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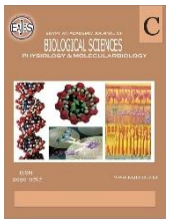

Indicators of Biochemistry and Hematology in Patients with Rheumatoid Arthritis Carrying the *HLA-DRB1*04 or HLA-DRB1*10* Gene Variants

Khalid E. K. Kheiralla[1,2*]

[1]Department of Basic Medical Sciences, Faculty of Applied Medical Sciences, Al Baha University, Al Baha, Saudi Arabia.

[2]Department of Biochemistry and Nutrition, Faculty of Medicine, University of Gezira, Wad Medani, Sudan.

***E-mail:** khatahir12@gmail.com

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ABSTRACT

Background: *HLA-DRB1* gene variation, especially *DRB1*04* and *DRB1*10*, was found to be associated with RA risk, and it may be involved in the alteration of biochemical and hematological indicators. RA patients with *HLA-DRB1*04* or **10* gene variants were evaluated for the aforesaid indicators. **Methods:** The biochemical and hematological indicators of 100 healthy controls and 120 RA patients with *HLA-DRB1*04* or *HLA-DRB1*10* gene variants were examined. The genotyping variants of all HLA genes were determined using PCR (specific primer). CBC, CRP, and ESR were measured using an automated analyzer and ACPA by ELISA. **Results:** RA patients have greater rates of *DRB1*04* and **10* gene variants than healthy controls (9.6% vs. 5.1%, $P = 0.038$ and 14.2% vs. 8.2%, $P = 0.042$). Compared with the healthy controls, PLT, LY, NE, MO, NLR, PLR, CRP, and ESR were remarkably higher in RA patients or those with *DRB1*04* or **10* gene variants ($P = 0.031$, < 0.001, 0.045, 0.002, 0.018, 0.011, < 0.001, and < 0.001). In contrast, RBC, Hb, MPV, and BA levels dropped ($P = 0.010$, 0.048, 0.021, and 0.039) in the patients. RA patients with the *HLA-DRB1*04* or **10* gene variants have considerably higher levels of LY, NE, NLR, PLR, CRP, and ESR than healthy controls ($P = < 0.001$, < 0.001, 0.036, 0.004, < 0.001, and < 0.001). In contrast, RBC, Hb, and BA decreased significantly ($P < 0.001$). **Conclusion:** The *HLA-DRB1*04* or **10* gene variants were associated with RA risk and contributed to the population's biochemical and hematological alterations.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that affects many parts of the body. The RA etiology remains enigmatic; nevertheless, genetic and environmental factors critically influence its pathogenesis. It has a complicated genetic background, especially when it comes to human leukocyte antigen (HLA) (Coenen *et al.,* 2009). RA is linked to the class II MHC complex, particularly *HLA-DRB1* alleles such as *HLA-DRB1*04* and *HLA-DRB1*10* among various ethnic groups (Van De Putte *et al.,* 1998; Xue *et al.,* 2022; Klimenta *et al.,* 2020; Chen *et al.,* 2020; Klimenta *et al.,* 2019). This showed the impact of genetic factors on the development of RA. Because of the inflammatory and systemic nature of RA, most affected patients have a pattern of progressive disease activity that varies in intensity over time.

There is widespread consensus in clinical practice that, to ensure patient safety, rheumatoid inflammation should be thoroughly and promptly managed and should also be maintained for as long as feasible (Van De Putte *et al.,* 1998). Quantitative assessment of disease activity should be a systematic and routine part of managing RA. There are lab tests that can be used along with clinical practice and X-rays to help doctors better figure out if someone has RA. Among these tests are biochemical ones like anti-cyclic citrullinated peptide autoantibodies (ACPA), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF). The hematological ones are red blood cells (RBC), hemoglobin (Hb), and hematocrit (HT). This is because these indicators (tests) are markedly altered during inflammation in RA. Many authors (Xue *et al.,* 2022; Klimenta *et al.,* 2020; Chen *et al.,* 2020; Klimenta *et al.,* 2019; Talukdar et al., 2017) have reported that the hematological and biochemical indices are associated with the activity of the RA disease. Different types of *HLA -DRB1* polymorphisms, such as *HLA-DRB1*01*,**04*, and **10*, are connected to disease severity in ACPA-positive individuals (Ali *et al.,* 2023; Bax *et al.,* 2011; de Vries *et al.,* 2011). Recent data shows that some hematological and biochemical indicators were significantly high in RA patients carrying the *HLA-DRB1*04* or *HLA-DRB1*03* gene variants (Klimenta *et al.,* 2020). Looking into the link between *HLA-DRB1* gene variants and changes in the hematological and biochemical profile of our RA population would help us learn more about the cause of RA and the best way to treat it.

