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# Comparison of Inflammatory Markers and White Blood Cell Levels between Diabetic Patients and Healthy Subjects

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## ABSTRACT

**Background and Objective:** Diabetes is a chronic metabolic disease and a global health problem, and the progression of complications of this disease can be prevented by identifying a series of biomarkers. Since white blood cell count, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) are inexpensive and accessible markers for assessing inflammation, the present study was conducted to compare white blood cell, CRP, and ESR between diabetic patients and healthy subjects.

**Methods:** This cross-sectional study was conducted on 376 healthy people and 72 diabetic people who were in the second phase of Kerman Coronary Artery Disease Risk Factors Cohort Study (KERCADRS). The collected data included age, sex, weight, height, waist circumference, BMI, duration of diabetes, blood pressure, FBS, HbA1c, TG, total cholesterol, LDL, HDL, CRP, ESR, WBC, and creatinine, which were then compared with healthy subjects without underlying disease.

**Findings:** Inflammatory markers ESR and CRP and white blood cells were significantly higher in diabetic subjects compared to healthy subjects. The mean age of diabetic subjects was 57.56±10.03 years. In regression analysis with adjustment for blood pressure, age and BMI, white blood cell count (OR=1.1), lymphocyte count (OR=1.22), neutrophil count (OR=1.07), CRP (OR=1.03) and ESR (OR=1.08) showed a statistically significant association with diabetes ($p<0.05$).

**Conclusion:** The results of the study demonstrated a chronic inflammatory state in diabetic patients compared to healthy subjects, which indicates the need for effective measures to control and treat the disease.

**Keywords:** *White Blood Cells, ESR, CRP, Diabetes.*

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## Introduction

Diabetes is a chronic metabolic disease that has become a global health problem. This disease is characterized by a relative or absolute deficiency of insulin secretion accompanied by chronic hyperglycemia and impaired carbohydrate and lipoprotein metabolism (1, 2). Recent epidemiological studies support a significant increase in the prevalence of diabetes, especially type 2, worldwide. The total population of diabetic patients is expected to increase from 171 million in 2000 to 366 million by 2030 (3). Atherosclerotic cardiovascular disease is currently the leading cause of mortality and morbidity in diabetic patients. Most studies have shown that only 25% of the increased risk of macrovascular complications can be explained by conventional risk factors such as dyslipidemia, hypertension, and smoking (4). A growing body of evidence suggests that low-grade inflammation is a key component in the pathophysiology of metabolic syndrome and type 2 diabetes (5). Infiltration of inflammatory cells into adipose tissue of obese individuals increases the secretion of inflammatory cytokines and may contribute to whole-body inflammation. Chronic inflammation is associated with an increased incidence of diabetes even in the absence of obesity. For example, in patients with rheumatoid arthritis, treatment with anti-inflammatory drugs significantly reduces the incidence of diabetes (6).

Previous studies show that an increase in the number of white blood cells in individuals is one of the risk factors for developing diabetes. In a study conducted by Twig et al., an increase in white blood cells in normal young men, including factors such as triglyceride levels and family history, body mass index (BMI), and fasting blood sugar (FBS), is one of the independent and separate factors determining the risk of developing diabetes (7). Another study by Mortazavi et al. showed that the number of white blood cells was significantly higher in the group with metabolic syndrome compared to the healthy group (1).

In meta-analyses, total peripheral WBC (a non-specific marker of inflammation) was associated with an increased risk of diabetes (8). In a study conducted in 2014, the number of T helper and T cytotoxic white blood cells was higher in patients with type 2 diabetes and the number of natural killer T cells was lower than in healthy individuals (9). A study by Kashi et al. investigating the association between periodontal disease and inflammatory factors (CRP, ESR) with gestational diabetes among 200 20-weeks or higher pregnant women showed that there was no association between gestational diabetes and inflammatory factors (ESR and CRP) (10). Moreover, in a study by Jonaidi Jafari et al., it was stated that inflammatory markers have significant values for predicting infection, but ESR can be introduced as the best independent marker for predicting infection due to its high sensitivity and specificity (11). Combining ESR with CRP or with white blood cell count can increase the accuracy of the predictive performance of these markers (11).

Currently, there are limited studies regarding the changes in white blood cell count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels in this population. Therefore, addressing this issue could be very important to find potential biomarkers that can facilitate early diagnosis, monitoring, and management of diabetes. Therefore, this study was conducted to compare white blood cell count, CRP, and ESR in diabetic patients compared to healthy subjects in Kerman.

## Methods

After approval by the Ethics Committee of Kerman University of Medical Sciences with the code IR.KMU.AH.REC.1396.2161, this cross-sectional study was conducted with the aim of comparing white blood cell, ESR, and CRP in diabetic patients and healthy subjects in Kerman.

