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Hematological parameters in patients with rheumatoid arthritis and gene variants *HLA-DRB1*04* and *HLA-DRB1*03*

Biljana Klimenta[1*], Hilada Nefić[2], Nenad Prodanović[3], Fatima Hukić[4], Aner Mešić[5]

[1]*Department of Laboratory Diagnostics, The Public Institution Health Centre of Sarajevo Canton Sarajevo, Sarajevo, Bosnia and Herzegovina*
[2]*Genetics and Clinical Biology, Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[3]*Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina*
[4]*Department of Biochemistry, Clinical University Centre of Tuzla,Tuzla, Bosnia and Herzegovina*
[5]*Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo,Bosnia and Herzegovina*

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***Correspondence**
E-mail: biljanaklimenta@hotmail.com

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Abstract

Rheumatoid arthritis is a polygenic disease of unknown etiology, occurs worldwide in both developed and underdeveloped countries and involves all races. The aim of this study is to determine the correlation between hematological parameters (DBC and ESR) and biomarkers of inflammation (CRP) in patients with RA predisposing gene variants *HLA-DRB1*04*or*HLA-DRB1*03*. This study analyzed the results of hematological and biochemical parameters of 33 patients diagnosed with RA, carriers ofgene variants of *HLA-DRB1*04* or *HLA-DRB1*03*, and 33 subjects of control group non-carriers for *HLA-DRB1*04* or *HLA-DRB1*03*. All hematological parameters (DBC) were analyzed on a Beckman Coulter DxH 800 hematology counter. The erythrocyte sedimentation rate was expressed in mm/h. The CRP biochemical test was performed on a Cobas c311 automatic analyzer. In groupof RA patients carriers of *HLA-DRB1*04*or*HLA-DRB1*03* gene variants, the values of HGB and HCTwere significantlylower($p < 0.05$) while the values of RDW, RDW-SD, MO, BA, MO#, BA#, ESR and CRPwere statistically increased($p < 0.05$) from the control group without these variants.

Introduction

Rheumatoid arthritis (RA) is a systemic chronic autoimmune connective tissue disease, with a slightly more frequent occurrence of the disease in women after age 50 (Silman et al., 1993; Jones et al., 1996; Harney et Workdsworth, 2002). This disease has a complex and multifactorial etiology, most

likely with an oligogenic predisposition that interacts with a "trigger from the environment" (Coenen et Gregersen, 2009). A healthy person's immune system controls and limits inflammatory processes, but in rheumatoid arthritis, certain disorders of the immune system support this process and lead to numerous clinical and pathological manifestations. The overlap of clinical symptoms, the diversity of immune and clinical parameters, the lack of a specific diagnostic test make it difficult to properly and timely diagnose and recognize the status of a rheumatic disease. The MHC major histocompatibility complex is the most polymorphic gene system in human whose products are antigen-presenting cells (APC) membrane proteins. The MHC human tissue matching gene and antigen complex is also called human leukocyte antigen (HLA), positioned on the short arm of chromosome 6 and encompasses about 3.5 megabases (Mb) of DNA, while antigens or products MHCs are found on different cells and in different amounts. HLA typing also allows the determination of the at-risk population, which can be of practical benefit in preventing the disease before it develops more severe symptoms and complications. The role of genetic factors in the etiology of RA was established by the discovery of an association of the disease with class II MHC complexes, more specifically with the *HLA-DRB1*04* gene variant (Stastny, 1978; Gregersen et al., 1987; Viatte et al., 2013; Klimenta et al., 2019). This association has been observed in many populations, while in other ethnic groups a link has been established between different variants of the HLA-DRB1 gene locus and RA, which occurs in association with *HLA-DRB1*04* or individually (Caffrey and James, 1973; Schlossteine et al., 1973).

Material and methods

The patient's blood samples for analysis were taken from the Public Primary Healthcare Unit of Sarajevo, Canton Sarajevo. Isolation of genomic DNA from the collected samples (Miller et al. 1988) was performed in a Genetics Laboratory, Faculty of Science, University of Sarajevo. The quality of isolated genomic DNA from peripheral blood cells was checked by electrophoresis on a 1% agarose gel. Polymerase chain reaction (PCR) with sequence-specific priming (SSP) primers was used for genotyping for selected HLA loci using HLA-Ready Gene DR kit (Inno-Train, Germany) (reference). The kit contains commercial Ready PCR with all additives (dNTPs, PCR buffer, cresol red, glycerin) necessary for the reaction to take place. The procedure involves performing a large number of PCR reactions per sample with specific primers - 24 reactions for genotyping low resolution allele of the HLA-DRB1 locus. The results are valid if internal controls are amplified. The following protocol was used to prepare the PCR mixture for amplification of the HLA-DRB1 gene locus: sterile deionized water (168 µl), amplification buffer (ReadyPCR; 84 µl), Taq Polymerase (5U/µl; 2.2 µl). An Eppendorf Mastercycler gradient PCR apparatus (Hamburg, Germany) was used to amplify the HLA-DRB1 gene locus. The results are evaluated by electrophoresis on 2% agarose gel stained with ethidium bromide in TBE buffer. In the electric field, the amplicons are separated according to their size and observed under UV light. In the SSP-PCR method, DNA is isolated from a sample of the person to be typed and amplified into multiple wells, each containing specific primers that are complementary to particular HLA alleles. The amplification product is formed only if the DNA probes are complementary to the HLA molecule sequences.