MATERIALS AND METHODS

Study Participants:

This cross-sectional hospital-based study was carried out at two tertiary hospitals (Academy Teaching Hospital and Ibrahim Malik) in Khartoum State, Sudan. One hundred twenty RA patients were diagnosed by board-certified rheumatologists following the 2010 classification criteria of the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) (Aletaha *et al.,* 2010). All RA patients who have had or are currently having any of the following conditions were not included in the study: autoimmune inflammatory diseases (such as systemic lupus erythematosus, psoriasis, or vasculitis); chronic diseases (such as diabetes mellitus, hypertension, dyslipidemia, thyroid dysfunction, or chronic renal failure); hematologic diseases; infections; cancers; aplastic anemia; and people who are taking steroids, anti-platelets, or anticoagulant drugs. This is because the aforementioned conditions, systemic disorders, and their treatment regimens may affect the hematological and biochemical indicators relevant to our study, regardless of the degree of RA disease activity. Age, sex, clinical details, and laboratory profiles are among the information gathered in the clinical interview form (Table 1). After clinical and laboratory testing, one hundred age- and sex-matched healthy controls were selected from other hospital departments. After informing study participants of the goals and aims of the research, written consent was obtained. The University of Gezira's research ethics committee gave the study approval (Ref. NO UGIREC/2021), and it was carried out under the Helsinki Declaration.

Blood Sampling and Laboratory Tests:

Ten milliliters (10 mL) of peripheral blood were taken from study participants to measure biochemical (inflammatory) indicators such as ACPA, CRP, and ESR and to do hematological tests like a complete blood count (CBC) and differential blood count (DBC). The CBC tests included the counts of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), platelets (PLT), and hematocrit (HCT). Other erythrocyte constants were assessed, including the means of cell hemoglobin (MCH), cell volume (MCV), and cell hemoglobin concentration (MCHC). The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were also

calculated. The percentages of lymphocytes (LY), neutrophils (NE), monocytes (MO), basophils (BA), and eosinophils (EO) were all included in the DBC. Three milliliters (3 mL) of blood were collected into a vacuum tube without the use of an anticoagulant to estimate ACPA and CRP. A vacuum tube containing the anticoagulant sodium citrate was used to collect 1.8 ml of blood for the erythrocyte sedimentation rate (ESR). Three milliliters (3 mL) of blood were mixed with the anticoagulant ethylenediaminetetraacetic acid (EDTA) in a vacuum tube for the hematological tests.

***HLA-DRB1* Genotyping:**

Genomic DNA was isolated from the whole blood using the QIA DNA kit (Qiagen, USA). To find out the genotype of *HLA class II DRB1*, the R.O.S.E. Europe GmbH (Steinbach/Taunus, Germany) provided the low-resolution kits along with sequence-specific primers (SSP) and polymerase chain reaction (PCR). The same company supplied HLA software for the HLA-DRB1 typing. An Applied Biosystem 9700 thermocycler was used to amplify DNA per the company's (ThermoFisher, UK) instructions. The DNA fragments that had been amplified were separated by electrophoresis using a 2% agarose gel stained with ethidium bromide and then shown in a gel documentation system under ultraviolet light.

Biochemical and Hematological Measurements:

Tests for antibodies against citrullinated proteins (ACPA) were done using Euro Diagnostic ELISA kits (Euro Diagnostic, Sweden). A positive concentration of ACPA was defined as more than 25 U/mL. Serum RF specific to IgM was measured using a rapid latex agglutination test (NS Bio-Tec, Egypt). Serum CRP levels were quantified using a Roche/Hitachi Cobas Mira chemistry analyzer (Basel, Switzerland), and the range between 5 and 20 IU/mL was considered normal. The usual Westergren method was applied to quantify ESR (Hashemi *et al.*, 2015). The CBC tests were measured using a fully automated hematological analyzer (Sysmex XP-300, Japan) per the manufacturer's instructions.

Statistical Analysis:

The SPSS 23.0 Mac OS program was used to analyze the data statistically. The frequencies and percentages were expressed for the categorical variables; the continuous variables were presented as mean±standard error of the mean. A chi-square (x^2) test was used to estimate the significance of the frequency of the *HLA-DRB1* gene variants between the study subjects. A student's *t*-test and one-way ANOVA test were used to estimate the significant differences in hematological and biochemical indicators between patient groups and healthy controls. The strength of the associations was estimated using odds ratios (ORs), and 95% confidence intervals and *P* values lower than 0.05 indicate a significant difference.

RESULTS

Study Patients:

The patient's laboratory results and in-hospital clinical characteristics are listed in Table 1. Following RF (28%), ACPA and CRP were positive in 62% of RA patients. The clinical picture of RA shows that morning stiffness accounts for 81.0% of cases. Among the patients, the most impacted joints were the wrists (90%), ankles (86%), and knees (85%), and the most common deformity was ulnar deviation (30%).

***DRB1* Allele Frequency and Biochemical and Hematological Indices**:

RA patients had significantly higher frequencies of *HLA-DRB1*04* and **10* gene variants compared to healthy controls (9.6% vs. 5.1%, $P = 0.038$ and 14.2% vs. 8.2%, $P = 0.042$), respectively. On the other hand, the frequency of *HLA-DRB1*07* was substantially higher ($P = 0.010$) in healthy controls (11.7%) compared to the patients (5.0%) (Table 2). The biochemical CRP and ESR levels in the RA patient groups were significantly higher than those in the healthy controls ($P < 0.001$). The hematological PLT ($P = 0.031$), LY ($P < 0.001$), NE ($P = 0.04$), MO ($P = 0.002$), NLR ($P = 0.018$), and PLR ($P = 0.011$) levels were also significantly higher than those in the healthy controls. On

the other hand, the patient's hematological tests revealed significantly low levels of RBCs ($P = 0.010$), Hb ($P = 0.048$), MPV ($P = 0.021$), and BA ($P = 0.039$) (Table 3). The same tested values were kept significant when comparing the patients with healthy controls (Table 4). Patients with the *DRB1* 04* or **10* gene variants had significantly higher levels of LY ($P < 0.001$), NE ($P < 0.001$), NLR ($P = 0.036$), PLR ($P = 0.004$), CRP ($P < 0.001$), and ESR ($P < 0.001$) than healthy controls, while the MPV, MO, and EO values didn't reach the relevant levels. Conversely, in comparison, significantly low levels of RBC, Hb, and BA were also noted ($P < 0.001$) **(**Table 5).

