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Original Research

To Assess and Evaluate Hematological Abnormalities (Anaemia, Thrombocytosis, Eosinophilia, and Haematological Malignancies) in Patients of Rheumatoid Arthritis: A Hospital Based Study

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***ABSTRACT*:**
Background: Rheumatoid arthritis (RA) is the paradigm of a systemic autoimmune disease characterized by inflammatory polyarthritis. The prevalence of rheumatoid arthritis (RA) in most populations is around 1% with an incidence in women three times that in men. Hence we planned to assess prevalence of anemia in patients of RA. We will also assess other hematological parameters like total leukocyte count (TLC), differential leukocyte count (DLC) platelet count, erythrocyte sedimentation rate (ESR). **Material & Methods:** This hospital based observation study was conducted among 50 patients of rheumatoid arthritis diagnosed according to 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis at Department of Pathology, Sardar Patel, Medical College &associated group of Hospitals, Bikaner. Evaluation of hematological parameters was done by collecting 5 ml of venous blood sample on EDTA prefilled vial and transporting it to the laboratory immediately. Pearson's coefficient was used to investigate the correlation between the two variables. Statistical significance was set at P value ≤ 0.05. **Results:** The mean age of anaemic patients was 36.58±13.79years, and that of non anaemic patients was 43.38±11.21 years. Difference in age in anaemic and non anaemic patients was not statistically significant (p>0.05). ACD (38%) was the commonest type of anaemia observed in our study group followed by IDA (16%) was the next most common. RF was positive in 40 (80%) patients. Among 34 anaemic patients 28 (82.35%) were RF positive and 6 (15%) were RF-ve. Higher RF positivity found in anaemic patients but this association between anaemia and RF positivity was not statistically significant(p>0.05). Thrombocytosis was found in 4 out of total 50 study subjects. Among these all patient were female. But the difference for sex among patients was not statistically significant (p>0.05). **Conclusion:** We concluded that 85 of cases of RA had anaemia. Higher RF positivity found in anaemic patients and RF positivity was more in patient with thrombocytosis.
Keywords: RA, Hematological parameters, Anemia, Thrombocytosis

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INTRODUCTION:

Rheumatoid arthritis (RA) is the paradigm of a systemic autoimmune disease characterized by inflammatory polyarthritis. Although RA is primarily considered a disease of the joints, abnormal systemic immune responses are evident and can cause a variety of extra-articular manifestations. These manifestations clearly show that RA has features of a systemic disease that can involve many organs.

The prevalence of rheumatoid arthritis (RA) in most populations is around 1% with an incidence in women three times that in men. This number was based on

many studies of population samples.[1-3] which varied among the surveys from 0.3% to 1.5%.
Worldwide annual incidence of RA is 3 cases per 10000 populations and prevalence is around 1%, increasing with age and peaking at ages of 35 and 50 years[4]. Women are affected approximately 3 times more often than men, but sex differences diminish in older age groups[4].
The pathogenesis of RA is not completely understood. An external trigger (eg, cigarette smoking, infection, or trauma) that triggers an autoimmune reaction, leading to synovial hypertrophy and chronic joint inflammation along with the potential for extra-articular manifestations, is theorized to occur in genetically susceptible individuals.
Haematological manifestations in RA can be broadly categorized into areas of anaemia, neutropenia, thrombocytopenia, thrombocytosis, eosinophilia, and haematological malignancies. Most common extra-articular manifestation is anemia. Most cases of RA-associated anemia (RA-anemia) are characterized as anemia of inflammation (AI), also known as anemia of chronic disease. Overall anaemia of chronic disease and iron deficiency anaemia are frequent causes of anaemia in RA patients[5], but in developing countries like India, nutritional deficiency may also be a major associated problem adding to the etiology in genesis of anaemia in RA patients.
Hence we planned to assess prevalence of anemia in patients of RA. We will also assess other hematological parameters like total leukocyte count (TLC), differential leukocyte count (DLC) platelet count, erythrocyte sedimentation rate (ESR).

MATERIAL & METHODS:
This hospital based observation study was conducted among 50 patients of rheumatoid arthritis diagnosed according to 2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis[6] at Department of Pathology, Sardar Patel, Medical College &associated group of Hospitals, Bikaner.
2010 ACR/EULAR Classification Criteria for Rheumatoid Arthritis[6] (Score-Based Algorithm for Classification in an Eligible Patient [Cut point for RA: ≥6/10]).

INCLUSION CRITERIA:-
1. All patients fulfilling 2010 ACR/EULAR Classification Criteria for RA

EXCLUSION CRITERIA:-
1. Patient with active apparent bleeding from any site of the body
2. Patient suffering from illnesses that can cause anaemia (other than RA) e.g. chronic kidney disease, chronic liver disease.
3. Patient suffering from infection or malignancy

METHODS:-
Eligible patients undergo detailed history and clinical examination. Evaluation of hematological parameters was done by collecting 5 ml of venous blood sample on EDTA prefilled vial and transporting it to the laboratory immediately. The analysis was done by the automated Analyzer. Peripheral smears were studied after staining with the field's and Leishman's stain.