For hematological tests - differential blood count, venepuncture samples were taken of peripheral venous blood 3ml, in a vacuum tube containing the anticoagulant ethylenediaminotetraacetic acid (EDTA). Haematological parameters were analyzed on an automated Beckman Coulter DxH 800 hematology analyzer. For hematological examination, erythrocyte sedimentation rates (ESRs) were taken 1.8 ml of blood into a vacuum tube with anticoagulant sodium citrate into which a graduated pipette (150mm) was placed. ESR reference values are from 1-10 mm / 1h. Samples for biochemical analysis C-reactive protein (CRP) were taken in a vacuum tube, centrifuged for 10 minutes at 3500rpm and serum separated. C-reactive protein was quantified on a Roche / Hitachi cobas c311 systems apparatus. The reference value of the C-reactive protein is set 0-5mg/l. Statistical analysis was performed using the software, Statistics 8, statistical methods t-test and Shapiro-Wilk W Test. Student's t-

test was used to determine statistically significant differences in hematological and biochemical parameters between patients with RA and control group. Values lower than p ≤0.05 were considered significant.

Results and Discussion

A particular aspect of our study was the analysis of hematological status of rheumatoid arthritis (RA) patients with confirmed either *HLA-DRB1*04* or *HLA-DRB1*03* allele variant (Table 1 and Table 2). For RA patients, ESR values were significantly increased when compared to the control group ($p<0.05$) (Table 3).

Serum CRP values were also increased in RA patients and were significantly different in comparison to the control ($p<0.05$). Numerous inflammatory conditions can cause elevated serum CRP concentrations, including RA. This inflammatory biomarker is synthesized in liver as a response to inflammatory processes activated by cytokines, mobilizing complement components. Persistance of a high CRP concentration, usually indicates poor prognosis that generally refers to the presence of an uncontrolled inflammation RA (Abbas et al., 2015; Wassuna et al., 1990). Some previous studies have demonstrated the correlation between rapid radiological progression and increased CRP/ESR values (Hassel et al., 1993; van Leeuwen et al., 1994; Plant et al., 2000). It has been suggested that both, DAS28 (CRP) and DAS28 (ESR) markers are useful for the assessment of condition in RA patients (Wells et al., 2009). CRP and ESR diagnostic tests could be valuable and reliable diagnostic indicators for disease monitoring. Recent research showed that up to 10% of patients have normal values of ESR, while the remaining patients exhibit increased CRP values, especially in acute stage of disease (Vrhovac et al., 2008). During chronic inflammatory processes, leukocyte count is an important diagnostic indicator for disease assessment, whereby in our study leukocyte number deviated from the reference values, but had no statistical power ($p>0.05$). In differential blood count, an increased number of neutrophils and trombocytes can be seen in active RA (Hochberg et al., 2015). The association of chronic inflammatory markers, neutrophils and lymphocytes ratio (NLR), as well as platelets (PLT) and platelets/lymphocyte ratio (PLR), was not detected in patients with *HLA-DRB1*04* or*HLA-DRB1*03* gene variants. In previous study, the correlation between PLR and RA, as well as between ESR and RA was demonstrated (Peng et al., 2015). Macrophage activation, during inflammatory reactions, enhances the innate immune response, thus increasing the number of monocytes in peripheral circulation. In the present study conducted on RA patients, monocyte values were significantly increased as compared to the control group ($p<0.05$), indicating the activity of inflammatory processes. Some authors found significantly lower mean platelet volume (MPV) values in patients with ankylosing spondylitis and rheumatoid arthritis, in a study investigating the role of MPV as an inflammatory marker (Kisacik et al., 2008). The results of our study, also revealed slightly reduced MPV values in RA patients in comparison to the control, but with no statistical significance ($p>0.05$). Furthermore, significant changes in hemoglobin concentration (HGB), hematocrit (HCT), red cell distribution width (RDW) and red cell distribution width standard deviation (RDW-SD), between RA patients and control group were detected ($p<0.05$) (Table 3). As for the, white blood cells (WBC), red blood cells (RBC), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) values, no significant differences between RA patients and control were observed ($p>0.05$). Most of the RA patients have normocytic and normochromic anemia of chronic disease (Vrhovac et al., 2008). Different inflammatory diseases, such as reumathoid arthritis, are accompanied by anemia of chronic disease. It is separated from other types of anemia by a characteristic disorder in iron metabolism, does not require therapy in itself, and is corrected by the succesful treatment of the underlying disease (Labar et al., 2007). The variety of clinical symptoms in which anemia occurs, has always the same characteristics that distinguish it as an entity, suggesting a common pathogenic mechanism related to the inflammatory and immune reaction (Labar et al., 2007). According to the American Rheumatism Association criteria (now the American College of

