ORIGINAL RESEARCH

CBC Parameters Including RDW, Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio, ESR and CRP at Diagnosis and After 8-Weeks of Methotrexate Therapy in Patients of Rheumatoid Arthritis

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ABSTRACT
Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease characterized by inflammation of synovial joints in a symmetrical pattern leading to destruction of cartilage and bone, joint deformities, permanent functional impairment and disability. RA is the most common inflammatory arthritis, with a lifetime prevalence of 1% worldwide.Rheumatoid arthritis being a chronic inflammatory disease is known to be associated with a variety of immune alterations. Neutrophil infiltration of synovial tissue with increased levels of inflammatory cytokines like TNF-alpha, IL-1, IL-6, oxidative damage, premature thymus aging and dysfunction, and lymphopenia with presence of abnormal senescent peripheral T cells due to selective destruction of some T cell subsets, microcirculatory thrombosis and reactive thrombocytosis due to inflammation induced anemia and increased erythropoietin levels and neutrophil induced platelet activation are recognized features of RA. Therefore, neutrophilia, lymphopenia and thrombocytosis are known immune alterations in RA which may be reflected in altered relative proportions of these cells in the peripheral blood. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to lymphocyte ratio (PLR) have come increasingly in focus as indicators of subclinical systemic inflammation which can be used to assess disease activity and prognosticate for a variety of infective, inflammatory, neoplastic and autoimmune conditions including RA. We studied complete blood count(CBC) parameters including Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio and RDW as markers of inflammation and disease severity in patients of rheumatoid arthritis after 8 weeks of methotrexate therapy. The study found that apart from DAS 28, RDW, PLR and NLR are emerging inflammatory biomarkers which could be used to evaluate disease activity in active RA patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease characterized by inflammation of synovial joints in a symmetrical pattern leading to destruction of cartilage and bone, joint deformities, permanent functional impairment and disability. RA is the most common inflammatory arthritis, with a lifetime prevalence of 1% worldwide. The onset can occur at any age, but peak between 30 to 50 years. In a large U.S. cohort, 35 percent of patients with RA had work disability after 10 years.The aetiology of RA is multifactorial. The genetic susceptibility is evident in familial clustering and monozygotic twin studies, with 50 percent of RA risk attributable to genetic factors. Genetic associations for RA include human leukocyte antigen DR45 and DRB1, and a variety of alleles called the shared epitope. Genome-wide association studies have

identified additional genetic signatures that increase the risk of RA and other autoimmune diseases, including *STAT4* gene and CD40 locus. Smoking is the major environmental trigger for RA, especially in those with a genetic predisposition. Although infections may unmask an autoimmune response, no particular pathogen has been proven to cause RA.

Rheumatoid arthritis being a chronic inflammatory disease is known to be associated with a variety of immune alterations. Neutrophil infiltration of synovial tissue with increased levels of inflammatory cytokines like TNF-alpha, IL-1, IL-6, oxidative damage, premature thymic aging and dysfunction, and lymphopenia with presence of abnormal senescent peripheral T cells due to selective destruction of some T cell subsets, microcirculatory thrombosis and reactive thrombocytosis due to inflammation induced anemia and increased erythropoietin levels and neutrophil induced platelet activation are recognized features of RA. Therefore, neutrophilia, lymphopenia and thrombocytosis are known immune alterations in RA which may be reflected in altered relative proportions of these cells in the peripheral blood. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to lymphocyte ratio (PLR) have come increasingly in focus as indicators of subclinical systemic inflammation which can be used to assess disease activity and prognosticate for a variety of infective, inflammatory, neoplastic and autoimmune conditions including RA. The patients with RA may be found with increasing platelet counts during active stages of the disease. The counts decrease upon remission of inflammation. The mechanism to explain the increased levels of PLR in RA is the presence of a chronic inflammatory state that affects the progressive damage of joint The reference value of NLR is 2.8 (ref. range 1.2-4.4) and PLR is 137 (ref. range 75-199). The NLR and PLR are calculated from the complete blood count (CBC) and are known to represent systemic inflammation.

Red cell distribution width (RDW), as part of a complete blood cell count,that estimates erythrocyte variability. Higher RDW values reflect greater heterogeneity in red cell sizes The RDW, may be useful to estimate the disease activity in RA. The high RDW in RA might be explained by the fact that RA is an autoimmune chronic disease which is often accompanied with anemia which may increase RDW. Another reason is that RDW may also be influenced by inflammation.

Methotrexate (MTX) is an immunosuppressive drug, analog to folic acid that competitively inhibits the binding of dihydrofolic acid (FH2) to the enzyme that is responsible for converting FH2 to folinic acid (FH4). Without FH4, the metabolism of purine and pyrimidine is impaired, and the synthesis of amino acids and polyamine is inhibited. It is an effective disease-modifying anti-rheumatic drug (DMARD). All people taking methotrexate require regular blood tests to monitor blood counts and liver function. Due to its relatively low cost and favourable efficacy/safety profile, MTX is currently considered the drug of choice for treating rheumatoid arthritis (RA), both as first-line monotherapy in treatment-naïve patients, and as an anchor drug, in MTX insufficient responders.

With this background the present study was planned to evaluate the changes in complete blood count (CBC) parameters including newer cell ratios with manual platelet count, ESR, CRP and RDW in patients of Rheumatoid arthritis at diagnosis and after 8 weeks of Methotrexate therapy.

