Iron Status in Rheumatoid Arthritis

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This project was carried out to evaluate disturbances of Iron metabolism in patients of Rheumatoid Arthritis. A total number of fifty patients were evaluated. The cases understudy were compared with twenty healthy controls severity of Rheumatoid Arthritis was grade according to the criteria of American Rheumatisum association into mild moderate and severe. There were five patients (10%) with mild 32(64%) moderate and 13(26%) severe disease. Moderate cases (64%) showed normocytic normochromic anaemias, where as Iron deficiency anaemia was seen in severe (26%) cases. Serum iron, TIBC, serum Ferritin and red cell ferritin levels were carried out in all the patients. Serum Iron level was lowered in patients but, TIBC was normal. 15(30%) patients had a high serum ferritin, where as red cell ferritin was normal. 13(26%) patients having iron deficiency also had low red cell ferritin levels. In this study we conclude that iron metabolism is disturbed in rheumatoid. Arthritis and red cell ferritin is better indicator of Iron stores as compared to the serum ferritin as red cell ferritin level is not influenced by inflammatory process of rheumatoid Arthritis.

Key words: Red cell, ferritin, RCF, total iron binding capacity (TIBC), rheumatoid arthritis(RA)

Anaemia in rheumatoid arthritis is a common extra-articular manifestation[1,2,3]. Mostly it is Normocytic and normochromic but microcytic and hypochromic anaemia is also seen in some patients[4] which is mostly due to the gastrointestinal bleeding due to ingestion of Nonsteroidal anti inflammatory drugs (NSAIDS)[5]. Severity of Anaemia is proportionate to severity of the disease. Severer is the disease, more severe is the anaemia[6]. According to the American Rheumatism Association (ARA), Rheumatoid arthritis can be divided functionally in mild, moderate and severe disease[7]. Being a chronic disorder, the iron metabolism is disturbed but the exact mechanism is not known. However there is defective transport of iron from reticulo-endothelial system (RES) to the erythroid cells in the bone marrow[8]. In Rheumatoid arthritis Lymphokines, Tumor necrosis factor-(TNF), Interleukin I^{IL} and IL-6 as well as relative deficiency of erythropoietin are thought to play a key role in the pathogenesis of anaemia in RA[17]. The impairment of iron transport to the erythroid cells is also accompanied by characteristic biochemical changes i.e. level of both the serum iron and TIBC are reduced but serum Ferritin is normal or elevated Serum ferritin being acute phase protein is not a reliable parameter of iron stores. Red Cell Ferritin (RCF) is a more reliable index of iron stores in rheumatoid arthritis as it is not influenced by inflammation[10]. The patients under such circumstances are also prone to develop iron deficiency due to ingestion of NSAIDS.

In present study, we evaluated types of anaemia in diagnosed patients of rheumatoid arthritis. Routine haematological investigations were performed to see the different types of anaemia along with the assessment of iron status and other abnormalities of iron metabolism.

Material and Methods

A total of 70 subjects were included in the study. There were 50 diagnosed patients of rheumatoid arthritis with anaemia having haemoglobin level less than 13 g/ dl in males and 12g/dl in females (25 males and 25 females). They were compared with 20 healthy age and sex matched controls. Depending upon the clinical severity of the disease the patients wee divided into mild, moderate, and severe category based on the criteria of American rheumatism association (ARA). None of the patients under study had taken oral or parental iron during the previous three months. Patients with history of intake of any disease modifying drugs like methortraxate were also excluded from the study. The subject were divided into the following groups: group I control, group II mild disease, group III moderate disease and group IV severe disease. Following Laboratory investigations were done in all the subjects which included Haemoglobin, Erythrocyte sedimentation rate (ESR), Differential leukocyte count (DLC), Total leukocyte count (TLC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH, Serum ferritin, Red cell ferritin (RCF) and stool for occult blood. Serum iron and TIBC were done coloremetrically, serum ferritin was measured by ELISA. For RCF estimation, Two ml EDTA blood was taken and buffy coat was made to remove leukocytes and platelets, then eluted red cells were washed three times by centrifugation and re-suspended in two ml phosphate buffer before being stored at (-20°C). Immediately prior to the assay, the samples were thawed , diluted 1:1 with distilled water, re-frozen and thawed to complete lysis. Stroma was removed by centrifugation at 3500 RPM for 15 minutes and the supernatant was assayed for ferritin estimation by ELISA. The haemoglobin of the supernatant was measured by cynamet haemoglobin method and MCH of eluted red blood cells was obtained. The red cell number was calculated from these values. Red cell ferritin contents was expressed as attogram per cell (Normal value: 4-44 attogram/cell). Statistical analysis was done using Analysis of variance (ANOVA) and Tukey honestly

significant difference (Tucky HSD).

Results

Out of 50 cases under study, 5 patients had mild disease (group II), 32 patients had moderate disease (group III) and 13 patients had severe disease (group IV) and results were compare with 20 cases (control) (group I)

Table 1. Functional classes of total subjects

Group	No. of Subject	Functional Class
I	20	Control
II	5	Mild
III	32	Moderate
IV	13	Severe

Mean age in different groups was between 40-50 years. Haemoglobin levels were decreased in all the patients under study and markedly decreased in severe disease (group IV). There was a significant difference in the values of patients group and normal in control (P<.0001). ESR was raised in patient group and normal in control (P>0001) (Table II), while no significant difference between patient and control group was noted in the values of TLC, DLC and platelets (Table II A+B).