Table 1. Clinical characteristics of patients and healthy controls.

Demographic and Lab Results		**Patients (n = 120) (mean±SEM)**		**Controls (n = 100) (mean±SEM)**
Age/years		44.82±1.29		45.65±1.81
Disease duration/years		2.19±0.24		NA
ACPA (U/ml)		603.80±81.47		NA
CRP (mg/l)		15.87±1.79		NA
ESR (mm/hr)		65.45±2.61		NA
		Yes (%)	**No (%)**	
Morning Stiffness		81	39	NA
ACPA$^+$		62	58	NA
RF$^+$		28	92	NA
CRP$^+$		62	58	NA
Joints effected by RA	**Fingers**	48	18	NA
	Hands	84	19	NA
	Wrists	90	12	NA
	Elbows	76	44	NA
	Shoulders	72	48	NA
	Neck	38	82	NA
	Back	31	89	NA
	Toes	62	58	NA
	Feet	77	43	NA
	Ankles	86	34	NA
	Knees	85	18	NA
	Hips	21	99	NA
Deformities	**Boutonniere Deformity**	16	104	NA
	Ulnar deviation	30	90	NA
	Swan-Neck Deformity	17	105	NA
	Z thumb	17	105	NA
	Presence of Rheumatic Nodules	8	114	NA

NA, not available; n, number; %, percentage; SEM, standard error of the mean

Table 2. *HLA-DRB1* allele frequencies in RA patients and healthy controls[a].

Allele HLA-DRB1	Healthy Controls n = 196		RA Patients n = 240		*P*-value
	n	AF	n	AF	
***01**	13	6.6	13	5.4	0.591
***03**	21	10.7	23	9.6	0.697
***04**	10	5.1	23	9.6	**0.038**
***07**	23	11.7	12	5.0	**0.010**
***08**	19	9.7	27	11.3	0.599
***09**	02	1.0	01	0.4	0.424
***10**	16	8.2	34	14.2	**0.042**
***11**	24	22.2	26	10.8	0.645
***13**	46	23.5	49	20.4	0.442
***14**	0	0	01	0.4	NA
***15**	21	10.7	30	12.5	0.564
***16**	01	0.5	01	0.4	0.698

NA, not available; AF, allele frequency; *n*, number of individuals, RA, rheumatoid arthritis. Significant associations are indicated in bold.

Table 3. Hematological and biochemical indices in healthy controls and RA patient groups.

Parameter	Controls (n = 100) (Mean±SEM)	RA Patients (n = 120) (Mean±SEM)	Patients with *HLA-DRB1*4* and **10* (n = 41) (Mean±SEM)	*P.* value
WBC (10^3/μl)	6.14±0.31	6.33±0.18	6.17±0.17	0.777
RBC (10^6/μl)	4.73±0.04	4.54±0.05	4.69±0.06	**0.010**
Hb (g/dl)	13.39±0.14	12.35±0.12	12.39±0.14	**0.048**
HCT (%)	40.44±0.34	39.48±0.45	40.38±0.58	0.185
MCV (fL)	87.10±0.64	85.68±0.62	86.32±0.82	0.271
MCH (pg)	27.71±0.58	26.63±0.25	27.12±0.33	0.166
MCHC (g/dl)	31.77±0.63	31.00±0.11	31.38±0.18	0.368
PLT (cell/cmm)	251.15±7.44	276.16±6.33	269.61±9.79	**0.031**
MPV (fL)	9.31±0.92	8.03±0.84	9.01±0.81	**0.021**
LY (%)	32.34±0.93	37.60±0.91	34.32±1.91	**< 0.001**
NE (%)	53.54±1.03	56.96±1.08	57.95±2.22	**0.045**
MO (%)	8.21±0.31	9.56±0.26	9.12±0.32	**0.002**
EO (%)	0.81±0.39	0.80±0.86	0.83±0.12	0.631
BA (%)	0.18±0.04	0.09±0.03	0.05±0.03	**0.039**
NLR	1.63±1.04	2.24±2.00	2.13±1.73	**0.018**
PLR	7.21±3.23	10.12±6.79	9.32±5.23	**0.011**
CRP (mg/L)	8.15±0.79	16.31± 1.83	17.98± 2.93	**< 0.001**
ESR (mm/hr)	28.59±0.97	66.02±2.62	70.90±4.83	**< 0.001**

SEM, Standard error of the mean; n, number; WBC, white blood cells; RBC, red blood cells; Hb, haemoglobin; HCT, Haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet count test; MPV, mean platelet volume; LY, lymphocytes; NE, neutrophils; MO, monocytes; EO, eosinophils; BA, basophils; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. Significant associations are indicated in bold.

Table 4. Hematological and biochemical indices in controls and RA patients.