AIMS AND OBJECTIVES

To study complete blood count(CBC) parameters including Neutrophil-Lymphocyte ratio, Platelet-Lymphocyte ratio and RDW as markers of inflammation and disease severity in patients of rheumatoid arthritis after 8 weeks of methotrexate therapy

MATERIALS & METHODS

This longitudinal study entitled “Study of Neutrophil-Lymphocyte Ratio, Platelet-Lymphocyte Ratio and RDW as surrogate marker of underlying inflammation in patients of Rheumatoid Arthritis on Methotrexate” was conducted on 30 subjects (total 36 subjects were chosen but 6 did not return on follow up) in Sir Sunderlal hospital, Varanasi from May-2018 to April-2019. All cases were selected based on inclusion and exclusion criteria. The study was approved by the institutional ethical committee. A detailed history, clinical examination and all required investigations were done for all study subjects.

Diagnosis of Rheumatoid arthritis- The diagnosis of Rheumatoid arthritis was made according to the American College of Rheumatology diagnostic criteria based on-

- History and clinical examination
- Blood investigations (Rheumatoid Factor and Anti-cyclic citrullinated peptide)

STUDY POPULATION

Selection of patients

Patients of Rheumatoid Arthritis were selected from patients admitted in the medicine ward and attending out patients in the Department of General Medicine and Rheumatology. Only those cases were registered who gave consent for their inclusion in the study. Registered cases were subjected to detailed examination which included general physical examination, careful systemic examination, blood investigations and DAS-28.

Source of data

- Patients attending General Medicine OPD of SS Hospital who has given consent to participate in the study

- IPD patients of General Medicine and Rheumatology who fulfilled the inclusion and exclusion criteria

Sample size- 30 cases after drop outs.

A. Inclusion criteria

- Newly diagnosed patients of seropositive RA who were treatment naïve and eligible for methotrexate therapy
- Previously diagnosed cases of seropositive RA presenting with acute flare who were eligible for methotrexate therapy and were not on steroid or any DMARD for more than six months

B. Exclusion criteria:

- Subjects with events that might cause reasonable alteration in CRP and CBC during the 8-week period from enrolment to reassessment shall be excluded from the study e.g. those who has undergone treatment for anemia, who have a recent hemorrhage, those treated for bacterial infections - LRTI, UTI, bronchitis, enteric fever, hepatitis etc.
- Subjects on concomitant therapy for other disease states where the disease or the drug was known to affect the parameters under consideration e.g. subjects who require steroid beyond two weeks following institution of therapy
- Subjects who have received drugs known to have confounding effects on the said parameters e.g. leflunomide within 105 days (its 5 half-lives) etc.
- Subjects whose qCRP titre increased but DAS-28 score improved at the end of 8 weeks of Methotrexate therapy were excluded from the study (as any such rise is attributable either to Methotrexate toxicity or to a new onset inflammation of unknown aetiology)

Methodology

- Detailed history and clinical examination was done regarding the diagnosis of RA, concomitant illnesses and past illnesses of recurrent or chronic nature after selection of subjects satisfying the inclusion and exclusion criteria
- CBC with manual platelet count, qCRP, RA Factor (q), Anti-CCP Ab and DAS-28 was assessed and on confirmation of RA, the subjects were put on Methotrexate if indicated
- The subjects who comply with therapy up to 8 weeks were subjected to detailed enquiry about any intercurrent illness during this period and intake of any medication that could cause changes in the parameters under study were excluded.
- The remaining subjects would undergo re-estimation of CBC with manual platelet count, qCRP, DAS-28 along with LFT, RFT with Calcium, Phosphate and Uric Acid
- Statistical correlation was studied between the two set of above mentioned parameters
- Statistical significance using students t-test should be calculated for the difference of means between the two set of qCRP, NLR, PLR and RDW.

Sample collection method

Serum and blood samples were collected from patients upon admission taking all aseptic precautions. About 5 ml of blood was drawn by venepuncture from a peripheral vein with a disposable syringe. The blood obtained was collected in one plain vial and one EDTA vial for estimation of CRP and ESR, CBC with manual platelet count. Care was taken to avoid hemolysis of the sample and early analysis.

STATISTICAL ANALYSIS

- The data obtained was collected, entered in Microsoft excel spreadsheet and then analysed using SPSS (version 20.0;SPSS Inc., Chicago, IL, USA).
- Data has been summarized using Descriptive analysis for determining frequencies, percentage, mean values and standard deviations for categorical variables.
- Intragroup and Intergroup comparisons were done using paired sample t-test and Pearson correlation statistical analysis.
- P-value<0.05 was considered for statistical analysis.

