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Early detection Rheumatoid arthritis disease is associated with a deficit in the "CD4 and CD25" in peripheral blood

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Abstract :

Objectives : is an autoimmune and inflammatory condition that causes swelling and pain in the affected areas of the body. (Rheumatoid arthritis) RA primarily targets the joints, frequently several joints at once. **Material and Methods :** 50 people with early-stage RA were included, along with 50 people in healthy controls. All subjects did not have other autoimmune disease except Rheumatoid arthritis , tumor, active infection, or a history of related drug administration. The isolation and analysis of peripheral blood mononuclear cells using flowcytometry. **Results :** The level of blood CD4 was Increase significantly in patients with RA in comparison with healthy group, 37.23 ± 7.87 versus 6.28 ± 1.46 respectively. The level of blood CD25 was decrease significantly in patients with RA in comparison with healthy group, 3.41 ± 1.07 versus 31.82 ± 7.32 respectively. **Conclusions :** Our research shows that people with Rheumatoid Arthritis have higher levels of CD4 T-cells, but lower levels of CD25 regulatory T-cells in their blood. This could lead to new ways of treating the condition and may be a reason why some people are more prone to developing Rheumatoid Arthritis.

Keyword : CD4 $^{+}$ CD25, regulatory T cell , Early Rheumatoid arthritis ,

الاكتشاف المبكر لمرض لالتهاب المفاصل الروموتيدي CD4 $^{+}$ CD25 المحيطي في الدم والارتباط مع خلل في

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الخلاصة

الأهداف : هي حالة من أمراض المناعة الذاتية والالتهابات التي تسبب التورم والألم في المناطق المصابة من الجسم. يستهدف التهاب المفاصل الروماتويدي المفاصل في المقام الأول، وفي كثير من

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الأحيان عدة مفاصل في وقت واحد. **المواد والطرق:** تم تضمين 50 شخصًا مصابًا بالتهاب المفاصل الروماتويدي في مرحلة مبكرة، بالإضافة إلى 50 شخصًا من الاصحاء . لم يكن لدى جميع الأشخاص أمراض مناعة ذاتية أخرى باستثناء التهاب المفاصل الروماتويدي أو الورم أو العدوى النشطة أو تاريخ تناول الأدوية ذات الصلة. عزل وتحليل خلايا الدم وحيدة النواة باستخدام قياس التدفق الخلوي. **النتائج :** ارتفع مستوى CD4 في الدم بشكل ملحوظ لدى مرضى التهاب المفاصل الروماتويدي مقارنة بالمجموعة السليمة، 37.23 ± 7.87 مقابل 6.28 ± 1.46 على التوالي. انخفض مستوى CD25 في الدم بشكل ملحوظ لدى مرضى التهاب المفاصل الروماتويدي مقارنة بالمجموعة السليمة، 3.41 ± 1.07 مقابل 31.82 ± 7.32 على التوالي **الاستنتاجات :** يظهر بحثنا أن الأشخاص المصابين بالتهاب المفاصل الروماتويد لديهم مستويات أعلى من خلايا CD4 T، ولكن لديهم مستويات أقل من خلايا T التنظيمية CD25 في دمائهم. قد يؤدي هذا إلى طرق جديدة لعلاج الحالة وقد يكون سببًا في جعل بعض الأشخاص أكثر عرضة للإصابة بالتهاب المفاصل الروماتويد.

Introduction:

Rheumatoid arthritis is an inflammatory condition that affects both large and small joints and has a systemic disease pattern. If left untreated, the condition is associated with a significant decline in patient quality of life and life expectancy without directly causing death (1). Swollen and tender joints are signs of the disease. With the exception of the cervical spine, the axial skeleton is typically unharmed. Investigations of the common symptoms of malaise, morning stiffness, and exhaustion show elevated acute phase reactants. Rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) are two examples of the serologic abnormalities. Joint space narrowing and bony erosion are visible on radiographs of the hands and feet. A lot of rheumatologists view ultrasound imaging as an extension of physical examination, and magnetic resonance imaging (MRI) is also helpful in the detection of early changes (2). Scientists are not sure about the main causes of RA even though they belie ve genetics and the environmental factor play a role A lot of things, including genes and things in our surroundings, can make it more likely for someone to get rheumatoid arthritis (3). CD4+Th Cells in the immune system are really important and complex. They multiply and spread to make other immune cells active. This helps the body to cause immediate immune responses (4). Activation of a certain type of immune cells called CD4 T cells is involved in the development of RA, a disease caused by problems with the immune system. The development of RA involves many molecules that regulate the immune system and are found on a type of white blood cell called CD4+ T cells (5). T-reg cells are a special type of cells that grow and get ready in the thymus gland to

control or suppress immune responses. T-reg cells are special cells found in both humans and mice. They have high levels of CD25 (IL2R) and also have a specific nuclear transcription factor called Foxp3. This factor is important for keeping the body's immune system in balance (6). The cytokines produced by T-regs cells maintain tolerance and stop the emergence of autoimmunity. The complex relationship between TH17 and T-reg cells in the pathogenesis of RA is crucial for maintaining a healthy immune system and preventing an autoimmune response (7).

Materials and Methods :

We selected 50 healthy control people who did not have Rheumatoid arthritis and 50 patients with early active Rheumatoid arthritis. According to clinical criteria and immunological tests reviewed by a specialized physician at the hospital's department of rheumatology and physiotherapy, RA was identified. Individuals were deemed clinically healthy after being cleared of any signs of RA or another autoimmune disease. Flowcytometric analysis All antibodies were employed at doses that were found to be ideal for staining in antibody titrations. Briefly, a 5 µL CD25 -FITCA was incubated with a sample of whole blood for 30 min. at room temperature. Following two rounds of phosphate-buffered saline washing, they were fixed at room temperature with 1% paraformaldehyde. Then, two-color immunofluorescence analysis was performed using MPL FC 500 (Beckman Coulter, Brea, CA). CXP software was used to analyze the data. Based on side scatter and forward scatter characteristics, a suitable gate was created around the lymphocyte population. After that, the expression of CD4 and CD25 in the gated cells was examined.

