ORIGINAL ARTICLE

Regulatory T cells in Active Systemic Lupus Erythematosus patients

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Abstract

Background: ***A complex autoimmune disease, systemic lupus erythematosus (SLE) manifests itself throughout the course of a long, relapsing-remitting illness with a broad spectrum of symptoms that can range from mild discomfort to complete disability. When it comes to autoimmune disorders like SLE, there's some indication that changes in Treg numbers or function could have a role in the development of the disease.***

The Aim of the work: ***evaluation of the proportions of CD 4+CD 25+CD 127- T regulatory cells in SLE patients in relation to disease activity.***

Patients and methods: ***One hundred patients with systemic lupus erythematosus were studied, with each patient divided into two groups based on disease activity as measured by the Systemic Lupus Erythematosus Disease Activity Index. Thirty healthy persons served as a control group.***

Results: ***When comparing the percentage value of (CD 4+CD 25+CD127-cells) between the active and control groups, as well as between the examined groups (inactive SLE and active SLE), there was a highly significant difference (P<0.001). Conversely, when looking at the absolute value of (CD4+CD25+CD127-cells), there was no statistically significant distinction (P=0.484) between the control group and the inactive SLE group.***

Conclusion: ***Tregs may be utilized as an activity biomarker due to the strong correlation between the percentage of Treg cells and SLE activity. More large-scale research is needed to determine the function of Tregs in SLE and to establish a connection between Tregs and disease parameters that impact mortality and morbidity.***

Keywords: SLE; T Regulatory cells(Tregs); (SELDAI)

1. Introduction

The autoimmune illness known as systemic lupus erythematosus (SLE) impacts several organ systems. It usually progresses in waves, with remission periods and subsequent flare-ups. Although anybody can get SLE, women between the ages of 15 and 44 are at increased risk. North America has a frequency of 241 per 100,000 individuals and an incidence of 23.2 per 100,000 person-years for SLE.[1] A subpopulation of CD4+ T cells called regulatory T cells (Tregs) is responsible for regulating the immune response and preserving tolerance.[2] There are two ways in which Tregs can carry out their role. Effector T cells (Teff) are directly impacted by suppressive cytokines secreted by Treg cells, which include IL-10, TGF-β, and IL-35.[3] In addition, they trigger cell death in their targets by producing perforin and granzyme.[4] In order to keep the immune system in check and stop autoimmune reactions, regulatory T cells (Tregs) are essential. There is some evidence that changes in Treg numbers or function may lead to the development of autoimmune illnesses such as Systemic Lupus Erythematosus (SLE). Tregs have a role in systemic lupus erythematosus (SLE) by preventing organ damage caused by complement activation and autoreactive antibody binding.[5] Disease initiation, therapeutic treatments, and disease flares determine the quantity and function of (Tregs) regulatory T cells, the last step in the breakdown of tolerance, in which B cells produce autoantibodies (autoreactive). [6]

The researchers in this study set out to determine how disease activity relates to the ratio of CD 4+CD 25+CD 127- T regulatory cells in SLE patients.

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2. Patients and methods

The research team from Al-Azhar University Hospitals' Clinical Pathology and Rheumatology & Rehabilitation departments worked together from April 15, 2023, to August 20, 2024. Based on their SELDAI scores, 100 patients with systemic lupus erythematosus were retrospectively divided into two groups: There are sixty active SLE patients in Group 1. Forty patients with relapsed SLE are part of Group 2. In contrast, the third group consisted of 30 healthy adults who were matched for age and sex. Every patient underwent a comprehensive evaluation that included a physical examination, a review of their medical history in accordance with the 2019 SLICC criteria for lupus, and the SELDAI-2K index. We used the SELDAI-2K calculator to get data for the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) from each patient's record. (If the description was written during the visit or during the past 10 days).

Inclusion criteria:

Age>16 years, Patients with active systemic lupus erythematosus(>3-4 SELDAI).

Exclusion criteria:

Individuals suffering from rheumatoid arthritis and other rheumatological conditions, Psoriasis, JIA, IBD, and overlap or mixed connective tissue diseases. Chronic specific infection(HBV, HCV, HIV), Endocrinopathies(hypothyroidism, hyperthyroidism, and type 1 DM). Malignancy. All patients with hypertension or DM.

All samples from all participants were submitted to the following:CBC, ESR, CRP, Creatinine, ALT, AST, Albumin/Creatinine ratio, ANA, Anti dsDNA, C3, and C4. Assessment of CD4+, CD25+ and CD127- cells level by flowcytometry Using a commercially available kit supplied by BD Biosciences, United States (kits was adapted on using Flow cytometer Becton Dickenson(BD) FACS Canto).

STATISTICAL ANALYSIS

Version 25 of SPSS, a statistical tool tailored for social science research, was used to examine the data. Standard deviation (SD) was used to express the quantitative data. In order to depict qualitative, percentages and frequencies were used. The midpoint of a collection of discrete integers is called the mean or average. To get it, take the total number of values and divide it by that number. As a measure of dispersion, the standard deviation (or SD) is applied to a set of data. Values that are highly dispersed across a larger range have a large standard deviation (SD), whereas values that are relatively near the mean have a small SD.