OBSERVATIONS AND RESULTS

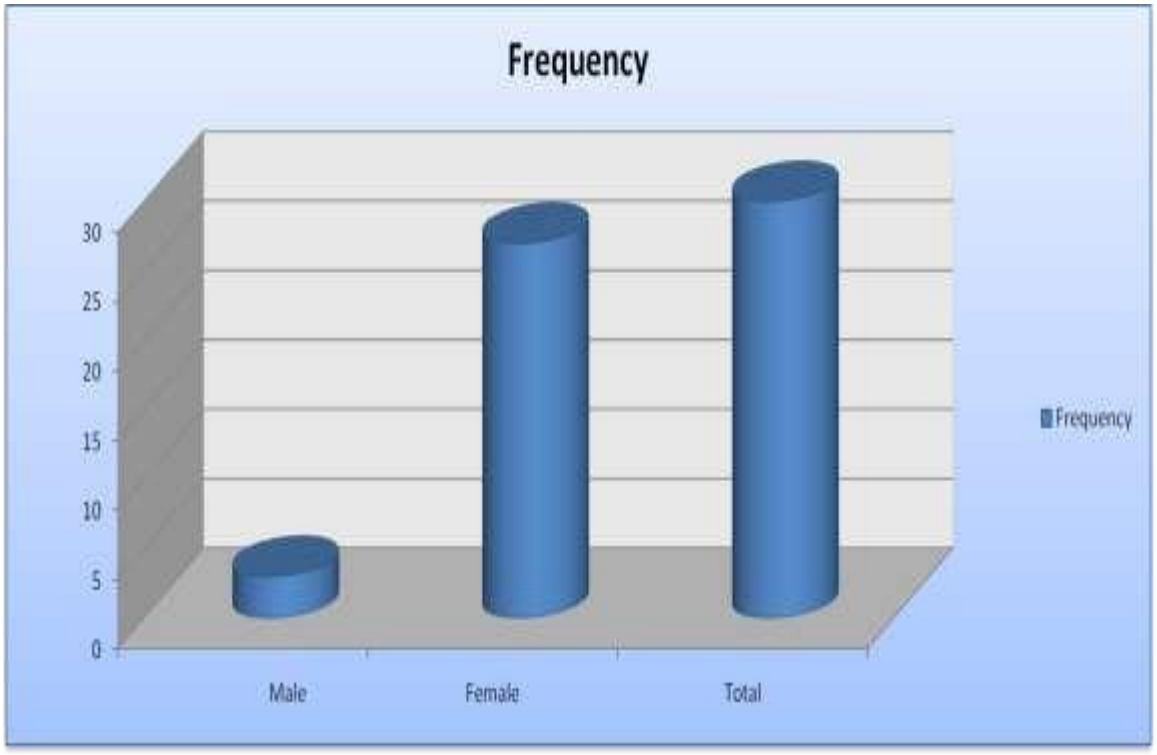
The present study was conducted to examine the mean values of various parameters i.e. Rheumatoid factor, Anticyclic-citrullinated peptide, Tender joint, Swollen joint, Global assessment scale, DAS 28, Neutrophil: Lymphocyte ratio, Platelet: Lymphocyte, ESR, CRP, and Red cell distribution width. The data obtained was subjected to statistical analysis using SPSS version 20.0. Descriptive statistics determining frequencies, percentage, mean values and standard deviations were calculated. Values were noticed for all the study subjects and mean values with standard deviation were calculated.

Table no. 1: Mean age of study subjects according to gender

Gender	Mean±SD
Male	34±8.023
Female	40±9.267
Total	39.40±8.593

Table no. 2: Gender distribution of study subjects

Gender	Frequency	Percent
Male	3	10.0
Female	27	90.0
Total	30	100.0

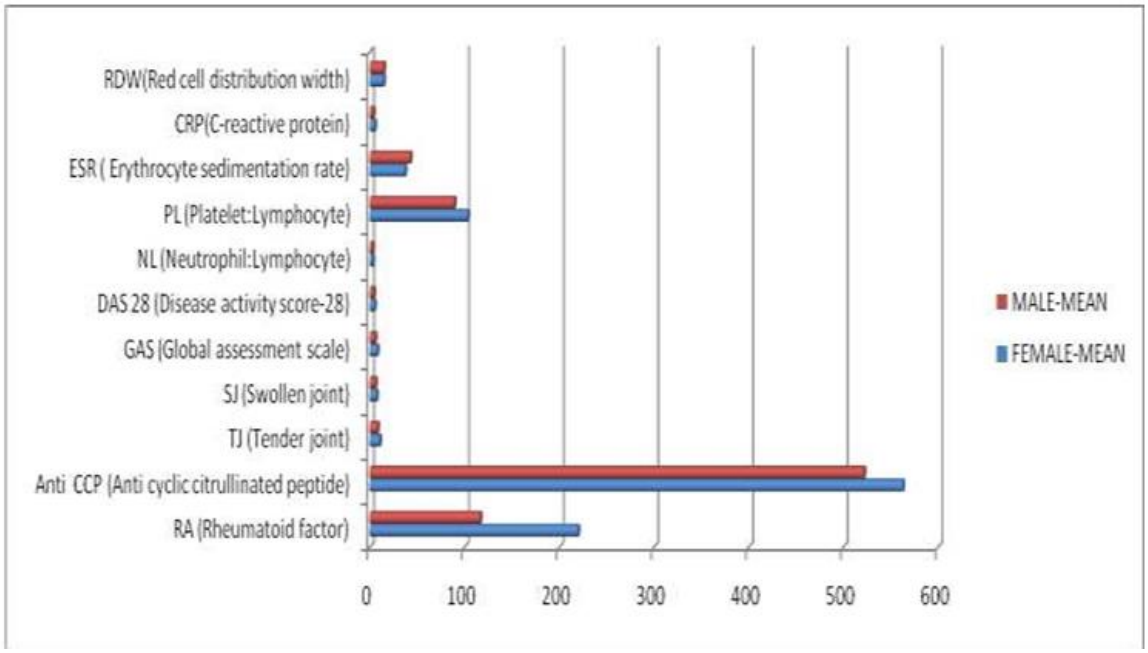


Graph no. 1: Gender distribution of study subjects

The study was conducted on a total of 30 study subjects, with 3 males and 27 females, having mean age of 39.40±8.593. The mean age of males and females was 34±8.023years and 40±9.267years respectively (**Table no. 1, 2 and Graph no. 1)**