Parameter	Controls (n=100) (Mean±SEM)	RA Patients (n=120) (Mean±SEM)	*P. value*	95% CI
WBC (10^3/µl)	6.14±0.13	6.33±0.18	0.523	-0.65-0.33
RBC (10^6/µl)	4.73±0.04	4.54±0.05	**0.003**	-0.06-0.32
Hb (g/dl)	13.39±0.14	12.35±0.12	**0.024**	-0.21-0.53
HCT (%)	40.44±0.34	39.48±0.45	0.085	-0.13-2.05
MCV (fL)	87.10±0.64	85.68±0.62	0.118	-3.19-0.36
MCH (pg)	27.71±0.58	26.63±0.25	0.076	-2.26-0.11
MCHC (g/dl)	31.77±0.63	31.00±0.11	0.191	-1.92-0.39
PLT (cell/cmm)	251.15±7.44	276.16±6.33	**0.011**	-5.89-44.14
MPV (fL)	9.31±0.92	8.03±0.84	**< 0.001**	-0.72-0.83
LY (%)	32.34±0.93	37.60±0.91	**< 0.001**	-2.66-7.85
NE (%)	53.54±1.03	56.96±1.08	**0.025**	0.43-6.40
MO (%)	8.21±0.31	9.56±0.26	**0.001**	0.55-2.14
EO (%)	0.81±0.39	0.80±0.86	**< 0.001**	-0.07-0.34
BA (%)	0.18±0.04	0.09±0.03	**0.048**	0.18-0.00
NLR	1.63±1.04	2.24±2.00	**0.006**	1.74-2.19
PLR	7.21±3.23	10.12±6.79	**< 0.001**	8.06-9.56
CRP (mg/L)	8.15±0.79	16.31± 1.83	**< 0.001**	-18.39-10.42
ESR (mm/hr)	28.59±0.97	66.02±2.62	**< 0.001**	-43.38-31.47

SEM, Standard error of the mean; n, number; CI, confidence intervals; WBC, white blood cells; RBC, red blood cells; Hb, haemoglobin; HCT, Haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet count test; MPV, mean platelet volume; LY, lymphocytes; NE, neutrophils; MO, monocytes; EO, eosinophils; BA, basophils; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. Significant associations are indicated in bold.

Table 5. Hematological and biochemical indices in healthy controls and RA patients carrying *HLA-DRB1*4* and **10* alleles.

Parameter	Controls (n = 100) (Mean±SEM)	Patients with *HLA-DRB1*4* and **10* (n = 41) (Mean±SEM)	*P.* value	95% CI
WBC (10^3/µl)	6.14±0.31	6.17±0.17	0.560	-0.89-0.50
RBC (10^6/µl)	4.73±0.04	4.69±0.06	**< 0.001**	-0.04-0.32
Hb (g/dl)	13.39±0.14	12.39±0.14	**< 0.001**	-0.22-0.78
HCT (%)	40.44±0.34	40.38±0.58	0.446	-0.68-2.49
MCV (fL)	87.10±0.64	86.32±0.82	0.493	-3.01-1.46
MCH (pg)	27.71±0.58	27.12±0.33	0.537	-2.46-1.29
MCHC (g/dl)	31.77±0.63	31.38±0.18	0.698	-2.36-1.58
PLT (cell/cmm)	251.15±7.44	269.61±9.79	0.164	-7.67-44.58
MPV (fL)	9.31±0.92	9.01±0.81	0.071	-0.81-0.97
LY (%)	32.34±0.93	34.32±1.91	**< 0.001**	-10.00-2.58
NE (%)	53.54±1.03	57.95±2.22	**< 0.001**	0.19-8.64
MO (%)	8.21±0.31	9.12±0.32	0.088	-0.14-1.96
EO (%)	0.81±0.39	0.83±0.12	0.748	-0.09-0.40
BA (%)	0.18±0.04	0.15±0.03	**< 0.001**	-0.26-0.00
NLR	1.63±1.04	2.13±1.73	**0.036**	1.71-2.13
PLR	7.21±3.23	9.32±5.23	**0.004**	8.12-8.63
CRP (mg/L)	8.15±0.79	17.98± 2.93	**< 0.001**	5.41-14.26
ESR (mm/hr)	28.59±0.97	70.90±4.83	**< 0.001**	35.52-49.10

SEM, Standard error of the mean; n, number; CI, confidence intervals; WBC, white blood cells; RBC, red blood cells; Hb, haemoglobin; HCT, Haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet count test; MPV, mean platelet volume; LY, lymphocytes; NE, neutrophils; MO, monocytes; EO, eosinophils; BA, basophils; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate. Significant associations are indicated in bold.