Table 1. The frequencies of the hematological and biochemical parameters in the control group non-carriers for *HLA-DRB1*04* or *HLA-DRB1*03* gene variants

Sample	Age	WBC (10^3/μL)	RBC (10^6/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	RDW-SD (fL)	PLT (10^3/μL)	MPV (fL)	NE (%)	LY (%)	MO (%)	EO (%)	BA (%)	NLR	PLR	NE# (10^3/μL)	LY# (10^3/μL)	MO# (10^3/μL)	EO# (10^3/μL)	BA# (10^3/μL)	ESR (mm/h)	CRP (mg/l)	Allele variant 1	Allele variant 2
1	38	6.7	4.2	12.2	38.2	91.2	29.3	32.1	13.0	41.1	225	8.2	52.2	36.7	7.2	2.8	1.1	1.40	90.00	3.5	2.5	0.5	0.2	0.1	6	2	DRB1*10	DRB1*16
2	49	7.2	4.7	13.3	41.5	88.4	28.3	32.0	12.4	38.1	301	8.7	48.6	39.0	9.5	2.6	0.3	1.25	107.50	3.5	2.8	0.7	0.2	0.0	5	1	DRB1*01	DRB1*15
3	35	7.6	5.0	14.7	44.6	89.8	29.5	32.9	13.0	40.7	273	7.4	68.5	24.2	6.8	0.1	0.4	2.89	151.67	5.2	1.8	0.5	0.0	0.0	4	4	DRB1*11	DRB1*16
4	33	5.0	4.4	12.7	38.9	88.1	28.8	32.7	12.6	38.5	232	9.0	68.1	24.6	5.9	0.9	0.5	2.83	193.33	3.4	1.2	0.3	0.0	0.0	6	2	DRB1*01	DRB1*07
5	51	7.4	4.7	13.4	41.6	88.8	28.7	32.3	13.2	40.3	256	8.8	55.1	31.3	6.9	5.9	0.8	1.78	111.30	4.1	2.3	0.5	0.4	0.1	8	2	DRB1*11	DRB1*13
6	58	8.3	4.2	12.2	38.1	90.8	29.1	32.1	13.8	43.8	214	10.3	46.7	41.2	8.1	3.4	0.6	1.15	62.94	3.9	3.4	0.7	0.3	0.1	8	4	DRB1*01	DRB1*07
7	56	6.5	4.8	14.3	44.5	92.2	29.6	32.2	12.8	41.1	311	8.6	62.1	25.2	10.5	1.2	1.0	2.50	194.38	4.0	1.6	0.7	0.1	0.1	9	1	DRB1*14	DRB1*16
8	36	5.9	5.0	14.5	44.9	90.1	29.2	32.4	12.9	40.7	282	7.8	52.6	35.1	9.1	2.5	0.7	1.48	134.28	3.1	2.1	0.5	0.1	0.0	6	1	DRB1*01	DRB1*08
9	41	7.5	4.1	12.0	37.3	91.1	29.4	32.2	14.5	45.9	220	9.1	69.1	23.0	6.0	0.7	1.2	3.06	129.41	5.2	1.7	0.4	0.1	0.1	8	2	DRB1*01	DRB1*16
10	37	9.4	5.1	14.8	45.5	89.5	29.1	32.6	13.5	42.4	240	9.0	56.3	35.3	5.6	2.1	0.7	1.61	72.73	5.3	3.3	0.5	0.2	0.1	5	3	DRB1*12	DRB1*16
11	55	6.3	4.7	14.6	45.8	96.7	30.9	32.0	12.9	43.3	244	8.1	49.4	38.3	7.3	4.2	0.8	1.29	101.67	3.1	2.4	0.5	0.3	0.1	8	1	DRB1*01	DRB1*07
12	57	7.3	5.1	14.8	46.1	90.5	29.1	32.2	14.1	43.8	207	10.4	55.6	30.7	9.6	3.2	0.9	1.82	94.09	4.0	2.2	0.7	0.2	0.1	5	4	DRB1*14	DRB1*16
13	52	8.5	4.5	13.2	41.5	91.8	29.1	32.1	13.1	41.6	289	8.2	63.9	27.4	4.2	3.5	1.0	2.35	125.65	5.4	2.3	0.4	0.3	0.1	6	1	DRB1*11	DRB1*16
14	50	7.1	4.6	13.3	41.7	90.4	28.8	32.2	12.7	39.8	182	9.6	51.3	37.5	7.6	3.1	0.5	1.38	70.00	3.6	2.6	0.5	0.2	0.0	4	3	DRB1*08	DRB1*15
15	37	7.7	5.1	14.6	46.0	88.6	28.1	32.4	14.7	45.1	287	8.9	45.8	40.6	9.8	2.7	1.1	1.13	92.58	3.5	3.1	0.8	0.2	0.1	9	1	DRB1*01	DRB1*15