The tests listed below were carried out:

Quantitative data normality was tested using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Two means were compared using the independent-samples t-test of significance for correctly distributed data. Non-parametric data were compared using the chi-square test. A one-way ANOVA analysis of variance(F) for continuous quantitative data comparing more than two groups. Post Hoc test: multiple group comparisons. ROC curve: used to determine cutoff value, sensitivity, specificity, PPV, NPV, and AUC. AUC: sums up test performance in discriminating between two groups. For probability (P-value), a value below 0.05 was deemed significant, a value below 0.001 was deemed extremely significant, and a value over 0.05 was deemed insignificant. When the disease is present, the likelihood of a positive test is known as sensitivity. A negative test result in the absence of illness is an example of specificity. When a test comes back positive, the positive predictive value indicates the probability of illness. When a test comes back negative, the negative predictive value indicates the probability that the disease is not present.

3. Results

In all, 130 people participated in the research. Three groups were formed from the subjects: Initially, there was Group I, which consisted of 60 individuals who were diagnosed with active SLE. The group consisted of sixty-five females. Their ages varied from seventeen to forty-one. From zero to twelve years is the typical length of the condition. Presented with inactive SLE were forty individuals who made up Group II. There were forty of them. They were all in the 19–45 age bracket. From one year to thirteen years, the condition can last. Thirty people of similar age and sex who seemed to be in good health made comprised Group III, the control group.

In terms of CD 4+CD 25+CD127-cells (percentage and absolute value), there was a highly significant (P<0.001) decline when comparing all groups that were analyzed using the ANOVA test. Group I had a significantly lower percentage of CD 4+CD 25+CD127 cells (0.4±0.4, range: 0-1.6) compared to group II (1.5±0.9, range: 0.6-4.7) and group III (1.1±0.3, range: 0.5-1.6), with a p-value of less than 0.001. In comparison to groups II and III, the absolute value of CD 4+ CD 25+ CD127-cells in group I was 7.1±10.1 with a range of 0-43.7, 27.3±19.4 with a range of 4.7-87.4, and 24.9±13 with a range of 8.9-49, respectively. Table (1)

Table 1. Evaluation of each group in terms of CD 4+CD 25+CD127-cells (absolute value and %).

CD 4+CD 25+CD127- cells		Group-I (n=60)	Group-II (n=40)	Group II (n=30)	F	P-value
Percentage	Mean±SD	0.4±0.4	1.5±0.9	1.1±0.3	48.48	<0.001 HS
	Min-Max	0-1.6	0.6-4.7	0.5-1.6		
Absolute value	Mean±SD	7.1±10.	27.3±19.	24.9±13	29.69	<0.001 HS
	Min-Max	0-43.7	4.7-87.4	8.9-49		

At Table 2 for the Post-Hoc test results: When looking at the proportion of cells that were CD4+CD25+CD127-, there was: Between the two

groups that were initially studied, there is a highly significant difference (P<0.001), between the two groups that were initially studied and the third, and between the two groups that were subsequently studied, there is a statistically important difference (P=0.004). The absolute value of CD 4+CD 25+CD127- cells did not show a statistically significant difference (P=0.484) between groups II and III, however there was an extremely significant distinction (P<0.001) between groups I and II and a highly significant variation (P<0.001) between groups I and III.

Table 2. Comparison of CD4+CD25+CD127-cell percentages and absolute values among patient groups: a post hoc test.

CD 4+CD 25+CD127- cell		Group-I Vs Group-II	Group I Vs Group-III	Group-II Vs Group-III
Percentage	LSD	-1.167	-0.7	0.4
	P-value	<0.001	<0.001	0.004
Absolute value	LSD	-20.2	-17.8	2.4
	P-value	<0.001	<0.001	0.484

Using roc curve, it showed that: Proportion of (CD 4+CD 25+CD127-cells) was excellent(AUC=0.945) in discriminating patients of group-I from patients of group II at cut off value of≤0.7 with sensitivity(83.3%), specificity(95%), PPV(96.2%), NPV(79.2%) and P-value<0.001.Table(3) Also, Absolute value of(CD 4+CD 25+CD127- cells) was very good(AUC=0.881) in discriminating patients of group I from patients of group-II at cut off value of≤10.75 with sensitivity(86.7%), specificity(85%), PPV(89.7%), NPV(81%) and (P-value <0.001).Table(4)

Table 3. Diagnostic performance of Percentage of(CD4+CD25+CD127-cells) in discriminating patients of Group-I from patients of Group-II.

CD 4+CD 25 CD127-cells	Cut of	AUC	Sensitivity	Specificit	PPV	NPV	p-value
Percentage	≤0.7	0.945	83.3%	95%	96.2%	79.2%	<0.001

Table 4. Diagnostic performance of absolute value of (CD4+ CD25+ CD127-cells) in discriminating patients of Group-I from patients of Group-II.

