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ORIGINAL PAPER

A study of several hematological and immunological parameters of patients with rheumatoid arthritis and their relationship with type 2 diabetes mellitus

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ABSTRACT

Introduction and aim. Rheumatoid arthritis (RA) is a systemic inflammation that damages the joints and causes disability. In RA, glucocorticoids reduce inflammation and peripheral insulin resistance. This study aimed to investigate hematological and immunological parameters, including interleukin-24 (IL-24), interleukin-32 (IL-32), and rheumatoid factor (RF), in patients with RA, type 2 diabetes mellitus, or both, and to assess their interrelationships.

Material and methods. A case-control study on RA and type 2 diabetes mellitus was conducted at Al-Nasiriyah Education Hospital with 100 blood samples collected from patients, divided into four groups. Complete blood counts (CBC), erythrocyte sedimentation rate (ESR), RF, IL-24, and IL-32 levels were measured using automated analyzers and enzyme-linked immunosorbent assay.

Results. Patients with both diseases showed elevated ESR ($p<0.001$) and RF ($p<0.01$). RA patients increased significantly in ESR and RF, but there was no statistically significant difference in RF in type 2 diabetic patients. IL-24 was not statistically significantly increased in RA patients. IL-32 levels increased significantly in type 2 diabetes ($p=0.02$), while RA showed no significant difference.

Conclusion. Patients with RA have elevated levels of IL-32 expression and has a positive correlation with indicators of RA activity indicators such as ESR and RF. An increase in IL-24 and IL-32 in RA patients indicates a positive correlation between IL-24 and IL-32. Diabetic patients exhibit significantly elevated pro-inflammatory properties of IL-32.

Keywords. interleukin, rheumatoid arthritis, type 2 diabetes mellitus

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease that primarily affects small joints. RA is characterized by symmetric peripheral polyarthritis. Morning stiffness that lasts more than an hour and discomfort in one or more joints that lasts weeks to months are common signs of RA.[1] The prevalence of RA around the world is estimated to be between 0.5% to 1%, with a prevalence rate that is four times higher in women than in males.[2] Patients with rheumatoid arthritis have peripheral insulin resistance that is associated with inflammatory indicators and returns to normal after treatment with glucocorticoids, which reduces the level of inflammation.[3] Systemic inflammation can increase the chance of getting diabetes in the future. C-reactive protein (CRP) and other indicators of active inflammation were linked to a higher risk of diabetes in patients with RA. Among RA-affected individuals, other convention-

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al risk factors for type 2 diabetes mellitus are also very common.[4]

Diabetes can be caused by an insufficient amount of insulin produced by the pancreas or an inappropriate response by the cells of the body to the insulin that is produced.[5] Insulin resistance is a pathological condition characterized by inadequate activation of insulin receptors (IR) by insulin. Insulin resistance is associated with diabetes. Under the influence of inflammatory cytokines, IRs are expressed ubiquitously on the cell surface of adipose tissue, muscle, and a great number of other cells, such as synovial cells and T-lymphocytes.[6] RA is frequently associated with organ dysfunction. In particular, persistent synovitis, which is the result of an abnormality in the immune system, causes abnormal bone and cartilage metabolism, in addition to a continuous increase in the production of inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which results in irreversible destruction of joint tissue. Therefore, treatment must begin as soon as possible and be appropriate.[7] Patients with RA have a high prevalence of anticitrullinated peptide antibodies and rheumatoid factor (RF), which not only provide information on how the immune system is dysregulated, but also signals for clinical diagnosis and therapy of the disease.[8] Variants of RF include IgG, IgM, IgA, and IgE autoantibodies that are directed against antigenic determinants in the Fc fraction of IgG molecules. RFs are also known as autoantibodies (auto-Abs).[9]

The primary cause is unknown, and it is unclear how this inflammatory disease was triggered and began. In RA, genetic, cognitive, and psychosocial factors interact, according to the multidimensional hypothesis. The immune system mediates the spread of the disease.[10] Alterations in the number and composition of circulating blood cells, such as normochromic anemia, thrombocytosis, and lymphopenia, typically accompany systemic inflammation. This is in addition to an increase in neutrophil count. Therefore, measurement of inflammatory activity could be possible using components of circulating blood cells.[11] During the active phases of the disease, platelet counts in RA patients can increase. Platelet counts decrease as inflammation subsides, but their precise function remains unclear.[12] The erythrocyte sedimentation rate (ESR) is a diagnostic marker. Several factors, including the size, morphology, and quantity of red blood cells, as well as other plasma components such as immunoglobulins, affect RA levels.[13]