16	56	5.0	4.1	13.3	41.1	96.8	32.8	32.4	13.0	45.5	196	9.4	53.3	35.7	8.2	2.0	0.8	1.50	108.89	2.7	1.8	0.4	0.1	0.0	2	1	DRB1*07	DRB1*11
17	53	8.4	4.3	12.9	40.1	94.4	30.3	32.1	12.8	42.0	295	10.3	55.5	31.3	6.5	6.1	0.6	1.81	113.46	4.7	2.6	0.5	0.5	0.1	5	3	DRB1*07	DRB1*15
18	56	8.3	4.7	13.6	42.1	90.0	29.0	32.2	13.4	42.0	279	9.2	67.2	20.4	6.8	4.8	0.8	3.50	174.38	5.6	1.6	0.6	0.4	0.1	4	3	DRB1*14	DRB1*15
19	37	9.5	4.7	13.0	41.2	88.6	28.0	32.4	14.1	43.8	272	8.6	54.4	36.4	7.1	1.7	0.4	1.48	77.71	5.2	3.5	0.7	0.2	0.0	3	1	DRB1*15	DRB1*16
20	33	5.8	4.7	13.3	41.7	88.3	28.1	32.1	12.8	39.4	307	8.1	48.6	33.8	10.6	6.1	0.9	1.40	153.50	2.8	2.0	0.6	0.4	0.1	5	2	DRB1*13	DRB1*15
21	41	5.9	4.7	15.2	46.1	97.0	32.5	32.9	13.8	46.8	206	8.8	46.7	43.6	7.9	1.4	0.4	1.04	79.23	2.7	2.6	0.5	0.1	0.0	7	1	DRB1*01	DRB1*07
22	40	5.5	4.6	13.2	41.6	90.5	28.8	32.2	13.2	41.1	228	9.3	49.6	34.9	8.0	6.6	0.9	1.42	120.00	2.7	1.9	0.4	0.4	0.0	6	4	DRB1*07	DRB1*10
23	36	7.0	4.8	13.3	42.9	89.2	27.6	32.1	14.9	45.1	199	9.3	52.2	37.5	8.7	1.2	0.4	1.42	76.54	3.7	2.6	0.6	0.1	0.0	3	1	DRB1*01	DRB1*15
24	58	6.2	4.6	12.1	38.7	84.5	27.5	32.1	14.4	42.4	163	9.9	51.7	37.7	6.5	3.8	0.3	1.39	70.87	3.2	2.3	0.4	0.2	0.0	6	1	DRB1*07	DRB1*16
25	56	6.8	5.0	14.2	45.3	89.8	28.1	32.1	13.8	40.7	204	10.3	44.3	45.4	8.8	1.3	0.2	0.79	60.00	2.7	3.4	0.6	0.1	0.0	5	1	DRB1*01	DRB1*01
26	47	5.7	5.0	14.3	44.8	90.5	28.9	32.1	13.8	43.3	244	10.4	68.4	29.0	1.8	0.1	0.7	3.00	174.28	4.2	1.4	0.1	0.0	0.0	9	1	DRB1*13	DRB1*16
27	45	7.8	5.1	14.5	44.7	88.4	28.8	32.5	13.5	41.6	272	10.2	54.8	34.0	7.3	3.4	0.5	1.59	100.74	4.3	2.7	0.6	0.3	0.0	4	1	DRB1*11	DRB1*16
28	36	4.8	5.5	15.7	48.5	88.6	28.6	32.3	13.3	41.1	262	9.4	60.9	31.6	5.2	2.0	0.3	1.93	174.67	2.9	1.5	0.2	0.1	0.0	2	1	DRB1*13	DRB1*15
29	48	6.9	5.2	15.6	48.7	94.0	30.2	32.1	13.7	44.2	262	8.4	64.6	25.1	7.7	2.2	0.4	2.65	154.12	4.5	1.7	0.5	0.2	0.0	5	2	DRB1*01	DRB1*15
30	55	8.3	4.5	13.7	42.3	94.2	30.5	32.4	14.0	45.5	245	10.3	49.6	37.0	7.5	4.7	1.2	1.32	79.03	4.1	3.1	0.6	0.4	0.1	6	3	DRB1*13	DRB1*14
31	51	9.8	4.5	14.3	44.7	98.6	31.5	32.0	12.8	43.3	256	9.2	59.8	30.4	6.9	2.1	0.8	1.97	85.33	5.9	3.0	0.7	0.2	0.1	3	0	DRB1*01	DRB1*15
32	52	6.7	4.8	13.6	43.0	89.4	28.3	31.7	13.8	42.9	299	9.1	61.8	28.1	6.2	3.4	0.5	2.16	157.37	4.1	1.9	0.4	0.2	0.0	3	2	DRB1*08	DRB1*14
33	52	4.7	4.8	13.8	43.4	90.1	28.8	31.7	13.0	41.1	213	8.9	61.0	29.6	6.8	1.6	1.0	2.07	152.14	2.9	1.4	0.3	0.1	0.0	4	1	DRB1*13	DRB1*15