DISCUSSION

HLA-DRB1 gene variants are just one of the many factors that have an impact on RA susceptibility. The *HLA-DRB1* locus has a significant degree of genetic variation and poses a greater susceptibility to rheumatoid arthritis (RA) compared to any other genetic locus (Reynolds et al., 2014). Some types of HLA-DRB1 gene variants, like *HLA-DRB1*01*, **04*, and **10*, were linked to a higher risk of getting RA (Balandraud et al., 2013). In this research, we found that *HLA-DRB1*04* (P = 0.038) and *HLA-DRB1* 10* (P = 0.042) were the most common gene variants in our cases. This indicates that they were risk variants and likely to cause RA development. This result was consistent with previous studies (Naqi *et al.,* 2011; Begum *et al.*, 2018; Sandoughi *et al.,* 2011; Mourad and Monem, 2018; Al-Swailem *et al.,* 2006; Louzada-Júnior *et al.,* 2008; Hughes *et al.,* 2008; Liu *et al.,* 2007). Some *HLA-DRB1* gene variants, like *HLA-DRB1* 01*, **04*, and **10*, have been strongly linked to the structural severity of the disease. They are located in the third hypervariable region of the *HLA-DRB1* chain locus, between positions 70 and 74, on the short arm of chromosome 6. Because they all share the same amino acid sequence (RRRAA, QRRAA, or QKRAA) in the HLA class II beta chain's antigen-binding site, these regions are known as shared epitopes (SE). The SE are likely connected to the production of ACPA through their effect on antigen presentation (de Vries, 2011; Bax *et al.,* 2011). In line with previous studies (Xue *et al.,* 2022; Dechanuwong and Phuan-Udom, 2021; Klimenta *et al.,* 2020; Mykola *et al.,* 2015; Sokolovic *et al.,* 2014), our data showed significantly higher CRP and ESR in RA patients than in healthy controls. Their significantly high levels remained between the RA groups when compared with the healthy controls. These results indicate that CRP and ESR as sensitive markers may play a role in the poor prognosis of RA, and most likely they may be involved in the occurrence of uncontrolled inflammation (Wassuna *et al.,* 1990; Abbas *et al.,* 2015). For this reason, the measurements of these two markers could allow early and aggressive RA treatments (Kim *et al.,* 2015). In DBC, RA groups exhibited a notable increase in the values of PLT, LY, NE, MO, NLR, and PLR. Our result was consistent with Hochberg *et al.'s* (2015) finding that patients with active RA have elevated levels of neutrophils and thrombocytes in addition to MO, which suggests the activity of inflammatory processes (Klimenta *et al.,* 2020). Early studies showed the involvement of PLT in the inflammatory process of RA (Olumuyiwa-Akeredolu *et al.,* 2019; Xue *et al.,* 2022), which is consistent with our result. As chronic inflammatory markers, NLR and PLR values were found to be significantly higher in RA patients than in healthy controls (Chandrashekara *et al.,* 2017; Erre *et al.,* 2019). These values were also found to be independently correlated with disease activity (Uslu *et al.,* 2015; Fawzy *et al.,* 2017; Dechanuwong and Phuan-Udom, 2021) and can be used as a marker of RA presence (Tekeoğlu *et al.,* 2016). Patients with *HLA-DRB1*04* or *HLA-DRB1*03* gene variants, on the other hand, did not have NLR or PLR found (Klimenta *et al.,* 2020). Consistent with other research (Hochberg *et al.,* 2015; Klimenta *et al.,* 2020; Okoroiwu *et al.,* 2016; Xue *et al.,* 2022), our data showed that RBC, Hb, and MPV levels were much lower in the RA groups than in the healthy controls. In many studies, it has been reported that RA patients with anemia are more likely to have severe structural damage, less joint function, and high disease activity compared to controls (Xue *et al.,* 2022; Klimenta et al., 2020; Klimenta *et al.,* 2019; Chen *et al.,* 2020). Also, both the Egyptian study (Farouk *et al.,* 2023) and the Cameron study (Atabonkeng *et al.,* 2018) showed that the levels of RBCs, Hb, and MPV in RA patients were much lower than the controls. However, our results contradicted other research (Al-Timimi *et al.,* 2014) that found no appreciable differences in RBCs and Hb between patients and controls. The controversial results seen in the above

studies could be due to several reasons, including the severity of the disease, the medicines the patients were taking, bone marrow dysfunction, or insufficient erythropoiesis (Harrison, 2001). Our results were similar to those of earlier studies that found lower levels of MPV in people with rheumatoid arthritis and ankylosing spondylitis (Kisacik *et al.,* 2008; Dechanuwong and Phuan-Udom, 2021). Tekeoğlu *et al.'s* (2016) study suggests that a low MPV in disease-active RA patients could indicate an absence of acute-phase disease. In contrast, it was found that while the MPV value was significantly lower in RA patients than in controls (Talukdar *et al.,* 2017), it had not reached a statistically significant level (Klimenta *et al.,* 2020; Moghimi et al., 2017). Our results suggest that *HLA-DRB1*04* and **10* gene variants are more common in RA patients and are linked to hematological and biochemical indicators. To validate these findings, however, additional research is required. Furthermore, the demonstration of a robust association between ESR, CRP, and RA can pinpoint the disease with more precision and begin treatment for RA at an earlier stage, when symptoms are less likely to manifest.

Conclusion:

In conclusion, ESR and CRP have a strong association with RA. The RA group had significantly greater DBC (LY, NE, MO, and EO) as well as PLT, NLR, and PLR values than the healthy controls. Conversely, the patients' RBC, Hb, and MPV values decreased. The biochemical and hematological indicators of RA patients who carried the *DRB1*04* or **10* gene variants in parallel differ significantly.

Declarations:

Ethical Approval: The University of Gezira Ethical Committee approved the study, and it was carried out under the Helsinki Declaration's ethical principles.

Conflict of interests: No conflict of interest to be declared.

Authors Contributions: I hereby verify that all authors mentioned on the title page have made substantial contributions to the conception and design of the study, have thoroughly reviewed the manuscript, confirm the accuracy and authenticity of the data and its interpretation, and consent to its submission.