CD 4+CD 25 CD127-cells	Cut of	AUC	Sensitivi	Specificit	PPV	NPV	p-value
Absolute valu	≤10.75	0.881	86.7%	85%	89.7%	81%	<0.001

4. Discussion

Salah et al.,[7] discovered that the percentage of regulatory T cells (Tregs) was noticeably lower in patients with systemic lupus erythematosus (SLE) compared to healthy controls (0.1% to 0.9% opposed to 0.9% to 2.1%, p<0.001). There was a negative connection (p<0.001) between the percentile of Treg cells and SLEDAI-2k with respect to SLEDAI-2k. Additionally, the damage index (SLICC/ACR DI) and the percentile of Treg cells exhibited a negative connection (p<0.001). They discovered a significantly positive connection (p<0.001) between SLEDAI-2 2K and the damage index (SLICC/ACR DI).

There was a highly significant (P<0.001) difference in the percentage of CD 4+CD 25+CD127-T regulatory cells between the active and inactive patient groups, a highly significant (P<0.001) difference between the active and control groups, and a statistically significant (P = 0.004) difference between the inactive and control groups. The findings from this study aligned with those from Crispin JC et al.,[8] which proves that sickle cell disease (SLE) patients have fewer CD4+CD25+T cells than healthy controls (P<0.001).

Additionally, research performed by Miyara et., al,[9] revealed a correlation between the clinical severity of the flare and the level of Tregs depletion. Another possible link between SLE physiopathology and a failure in Treg homeostatic regulation was highlighted in this study.

As well as Zhao et al.,[10] discovered a negative correlation between the SLE disease activity index and the quantities of three cell types: CD4+CD25+CD127(low/-) T cells, CD4+CD25high T cells, and CD4+CD25+FOXP3+ T cells, together with their ratios to CD4+CD25+ T cells (P<0.05).

In addition, research carried out by Atfy et al.,[11] showed that the number of CD4+CD25high cells in peripheral blood were shown to be significantly lower in active lupus patients compared to inactive lupus patients and healthy controls. Consequently, research carried out by Habibagahi et., al[12] found that active patients had far lower levels of CD4+CD25hi Tregs than healthy persons. In patients with active disease, there was an inverse correlation between the percentage of CD4+CD25hi cells and the SLEDAI disease score.

Likewise, research carried out by Ma et al.,[13] found a negative correlation between the values of the SLE disease activity index (SLEDAI) and the counts of CD4(+) CD25(+) FoxP3(+) T cells. In conjunction with Tselios et al.,[14] Results demonstrated a marked decrease in regulatory T cells (Tregs) in patients with very active disease as compared to healthy controls, patients with no disease activity, patients with mild disease activity, and patients with moderate disease.

Furthermore, Żabińska et al.[15], The study found that the percentage and absolute number of CD4+CD25+Foxp3+ lymphocytes varied significantly between the groups with active lupus nephritis and those without. Also included, Maher et al[16]. Demonstrated that the Th17/Treg ratio was markedly elevated in the active SLE and juvenile SLE cohorts relative to the inactive SLE and juvenile SLE cohorts, as well as the control groups. Treg cells had a favorable correlation with absolute neutrophil count, platelets, C3, and C4 in both pediatric and adult systemic lupus

erythematosus (SLE.

On the other hand, Zhang et al.,[17] determined that the frequency of CD4(+) CD25(+) Foxp3(+) T cells did not differ significantly between normal controls and persons with active or dormant SLE.

Moreover, Hu S et al.,[18] discovered that there was a substantial decrease in Treg levels (P<0.01) throughout the active SLE and inactive SLE groups when compared to the normal control group. Concerning Treg levels, there was no statistically significant variation among the active and inactive individuals (P>0.05).

Also, Yates et al.,[19] examined 21 patients with SLE and 6 patients with dormant illness in terms of prevalence, phenotype, and function. Although the fraction of CD4(+) CD25(+) T cells increased in patients with active illness, they did not observe a decrease in the frequency of the CD25(hi) subset.

The most reliable protein indicator for identifying Tregs up to this point is the nuclear transcription factor Foxp3+, which plays a crucial role in Treg formation and activity. Evidence suggests that CD127 can replace Foxp3 in several ways. A popular and effective method for isolating live cells is to co-stain CD25 and CD127; this allows one to easily identify Tregs from other T cells. Another potential source of variation is the selection of SLEDAI thresholds for distinguishing between active and inactive SLE. A smaller percentage of Tregs was often achieved when the SLEDAI score, used to characterize active SLE, was higher.

There were inconsistencies in the results since the diagnostic criteria used in the eligible publications were also inconsistent.

4. Conclusion

The results show that there is a strong correlation between the percentage of regulatory T cells (Tregs) and SLE activity, suggesting that Tregs could be employed as a damage marker and activity biomarker. Additionally, Tregs have the potential to aid in evaluating disease status in contentious situations and preventing additional irreversible harm as activity and damage biomarkers. Further evidence of its protective function in SLE patients against the development of activity comes from the finding of a decreased amount of Tregs in active SLE.

Disclosure

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