Table 2. The frequencies of the hematological and biochemical parameters in the group of patients with RA carriers of*HLA-DRB1*04* or *HLA-DRB1*03* gene variants

Sample	Age	WBC (10^3/μL)	RBC (10^6/μL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW (%)	RDW-SD (fL)	PLT (10^3/μL)	MPV (fL)	NE (%)	LY (%)	MO (%)	EO (%)	BA (%)	NLR	PLR	NE# (10^3/μL)	LY# (10^3/μL)	MO# (10^3/μL)	EO# (10^3/μL)	BA# (10^3/μL)	ESR (mm/h)	CRP (mg/l)	Allele variant 1	Allele variant 2
1	58	10.5	4.6	12.9	40.6	87.4	27.8	31.8	15.9	48.1	196	8.0	71.8	18.8	6.3	2.1	1.0	3.75	98.00	7.5	2.0	0.7	0.2	0.1	25	10	DRB1*04	DRB1*16
2	40	5.3	4.5	13.2	40.5	90.3	29.5	32.7	13.6	42.0	212	10.3	52.4	36.1	8.6	2.4	0.5	1.47	111.58	2.8	1.9	0.5	0.1	0.0	6	2	DRB1*03	DRB1*16
3	59	6.5	4.8	13.1	40.9	85.1	27.1	31.9	16.5	48.1	184	9.4	52.3	29.2	13.1	3.9	1.5	1.79	96.84	3.4	1.9	0.9	0.3	0.1	11	1	DRB1*04	DRB1*13
4	50	10.0	4.9	13.8	39.3	90.2	29.1	31.5	13.9	43.2	323	8.9	56.3	27.3	7.3	7.5	1.6	2.07	119.63	5.6	2.7	0.7	0.8	0.2	21	8	DRB1*01	DRB1*03
5	57	10.4	5.1	15.4	47.3	92.8	30.3	32.6	13.6	43.8	181	10.6	50.5	35.0	9.6	4.1	0.8	1.44	50.28	5.2	3.6	1.0	0.5	0.1	4	3	DRB1*04	DRB1*11
6	56	8.4	4.2	12.3	37.5	89.9	29.5	32.9	15.0	46.4	341	8.8	66.9	22.4	7.8	2.2	0.7	2.95	179.47	5.6	1.9	0.7	0.2	0.1	18	3	DRB1*03	DRB1*04
7	59	4.0	4.2	13.7	40.8	96.3	32.3	33.5	12.7	42.9	123	8.0	47.8	44.7	6.1	0.3	1.1	1.06	68.33	1.9	1.8	0.2	0.0	0.0	21	1	DRB1*03	DRB1*15
8	42	8.6	4.3	13.1	40.4	93.4	30.3	32.5	12.7	41.1	193	11.1	56.0	33.0	6.9	3.0	1.1	1.71	68.93	4.8	2.8	0.6	0.3	0.1	5	1	DRB1*03	DRB1*15
9	51	6.6	4.4	13.2	40.3	92.0	30.0	32.6	13.6	43.3	227	7.1	61.4	29.6	7.1	1.1	0.8	2.05	113.50	4.1	2.0	0.5	0.1	0.1	15	3	DRB1*04	DRB1*15
10	60	8.2	4.4	12.4	38.1	85.9	28.0	32.6	13.4	39.8	228	10.3	51.6	32.3	11.9	3.4	0.8	1.62	87.69	4.2	2.6	1.0	0.3	0.1	8	4	DRB1*03	DRB1*08
11	59	7.5	4.8	14.3	43.7	91.8	29.8	32.7	13.3	42.0	233	8.8	55.6	29.7	9.2	2.7	2.8	1.86	105.91	4.1	2.2	0.7	0.2	0.2	3	1	DRB1*03	DRB1*16
12	56	10.0	4.2	11.5	36.6	87.8	27.5	31.4	16.8	50.8	393	9.3	57.0	23.1	9.8	9.0	1.1	2.48	170.87	5.7	2.3	1.0	0.9	0.1	48	28	DRB1*04	DRB1*04
13	59	6.7	5.4	16.6	50.5	94.1	31.0	32.9	13.0	42.4	191	8.8	46.0	39.6	9.8	3.6	1.0	1.19	73.46	3.1	2.6	0.6	0.2	0.1	1	1	DRB1*03	DRB1*11
14	50	6.5	4.6	13.4	42.0	92.0	29.4	32.0	14.2	45.5	254	9.4	43.3	39.0	9.9	6.6	1.2	1.12	101.60	2.8	2.5	0.6	0.4	0.1	5	8	DRB1*04	DRB1*11
15	56	6.4	4.3	13.3	40.4	94.5	31.1	33.0	12.4	40.7	226	10.5	53.8	32.4	9.2	3.5	1.1	1.67	107.62	3.5	2.1	0.6	0.2	0.1	4	1	DRB1*04	DRB1*07