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REFERENCES

Abbas, A.K., Lichtman, A.H., and Pillai, S. (2015). Cellular and Molecular Immunology. 8th edition. Elsevier Saunders, Philadelphia.

Aletaha, D., Neogi, T., Silman, A.J., Funovits, J., Felson, D.T., Bingham, CO 3rd, Birnbaum, N.S., Burmester, G.R., Bykerk, V.P., Cohen, M.D., Combe, B., Costenbader, K.H., Dougados, M., Emery, P., Ferraccioli, G., Hazes, J.M., Hobbs, K., Huizinga, T.W., Kavanaugh, A., Kay, J., Kvien, T.K., Laing, T., Mease, P., Ménard. H.A., Moreland, L.W., Naden, R.L., Pincus, T., Smolen, J.S., Stanislawska-Biernat, E., Symmons, D., Tak, P.P., Upchurch, K.S., Vencovský, J., Wolfe, F., and Hawker, G. (2010). Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis and Rheumatism*, 62(9):2569-81. doi: 10.1002/art. 27584.

Ali, A.A., Khalid, K.E., Mohammed, S.E. Akhtar, M.S., and Saeed, O.K. (2023). Association of Human Leukocyte Antigen (HLA) class II (DRB1 and DQB1) alleles and haplotypes with Rheumatoid Arthritis in Sudanese patients.

Frontiers in Immunology, 14:1178546. doi: 10.3389/fimmu. 2023.1178546.

Al-Swailem, R., Al-Rayes, H., Sobki, S., Arfin, M., and Tariq, M. (2006). HLA-DRB1 association in Saudi rheumatoid arthritis patients. *Rheumatology International*, 26(11):1019-24. doi: 10.1007/ s00296-006-0119-2

Al-Timimi, D.J., Rasool, M.T., and Sulaiman, D.M. (2014). HLA-DR/DQ Genotypes in Kurd Patients with Rheumatoid Arthritis: Relation to Disease Activity. J*ournal of Clinical and Diagnosis Research*, 8(5): CC01-4. doi: 10.7860/JCDR/ 2014/ 8112.4349.

Atabonkeng, E.P., Claire, O.A.M., Singwe-Ngandeu, M., Nwobegahay, J., Bonsi, S.T., Fokou, P.V.T., Tsague, F.P.M., Tchakounte, C., and Tume, C. (2018). High C-reactive Proteins Levels, Rheumatoid Anemia, Alpha-1 Globulin Deficiency, and Hypergammaglobulinemia in Rheumatoid Arthritis Patients from Yaounde, Cameroon. *Biomedical and Biotechnology Research Journal (BBRJ)*, 2(3):203-207. doi: 10.4103/bbrj.bbrj_91_18.

Balandraud, N., Picard, C., Reviron, D., Landais, C., Toussirot, E., Lambert, N., Telle, E., Charpin, C., Wendling, D., Pardoux, E., Auger, I., and Roudier, J. (2013). HLA-DRB1 genotypes and the risk of developing anti citrullinated protein antibody (ACPA) positive rheumatoid arthritis. *PLoS One*, 8(5): e64108. doi: 10.1371/journal.pone.0064108

Bax, M., van Heemst, J., Huizinga, T.W., and Toes, R.E. (2011). Genetics of rheumatoid arthritis: what have we learned? Immunogenetics, 63(8): 459-66. doi: 10.1007/s00251-011-0528-6.

Begum, M., Sattar, H., Haq, S.A., Islam, M.N., Saha, S.K., Hassan, M.M., Saha, R., and Kabir, M.S. (2018). Study on association of human leukocyte antigen-DRB1 alleles amongst Bangladeshi patients with rheumatoid arthritis. *International Journal of Rheumatic Diseases*, 21(8):1543-1547. doi: 10.1111/ 1756-185X.13291

Bereir, R.E., Hassan, H.Y., Salih, N.A., Underhill, P.A., Cavalli-Sforza, L.L., Hussain, A.A., Kwiatkowski, D., and Ibrahim, M.E. (2007). Co-introgression of Y-chromosome haplogroups and the sickle cell gene across Africa's Sahel. *European Journal of Human Genetics*, 15(11):1183-5. doi: 10.1038/sj. ejhg. 5201892

Bos, W.H., van de Stadt, L.A., Sohrabian, A., Rönnelid, J., and van Schaardenburg, D. (2014). Development of anti-citrullinated protein antibody and rheumatoid factor isotypes prior to the onset of rheumatoid arthritis. *Arthritis Research and Therapy*, 16(2):405-406. doi: 10.1186/ar4511

Chandrashekara, S., Mukhtar Ahmad, M., Renuka, P., Anupama, K.R., and Renuka, K. (2017). Characterization of neutrophil-to-lymphocyte ratio as a measure of inflammation in rheumatoid arthritis. *International Journal of Rheumatic Diseases*, 20(10):1457-1467. doi: 10.1111/ 1756-185X.13157.

Chen, Y.F., Xu, S.Q., Xu, Y.C., Li, W.J., Chen, K.M., Cai, J., and Li, M. (2020). Inflammatory anemia may be an indicator for predicting disease activity and structural damage in Chinese patients with rheumatoid arthritis. *Clinical Rheumatology*, 39(6):1737-1745. doi: 10.1007/ s10067-019-04873-y.