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The sample size was estimated to be at least 100 people (50 people in the case group and 50 people in the control group) based on a study by Elimam et al. (12). In this study, simple random sampling was performed on people who were in the second phase of the KERCADRS project to investigate cardiovascular risk factors in Kerman. Diabetes was defined as HbA1c higher than 6.5%, fasting blood sugar 126, or two-hour postprandial glucose higher than 200 (13). The subjects contacted the Physiology Research Center by phone, and the project manager randomly selected a number of eligible applicants based on a medical history and physical examination. After filling out a questionnaire and personal information form, a blood sample was taken for testing and sent to the laboratory.

Patients with heart failure class III and IV, GFR less than 60, history of malignancy and infectious and inflammatory diseases, pregnant women, asthma and active pulmonary disease, use of anti-inflammatory drugs and glucocorticoids, and unwillingness or lack of cooperation of the patient in performing the test were excluded from the study based on their profile and clinical examination.

In the present study, data collection was done through library and field methods using available statistics and documents. Field method was used by visiting the hospitals under study and using forms that contained the information needed for this study. This information included: age, gender, weight, height, waist circumference, BMI, duration of diabetes, blood pressure, FBS, HbA1c, TG, total cholesterol, LDL, HDL, CRP, ESR, WBC, and Creatinine.

After data collection, the data were analyzed using SPSS 24 and after determining descriptive, central, and dispersion indices. In addition, non-parametric Spearman and independent samples t-test and parametric Pearson and Chi-square tests were used to examine the differences between groups, and $p<0.05$ was considered significant.

## Results

A total of 72 participants in the diabetic group and 376 controls were included in this study. The mean age of the diabetic group was 57.5±10.03 years and the mean age of the non-diabetic group was 47.7±15.3 years, indicating a significant difference ($p<0.05$). No significant difference was observed in terms of gender between the two groups. 19 people in the diabetic group and 106 people in the healthy group were male and the rest were female.

In this study, the mean weight was significantly higher in patients with diabetes compared to the control group (13.73±16.05 vs. 53.69±12.07).

Height, waist circumference, and cholesterol levels did not show a statistically significant difference between the diabetic and non-diabetic groups. Also, the mean systolic and diastolic blood pressure were significantly higher in the diabetic group ($p=0.01$) (Table 1).

In terms of medication use, 97.22% of diabetic participants (n=65) were taking oral diabetes control medications and 7 were taking insulin. Triglyceride (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were not significantly different between the two groups. However, glycosylated hemoglobin (HbA1c), C-reactive protein (CRP), white blood cell count (WBC), lymphocyte count (LY), neutrophil count (NE), erythrocyte sedimentation rate (ESR), and body mass index (BMI) were significantly higher in the diabetic group compared to the non-diabetic group ($p<0.05$) (Table 1).

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Overall, these findings indicate significant differences in age, weight, blood pressure, medication use, duration of diabetes, fasting blood glucose levels, HbA1c, WBC, lymphocyte count, neutrophil count, CRP, and ESR between diabetic and non-diabetic individuals.

**Table 1. Comparison of demographic variables and clinical and laboratory parameters in diabetic and healthy groups**

| Variable | Diabetic (n=72) Mean±SD | Healthy (n=376) Mean±SD | p-value |
|---|---|---|---|
| Age (years) | 57.56±10.03 | 47.76±15.33 | <0.01 |
| Weight (kg) | 73.13±16.05 | 69.53±12.07 | 0.030 |
| BMI | 30.26±3.19 | 27.25±4.25 | 0.086 |
| Waist circumference (centimeters) | 93.68±9.72 | 91.62±13.57 | 0.127 |
| **Blood Pressure** | | | |
| Systolic | 127.32±14.01 | 118.15±16.68 | <0.01 |
| Diastolic | 78.83±10.03 | 75.67±9.92 | 0.014 |
| FBS | 170.43±53.13 | 112.39±62.83 | <0.01 |
| Cholesterol | 184.69±39.74 | 181.15±42.03 | 0.511 |
| Triglycerides | 151.34±60.46 | 134.57±61.65 | 0.036 |
| HDL | 51.21±12.23 | 53.80±13.02 | 0.106 |
| LDL | 104.08±33.36 | 97.8±29.29 | 0.113 |
| HbA1c | 7.71±1.9 | 6.6±0.32 | <0.01 |
| WBC | 7.4±1.68 | 6.3±4.09 | 0.027 |
| Lymphocyte | 38.87±6.45 | 36.8±7.45 | 0.030 |
| Neutrophil | 57.01±10.2 | 54.74±8.48 | 0.047 |
| ESR | 20.35±11.18 | 16.26±13.70 | 0.018 |
| CRP | 3.87±4.58 | 2.26±4.75 | <0.01 |

Binary logistic regression analysis was performed to determine the association between different factors and the risk of developing diabetes and the results are presented in Table 2. In this model, the regression was adjusted for systolic and diastolic blood pressure, age and BMI. Among the variables analyzed, cholesterol showed a positive and significant association with being diabetic (B=0.029, p=0.045). The odds ratio was 1.02, indicating that for every unit increase in cholesterol, the chance of being diabetic increased by approximately 2.9%. The lower and upper 95% confidence intervals were 1.0006 and 1.0586, respectively. Triglyceride (TG) did not show a statistically significant association with being diabetic (B=0.005, p=0.177). High-density lipoprotein (HDL) was negatively associated with being diabetic (B=-0.046, p=0.013). The odds ratio was 0.95, indicating that for every unit increase in HDL level, the odds of diabetes decreased by approximately 4.6%. The lower and upper 95% confidence intervals were 0.919 and 0.990, respectively. A positive association was also observed between diabetes and lymphocytes, neutrophils, WBC, ESR, and CRP (Table 2).

