (median 7.89 ng/ml). Ferritin levels of the IDA patients were significantly lower than that of the healthy controls (median 20.87 ng/ml), RA group (median 110.8 ng/ml) and CRF group ( $p=0.032$ ,  $p<0.0001$ , and  $p<0.0001$ , respectively), and the increase in the ferritin levels in CRF patients was found to be significant in comparison to that in controls ( $p=0.006$ ). Serum iron levels were significantly low in all groups ( $p=0.038$ ), IDA patients having a significantly lower level than that of CRF patients ( $p<0.0001$ ). TIBC was detected in the highest levels in the IDA group ( $p=0.006$ ). ESR had significant variance among the groups ( $p<0.0001$ ), with a significantly higher level in the CRF group than both IDA patients and healthy individuals ( $p=0.0001$  and  $p=0.009$ , respectively). Although CRP levels were higher in the group composed of CRF and RA than those in the IDA group, the difference was not significant ( $p>0.05$  and  $p>0.05$ , respectively).

Among the groups, serum IL-6 levels varied ( $p<0.0001$ ) such that, RA patients had a higher level than IDA patients ( $p=0.011$ ), CRF patients, likewise, had a higher value than IDA patients ( $p<0.0001$ ), and both CRF and RA patients had higher values than that of healthy controls ( $p<0.0001$  and  $p=0.017$ , respectively). Serum TNF- $\alpha$  levels were measured at comparable values in all 4 groups ( $p>0.05$ ). Serum EPO levels, on the other hand, were different among the groups ( $p=0.006$ ) with the lowest values detected in the control group and a higher value in IDA group when compared to that of CRF and RA group. EPO levels in IDA patients are significantly higher ( $p=0.008$ ) than healthy individuals, but no difference was found among other groups ( $p>0.05$ ). Although serum hepcidin levels were higher in the control and IDA groups than those in CRF and RA groups, no significant difference was determined ( $p>0.05$ ). Iron parameters, ferritin, acute phase mediators, and IL-6, TNF- $\alpha$ , EPO, and hepcidin levels of the patients and healthy control group are given in Table 2.

Based on our assessment to find out whether their laboratory values are correlated to the ferritin levels, serum IL-6, TNF- $\alpha$ , hepcidin, and EPO values of the patients; a significant correlation was identified between the following parameters: ferritin level was positively correlated to IL-6 ( $r=0.57$ ,  $p<0.0001$ ), age ( $r=0.69$ ,  $p<0.0001$ ), serum Cr ( $r=0.62$ ,  $p<0.0001$ ), fibrinogen ( $r=0.46$ ,  $p=0.040$ ), MCV ( $r=0.547$ ,  $p<0.0001$ ), MCH ( $r=0.49$ ,  $p<0.0001$ ), MCHC ( $r=0.27$ ,  $p=0.014$ ), WBC ( $r=0.34$ ,  $p=0.002$ ), ANC ( $r=0.38$ ,  $p=0.001$ ), and ESH ( $r=0.64$ ,  $p<0.0001$ ) while negatively correlated to albumin ( $r=-0.47$ ,  $p<0.0001$ ), erythrocyte ( $r=-0.46$ ,  $p<0.0001$ ), absolute lymphocyte count ( $r=-0.25$ ,  $p=0.027$ ), platelet ( $r=-0.28$ ,  $p=0.012$ ), and TIBC ( $r=-0.72$ ,  $p<0.0001$ ). IL-6 was detected to exhibit a positive correlation with age ( $r=0.50$ ,  $p<0.0001$ ), serum Cr ( $r=0.37$ ,  $p=0.002$ ), CRP ( $r=0.40$ ,  $p=0.008$ ), RDW ( $r=0.23$ ,  $p=0.029$ ), WBC ( $r=0.40$ ,  $p<0.0001$ ), ANC ( $r=0.34$ ,  $p=0.001$ ), and ESH ( $r=0.68$ ,  $p<0.0001$ ) while negatively correlated to albumin ( $r=-0.55$ ,  $p<0.0001$ ), haemoglobin ( $r=-0.55$ ,  $p=0.011$ ), haematocrit ( $r=-0.28$ ,  $p=0.005$ ), erythrocyte ( $r=-0.34$ ,  $p<0.0001$ ), and TIBC ( $r=-0.61$ ,  $p=0.001$ ). Serum hepcidin was negatively correlated to ferritin ( $r=-0.24$ ,  $p=0.029$ ), serum iron ( $r=-0.46$ ,  $p=0.049$ ), and transferrin saturation ( $r=-0.543$ ,  $p=0.016$ ). A positive correlation of serum EPO level with RDW ( $r=0.585$ ,  $p<0.0001$ ) whereas a negative correlation with Hb ( $r=-0.502$ ,  $p<0.0001$ ), HCT ( $r=-0.455$ ,  $p<0.0001$ ), MCH ( $r=-0.313$ ,  $p<0.0001$ ), and MCHC ( $r=-0.461$ ,  $p<0.0001$ ) was identified.