Coenen, M.J., and Gregersen, P.K. (2009). Rheumatoid arthritis: a view of the current genetic landscape. *Genes and Immunity*, 10(2):101-11. doi: 10.1038/gene.2008.77. Epub 2008 Nov 6.

de Vries R. (2011). Genetics of rheumatoid arthritis: time for a change!. *Current Opinion in Rheumatology*, 23(3):227-32. doi: 10.1097/BOR. 0b013e3283457524.

Dechanuwong, P., and Phuan-Udom, R. (2021). Hematological parameters as a predictor of disease remission in patients with rheumatoid arthritis. *Annals of Medicine and Surgery (Lond)*, 19;72:103085. doi: 10.1016/ j.amsu.2021.103085.

Erre, G.L., Paliogiannis, P., Castagna, F., Mangoni, A.A., Carru, C., Passiu, G., and Zinellu, A. (2019). Meta-analysis of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratio in rheumatoid arthritis. *European Journal of Clinical Investigation*, 49(1): e13037. doi: 10.1111/eci.13037.

Farouk, A.M., Abdel Rahman, S.M., Abou Elwafa, M.Z., and Aboud, F.M. (2023). Hematological parameters in rheumatoid arthritis and their relationship with disease activity. *Ain Shams Medical Journal*, 74 (2): 493-503.

Fawzy, R.M., Said, E.A., and Mansour, A.I. (2017). Association of neutrophil to lymphocyte ratio with disease activity indices and musculoskeletal ultrasound findings in recent onset rheumatoid arthritis patients. *Egyptian Rheumatologist*, 39, 203-206.

Harrison, E.D. (2001). Chemical features of rheumatoid arthritis, Textbook of Rheumatology. 5th edition W. B. Saunders Philadelphia.

Hashemi, R., Majidi, A., Motamed, H., Amini, A., Najari, F., and Tabatabaey, A. (2015). Erythrocyte Sedimentation Rate Measurement Using as a Rapid Alternative to the Westergren Method. *Emerg (Tehran)*, 3(2):50-3.

Hochberg, M.C., Silman, A.J., Smolen, J.S., Weinblatt, M.E., Weisman, M.H. (2015). Rheumatology. 6th edition, Mosby Alesevier Ltd. Philadelphia.

Hughes, L.B., Morrison, D., Kelley, J.M., Padilla, M.A., Vaughan, L.K., Westfall, A.O., Dwivedi, H., Mikuls, T.R., Holers, V.M., Parrish, L.A., Alarcón, G.S., Conn, D.L., Jonas, B.L., Callahan, L.F., Smith, E.A., Gilkeson, G.S., Howard, G., Moreland, L.W., Patterson, N., Reich, D., and Bridges, S.L. Jr. (2008). The HLADRB1 shared epitope is associated with susceptibility to rheumatoid arthritis in African Americans through European genetic admixture. *Arthritis and Rheumatism*, 58(2): 349-58. doi: 10.1002/art. 23166

Kim, K.W., Kim, B.M., Moon, H.W., Lee, S.H., and Kim, H.R. (2015). Role of C-reactive protein in osteoclastogenesis in rheumatoid arthritis. *Arthritis Research and Therapy*, 17(1):41.

Kisacik, B., Tufan, A., Kalyoncu, U., Karadag, O., Akdogan, A., Ozturk, M.A., Kiraz, S., Ertenli, I., and Calguneri, M. (2008). Mean platelet volume (MPV) as an inflammatory marker in ankylosing spondylitis and rheumatoid arthritis. *Joint Bone Spine*, 75(3):291-4. doi: 10.1016/j. jbspin.2007.06.016.

Klimenta, B., Nefic, H., Prodanovic, N., Hukic, F., and Mesic, A. (2020). Hematological parameters in patients with rheumatoid arthritis and gene variants HLA-DRB1*04 and HLA-DRB1*03. *Genetics and Applications*, 4(1): 1-8.

Klimenta, B., Nefic, H., Prodanovic, N., Jadric, R., and Hukic, F. (2019). Association of biomarkers of inflammation and HLA-DRB1 gene locus with risk of developing rheumatoid arthritis in females. Rheumatol Int, 39(12):2147-2157. doi: 10.1007/s00296-019-04429-y.

Konda Mohan, V., Ganesan, N., Gopalakrishnan, R., and

Venkatesan, V. (2017). HLA-DRB1 shared epitope alleles in patients with rheumatoid arthritis: relation to autoantibodies and disease severity in a south Indian population. *International Journal of Rheumatic Diseases*, 20(10):1492-1498. doi: 10.1111/1756-185X.12948

Liu, S.C., Chang, T.Y., Lee, Y.J., Chu, C.C., Lin, M., Chen, Z.X., Liu, H.F., Dang, C.W., Chang, S.C., Lee, C.S., Chen, T.L., and Huang, C.H. (2007). Influence of HLADRB1 genes and the shared epitope on genetic susceptibility to rheumatoid arthritis in Taiwanese. *The Journal of Rheumatology*, 34(4):674-80.