Results :

Table 1 displays the demographic information of the patients and controls who participated in this study. Man age of early diagnosis Rheumatoid arthritis patients was 51.22±10.15 years and that of healthy group was 48.52 ±8.33 years and the range between 25 and 60 years old. Evaluation of CD4 and CD25 concentration in patients with early diagnosis RA compared with healthy group is shown in table 2. The main concentration of blood CD4 was high significant in patients with early diagnosis RA in comparison with control group, 37.23 ± 7.87 versus 6.28 ± 1.46 respectively, while the level of blood CD25 was decrease significantly in patients with early diagnosis RA in comparison with healthy group, 3.41 ± 1.07 versus 31.82 ± 7.32 respectively. Table 3 displays the correlation between blood CD4 and CD25

levels and various traits. CD4 and CD25 significantly negative correlated with ESR,CRP Anti-CCP and RF, while the blood CD4 and CD25 non significantly correlated with age.

Table 1: Distribution of studied groups according to age and sex

Characteristic	Patient (early diagnosis RA) n=50	Control n=50	Total
Mean	51.22	48.52	50.21
SD	10.15	8.33	10.57
Male , n (%)	14 (28.0%)	25 (50.0%)	39 (39.0%)
Female , n (%)	36 (72.00%)	25 (50.0%)	100 (61.0%)

Table 2 : Mean levels of CD4 and CD25 in Rheumatoid arthritis patients compared to control group

Markers	Control Mean ±SD	Early diagnosis RA Mean ± SD
CD4	6.28 ± 1.46	37.23 ± 7.87
CD25	31.82 ± 7.32	3.41 ± 1.07

Table -3: Relationship between CD4 and CD2 with other traits

Characteristic	CD25	CD4

Age	*r*	-0.133	0.114
	P	0.106	0.163
ESR	*r*	-0.766**	0.707**
	P	0.000	0.000
CRP	*r*	-0.658**	0.654**
	P	0.000	0.000
RF	*r*	-0.821**	0.797**
	P	0.000	0.000
Anti-CCP	*r*	-0.882**	0.803**
	P	0.000	0.000

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION:

The current study is in accordance with Sura *et al.,* (2021), Cope, (2019), and Al-Rubaye *et al.,* (2017). Usually, people get rheumatoid arthritis between the ages of 30 and 70 (8). It is more common among people aged 45 to 75 (9). Most of the patients with RA are between the ages of 40 and 59 (10). This study found that the average age of patients with RA is 45. 44±1139 This finding agrees with what Fathi et al., (2018) found the average age was 49.1±13 (11). according to a study, More women than men have RA, which means that female hormones may contribute to the disease getting worse. Around the time of women's menopause, in the fifth decade of life, is when Rheumatoid arthritis incidence peaks. For instance, it has been suggested that oestrogen causes inflammation . In this study, we found that patients with early diagnosis of rheumatoid arthritis had higher levels of blood CD4 compared to a group of healthy people. The CD4 levels were 37. 23 ± 787 in the patients and 6. 28 ± 146 in the healthy group. This finding supports the research by Symons et al., (1991). That indicated to Intriguingly, Release of soluble CD4 indicates T helper cells and macrophages are involved in the pathogenesis of joint inflammation, particularly in RA (12).

specifically in RA The bimodality of the spread of CD4+CD28- T cell frequencies suggests that genetics plays a role in producing these strange T cells. In people with rheumatoid arthritis, a type of immune cell called CD4+CD28- T cells increase the risk of developing inflammation in tissues

outside of the joints. These cells are not a side effect of the disease, but rather contribute to the development of these inflammatory lesions.

This study found that people with rheumatoid arthritis had much lower levels of CD25 in their blood compared to the control group. The levels were 3. 41 ± 107 for the rheumatoid arthritis group and 31. 82 ± 732 for the healthy group. This result matches a study done by Carbone et al., (2014) it has been found that people with autoimmune disorders have less active immune systems and lower levels of a type of immune cell called Treg cells in their bloodstream (13). Regulatory T cells (Tregs) are very important for keeping our body's systems in balance and preventing self-attack. Another study conducted by Buckner, (2010). also consistent with our study, we found that people with rheumatoid arthritis have lower levels of blood CD25 compared to people without the condition. In both mice and humans, a lack of T-Reg cells that have CD4, CD25, and FOXP3 leads to severe autoimmune disease. Since the discovery of T-Reg cells, scientists have been studying how they protect the body from autoimmune diseases (14). In this study indicate to blood CD4 and CD25 significantly negative correlated with ESR,CRP Anti-CCP and RF .This result agreement with Xu, T. et.al.,2022. The $CD4^{+}CXCR5^{+}CD40L^{+}$ T fh cells were related to rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) (15).

Conclusion:

The study showed that patients with Rheumatoid arthritis had more CD4 cells in their blood compared to healthy people. People who have active RA might also develop an autoimmune disorder. CD4 can show how likely it is for a disease to get worse in people with active Rheumatoid arthritis. This study discovered that people with Rheumatoid arthritis have lower levels of CD25 in their blood. This could lead to new ways to treat RA and may make people more likely to develop the disease.

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Ethical approval.

Research was carried out in accordance with the ethical principles outlined in the Helsinki Declaration. A local Ethics Committee reviewed and approved the study protocol, according to the document with the number 177/30 in the date January / 17 / 2023.

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