## Discussion

Most commonly encountered forms of anemia in the community are anemia of chronic disease and iron deficiency anemia (1) Pro-inflammatory factors such as CRP, IL-6, and TNF- $\alpha$  and the hepcidin hormone were found to contribute in the pathogenesis of ACD [3,5,6]. While increased levels of hepcidin were detected in ACD, hepcidin levels were, on contrary, measured to be lower in IDA [21]. There are a

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number of studies investigating any relationship between IDA and cytokines which have documented lower IL-6 levels in IDA patients than in ACD patients [1,22]. In a similar manner, we have investigated the serum IL-6, TNF- $\alpha$ , hepcidin, and EPO levels in the ACD group composed of CRF and RA patients and in the IDA group and identified IL-6 level as the most determinative cytokine in differential diagnosis of anemia between our groups. In particular, our ACD group made of CRF and RA patients had higher IL-6 levels than both IDA patients and the healthy controls. Our results are similar to the results available in the literature and points out that besides the ferritin and acute phase reactants, the routine laboratory tests used in the diagnosis of ACD, serum IL-6 level may also be employed.

In a study which has evaluated 76 CRF patients on peritoneal dialysis, efficacy of the dialysis and inflammation was assessed using CRP and an elevated level of inflammatory molecules such as CRP or IL-6 was detected in patients with impaired renal functions [12]. IL-6 values were significantly higher in our CRF patient group than that of both the healthy control and IDA group. When the patients were evaluated based on their CRF stage, however, no difference occurred in IL-6 levels by the advanced stage of CRF.

In a study investigating serum pro-hepcidin levels in 57 patients on dialysis who are resistant to EPO treatment, hepcidin levels were found to be lower in IDA while higher in CRF than healthy individuals stating that multiple factors, besides hepcidin, contribute into EPO-resistant anemia [15,22]. Recent studies have generated controversial results regarding hepcidin concentrations involved in the iron metabolism in ACD and ACD/IDA. In the literature, on contrary to the studies demonstrating higher hepcidin levels in CRF patients compared to the IDA patients, studies with lower hepcidin levels in CRF than IDA are also available. In such studies both negative and positive correlation were shown between hepcidin levels and ferritin [1,15,23-25]. Unlike most of the studies in the literature, and in a similar manner to certain studies hepcidin levels in our study were determined, unexpectedly, to be lower in our ACD patient group made of RA and CRF patients, while high in IDA and healthy control group. In correlation with our results, we have identified higher serum hepcidin values in those with low ferritin levels. In line with the literature results, individuals with high hepcidin levels were found to have low serum iron and transferrin saturation in our study. Our results suggest the role of various factors in ACD etiology other than hepcidin, and the likelihood of suppressed hepcidin expression due to anemia-induced functional erythropoiesis in the bone marrow.