Staining Thick Films by the field's stain
Field's method of staining is quick and usually satisfactory for thick films, but the method is not practical for staining large numbers of films; for this purpose the Giemsa, Leishman or azure B–eosin Y methods are more suitable. Careful attention to pH is critical for satisfactory staining of parasites

Rapid Staining Method
The field's method was introduced to provide a quick method for staining thick films for malaria parasites. With some modifications, it can be used fairly satisfactorily for the rapid staining of thin films.

Serum ferritin level-
In patients of Rheumatoid arthritis with microcytic hypochromic anemia serum ferritin level will be measured. Serum Ferritin will be estimated by enzyme linked fluorescent assay (ELFA) on MINIVIDAS auto analyzer (Biomerieux). 2 ml venous blood will be drawn in plain vial and allowed to clot. Then sample will be centrifuged to separate serum for measurement of serum ferritin.

Serum vitamin B 12 and folate level-
In patients of Rheumatoid arthritis with macrocytic anemia, serum vitamin B12 and serum folate levels will be measured. For that 2 ml blood will be drawn in plain vial and serum will be separated by centrifugation. Serum will be used for measuring vitamin B12 and folate by chemiluminescence immunoassay (CLIA) method.

2. Laboratory investigations:-

a. Complete blood count (CBC):
The CBC consists of haemoglobin concentration, haematocrit (packed cell volume), mean corpuscular haemoglobin (MCH), MCH concentration, total erythrocyte count, total leucocyte count and platelet count. CBC was performed using automated analyzer. The automated instrument uses two basis technologies for routine CBC measurement: (1) electric impedance and (ii) light scatter.[7] In our set up, CBC measurement is done by the Symex (^{R}) SF-3000, a technically advanced, computerized fully automatic hematology

analyzer. The analyzer is developed by TOA Medical Electronics Co. Ltd. (SYSMEX). Despite its compact size the SF-3000 incorporates Direct current detection, calorometric determination and flow cytometry using a semiconductor laser to produce the parameter of standard CBC with associated cell size distribution analysis.

b. peripheral blood film-
To be stained by the leishman stain and will be evaluated for red cell morphology, platelet count and white cell morphology by 2 hematologists who were blinded to the clinical state of the patients. Anaemic patients were classified according to PBF picture into normocytic normochromic, microcytic hypochromic, macrocytic hypochromic and mixed picture.

c. Rheumatoid factor:
Measured by nephelometrytest**.** This test mixes the blood being tested with antibodies that cause the blood to clump if RF is present. A laser light is shined on the tube containing the mixture and the amount of light blocked by the blood sample is measured. As levels of RF increase, more clumping occurs, causing a cloudier sample and less light to pass through the tube.

d. C-reactive protein (CRP): Measured by nephelometry.

e. Serum folate and cobalamine levels: The vitamines will be assayed using IMMULITE2000 analyser (two site Chemi Luminescent Immunometric Assay).

STATISTICAL ANALYSIS
Continuous variables were expressed as mean ± standard deviation. Unpaired Student's t test and Chi-Square test were used to determine statistical difference between variables. Pearson's coefficient was used to investigate the correlation between the two variables. Statistical significance was set at P value ≤ 0.05.

RESULTS:
The present study showed that maximum no. of cases were seen in above 30 years of age group (table 1).The mean age of anaemic patients was 36.58±13.79years, and that of non anaemic patients was 43.38±11.21 years. Difference in age in anaemic and non anaemic patients was not statistically significant (p>0.05) (table 2).
ACD (38%) was the commonest type of anaemia observed in our study group followed by IDA (16%) was the next most common (table 3).
RF was positive in 40 (80%) patients. Among 34 anaemic patients 28 (82.35%) were RF positive and 6 (15%) were RF-ve. Higher RF positivity found in anaemic patients but this association between anaemia and RF positivity was not statistically significant (p>0.05) (table 4). Thrombocytosis was found in 4 out of total 50 study subjects. Among these all patient were female. But the difference for sex among patients was not statistically significant (p>0.05) (table 5).