16	60	7.5	3.9	11.3	35.6	92.0	29.1	31.6	15.9	50.3	241	9.5	55.6	32.0	8.6	3.4	0.4	1.75	100.42	4.2	2.4	0.6	0.3	0.0	44	8	DRB1*03	DRB1*04
17	59	5.4	4.9	14.7	45.0	91.5	29.9	32.7	15.4	48.1	174	7.5	48.9	34.5	12.0	3.9	0.7	1.44	96.67	2.6	1.8	0.6	0.2	0.0	17	8	DRB1*01	DRB1*04
18	55	9.6	5.3	14.1	44.6	84.2	26.7	31.7	15.2	43.8	300	8.6	59.3	29.0	7.5	3.5	0.7	2.04	107.14	5.7	2.8	0.7	0.3	0.1	34	18	DRB1*03	DRB1*04
19	59	8.0	4.5	12.3	38.4	86.1	27.7	32.1	14.2	42.0	244	9.0	62.2	27.3	9.0	0.8	0.7	0.26	106.09	4.6	2.3	0.5	0.1	0.1	7	1	DRB1*04	DRB1*16
20	54	6.4	4.8	13.4	42.6	89.3	28.0	31.4	15.5	47.3	228	10.3	58.7	28.9	7.7	3.7	1.0	2.06	126.67	3.7	1.8	0.5	0.2	0.1	30	9	DRB1*04	DRB1*04
21	37	7.7	5.0	14.3	44.6	88.8	28.4	32.0	12.6	38.5	295	8.9	41.6	38.6	10.5	8.1	1.2	1.07	98.33	3.2	3.0	0.8	0.6	0.1	9	1	DRB1*03	DRB1*11
22	60	7.4	4.7	13.8	43.2	92.8	29.6	31.8	14.9	47.7	229	8.8	67.4	19.4	8.2	4.4	0.6	3.57	163.57	5.0	1.4	0.6	0.3	0.0	47	11	DRB1*04	DRB1*12
23	59	6.1	5.3	14.3	45.3	86.2	27.1	31.5	13.5	40.3	131	8.8	45.5	38.7	13.9	0.8	1.1	1.22	56.96	2.8	2.3	0.8	0.0	0.1	20	2.8	DRB1*03	DRB1*11
24	59	9.4	5.0	10.6	35.3	70.7	21.3	30.1	18.7	45.9	409	8.2	57.6	30.9	9.8	1.5	0.2	1.86	141.03	5.4	2.9	0.9	0.1	0.0	16	2.8	DRB1*03	DRB1*15
25	39	6.3	4.9	11.2	35.5	73.3	23.0	31.4	16.3	41.6	312	7.9	65.7	21.5	6.7	5.8	0.3	3.15	240.00	4.1	1.3	0.4	0.4	0.0	13	2	DRB1*01	DRB1*04
26	56	10.2	5.1	14.9	46.9	91.2	29.1	31.9	14.8	45.9	320	8.6	40.7	48.4	8.1	2.0	0.8	0.84	65.31	4.1	4.9	0.8	0.2	0.1	19	1	DRB1*03	DRB1*04
27	48	8.2	4.6	10.2	35.0	76.4	22.2	29.0	20.8	54.3	338	9.0	58.5	30.0	7.0	3.4	1.1	1.92	135.20	4.8	2.5	0.6	0.3	0.1	46	3	DRB1*03	DRB1*03
28	52	5.7	4.3	13.0	41.0	95.4	30.3	31.8	12.9	42.4	214	10.4	59.9	25.2	9.2	4.7	1.0	2.43	152.86	3.4	1.4	0.5	0.3	0.1	28	2	DRB1*03	DRB1*03
29	49	5.3	4.1	12.6	40.5	94.3	30.5	31.5	12.1	42.1	208	10.1	59.3	25.8	9.5	4.3	1.1	2.38	160.00	3.1	1.3	0.5	0.3	0.1	25	1	DRB1*03	DRB1*03
30	56	4.7	4.3	11.9	37.3	86.3	27.7	32.1	17.7	52.5	358	8.0	57.5	35.5	4.9	1.8	0.3	1.59	210.59	2.7	1.7	0.2	0.1	0.0	88	47	DRB1*03	DRB1*04
31	58	4.9	4.5	13.9	42.0	93.7	31.1	33.1	14.4	46.4	336	8.0	45.3	42.5	8.2	2.9	1.1	1.05	160.00	2.2	2.1	0.4	0.1	0.1	10	2	DRB1*04	DRB1*15
32	46	16.0	4.5	12.1	38.6	86.0	27.0	31.5	15.1	45.1	471	9.3	66.3	17.3	9.7	5.7	1.0	3.78	168.21	10.6	2.8	1.6	0.9	0.2	28	24	DRB1*04	DRB1*04
33	56	5.9	4.3	13.0	41.0	95.8	30.5	31.8	12.8	41.9	216	10.6	59.3	25.8	9.8	4.8	1.1	2.57	154.28	3.6	1.4	0.5	0.3	0.1	18	1	DRB1*04	DRB1*15