Table no. 3: Mean values of various parameters for both the genders at baseline

Parameters	Mean±SD			
	FEMALE		MALE	
	MEAN	SD	MEAN	SD
RA (Rheumatoid factor)	220.1185	217.47925	116.6667	111.18153
Anti CCP (Anti cyclic citrullinated peptide)	562.9111	385.72038	522.0000	284.66823
TJ (Tender joint)	10.5926	7.40717	8.3333	3.51188
SJ (Swollen joint)	7.2963	6.46776	6.0000	2.00000
GAS (Global assessment scale)	8.0370	1.89090	5.6667	1.15470
DAS 28 (Disease activity score-28)	5.0048	1.13298	4.3667	.43016
NL (Neutrophil:Lymphocyte)	3.3656	1.32711	3.1667	.94241
PL (Platelet:Lymphocyte)	103.7211	36.13513	89.4367	8.25200
ESR (Erythrocyte sedimentation rate)	37.4815	13.52880	43.0000	31.95309
CRP(C-reactive protein)	5.4544	6.36368	3.8633	.75036
RDW(Red cell distribution width)	14.7148	1.14613	14.9667	1.00167



Graph no. 2: Mean values of various parameters for both the genders

Mean value of Rheumatoid factor was higher in females (220.1185±217.47925) than in males (116.6667±111.18153). Mean values of Anti cyclic citrullinated peptide were calculated for males (522.820±284.66)and females (562.9111±385.72038). In males, mean values for tender and swollen joints were observed to be 8.33±5.11 and 6.00±2.00 respectively. Whereas in females, mean values for tender and swollen joints were noticed to be higher than in males 10.59±7.407 and 7.29±6.46 respectively. Mean of Global assessment scale was observed to be 5.66±1.155 in males and 1.03±1.89 in females. The mean DAS 28 (Disease activity score-28) in males was calculated to be 4.36±.43, whereas in females it was 5.00±1.132. Ratio of neutrophills and platelets with lymphocytes was calculated. Neutrophil:Lymphocyte ratio was 3.16±0.942 in males and 3.36±1.32 in females. Mean values for Platelet:Lymphocyte ratio was calculated as 89.43±8.252 in males and 103.72±36.135 in females. Mean Erythrocyte sedimentation rate was found to be less in females (37.48±13.528) than in males (43.00±31.953). Mean value of CRP(C-reactive protein) was 3.86±0.75 in males and 5.457±6.36 in females. RDW (Red cell distribution width) was observed to be more in males (14.960±1.00) than in females (14.710±1.146), (**Table no. 3, Graph no. 2**). Results of present study revealed that mean values for all the parameters were more in females than in males, except for parameter ESR and RDW.

Table no. 4: Intergroup comparison between both the genders at baseline using paired sample t-test

MALE VS FEMALES (AT BASELINE)		Paired Differences					t	df	Sig. (2-tailed)*
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	RA	3.66667	13.79613	7.96520	-30.60483	37.93817	.460	2	.690
Pair 2	Anti CCP	313.20000	595.66276	343.90605	-1166.50832	1792.90832	.911	2	.459
Pair 3	TJ (Tender joint)	8.00000	7.00000	4.04145	-9.38896	25.38896	1.979	2	.186
Pair 4	SJ (Swollen joint)	7.00000	11.53256	6.65833	-21.64847	35.64847	1.051	2	.403
Pair 5	GAS	2.33333	3.05505	1.76383	-5.25583	9.92250	1.323	2	.317
Pair 6	DAS 28	1.4633	1.196	.69052	-	4.43438	2.119	2	.168

		3	01		1.50771				
Pair 7	NL	-1.25667	1.61939	.93496	-5.27946	2.76613	-1.344	2	.311
Pair 8	PL	-15.79333	20.27548	11.70606	-66.16043	34.57376	-1.349	2	.310
Pair 9	ESR	2.33333	21.57159	12.45436	-51.25346	55.92012	.187	2	.869
Pair 10	CRP	4.77000	10.97049	6.33382	-22.48221	32.02221	.753	2	.530
Pair 11	RDW	.00000	.75498	.43589	-1.87548	1.87548	.000	2	1.000

*p-value>0.05 is insignificant

Intergroup comparison was done between both the genders for all study parameters at baseline using paired sample t-test. It was observed that comparisons for all the parameters between males and females were insignificant (p-value>0.05), (**Table no. 4**).