Louzada-Júnior, P., Freitas, M.V., Oliveira, R.D., Deghaide, N.H., Conde, R.A., Bertolo, M.B., and Donadi, E.A. (2008). A majority of Brazilian patients with rheumatoid arthritis HLA-DRB1 alleles carry both the HLA-DRB1 shared epitope and anti-citrullinated peptide antibodies. *Brazilian Journal of Medical and Biological Research*, 41(6):493-9. doi: 10.1590/s0100-879x 2008005000021

Moghimi, J., Ghahremanfard, F., Salari, M., and Ghorbani, R. (2017). Association between mean platelet volume and severity of rheumatoid arthritis. *The Pan African medical journal*, 27:276. doi: 10.11604/ pamj.2017.27.276.12228.

Mourad, J., and Monem, F. (2013). HLA-DRB1 allele association with rheumatoid arthritis susceptibility and severity in Syria. *Revista Brasileira de Reumatologia*, 53(1): 47-56.

Mykola, V., Ganna, S., and Gennadiy, T. (2015). Hematological Abnormalities in Ukrainian Patients with Rheumatoid Arthritis. *Journal of Arthritis*, 4(1): 1000146. doi:10.4172/2167-7921.1000146.

Naqi, N., Ahmed, T.A., Malik, J.M., Ahmed, M., and Bashir, M.M. (2011). HLA DRβ1 alleles in Pakistani patients with rheumatoid arthritis. *Journal of the College of Physicians and Surgeons--Pakistan*, 21(12):727-30.

Okoroiwu, I.L., Obeagu, E.I., and Obeagu, G.U. (2016). Hematological variables in rheumatoid arthritis patients in Imo state Nigeria. *International Journal of Advanced Research in Biological Sciences*, 3(4): 91-98.

Olumuyiwa-Akeredolu, O.O., Page, M.J., Soma, P., and Pretorius, E. (2019). Platelets: emerging facilitators of cellular crosstalk in rheumatoid arthritis. *Nature Reviews. Rheumatology*, 15(4):237–248. doi: 10.1038/s41584-019-0187-9.

Priehodová, E., Austerlitz, F., Čížková, M., Nováčková, J., Ricaut, F.X., Hofmanová, Z., Schlebusch, C.M., and Černý, V. (2020). Sahelian pastoralism from the perspective of variants associated with lactase persistence. *American Journal of Physical Anthropology*, 173(3):423-436. doi: 10.1002/ajpa.24116

Reynolds, R.J., Ahmed, A.F., Danila, M.I., Hughes, L.B.; Consortium for the Longitudinal Evaluation of African Americans with Early Rheumatoid Arthritis Investigators, Gregersen, PK., Raychaudhuri, S., Plenge, R.M., and Bridges, S.L. Jr. (2014). HLA-DRB1-associated rheumatoid arthritis risk at multiple levels in African Americans: hierarchical classification systems, amino acid positions, and residues. *Arthritis and Rheumatology*, 66(12):3274-82. doi: 10.1002/art.38855

Sandoughi, M., Fazaeli, A., Bardestani, G., and Hashemi, M. (2011). Frequency of HLA-DRB1 alleles in rheumatoid arthritis patients in Zahedan, southeast Iran. *Annals of Saudi Medicine*, 31(2):171-3. doi: 10. 4103/0256-4947.78205

Sokolovic, S., Dervisevic, V., and Fisekovic, S. (2014). Mental health status can

reflect disease activity in rheumatoid arthritis. *European Journal of Rheumatology*, 1:55-57.

Talukdar, M., Barui, G., Adhikari, A., Karmakar, R., Ghosh, U.C., and Das, T.K. (2017). A Study on Association between Common Haematological Parameters and Disease Activity in Rheumatoid Arthritis. *Journal of Clinical and Diagnostic Research*, 11(1):EC01-EC04. doi: 10.7860/ JCDR/2017/23524.9130.

Tekeoğlu, İ., Gürol, G., Harman, H., Karakeçe, E., and Çiftçi, İ.H. (2016). Overlooked hematological markers of disease activity in rheumatoid arthritis. *International Journal of Rheumatic Diseases*, 19(11):1078-1082. doi: 10.1111/1756-185X. 12805.

Uslu, A.U., Küçük, A., Şahin, A., Ugan, Y., Yılmaz, R., Güngör, T., Bağcacı, S., and Küçükşen S. (2015). Two new inflammatory markers associated with Disease Activity Score-28 in patients with rheumatoid arthritis: neutrophil-lymphocyte ratio and platelet-lymphocyte ratio. *International Journal of Rheumatic Diseases*, 18(7):731-5. doi: 10.1111/ 1756-185X.12582.

Van De Putte, L.B., Van Gestel, A.M., and Van Riel, P.L. (1998). Early treatment of rheumatoid arthritis: rationale, evidence, and implications. *Annals of the Rheumatic Diseases*, 57(9): 511-12.

Wasunna, A., Whitelaw, A., Gallimore, R., Hawkins, P.N., and Pepys, M.B. (1990) C-reactive protein and bacterial infection in preterm infants. *European Journal of Pediatrics*, 149(6):424-427.

Xue, L., Tao, L., Sun, H., Wang, Y., Zhang, Y., Lei, N., Liu, Z., Zhang, H., Jin, L., Zhang, T., Zhang, J., Meng, H., Huang, F., Geng, Y., and Li, M. (2022). Association Between Blood PLT and RBC Related Indices and Disease Activity in Patients with Rheumatoid Arthritis. *International Journal of General Medicine*, 15:573-581.doi: 10.2147/IJGM. S351505. eCollection 2022.