Table 3. The comparison of the hematological and biochemical parameters between the control group and RA patients group carriers of *HLA-DRB1*04* or *HLA-DRB1*03* gene variants using Student t-test

Parameters	Normal values for women	Control (N = 33)			RA patients with *HLA-DRB1*4* or *HLA-DRB1*3* allele variants(N = 33)			t-value	P
		Mean ± SD	Median (Min–Max)	V%	Mean ± SD	Median(Min–Max)	V%		
WBC (10^3/μL)	3.4 - 9.7	7.02±1.35	**7.0** (4.7–9.8)	9.80	7.58±2.35	**7.4**(4.0–16.0)	30.97	–1.21	0.23
RBC (10^6/μL)	3.86 - 5.08	4.71±0.33	**4.69**(4.06–5.48)	7.01	4.62±0.38	**4.57**(3.87–5.37)	8.19	1.05	0.30
HGB (g/dL)	11.9 - 15.7	13.76±0.98	**13.6**(12.00–15.7)	7.14	13.16±1.36	**13.2**(10.2–16.6)	10.36	2.11	**0.04***
HCT (%)	35.6 - 47.0	42.94±2.91	**42.9**(37.30–48.7)	6.78	40.95±3.71	**40.60**(35.0–50.5)	9.06	2.43	**0.02***
MCV (fL)	83.0 - 97.2	90.94±3.07	**90.1**(84.50–98-60	3.37	89.02±6.05	**90.3**(70.7–96.3)	6.79	1.63	0.11
MCH (pg)	27.4 - 33.9	29.25±1.25	**29.0**(24.3–32-8)	4.27	28.54±2.48	**29.1**(21.3–32.3)	8.70	1.47	0.15
MCHC (g/dL)	32.0 - 34.5	32.24±0.27	**32.2**(30–32.9)	0.85	31.99±0.87	**31.9**(29.0–33.5)	2.71	1.57	0.12
RDW (%)	9.0 - 15.0	13.43±0.64	**13.3** (12.40–14.9)	4.79	14.65±1.94	**14.2**(12.1–20.8)	13.28	–3.41	**0.00***
RDW-SD (fL)	36.5 - 45.9	42.36±2.16	**42.0**(38.1–46.8)	5.11	44.73±3.78	**43.8** (38.5–54.3)	8.46	–3.12	**0.00***
PLT (10^3/μL)	158 - 424	247.42±39.21	**245.0**(163.0–311)	15.85	258.45±80.88	**229.0**(123.0–471.0)	31.29	–0.70	0.48
MPV (fL)	6.8 - 10.4	9.13±0.81	**9.1**(7.4–10.4)	8.90	9.12±1.01	**8.9**(7.1–11.1)	11.07	0.05	0.96
NE (%)	44.0 - 72.0	56.05±7.43	**54.8** (44.30–69.10)	13.26	55.52±7.81	**56.3** (40.70–71.80)	14.07	0.29	0.78
LY (%)	20.0 - 46.0	33.08±6.16	**34.0** (20.40–45.40)	18.62	31.02±7.48	**30.0** (17.30–48.40)	24.11	1.22	0.23
MO (%)	0.0 - 12.0	7.35±1.78	**7.3** (1.80–10.60)	24.27	8.88±1.98	**9.0** (4.90–13.90)	22.27	–3.29	**0.00***
EO (%)	0.0 - 7.0	2.83±1.73	**2.6** (0.10–6.60)	61.05	3.66±2.07	**3.5** (0.30–9.00)	56.57	–1.77	0.08
BA (%)	0.0 – 2.0	0.69±0.029	**0.7** (0.06–1.20)	45.54	0.95±0.46	**1.0** (0.20–2.80)	48.51	–2.75	**0.00***
NE# (10^3/μL)	1.1 - 7.0	3.93±0.95	**3.9**(2.7–5.9)	24.28	4.25±1.68	**4.1**(1.9–10.6)	39.50	–0.94	0.35