The cytokine known as IL-24 regulates cellular responses. IL-24 is associated with an increased risk of developing a number of autoimmune disorders, including psoriasis and rheumatoid arthritis (RA). IL-24 also exerts actions that are inflammatory in nature.[14] IL-24 is able to block the production of pro-inflammatory cytokines by increasing the expression of proteins that decrease the signaling of cytokines.[15] IL-24 and IL-20 are cytokines that are produced by leukocytes; nevertheless, these cytokines act most effectively in nonhematopoietic cells, particularly epithelial cells.[16] The antitumor, antibacterial, tissue remodeling and wound healing properties of IL-24 It can depend on the type of cell, the target and the stage of the immune response; the action of this cytokine may cause additional complications. Diabetes mellitus is one of the diseases influenced by IL-24 due to the effects of infections and inflammation.[17] Among the immune cells that generate IL-24 are T cells, B cells, natural killer (NK) cells, monocytes, and macrophages.[18]

IL-32 is a new pro-inflammatory cytokine that plays a crucial role in immune regulation.[19] IL-32 plays a crucial role in inflammatory disorders such as rheumatoid arthritis, ulcerative colitis, and Crohn's disease, according to previous research.[20] IL-32, also known as natural killer cell protein 4 (NK4),[21] has nine alternative transcript variants of the IL-32 gene. It was produced by epithelial cells and immune cells such as NK cells, T cells, and monocytes. Although the mechanism(s) by which IL-32 exerts its signaling capabilities are not fully understood,[22] IL-32 is capable of exerting its effects. IL-32 is an endogenous regulator that controls cytokine production, stimulating TNF-α production by macrophages. Repression of IL-32 decreased TNF production in human monocytes, demonstrating the essential pro-inflammatory properties of IL-32 and its close relationship with TNF-α.[23]

Aim

The study sought to explain differences in CBC values among patients with RA and type 2 diabetes. It also looked at the levels of IL-24 and IL-32 in patients with both disorders, as well as the sensitivity to RF in those with RA.

Material and methods

Study design

The case-control study at the Al-Nasiriyah Teaching Hospital in Thi-Qar province, Iraq, was collecting one hundred blood samples from patients. The study period was September 2022 to February 2023. They were divided into four groups, with each group of 25 patients (n=25) as follows: the first group of patients with RA, the second group of patients with type 2 diabetes, the third group of RA patients in addition to type 2 diabetes patients, and a control group that included 25 healthy people. Ethical clearance was obtained from the Dhi-Qar Health Department in Dhi-Qar city, Iraq. The reference number for ethical approval is 1461/11/3.

The study considered patients suitable for participation if they disclosed a positive diagnosis of RA. They had an expert in rheumatology and joint problems per-

form the diagnostics. Patients were not allowed to participate in the study if they were pregnant, had a history of cancer, or had a disease caused by bacteria or a virus. Each patient had a biochemical examination of their blood as part of the diagnostic process. Demographic information of the patients, such as their gender, age, family history, smoking habit, chronic illness, and occupation.

Blood samples (5 mL) were collected and divided into two parts: the first part was 2 mL in EDTA tubes, which was used to determine hematological parameters such as red blood cell count (RBC), platelet (PLA) count, hemoglobin (Hb) and total white blood cells (WBC) using a complete automated hematology analyzer (Mindray BC-5000), and ESR was measured by using a Westergren method.[24] Blood is drawn and mixed with an anticoagulant so that it remains fluid, and the red cells will gradually settle to the bottom of the Westergren container. The second part was 3 mL of blood collected in gel tubes and centrifuge tubes at room temperature at 4000 rpm for 5 minutes. The serum was separated and analyzed to determine the concentration of immunological parameters. A sandwich enzyme-linked immunosorbent assay was used to measure IL-24 and IL-32. The plate has been precoated with the human immunological parameters antibody. The immunological parameters present in the sample are added and bind to antibodies coated in the wells. A biotinylated human immunological parameters is added and binds to the immunological parameters in the sample. Then streptavidin-HRP is added and binds to the antibody with biotinylated immunological parameters. After incubation, the unbound streptavidin-HRP was washed away during a washing step. The substrate solution is then added, and the color develops in proportion to the amount of human immunological parameters. The reaction is terminated by the addition of an acidic stop solution, and absorbance is measured at 450 nm by a semiautomated ELISA reader (ELx800), company and origin Bio Tek (USA). A fully automatic clinical chemistry analyzer (Dirui, CS-T180) was used to measure RF.

