Anemia, Iron Status and Calcium-Phosphorus levels in Rheumatoid Arthritis Patients

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Abstract: The occurrence of anemia, iron status and calcium-phosphorus levels were studied in 70 rheumatoid arthritis (RA) patients. Anemia was found in 52.8% of the patients, 43.24 % of the anemic patients had iron deficiency anemia (microcytic hypochromic anemia and decreased serum iron and ferritin) and 56.76 % had anemia of chronic disease (normocytic normochromic anemia and normal serum ferritin level). Calcium/phosphorus ratio was found disturbed in RA patients as compared to controls and more disturbed in anemic RA patients than RA without anemia.

Keywords: Anemia, Iron, Calcium, Phosphorus, Rheumatoid Arthritis.

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory polyarthritis, leading to joint destruction, deformity and loss of function. Extra-articular features and systemic symptoms usually occur and may accompany the onset of joint symptoms **(Majhi and Srivastava, 2010**).
RA is a source of significant morbidity and decreased quality of life as it was found that 20 to 30 % of RA patients will become permanently disabled within three years of diagnosis if left untreated (**Sokka *et al.,* 2003**).

Anemia is the most common extra-articular manifestation of RA, estimated to occur in 30 % to 60 % of patients. Anemic RA patients have more severe disease evidenced as more involved joints and higher levels of functional disability and pain (**Bear *et al.,* 1987, Peeters *et al.,* 1996, Tanaka *et al.,* 1999 and Wilson *et al.,* 2004**).

The main types of anemia occurred in RA are iron deficiency anemia and anemia of chronic disease (**Majhi and Srivastava, 2010**). Iron deficiency anemia is usually microcytic hypochromic while anemia of chronic disease is normocytic normochromic (**Hove *et al.,* 2000**).
Iron is a vital mineral to the human being required for erythropoiesis, oxygen transport, DNA synthesis and electron transport (**Conrad *et al.,* 1993 and Goodnough, 2011**).

The human body contains approximately 3 to 4 gm of iron, with hemoglobin accounting for 60 % of the body's total iron. In humans 80 % of the iron demand is related to the daily production of 200 billion new erythrocytes, require about 20 to 24 mg of iron for the synthesis of hemoglobin. Most of this iron is provided by macrophages through the catabolism of hemoglobin of senescent erythrocytes (**Cavill, 2003**).

Calcium is the fifth most common element in the body and the most prevalent cation. The skeleton contains 99% of the body's calcium, predominantly as extracellular crystals of unknown structure with a composition approaching that of hydroxyapatite $[Ca_{10}(PO_4)_5(OH)_2]$ (**Endres and Rude, 2008**).

Phosphorus in the form of organic and inorganic phosphate is important and widely distributed element in the human body. Inorganic Phosphate is the fraction measured in serum and plasma by clinical laboratories. Organic phosphate is a major component of hydroxyapatite of bone (**Endres and Rude, 2008**).

Calcium / phosphorus ratio is very important for the formation of bone (**Walwadkar *et al.,* 2006**). RA is associated with localized or generalized osteoporosis. Periarticular osteoporosis has been recognized as one of the earliest radiological signs of RA and represents an important criterion for the diagnosis of RA (**Lange *et al.,* 2000 and Ramprasath *et al.,* 2006**).

The aim of the present work is to study the occurrence of anemia, iron status (serum iron, total iron binding capacity and serum ferritin) and calcium-phosphorus levels in rheumatoid arthritis patients.

2. Materials and Methods
Subjects:

The study included 70 RA patients (20 ♂ and 50 ♀, their ages ranged from 40 to 60 years and the duration of their disease ranged from 2 to 8 years), satisfied the criteria of the American College of Rheumatology / European League Against Rheumatism Collaborative initiative for the classification of rheumatoid arthritis (**Aletaha *et al.,* 2010**), recruited from the Department of Rheumatology , Sohag University Hospital and 20 healthy control subjects, their age and sex were matched to the patients The control subjects and the patients were not on vitamin, mineral or any drug or supplementation that may alter minerals level. Complete blood picture using automated coulter which show hemoglobin concentration and RBCs indices was done for all the participants. For the assay of minerals, approximately 3ml venous blood samples were collected from the patients and the controls under fasting conditions and non hemolysed sera were used.

Chemicals

All the chemical and the reagents were of high analytical grade.

Methods

Serum iron was estimated by a test kit supplied by Spectrum Diagnostics (Cat. No. 271002) based on the dissociation of iron from its carrier protein transferrin and its reduction to ferrous form, the ferrous ions react highly specific with the chromogen Nitro-PAPS (2-(5-Nitro-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropyl-amino) phenol Na salt) to form a colored complex and the absorption is read at 578 nm.