LY# (10^3/μL)	0.7 - 4.5	2.31±0.65	**2.3**(1.2–3.5)	28.17	2.27±0.72	**2.2**(1.3–4.9)	31.83	0.23	0.82
MO# (10^3/μL)	0.0 - 1.2	0.51±0.16	**0.5**(0.1–0.8)	30.39	0.66±0.26	**0.6**(0.2–1.6)	39.50	–2.81	**0.01***
EO# (10^3/μL)	0.0 - 0.7	0.21±0.13	**0.2** (0.0–0.5)	62.97	0.29±0.22	**0.3** (0.0–0.9)	76.52	–1.94	0.06
BA# (10^3/μL)	0.0 - 0.2	0.05±0.05	**0.1** (0.0–0.1)	111.24	0.08±0.06	**0.1** (0.0–0.2)	66.68	–2.21	**0.03***
NLR		1.83±0.67	**1.59**(0.79–3.5)	36.79	1.92±0.83	**1.79**(0.26–3.78)	43.49	–0.46	0.64
PLR		116.48±39.95	**108.89**(60–194.38)	34.30	121.12±44.36	**107.62**(50.28–240.0)	36.63	–0.45	0.66
ESR (mm/h)	0.0 - 10.0	5.42±2.0	**5.0** (2.0–9.0)	36.88	21.03±17.99	**18** (1.0–88.0)	85.56	–4.95	**0.00***
CRP (mg/l)	0.0 - 5.0	1.85±1.12	**1.0** (0.0–4.0)	60.67	6.65±9.82	**2.8** (1.0–47)	147.53	–2.79	**0.01***

*P-values ≤ 0.05 were significant (bold).

Rheumatology), after a diagnosis of rheumatoid arthritis has been established, additional examinations indicate to complications or unexpected outcomes of the disease. Analysis of blood counts and differential blood counts indicates that in most people mild anemia is present, while neutropenia is found in 1 – 2% of cases. Acute-phase reactants, accelerated sedimentation and high C-reactive protein, indicate the maintenance of process activity in patients with rheumatoid arthritis (Popovic et al, 2000). It is important to point out that differential diagnostic tests were established in 2010 in collaboration with the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) (Hochberg et al., 2015).

Conclusion

The RBC, HGB, HCT, MCV, MCH, MCHC, MPV, NE, LY, LY# values were lower in the group of patients with RA carriers of*HLA-DRB1*04* or *HLA-DRB1*03* gene variants compared to the control group without these variants. Differences for HGB and HCT were statistically significant. Nevertheless, the WBC, RDW, RDW-SD, PLT, MO, EO, BA, NE#, MO#, EO#, BA#, NLR, PLR, ESR, and CRP values were increased in the group of patients with RA in relation to the control group. The values of RDW, RDW-SD, MO, BA, MO#, BA#, ESR and CRP were significantly increased.

Conflict of Interest

The authors declare no conflict of interest.

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