Statistical analysis

All data in the current study were statistically analyzed with Microsoft Windows Excel (version 2019) and SPSS version 26 (IBM, Armonk, NY, USA) using one-way analysis of variance, least significant difference and independent sample t tests with p values of 0.05 and 0.01.

Results

According to the current results, there was no identifiable difference in WBC count, neutrophils, Hb, or PLT between the groups compared to the control group; the percentage of monocytes decreased significantly in type 2 diabetic patients compared to the control group; LYM decreased significantly in rheumatoid patients compared to control groups; and RBC decreased significantly in rheumatoid patients when compared to the control groups. The level of ESR increased significantly in patients who had both conditions. This was followed by a significant increase in patients with rheumatoid disease, which was then followed by patients who were type 2 diabetic compared to the control group. at $p<0.05$, as shown in Table 1.

Table 1. Estimate of hematological parameters in studded groups[#]

Groups Parameters	RA	T2DM	RA and T2DM	Control	p	LSD	F
	Mean±SD						
WBC (10^3/µL)	7.82±1.91	8.45±2	8.70 ±2.2	7.79±1.69	0.267	Non-sig	1.33
Neutrophils (%)	63.7±10.2	60.4 ±5.88	57.0 ±14.1	58.2±6.89	0.092	Non-sig	2.21
Monocytes (%)	6.95±1.87^{ab}	6.08 ±1.79^{b}	6.98 ±1.91^{ab}	7.61±1.98^{a}	0.045*	1.06	2.78
Lymphocytes (%)	26.1±6.78^{b}	30.8 ±6.79^{a}	31.2 ±8.40^{a}	32.5±6.30^{a}	0.011*	3.98	3.91
RBC (10^6/µL)	4.47±0.51^{b}	4.76 ±0.54^{ab}	4.60 ±0.74^{b}	4.95±0.54^{a}	0.036*	0.33	2.97
Hb (mg/dL)	11.9±1.51	12.8 ±1.31	12.4 ±1.76	12.5±1.34	0.258	Non-sig	1.36
PLT (10^3/µL)	260.2±80.1	271.3 ±66.4	254.2 ±67.5	300.7±68.2	0.101	Non-sig	2.13
ESR (mm/h)	30.5±9.95^{b}	17.4 ±5.98^{c}	43.9 ±10.6^{a}	4.56±1.26^{d}	<0.001**	4.44	114.5

[#] F table for DF 96=2.305; each p-value has two stars indicating a high significant at p value 0.01, p. value has one star indicate significant at 0.05, while p-value without star indicates a nonsignificant difference, similar small letters above the means indicate the non-significant differences, while different letters indicate the significant differences, the LSD value is used to determine the significant differences between means in the ANOVA test, where we subtract any two means from the table and compare the result of the subtraction with the LSD value, if the value of the subtraction is equal to or higher than the LSD value, it indicates a significant difference, while if it is less, it indicates that there is non-significant difference, T2DM – type 2 diabetes mellitus

According to the most recent findings, there is a statistically significant increase in the level of RF in patients who have both diseases, followed by a statistically significant increase in patients with rheumatoid arthritis compared to the control group; however, there is no statistically significant difference between patients who only have type 2 diabetes compared to the control group. IL-24 did not statistically significant increase in rheumatoid patients compared to the control group. IL-24 also did not show a statistically significant decrease in diabetic patients compared to the control

group. IL-32 levels showed a significant increase in type 2 diabetes patients, but individuals with rheumatoid arthritis showed a nonsignificant difference compared to the control group at a $p<0.05$, as shown in Table 2.