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**Table 2. Multivariate analysis of the association of blood and inflammatory factors with diabetes compared to the control group.**

| Variable | B | OR | Lower 95% confidence level | Higher 95% confidence level | p-value |
|---|---|---|---|---|---|
| Cholesterol | 0.029 | 1.029 | 1.001 | 1.059 | 0.045 |
| Triglycerides | -0.006 | 0.994 | 0.986 | 1.003 | 0.177 |
| HDL | -0.047 | 0.954 | 0.919 | 0.99 | 0.013 |
| LDL | 0.035 | 0.966 | 0.938 | 0.994 | 0.017 |
| WBC | 0.1 | 1.105 | 1.032 | 1.185 | 0.024 |
| Lymphocyte | 0.032 | 1.221 | 1.125 | 1.324 | <0.01 |
| Neutrophil | 0.052 | 1.075 | 1.014 | 1.139 | 0.018 |
| CRP | 0.035 | 1.036 | 1.046 | 1.690 | <0.01 |
| ESR | 0.028 | 1.087 | 1.004 | 1.014 | 0.036 |

## Discussion

The results of this study showed that the levels of C-reactive protein (CRP), white blood cell count (WBC), lymphocyte count (LY), neutrophil count (NE), erythrocyte sedimentation rate (ESR), and cholesterol, body mass index (BMI) were significantly higher in the diabetic group compared to the non-diabetic group. However, the difference in triglycerides between the two groups was not significant.

Previous studies have shown that an increased white blood cell count is a risk factor for diabetes. In a study by Twig et al., an increased white blood cell count in normal young men was an independent and separate determinant of the risk of diabetes (7). Factors such as BMI, FBS, triglyceride levels, and family history were included in the present study population, 72% of whom were women and 28% were men. The mean age of diabetic subjects was 56.57±10.03 years, with the youngest subject being 17 years old and the oldest being 80 years old. Our study generally showed that the levels of ESR, CRP and WBC in healthy subjects were significantly different from diabetic subjects. In a study conducted by Menart-Houtermans et al. in 2014, white blood cell count in patients with type 2 diabetes was higher than in healthy subjects, and the percentage of T-Helper Cytotoxic cells was higher and the ratio of Natural killer cells was lower (9). Furthermore, a systematic review and meta-analysis by Gkrania-Klotsas et al. showed that the risk of diabetes was 1.6 times higher in higher white blood cell count (8). A study by Lorenzo et al. also showed similar results (1.8 times higher chance of developing diabetes) (14).

The close association between WBC count and microvascular and macrovascular complications suggests that inflammation may be a common unifying factor. In support of this concept, the inflammatory process is now recognized as a major component of atherosclerosis (15). Mononuclear leukocytes are recruited to the site of endothelial injury and form foam cells in the plaque (16). Neutrophil activation leads to changes in rheological properties and adhesion to the endothelium, all of which lead to capillary occlusion and tissue ischemia (17). In addition, various cytokines and growth factors, such as interleukins, tumor necrosis factor-α, and transforming growth factor-β1 (TGF-β1), are released from activated leukocytes (18, 19) to cause endothelial dysfunction (20, 21). In this regard, leukocyte count has been shown to be an independent predictor of endothelium-dependent as well as endothelium-independent vasodilation in type 2 diabetic patients (22). Furthermore, increased secretion of TGF-β1 by mononuclear cells has been shown in patients with diabetic nephropathy (23). Increased levels of TGF-β1 in the glomeruli stimulate the proliferation of mesangial and epithelial cells, leading to the development of the typical matrix of glomerulosclerosis (24, 25). Moreover, activated leukocytes can release superoxide radicals and proteases, all of which increase

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oxidative stress. The latter can activate the transcription factor NF-κB in peripheral blood mononuclear cells. All of these pathways can lead to diabetic nephropathy (26). In general, chronic low-grade inflammatory responses can interact with other risk factors to cause widespread vascular damage, endothelial dysfunction, increased oxidative stress, and increased production of growth factors and cytokines to cause micro- and macrovascular complications in type 2 diabetes.

According to the results of this study, inflammatory markers ESR, CRP, and white blood cells in diabetic patients are significantly higher than healthy subjects. This shows a chronic inflammatory state in these patients compared to healthy subjects, which indicates the need for effective measures to control and treat inflammation and oxidative stress.

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