Main control mechanism on EPO production is oxygen related feedback in addition to turnover rate and inflammatory cytokines acting as other effective factors in erythroid cells. The lowest the Hb level, the higher, in response, gets the serum EPO level [26,27]. Coming after the IL-6, EPO was identified as one of the most effective factors on anemia in our patient groups. Serum EPO levels were measured to be highest in IDA and lowest in the control group and between those levels in

ACD patients, in a study which have enrolled IDA patients as well as anemia patients with unknown cause and with renal failure [28]. We have detected a significantly higher EPO level in our IDA and ACD (CRF and RA) groups than that in control group, in a similar manner to the literature. Moreover, a positive correlation of serum EPO level with RDW whereas a negative correlation with Hb, HCT, MCH, and MCHC was identified. In anemic patients, the lower the HB value, in response, the higher gets the serum EPO level. In parallel with the lowest Hb level in IDA group, EPO level was determined to be highest.

One of its most predominant complications of RA is anemia of inflammation. In particular, pro-inflammatory cytokines TNF- $\alpha$ , IL-6 and IL-1 were documented to be effective in the pathogenesis of RA and in the development of RA-associated anemia by means of inhibiting iron metabolism and erythropoiesis taking place in the bone marrow [18,19]. Studies on iron parameters of RA patients have concluded low iron, normal to low transferrin, and normal to high serum ferritin levels [15]. In our RA patients, serum iron levels were lower than normal, transferrin saturation was also lower, whereas TIBC was in usual range and ferritin was higher, in compliance with the literature. Furthermore, RA patients in our study had EPO levels lower than that of IDA patients but higher than that of healthy controls; IL-6 levels were detected to be higher than IDA and healthy controls, while no difference was detected in their TNF- $\alpha$  levels. Serum EPO levels in the RA patients were lower than in the control subjects. When RA patients were divided into 2 based on existence or nonexistence of ACD, ACD group was concluded to have higher CRP and ESR but a lower serum EPO level [21,29]. In our inter-comparison within the RA patients with ACD based on their DAS-28 disease activity, patients with a high DAS-28 score were detected, in a similar manner to the literature, to have high CRP and ESR levels. Their high IL-6 levels, despite the fact that a majority of our RA patients were in remission or featuring with low disease activity, imply on-going subclinical inflammation and associated ACD. This result suggests IL-6 receptor antagonists as a good alternative to conventional synthetic DMARD therapies in the event of ACD in RA patients.

Although ACD is a complex phenomenon, high values of inflammatory mediators CRP, ESR, ferritin and leukocyte which are secreted by the influence of IL-6 and a low value of albumin are expected results in ACD patients [1,28,30]. A positive correlation of ferritin level with IL-6, fibrinogen, WBC, ANC, and ESR whereas a negative correlation with serum hepcidin, albumin, and platelet values was identified in our patients. We have determined a positive correlation of IL-6 level with CRP, WBC, ANC, and ESR whereas a negative correlation with albumin, haemoglobin, haematocrit, erythrocyte, and TIBC. Our results comply with the literature findings which have established IL-6 as the mediator that is responsible for the leukocytosis, neutrophilia, increased CRP, ESR, and ferritin levels, and decreased albumin levels. Our results support the previous literature findings which have also demonstrated IL-6 as the most important cytokine in inflammatory response.

## Conclusion

We have determined that ACD patients have increased IL-6 levels compared to IDA patients and healthy controls, lower EPO levels than that of IDA patients, but indifferent serum hepcidin and TNF- $\alpha$  value. Our results indicate that serum hepcidin and TNF- $\alpha$  values are not useful parameters to differentiate CRF and IDA while serum iron, TIBC, ferritin, in addition to inflammation markers CRP, ESR, and albumin as well as IL-6 and EPO are the key cytokines.

## Study Limitations

The patient sample size was a small and a larger population would be necessary to validate our findings.

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