Table no. 5: Mean values of all parameters for baseline and follow-up

Paired Samples Statistics					
		Mean	**N**	**Std. Deviation**	**Std. Error Mean**
RA	BASELINE	209.7733	30	210.36477	38.40718
	AT 2 months	139.2500	30	164.51830	30.03679
ANTI-CCP	BASELINE	558.8200	30	373.00627	68.10132
	AT 2 months	343.7833	30	265.45704	48.46560
TJ	BASELINE	10.3667	30	7.10747	1.29764
	AT 2 months	1.8667	30	1.94286	.35472
SJ	BASELINE	7.1667	30	6.15928	1.12453
	AT 2 months	1.6667	30	1.86313	.34016
GAS	BASELINE	7.8000	30	1.95466	.35687
	AT 2 months	1.1333	30	1.13664	.20752
DAS 28	BASELINE	4.9410	30	1.09614	.20013
	AT 2 months	2.3367	30	.86982	.15881
NL	BASELINE	3.3457	30	1.28217	.23409
	AT 2 months	3.2690	30	1.25538	.22920
PL	BASELINE	102.2927	30	34.55957	6.30969
	AT 2 months	96.3175	30	29.36467	5.36123
ESR	BASELINE	38.0333	30	15.40596	2.81273
	AT 2 months	32.0333	30	15.63922	2.85532
CRP	BASELINE	5.2953	30	6.04828	1.10426
	AT 2 months	2.2857	30	4.57347	.83500
RDW	BASELINE	14.7400	30	1.11930	.20435
	AT 2 months	14.5400	30	.87675	.16007

Table no. 5 revealed values of all parameters at baseline and at 2months follow up. Values of RA was decreased at 2months (139.25) from baseline value (209.77). Anti-CCP was decreased at 2months (343.78) from baseline value (558.82). Tender joint values were more at baseline (10.36) than at 2months (1.86). Swollen joint values were also observed to decrease at 2months (1.66) from baseline (7.16). GAS values were more at baseline (7.80) than 2months (1.13). DAS28 value was 4.94 at baseline and 2.3367 at 2months. Neutrophil: leukocyte ratio was 3.34 at baseline and 3.26 at 2months. Platelet:leukocyte ratio was 102.29 at baseline and 96.31 at 2months. ESR was 38.03 at baseline and 32.03 at 2months. CRP values were 5.29 and 2.28 at baseline and at 2months respectively. RDW values at baseline and 2months were 14.74 and 14.54 respectively. Thus, values from our study revealed that values of all parameters decreased at 2months from baseline.

Table no. 6: Intragroup comparison between all parameters at baseline using paired sample t-test

BASELINE VS FOLLOW UP AT 2 months		Paired Differences					t	df	Sig. (2tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	RA	70.52333	115.26677	21.04474	27.48201	113.56465	3.351	29	.002*
Pair 2	Anti CCP	215.03667	217.66716	39.74040	133.75841	296.31492	5.411	29	.000*
Pair 3	TJ (Tender joint)	8.50000	6.83677	1.24822	5.94711	11.05289	6.810	29	.000*
Pair 4	SJ (Swollen joint)	5.50000	5.99856	1.09518	3.26010	7.73990	5.022	29	.000*
Pair 5	GAS	6.66667	2.15492	.39343	5.86201	7.47133	16.945	29	.000*
Pair 6	DAS 28	2.60433	1.13219	.20671	2.18157	3.02710	12.599	29	.000*
Pair 7	NL	.07667	.89411	.16324	-.25720	.41053	.470	29	.642**
Pair 8	PL	5.97520	24.96031	4.55711	-3.34513	15.29553	1.311	29	.200**
Pair 9	ESR	6.00000	9.47592	1.73006	2.46163	9.53837	3.468	29	.002*
Pair 10	CRP	3.00967	3.36892	.61508	1.75169	4.26764	4.893	29	.000*
Pair 11	RDW	.20000	1.07543	.19635	-.60157	.20157	1.019	29	.317**

*p-value<0.05 is significant; **p-value>0.05 is insignificant.

Table no. 6 revealed intragroup comparison between baseline and follow up for each parameter using paired sample t-test. It was observed that for all parameters except NL, PL and RDW, a significant difference (p-value<0.05) was observed between baseline and 2months. Thus it can be concluded that after 2months of time period, a significant difference (p-value<0.05) was observed in readings of all parameters except NL, PL and RDW.

Table no. 7: Intergroup comparison between all study parameters at BASELINE

		RA	CCP	GAS	DAS1	NL	PL	ESR	CRP	RDW
RA	Pearson Correlation	1	.122	.373*	.376*	.255	.177	-.046	-.046	.066
	Sig. (2-tailed)		.519	.043	.041	.174	.350	.811	.809	.730
	N	30	30	30	30	30	30	30	30	30
CCP	Pearson Correlation	.122	1	.640**	.472**	-.021	.167	.019	-.053	.121
	Sig. (2-tailed)	.519		.000	.008	.911	.377	.920	.780	.523
	N	30	30	30	30	30	30	30	30	30
GAS	Pearson Correlation	.373*	.640**	1	.625**	.037	.256	.024	.023	-.042
	Sig. (2-tailed)	.043	.000		.000	.847	.172	.899	.905	.826
	N	30	30	30	30	30	30	30	30	30
DAS 1	Pearson Correlation	.376*	.472**	.625**	1	.007	.271	-.049	.196	.011
	Sig. (2- tailed)	.041	.008	.000		.969	.148	.799	.300	.953
	N	30	30	30	30	30	30	30	30	30
NL	Pearson Correlation	.255	-.021	.037	.007	1	.497**	-.219	.041	-.182
	Sig. (2-tailed)	.174	.911	.847	.969		.005	.246	.830	.336
	N	30	30	30	30	30	30	30	30	30
PL	Pearson Correlation	.177	.167	.256	.271	.497**	1	-.051	.458*	.023