Table 2. Assessment of immune markers, RF, IL-24, and IL-32 in studied groups[#]

Immune parameters	RF (IU/mL)	IL-24	IL-32
Groups		Mean±SD	
RA	10.9±3.52^{b}	249.0±59.8^{ab}	37.3±7.82^{ab}
T2DM	5.88±0.21^{c}	226.4±52.5^{b}	40.6±12.2^{a}
RA and T2DM	15.8±4.85^{a}	228.9±52.4^{b}	35.2±9.07^{b}
Control	5.81±0.73^{c}	266.1±65.5^{a}	32.3±7.70^{b}
p	<0.01**	0.047*	0.020*
LSD	1.69	32.4	5.27
F	63.25	2.598	3.443

[#] F table for DF 96=2.305, T2DM – type 2 diabetes mellitus

Discussion

According to Table 1, the results are consistent with Korkmaz et al. The study results did not show a clear difference between the mean counts of leukocytes, neutrophils, or lymphocytes among the hemogram parameters. The results of the study agree in WBC and neutrophils, but differ for lymphocytes.[25] As acute inflammation subsides or becomes chronic, peripheral leukocyte counts fall. During the active phase of the disease, the total leukocyte count (TLC) may grow or remain normal. Chronic inflammation can induce these effects. Hormonal changes, stress, food, lifestyle and sex might affect the total leukocyte count. Due to these factors, TLC values alone cannot predict chronic inflammatory activity.[26] The impacts of various pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, and IFN-, are involved in the pathways that can be responsible for the enhancement of abnormalities in the levels of certain hematological parameters when chronic inflammation is present.[27] The results disagree with the findings of Badawy et al.[28] According to the findings of another study by Larasati et al., about patients with type 2 diabetic patients, the result did not agree with this study.[29] According to Table 1, monocytes and macrophages were the primary or possibly the only, responder cells involved in the production of TNF-α in the systems. Most disease-modifying medicines used to treat RA are designed to reduce monocytes and cytokines that are produced from monocytes. Recent research has shown that monocytes and macrophages, as monocyte-generated well as dendritic cells, play an essential part in the generation of cytokines in RA joints after being stimulated by an immune complex.[30] The important part that TNF-α plays in causing leukocyte infiltration into an inflammatory joint: TNF inhibitors were responsible for a reduction in joint leukocyte counts, resulting in a decrease in clinical scores.[31]

The current result in Table 1 did not agree with the findings of another study with the PLT, but agreed in neutrophils and lymphocytes carried out by Fu et al., where the number of neutrophils and platelets detected in the blood of RA patients was found to be much higher, while the number of lymphocytes was significantly lower.[32] The inflammatory state of RA, which is characterized by elevated serum levels of IL-6 and TNF-α as well as other inflammatory substances, may be responsible for the increase in the number of neutrophils and platelets.[33] ; these inflammatory substances can encourage the development of neutrophils and platelets in the bone marrow as well as their release.[34] Crosstalk between coagulation markers and the inflammatory system suggests that platelets may play a significant role in inflammation and immunological regulation. This hypothesis is based on the observation that platelets are present when coagulation markers are present. Platelets, when activated, release pro-inflammatory platelet microparticles. These microparticles then interact with leucocytes, which ultimately results in joint and systemic inflammation in RA patients.[35]

The results in Table 1 agree with the finding of Chen et al. and are inconsistent with the finding of Mohammed et al.[36,37] Both patients who had an active form of the disease and patients who had a latent form of the condition had lower levels of hemoglobin. Anemia may be brought on by rheumatoid arthritis for a number of reasons, including a diminished response to erythropoietin, a pathologic iron homeostasis brought on by hepcidin, and a reduced lifespan for red blood cells. Cytokines are also known to have a direct harmful effect on erythropoietin.[38]

The results of the current study are shown in Table 1. These findings are consistent with the studies conducted by Khadim and Al-Fartusie.[39] Age is correlated with a higher ESR reading. ESR increases with patient age.[40] Al-Nimer and Ratha show in this study that ESR values are significantly higher in diabetic patients.[41] RBC can keep their distinctive shapes thanks to the assistance of a phospholipid bilayer and several transmembrane proteins. Proteins that act as receptors or transporters are responsible for the elasticity and structure of RBC, as well as their interactions with the biochemical environment. Proteins also play a role in how RBC respond to stimulation. Some of these proteins, such as calpastatin, have the potential to trigger inflammatory activities in other cells or become antigens in the autoimmune environment present in the physiology of an RA patient. Due to this, the structure and contacts of RBCs may be hampered by the transmembrane protein alterations, which is a potential explanation of the recognizable phenomena of increased sedimentation rates in plasma.[42] ESR is affected by blood fibrinogen levels; therefore, even non-inflammatory situations can elevate ESR: pregnancy, diabetes, end-stage renal illness, and heart disease. In multiple myeloma, monoclonal immuno-

globulin concentrations increase significantly, which also causes increased sedimentation.[43]