For the estimation of total iron binding capacity (TIBC), serum transferrin is saturated with an excess of ferric iron and the unbound portion is precipitated with magnesium carbonate and the total iron binding capacity is then estimated. The difference between the total iron binding capacity the initial serum iron represents the unsaturated iron binding capacity (**Baadenhuinjsen, 1998**). Transferrin saturation is the ratio of the serum iron and the total iron binding capacity expressed as a percentage. Serum ferritin was estimated using a test kit ST AIA-PACK FER, Cat. No.0025253 on TOSOH AIA System Analyzers.

Serum Calcium was estimated using a test kit supplied by Reactivos GPL, Spain Cod. SU007, based on Arsenazo 111-colorimetric method, and serum phosphorus was estimated by ammonium molybdate – UV method (**Atkinson *et al.,* 1973**).

Statistical analysis:

Data were expressed as mean ± SD and analyzed using unpaired t-test, and Spearman correlation test; values lower than 0.05 were considered significant.

3. Results

According to World Health Organization, anemia is diagnosed in males when hemoglobin (Hb) is < 13 g /dL and in females when Hb is < 12 g /dL. Anemia is classified as microcytic hypochromic when MCV (mean cell volume) is < 78 fL, normocytic normochromic when MCV lies between 78-100 fL and macrocytic when MCV >100 fL (**Hove *et al.,* 2000**).

Table(1) The parameters measured in the study group.

Parameter	Controls No. (20)	Non anemic RA No. (33)	RA with IDA No. (16)	RA with ACD No. (21)
ESR (mm/1sth)	12 ± 5	50 ± 16	81 ±20.2	89 ± 15
CRP (mg/dL)	1.5 ± 0.3	3. 5 ± 1.3	5.6 ± 2.1	5.8 ± 1.9
Hb (g/dL)	14.36 ± 1.16	14.26 ± 1.14	10.33 ± 1.22	9.73 ± 0.96
MCV(fL)	91 ± 9	90 ± 7	65 ± 3	89 ± 8
Iron (µmol/L)	20.1 ± 1.2	19.9 ± 1.8	12.32 ± 1.34[**]	12.54 ± 1.84[**]
TIBC (µmol/L)	60.5 ± 6.32	61.1 ± 5.47	104.57 ± 14.6[**]	101.92 ± 12.7[**]
Transferrin sat. (%)	32.9 ± 2.4	32.7 ± 2.7	12.08 ± 1.7[**]	12.46 ± 1.8[**]
Ferritin (µg/L)	123.3 ± 12.5	120.2 ± 13.18	49.83 ± 9.36[**]	121.1 ± 11.66[**]
Calcium (mg/dL)	9.26 ± 0.61	7.8 ± 1.6[*]	6.78 ± 1.37[**]	6.98 ± 1.2[**]
Phosphorus (mg/dL)	2.7 ± 0.3	3.2 ± 056[*]	4.37 ± 0.72[**]	3.67 ± 0.58[**]
Calcium/Phosphorus ratio	3.43 ± 0.43	2.84 ± 0.23[*]	1.56 ± 0.27[**]	1.96 ± 0.45[**]

Values are expressed as mean ± SD; [*]*P* < 0.01, [**]*P* < 0.001.

The results showed that (Table.1) , 37 out of 70 RA patients were anemic representing 52.8% of the patients. 16 anemic RA patients (representing 43.24 % of the anemic patients) had mirocytic hypochromic anemia and decreased serum iron and serum ferritin and increased TIBC, indicating iron deficiency anemia and 21 anemic RA patients (representing 56.76 % of the anemic patients) had normocytic normochromic anemia, decreased serum iron, increased TIBC but normal serum ferritin indicating anemia of chronic disease. Serum calcium levels decreased significantly , phosphorus levels increased significantly and Calcium / Phosphorus ratio decreased significantly in RA patients compared to the controls ($P < 0.01$ in non anemic RA patients and $^{**}P < 0.001$ in case of anemic RA patients). There was a negative correlation between Hb concentration and serum iron and both of ESR and CRP in anemic RA patients, there was a positive correlation between TIBC and serum ferritin and ESR and CRP (Table.2).

Table (2)Correlation between the parameters in RA patients

	Correlation between the parameters	r-value
1-	Correlation between Hb conc. and ESR	-0.57
2-	Correlation between Hb conc. and CRP	-0.61
3-	Correlation between serum iron and ESR	-0.51
4-	Correlation between serum iron and CRP	-0.59
5-	Correlation between TIBC and ESR	0.66
6-	Correlation between TIBC and CRP	0.67
7-	Correlation between serum ferritin and ESR	0.62
8-	Correlation between serum ferritin and CRP.	0.56

4. Discussion

This study revealed that anemia was present in 52.8 % of RA patients, 43.24 % of the anemic patients had iron deficiency anemia (microcytic hypochromic , low serum iron and serum ferritin and increased in total iron binding capacity) and 56.76 % had anemia of chronic disease (normocytic normochromic anemia, low serum iron, an increase in total iron binding capacity and normal serum ferritin level). Serum calcium levels decreased significantly, phosphorus levels increased significantly and Calcium / Phosphorus ratio decreased significantly in RA patients compared to the controls.