	Sig. (2-tailed)	.350	.377	.172	.148	.005		.788	.011	.902
	N	30	30	30	30	30	30	30	30	30
ESR	Pearson Correlation	-.046	.019	.024	-.049	-.219	-.051	1	.442*	.159
	Sig. (2-tailed)	.811	.920	.899	.799	.246	.788		.015	.402
	N	30	30	30	30	30	30	30	30	30
CRP	Pearson Correlation	-.046	-.053	.023	.196	.041	.458*	.442*	1	.173
	Sig. (2-tailed)	.809	.780	.905	.300	.830	.011	.015		.361
	N	30	30	30	30	30	30	30	30	30
RDW	Pearson Correlation	.066	.121	-.042	.011	-.182	.023	.159	.173	1
	Sig. (2-tailed)	.730	.523	.826	.953	.336	.902	.402	.361	
	N	30	30	30	30	30	30	30	30	30

Table no. 7revealed intergroup comparison between all study parameters at baseline using Pearson correlation statistical analysis. It was observed that DAS28 was positively correlated with all study parameters except for ESR. It was observed that all parameters were insignificantly correlated with DAS 28 at baseline except for RA, CCP and GAS. Few other parameters were also found to be in significant relation with each other (RA vs GAS; CCP vs GAS; PL vs NL and CRP; and ESR vs CRP)

Table no. 8: Intergroup comparison between all study parameters at 2 months

		RA	**CCP**	**GAS**	**DAS**	**NL**	**PL**	**ESR**	**CRP**	**RDW**
RA	Pearson Correlation	1	.095	.580**	.557**	.291	.427*	-.232	-.060	.327
	Sig. (2-tailed)		.618	.001	.001	.119	.019	.217	.754	.078
	N	30	30	30	30	30	30	30	30	30
CCP	Pearson Correlation	.095	1	.051	.161	-.095	.086	-.086	-.117	.090
	Sig. (2-tailed)	.618		.788	.395	.616	.651	.650	.537	.636
	N	30	30	30	30	30	30	30	30	30
GAS	Pearson Correlation	.580**	.051	1	.548**	.206	.349	.196	.060	.590**
	Sig. (2-tailed)	.001	.788		.002	.275	.059	.300	.753	.001
	N	30	30	30	30	30	30	30	30	30
DAS1	Pearson Correlation	.557**	.161	.548**	1	.003	.292	.167	.541**	.461*
	Sig. (2-tailed)	.001	.395	.002		.987	.117	.377	.002	.010
	N	30	30	30	30	30	30	30	30	30
NL	Pearson Correlation	.291	-.095	.206	.003	1	.623**	-.311	-.146	-.244
	Sig. (2-tailed)	.119	.616	.275	.987		.000	.094	.440	.194
	N	30	30	30	30	30	30	30	30	30
PL	Pearson Correlation	.427*	.086	.349	.292	.623**	1	-.039	.194	-.058
	Sig. (2-tailed)	.019	.651	.059	.117	.000		.839	.305	.760
	N	30	30	30	30	30	30	30	30	30
ESR	Pearson Correlation	-.232	-.086	.196	.167	-.311	-.039	1	.512**	.295
	Sig. (2-tailed)	.217	.650	.300	.377	.094	.839		.004	.113
	N	30	30	30	30	30	30	30	30	30
CRP	Pearson Correlation	-.060	-.117	.060	.541**	-.146	.194	.512**	1	.134
	Sig. (2-tailed)	.754	.537	.753	.002	.440	.305	.004		.479
	N	30	30	30	30	30	30	30	30	30
RDW	Pearson Correlation	.327	.090	.590**	.461*	-.244	-.058	.295	.134	1
	Sig. (2-tailed)	.078	.636	.001	.010	.194	.760	.113	.479	
	N	30	30	30	30	30	30	30	30	30

Table no. 8 revealed intergroup comparison between all study parameters at 2months using Pearson correlation statistical analysis. It was observed that DAS28 was positively correlated with all study parameters. It was observed that all parameters were insignificantly correlated with DAS 28 at 2months except for RA, GAS, CRP and RDW. Few other parameters were also found to be in significant relation with each other (RA vs GAS and PL; RDW vs GAS; PL vs NL; and ESR vs CRP).

Table no. 9: Intergroup comparison between all study parameters for mean difference between 2months and baseline