The results in Table 2 show consistency with the findings of Kragstrup et al., who discovered that the levels of IL-24 in RA patients' plasma were considerably higher than in normal people's plasma, are higher than those of the current study.[44] Another study by Kragstrup et al., which discovered that plasma concentrations of IL-20 and IL-24 were higher in early RA patients, was also higher than the current study.[45] This provides more evidence in favor of the concept that changes in IL-20 and IL-24 production occurring outside the synovial joints are associated with rheumatic disease. Peripherally activated mononuclear cells that have infiltrated the synovial joint might be a source of IL-20 and IL-24 that is complementary to other sources.[46] The current result agrees with the findings of Eswadi and AL-Hellawi's study, which demonstrated a fall in serum IL-24 level in diabetic patients as compared with the controls.[47] IL-24 deficiency in diabetic patients is dangerous. Despite neuropathies, nerve injuries, and wound nonhealing, this interleukin drop may lower patient immunity. IL-24 induces innate defense mechanisms in epithelial tissues during infection and inflammation to preserve tissue homeostasis.[48] IL-24 levels recorded in a prior investigation were not the same as the levels in the current study[49] The opposite findings were caused by a number of factors, including the smaller sample size for patients with RA in the current study and the fact that half of the RA patients chosen were inactive. In addition, variations in the types and dosages of biological treatments may result in changes in IL-24 levels.[50]

The current results in Table 2 are consistent with the finding of Mohammed et al. that RA patients can benefit from a decrease in IL-32 activity, and the pathophysiology of the disease depends heavily on the quantity of cytokines, and are inconsistent with Gui et al.[51,52] The fact that IL-32 expression is related to inflammatory indicators such as ESR provides further evidence that IL-32 has an important effect on the production of inflammation in joints that are afflicted by RA.[53] According to the study of conclusions of the Abid et al., IL-32 levels were considerably higher in diabetic patients than in the control group.[54] The findings are consistent with the study.

The findings in Table 2 are consistent with those of Al-Taee et al. and Hassoon et al., who reported that the RF titer in patients with RA was substantially greater than in the control group.[55,56] Because it is well known that rheumatoid factors contain two antigen-binding sites, when B cells are stimulated by an antigen, they begin to develop into antibody-producing plasma cells. These plasma cells can produce IgM, IgG, IgA, or IgE, depending on the stimulating antigen and the location of the infection.[57] Patients with RA who have a positive RF have immune complexes in their synovial fluids. This causes the immune complexes to attach to Fc receptors on the surfaces of macrophages or toll-like receptors, which in turn activates the production of TNF-α. This cytokine, in turn, supports the synthesis of a number of other molecules and cytokines that contribute to the development of inflammation. Ultimately, this cytokine is responsible for the development of inflammation.[30] The current study agrees with the finding of Ali et al. that individuals with RA with hyperinsulinemia had highly significant RF.[58]

Study limitations

The study has several limitations. One of them is that the patients included in the study take medications that can influence the results. In addition, some patients have underlying viral or cancerous infections, which could affect outcomes. Most patients also lead an unhealthy lifestyle, which is another factor to consider. Furthermore, the presence of pregnant women among study participants introduces additional variability that can impact the findings.

Conclusion

Patients with RA have higher expression of IL-32, which is positively with RA activity markers such as ESR and RF. A rise in patients with IL-24 and IL-32 in RA suggests a positive association between the two. Furthermore, diabetic patients have significantly higher pro-inflammatory levels of IL-32. Therefore, rheumatoid arthritis should be diagnosed using IL-24 and IL-32. Furthermore, IL-32 can help in the diagnosis of diabetes.

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Declarations

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Author contributions

Conceptualization, G.O.A.; Methodology, G.O.A. and B.R.A.; Software, B.R.A.; Validation, G.O.A. and B.R.A.; Formal Analysis, G.O.A. and B.R.A.; Investigation, G.O.A.; Resources, G.O.A.; Data Curation, G.O.A.; Writing – Original Draft Preparation, B.R.A.; Writing – Review & Editing, B.R.A.; Visualization, G.O.A. and B.R.A.; Supervision, B.R.A.; Project Administration, B.R.A.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Data availability

The data sets used and analyzed in this study have been made available from the corresponding author on a reasonable request.

Ethics approval

Ethical clearance was obtained from Dhi-Qar health department in Dhi-Qar city, Iraq. The reference number for ethical approval is 1461/11/3.

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