Obtained results regarding the prevalence of anemia in RA were in accordance with those obtained by (**Bear *et al.,*1987, Hochberg *et al.,* 1988, Peeters *et al.,*1996, Tanaka *et al.,* 1999, Wilson *et al.,* 2004 and van Santen *et al.,* 2011**). In addition, in both anemia of chronic disease and iron-deficiency anemia, the serum concentration of iron and transferrin saturation are reduced, reflecting absolute iron deficiency in iron-deficiency anemia and hypoferremia due to acquisition of iron by the reticuloendothelial system in anemia of chronic disease (**Weiss, 2002 and Means, 2003).** Anemia in RA patients may be due to; improper iron utilization with decreased serum iron and transferrin concentrations, reduced erythropoietin levels, decreased bone marrow response to erythropoietin, premature destruction of red blood cells or due to the drugs used in the treatment of RA patients as non steroidal anti-inflammatory drugs and methotrexate.(**Porter *et al.,* 1994 and Ravindran *et al.,* 2008**). The degree of anemia in RA is related to disease activity and inflammation. Treatment of disease activity and erythropoietin therapy usually improve the anemia. The anemia of chronic disease (ACD) will not respond to iron. It is usually normochromic and normocytic (**Porter *et al.,* 1994 and Weiss *et al.,* 2005**). A hallmark of ACD is the development of disturbances of iron homeostasis, with increased uptake and retention of iron within cells of the reticuloendothelial system. This leads to a diversion of iron from the circulation into storage sites of the reticuloendothelial system, unavailability of iron for erythropoiesis, and iron-restricted erythropoiesis. The uptake of iron by macrophages most prominently takes place through erythrophagocytosis and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT1) (**Weiss *et al.,* 2005 and van Santen *et al.,* 2011**). In addition the inflammation associated with RA increases the production of inflammatory cytokines resulting in decreased availability of erythropoietin, decreased erythropoietic response in the bone marrow and inadequate erythropoiesis. Numerous cytokines included in the pathogenesis of RA (**Brennan and Beech,**

3

2007), like tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-10 (IL-10), Interferon-γ and interleukin-6 (IL-6), mediate ACD (**Kheansaard et al., 2012**). It was found that injection of mice with TNF-α and IL-1 caused hypoferremia and anemia (**Alvarez-Hernandez et al., 1989**). Interferon-γ and TNF-α increase the expression of DMT1, causing an increase in uptake of iron by activated macrophages (**Ludwiczek et al., 2003**). These proinflammatory stimuli also induce the retention of iron in macrophages by decreasing the expression of ferroportin, thus blocking the release of iron from these cells (**Ludwiczek et al., 2003**). Ferroportin is a transmembrane exporter of iron, a process that is believed to be responsible for the transfer of absorbed ferrous iron from duodenal enterocytes to the circulation. (**Pietrangelo et al., 2002**). Interleukin-10 can induce anemia through the stimulation of transferrin-mediated acquisition of iron by macrophages and by translational stimulation of ferritin expression (**Weiss et al., 2005**). IL-6 induces the expression of hepcidin, a 25 amino acid, iron-regulated acute-phase protein, produced by hepatocytes and macrophages and causes iron accumulation in macrophages (**Layoun et al., 2012**).

This investigation revealed that serum calcium levels decreased significantly, phosphorus levels increased significantly and Calcium / Phosphorus ratio decreased significantly in RA patients compared to the controls. Several studies revealed altered calcium and phosphorus levels in RA patients (**Scott el al.,1981, Star and Hochber, 1994, Langue et al., 2000, Walwadkar et al., 2006 and Makhdoom et al., 2009**). RA is usually associated with localized or generalized osteoporosis, erosions and hand and generalized bone mineral density (BMD) loss resulting in functional disability and increased risk of clinical fractures (**Güler-Yüksel et al., 2009**). Inflammatory mediators, cytokines and oxidative stress are the most likely causes (**Ramprasath et al., 2006 and Walwadkar et al., 2006**). corticosteroids decrease generalised BMD loss by suppression of inflammatory activity, but as a side-effect, increase BMD loss (**Güler-Yüksel et al., 2009**). In conclusion, anemia is a common problem in RA patients and is of two types iron deficiency anemia and anemia of chronic disease and anemia signs were correlated to parameters of inflammation. Also we concluded that calcium/phosphorus levels were disturbed in RA patients and more disturbed in anemic RA

patients. So, supplements of calcium, iron and vitamin D are needed for RA patients.

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