		RA	CCP	GAS	DAS	NL	PL	ESR	CRP	RDW
RA	Pearson Correlation	1	.329	-.511**	-.411*	-.719**	.401*	.463**	-.724**	.710**
	Sig. (2-tailed)		.076	.004	.024	.000	.028	.010	.000	.000
	N	30	30	30	30	30	30	30	30	30
CCP	Pearson Correlation	.329	1	-.015	.149	-.240	.170	.225	-.163	.312
	Sig. (2-tailed)	.076		.937	.432	.201	.370	.232	.390	.093
	N	30	30	30	30	30	30	30	30	30
GAS	Pearson Correlation	-.511**	-.015	1	.826**	.826**	-.319	-.263	.732**	-.789**
	Sig. (2-tailed)	.004	.937		.000	.000	.086	.160	.000	.000
	N	30	30	30	30	30	30	30	30	30
DAS	Pearson Correlation	-.411*	.149	.826**	1	.644**	.371*	.245	.557**	.583**
	Sig. (2-tailed)	.024	.432	.000		.000	.043	.192	.001	.001
	N	30	30	30	30	30	30	30	30	30
NL	Pearson Correlation	-.719**	-.240	.826**	.644**	1	-.435*	-.568**	.831**	-.975**
	Sig. (2-tailed)	.000	.201	.000	.000		.016	.001	.000	.000
	N	30	30	30	30	30	30	30	30	30
PL	Pearson Correlation	.401*	.170	-.319	-.371*	-.435*	1	.109	-.168	.466**
	Sig. (2-tailed)	.028	.370	.086	.043	.016		.565	.376	.010
	N	30	30	30	30	30	30	30	30	30
ESR	Pearson Correlation	.463**	.225	-.263	-.245	-.568**	.109	1	-.389*	.518**
	Sig. (2-tailed)	.010	.232	.160	.192	.001	.565		.034	.003
	N	30	30	30	30	30	30	30	30	30
CRP	Pearson Correlation	-.724**	-.163	.732**	.557**	.831**	-.168	-.389*	1	-.779**
	Sig. (2-tailed)	.000	.390	.000	.001	.000	.376	.034		.000
	N	30	30	30	30	30	30	30	30	30
RDW	Pearson Correlation	.710**	.312	-.79**	-.583**	-.975**	.466**	.518**	-.779**	1
	Sig. (2-tailed)	.000	.093	.000	.001	.000	.010	.003	.000	
	N	30	30	30	30	30	30	30	30	30

Table no. 9 revealed intergroup comparison between all study parameters for mean difference between time interval using Pearson correlation statistical analysis. It was observed that all parameters were insignificantly correlated with DAS 28 except for RA, GAS, NL, PL,CRP and RDW. Few other parameters were also found to be in significant relation with each other (RA vs GAS,DAS,NL, PL,ESR, CRP and RDW; GAS vs RA, NL, CRP, RDW; NL vs RA, GAS, PL, ESR, CRP and RDW; PL vs RA, NL and RDW).

DISCUSSION

The present study was conducted on 30 subjects (Total=36, 6 patients lost to follow up) suffering with Rheumatoid Arthritis in Sir Sunderlal hospital, BHU, Varanasi. We studied Neutrophil-Lymphocyte Ratio, Platelet:Lymphocyte Ratio and RDW as surrogate markers for underlying inflammation in patients being treated with Methotrexate. Patients were subjected to routine examination and laboratory investigations like CBC with manual platelet count, qCRP, RA Factor (q), Anti-CCP Ab and DAS-28 and after confirmation of RA, the subjects were started on Methotrexate. The present study determined the mean values of and changes in various parameters i.e Rheumatoid factor, Anti cyclic citrullinated peptide, Tender joint, Swollen joint, Global assessment scale, DAS 28, Neutrophil:Lymphocyte ratio, Platelet:Lymphocyte, ESR, CRP, and Red cell distribution width over a period of 8 weeks.

The study comprised of 3 males and 27 females with mean age of 39.40±8.593. Majority of subjects were of 31-40 years old. Similar to our study, Abd-Elazeem MI *et al*.[1] also observed that mean age of patients with RA was 40.7 ± 10.1 years. Study by Jung JY *et al*.,[2]and Lin F et al.[3], also observed that females were more affected with RA than males, similar to our study. It has been observed for rheumatoid arthritis (RA), which the sex ratio is typically around 3:1.[4]

In our study, the mean value of Rheumatoid factor was found to be higherin females (220.1185±217.47925) than in males (116.67±111.18).Mean values of Anti cyclic citrullinated peptide was found to be more in females than males. In females, mean values for tender and swollen joints were observed to be more (10.59±7.407 and 7.29±6.46 respectively) than in males. The mean values were found to be in similar range as observed in study by Jung JY *et al*.[2]Mean of Global assessment scale was observed to be more in females (8.037±1.891) than males (5.66±1.155). The mean DAS 28 (Disease activity score-28) in females (5.00±1.132) was calculated to be more than males. Similar findings were observed in study by Bhatnagar M et al.[5] Ratio of neutrophils to lymphocytes (3.36±1.32) and Platelet to Lymphocyte ratio(103.72±36.135) was more in females than in males. Results of our study were in accordance with study conducted by Peng YF et al. and Jung JY *et al*. Mean Erythrocyte sedimentation rate was found to be more in males (43.00±31.953), whereas CRP(C-reactive protein) was found to be more in females (5.457±6.36). RDW (Red cell distribution width) was observed to be more in males (14.960±1.00) than in females. The range of mean values of various parameters in RA patients were similar to as observed in studies by Sargin G et al.[6] and Gasparyan AY et al.[7]Results of present study revealed that mean values for all the parameters were more in females than in males, except for parameter ESR and RDW.

The present study showed that all parameters decrease after MTX therapy at 2months from baseline. Methotrexate (MTX) is currently the most frequently used and first line DMARD in the treatment of rheumatoid arthritis (RA)[8]. In a systematic comparison, the traditional DMARDs have recently been compared regarding efficacy and safety (Aletaha*et al.*2002).[9] MTX was the most commonly administered drug in patients with RA, followed by chloroquine.