Table II A. Mean Values of Haemoglobin g/d, ESR, mm/hour in patients and controls

Group	No. of Subjects	Haemoglobin g/dl	ESR mm/hour
I	20	13.4 ± 0.8 (12.0 – 14.8)	6.5 ± 2.7 (2 – 11)
II	5	10.2 ± 0.2 (9.9. – 10.5)	56.8 ± 11.2 (39 - 69)
III	32	9.6 ± 0.5 (8.8 – 10.4)	68.8 ± 9.1 (36 – 110)
IV	13	7.9 ± 0.4 (7.1 - 8.6)	92.2 ± 16.3 (66 – 140)
P value		<.001	<.001

<.05 significant

<.001 – very highly significant

Table II B Comparison of 'P' values of Haemoglobin, ESR, In controls and patients

Group	Haemoglobin	ESR
I vs II	< .0001	< .0001
I vs III	< .0001	< .0001
I vs IV	< .0001	< .0001
II vs III	> .05	> .05
II vs IV	< .0001	< .0001
III vs IV	< .0001	< .0001

'P' Values *Not Significant *** significant

Iron status was evaluated in all the subjects understudy by doing serum iron, TIBC, serum ferritin and RCF. Serum iron was low in patients group (II, III, IV) while TIBC was normal in all the subjects. (I-IV) in these investigations significant statistical difference was seen between control (I) and patients group (II-IV) (P < 0.01). Serum ferritin was raised in 15 (30%) subjects while it was low in 13 (26%) subjects. Serum ferritin levels less than 55ng/ml in rheumatoid arthritis was considered to have iron deficiency. RCF levels were within normal range in mild (group II) and in moderates disease (group III) but decreased in 13 patients of group IV. Among these patients of group IV has iron deficiency anaemia and serum ferritin levels were less than 55ng/ml (Table II). These patients had raised serum ferritin level but RCF was normal. Stool for occult blood was positive in 8 (16%) patients.

Table III Mean value of serum Iron (µg/dl), TIBC (ug/d1), Serum Ferritin (µg/d1) and Red Cell Ferritin (att/cell) in Different groups

Gp	No. of subjects	Serum Iron (ug/dl)	TIBC (µg/dl)	Serum ferritin (µg/ml)	RCF (Att/cell)
I	20	92.3±9.3 (77-112)	319.7+22.5 (292-390)	101.7+21.3 (76.6-148.1)	20+3.8 (13.6-299)
II	5	59.2 ± 3.9 (52 65)	388.1± 17.5 (271– 321)	189.7±75.9 (131-310)	15.9 ± 4.6 (11.1–22)
III	32	48.3± 12.4 (25 - 72)	292..9 ±15.7 (268– 322)	204±129.2 (386 -484)	17.7 ± 8.0 (6.3–32.3)
IV	13	37.7 ± 7.8 (26.2 - 52.0)	335.4 ± 31.0 (271-376)	76.4 ± 20.0 (48.7- 98.2)	3.6 ± 1.4 (2.2-7.5)
P values		<.0001***	<.0001****	<.0001****	<.0001****

***Highly significant

Table III B. Comparison of P Values serum Iron, TIBC, Serum ferritin and Red cell ferritin in controls and patients

Group	Serum Iron	TIBC	Serum ferritin	RCF
I vs II	<001****	>05*	>05*	>05*
I vs III	<001****	<.001*	<.001*	>05*
I vs IV	<.001***	<.001***	<001***	<001***
II vs III	>05*	>.05*	>05*	>05*
IIvs IV	<.001***	<.001***	<.001***	<001***
IIIvsIV	<.001***	>05*	<001***	<001***

'P' Values * Significant, **** Highly significant

Discussion

The current study was carried out in 70 subjects, 20 control and 50 patients. The patients were diagnosed cases of rheumatoid arthritis having haemoglobin level less than 13g/d1 in males and 12g/dl in females. On the basis of functional classification, 5(10%) patients had mild disease, 32(64%) had moderate disease (group III) and 13(26%) had severe disease (group IV). Hafeez et al in 1989 reported maximum number of patients having moderate disease. Same was reported by Moreland et al in 1999. This was similar with the present study. Mean age in the present study was in the fourth decade of life. Same was reported by Hafeez at al in 1989 and Campbell et al in 1999. ESR was raised in all the patients same results were reported by Gudjursonberg et al in 1992.

Haemoglobin was decreased in patients and markedly decrease in severe disease. Anaemia appears to the commonest extra -articular feature of rheumatoid arthritis. The similar results were observed by Mowat et al in 1988, Krantz et al in 1995. TLC, DLC and platelets were not significantly different between control and patients. This was in accordance with the study of Stockes et al in 1992. Serum iron was low in patient as compared to control. Das and Sattar observed similar findings in 1989. Serum ferritin was raise in 15 (30%) patients their RCF were within normal range[10]. Serum ferritin was low in 13(26%) patient and there RCF level were also decreased indicating iron deficiency. They all had iron deficiency anaemia. Raised serum ferritin levels with normal RCF levels show that RCF is more reliable indicator of iron stores in the rheumatoid arthritis as compared to serum ferritin because RCF is not influenced by inflammation. Low serum iron-levels and normal or raised serum ferritin levels showed impaired transport of iron from reticulo-endothelial system. Thus it may be concluded that the pattern of rheumatoid arthritis in this part of world is the same as seen in the west.

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