Table 1: Age groups wise distribution of male and female

Age group (yrs)	Male	Female	Total
15-30 yrs	1 (10%)	11 (27.5%)	12 (24%)
30-40 yrs	1 (10%)	12 (30%)	13 (26%)
40-50 yrs	2 (20%)	11 (27.5%)	13 (26%)
>50 yrs	6 (60%)	6 (15%)	12 (24%)
Total	10 (20%)	40 (80%)	50 (100%)

Table 2: Comparison of data in anemic and non-anemic patients

Parameters	Anemic (N=34)	Non-anemic (N=16)	p-value
Age (yrs) (mean±SD)	36.58±13.79	43.38±11.21	>0.05
Gender			
Male	6 (17.64%)	4 (25%)	>0.05
Female	28 (82.35%)	12 (75%)	

Table 3: Distribution of male and female according to type of anemia

Type of anemia	Male (N=10)	Female (N=40)	Total
Non-anemia	4	12	16
ACD	5	14	19
IDA	0	8	8
Vit. B_{12} deficiency	1	1	2
IDA+ Vit. B_{12} deficiency	0	3	3
AIHA	0	1	1
Folate deficiency	0	1	1

Table 4: **Distribution of study subjects according RF and Anaemia**

Rheumatoid factor	Anemia		Total	p-value
	Present	Absent		
Present	28	12	40	>0.05
Absent	6	4	10	
Total	34	16	50	

Table 5: **Distribution of study subjects according RF and platelets**

Rheumatoid factor	Platelets		Total	p-value
	Normal	Elevated		
Present	38	2	40	>0.05
Absent	8	2	10	
Total	46	4	50	

DISCUSSION:

Rheumatoid arthritis (RA) is the paradigm of a systemic autoimmune disease characterized by inflammatory polyarthritis. The mean age of anaemic patients was 36.58±13.79years, and that of non anaemic patients was 43.38±11.21 years. Difference in age in anaemic and non anaemic patients was not statistically significant (p>0.05). Munevver Serdarogluet al[8] reported mean age of 48.3± 12.38 years in their study and all of them were females. Another study done by Mansoor Karimifar et al[9] found mean age of 49.5±15.5 years in 90 patients of RA. The mean age of study subjects in Ibrar Ahmed et al[10] study was 41.77±8.68 years. These finding are close to our study.

In our study out of total 50 patients, 40 (80%) were females and 10 (20%) were males. In a study conducted by Ronnelid et al[11] Male: female ratio in RA cases was 3:7. Forslind[12] et al reported Male : female ratio in RA cases 7:13.

Our results were similar to the Adriana Sabau at el[13] who found 70.6% patients anaemic and 29.4% patients nonanemic in their study. In anaemic group 78.9% patients were females and 21.1% patients were males. In nonanaemic group 81.2% patients were females and 19.8% patients were males. Sex difference among anaemic and nonanaemic patients was not statistically significant (p>0.05).

So our study shows that although anaemia is more common in females, association of sex difference and anaemia is not statistically significant (p>0.05).

In our study mean age of anaemic patients was 36.58±13.79 years, and that of non anaemic patients was 43.38±11.21 years. Difference in age in anaemic and nonanaemic patients was not statistically significant (p>0.05). Helen A.et al[14] also observed no statistically significant difference in age in anaemic (48years) and nonanaemic (44years). (p>0.05).

In the study by Adriana Sabauet al[13],mean age in anaemic group was 58.26±14.24years and 52.25±15.96years respectively and it was statistically not significant.(p>0.05). So it can be stated that although anaemic patients presents at a younger age as compared to nonanaemic patients with RA, association between and anaemia and age of RA patients is not statistically significant.

In the study by Adriana Sabauet al[13] 70.4% patients were anaemic in which IDA was presented in 47.36% patients with and the rest of 52.63% patients had ACD. Helen A. at el[7]had observed 57.5% patients anaemic out of which 37.5% patients had ACD and 20% patients had IDA. Agrawal S. et al[4] reported70.6% of their cases to be anemic, of which ACD was present in 51.6% patients and IDA was present in 48.4%patients. So results of all these studies were similar to our study. So results of our study showed that ACD is the most common type of anaemia seen in RA patients followed by IDA being next common type.

RF was positive in 40 (80%) patients. Among 34 anaemic patients 28 (82.35%) were RF positive and 6 (15%) were RF-ve. Higher RF positivity found in anaemic patients but this association between anaemia and RF positivity was not statistically significant(p>0.05).

Our results were similar to Helen A. Papadaki at el[14] who reported 60.87% RF positivity in anaemic patients as compared to 52.94% RF positivity in non-anaemic patients and this difference was not statistically significant. Similar to our study, D J Borah at el[15] had also observed higher proportion of RF positivity in anaemic patients 90% as compared to non-anaemic 54.55%, but this association was not statistically significant(p>0.05). Due to limitation of resources, we could not measure quantitative value of RF in our patients which could have given a better idea of correlation between RF and level of haemoglobin.

Thrombocytosis was found in 4 out of total 50 study subjects. Among these all patient were female. But the difference for sex among patients was not statistically significant (p>0.05). RF indicates aggressiveness in RA, not the activity of RA. Thrombocytosis is associated with active RA so this might be the cause that thrombocytosis is not associated with RF positivity.

CONCLUSION:

We concluded that 85 of cases of RA had anaemia. Higher RF positivity found in anaemic patients and RF positivity was more in patient with thrombocytosis.

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