The present study showed that there was a statistically significant difference between NLR and PLR before and after 8 weeks of treatment with methotrexate. A positive correlation was observed between various parameters (except ESR) with DAS 28-CRP. Results were in accordance to study conducted by Fawzy RM et al who also reported a positive and significant correlation between various parameters and DAS-28 score ($p<0.005$). Mercan et al and Tekeglu et al[10] also reported a significant correlation between NLR and DAS-28 score. Ghang B et al[11] and Koiwa et al[12]while studying RA patients on biological DMARDS also found that NLR was significantly elevated in RA patients during flares and decreased with treatment concluding that NLR was a more reliable criterion than ESR or CRP to assess disease activity. In India, Chandrashekhar et al1[13] found that NLR is useful in prediction of sustained remission in RA disease along with patients' perception of pain. Uslu et al[14] concluded that there was a significant correlation between NLR and DAS-28 ($r=0.345$, $p=/<0.0001$). These findings reflect the immune alterations. Neutrophil infiltration of synovial tissue with increased levels of inflammatory cytokines like TNF-alpha, IL-1, IL-6, oxidative damage, premature thymic aging and dysfunction, and lymphopenia with presence of abnormal senescent peripheral T cells due to selective destruction of some T cell subsets and reactive thrombocytosis due to inflammation induced anemia and increased erythropoietin levels and neutrophil induced platelet activation are recognized features of RA. Therefore, neutrophilia, lymphopenia and thrombocytosis are known immune alterations in RA which may be reflected in altered relative proportions of these cells in the peripheral blood.Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio have come increasingly in focus as indicators of subclinical systemic inflammation which can be used to assess disease activity and prognosticate for a variety of infective, inflammatory, neoplastic and autoimmune conditions including RA.

Our study used neutrophil-lymphocyte ratio and platelet-lymphocyte ratio as markers of RA disease activity, similar to study Uslu AU et al.[14], who also utilized similar biomarkers as RA indicators. However, the study did not take into account the effect of DMARDs on these ratios and changes in the ratio with longitudinal follow up in correlation with disease activity. Methotrexate, the most commonly used DMARDs, has hematologic changes of its own

and our study aimed to observe if the NLR and PLR hold as markers of disease severity when methotrexate is used concomitantly on longitudinal follow up.

In our study, the mean change in DAS28-CRP was significantly correlated to the mean change of NLR ($p<0.001$), PLR ($p=0.043$) and RDW ($p=0.001$) over a period of 2-month with concomitant methotrexate therapy.

NLR being a dynamic entity, has various factors which cause inter-individual (Age, Heritability, smoking, BMI etc) as well as intra-individual (Temperature, season, etc)variations. RDW is also changed in the presence of any abnormalities such as thyroid disease, renal or hepatic dysfunction, medications, nutritional deficiency (*i.e.* iron, vitamin B_{12}, and folic acid) genetic factors, gender differences and the ethnicity. Considering these factors, it might be difficult to ascertain an absolute value of NLR, PLR and RDW for grading the severity of inflammation. A more practical approach may be to assess the improvement of inflammation by assessing the change in the NLR, PLR and RDW. By this approach the inter-individual differences can be minimized, if not completely eliminated i.e. for an individual patient, assessing the change in NLR, PLR and RDW, over a period of time will eliminate the confounding effects of age, sex, heritability, BMI, smoking etc if no other intervention is carried out for the concerned variables.

There is a particular concern about the independent alteration of NLR, PLR and RDW due to administration of methotrexate as a part of its haematological effects independent of the degree of control of inflammation by the drug. The mechanism of action of methotrexate to reduce inflammation in rheumatoid arthritis as Disease modifying agent is subject to various controversies. Currently, adenosine signalling is probably the most widely accepted explanation for the methotrexate mechanism in RA given that methotrexate increases adenosine levels and on engagement of adenosine with its extracellular receptors, an intracellular cascade is activated promoting an overall anti-inflammatory state, which interferes with neutrophil adhesion and activation, rather than decreasing its number. At the same time, methotrexate induces depletion of lymphocyte subsets by inducing apoptosis of T-cells. This is consistent with the observations of reductions in peripheral blood T and B lymphocyte populations after short-term methotrexate treatment. This mechanism was expected to raise the NLR and PLR independent of the reduction of degree of inflammation and thus interfere with the results of the study. But after a follow up period of 2 months on methotrexate, significant reduction in the NLR and PLR was seen which was in correlation with the degree of reduction of DAS28-CRP. This observation further leaves the discussion open for additional or alternative mechanism of action of methotrexate as a disease modifying agent in methotrexate.

CONCLUSION

This study found that DAS 28, CRP, RDW, PLR, NLR, RA, ESR are emerging inflammatory biomarkers which could be used to evaluate disease activity in active RA patients. Studies employing larger sample size are required to establish whether DAS28 could be used routinely to diagnose and monitor patients of rheumatoid arthritis especially with the increasing use of biological and non-biological DMARDS in these patients. The design of future studies should take into account confounding effects of numerous clinical, drug-related, and pre-analytical factors that affect the variability of various biomarkers. Prospective enrolment of subjects in these studies and standardized measurements of all laboratory parameters at a single time point are advisable. Race, age and sex of the subjects, among many other confounders, should be considered when interpreting results.

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