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Evaluation of CD4/CD8 Ratio in Vitiligo Patients and Its Correlation to Activity and Severity of the Disease

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ABSTRACT

Background: The loss of melanocytes from the epidermis causes vitiligo, a common acquired skin condition that shows up as distinct white spots on the body. Its pathophysiology is closely linked to autoimmunity. The imbalance of peripheral blood lymphocytes, mostly cytotoxic T cells (CD8+ cells) and helper T cells (CD4+ cells), is associated with the death of melanocytes.

Aim: This study aimed to determinate CD4+/CD8+ ratio in vitiligo patients and control, to assess the correlation between CD4+/CD8+ ratio and activity and severity of vitiligo and to correlate between the levels of these T-cell subsets with activity and severity of the disease.

Patients and methods: This case control study was conducted at Dermatology Department, Suez Canal University hospital from May 2023 to June 2024. This study included 44 vitiligo patients and 44 apparently healthy controls. All participants underwent full history taking, full physical examination and investigations including Complete blood count and flowcytometry analysis for memory T cells (CD3, CD4 and CD8).

Results: A significant correlation was detected between CD3+/4+ (absolute) and vitiligo area severity index (VASI) score, lymphocytes % and absolute lymphocytes in addition. There was a significant correlation between CD3+/8+ (absolute) and lymphocytes % and absolute lymphocytes in patients group. However, no significant difference was recorded between active and non-active patients regarding relative and absolute CD3+ and absolute CD3+/4+.

Conclusion: This study demonstrated that CD3+, CD4+ and CD8+ may be implicated in the pathogenesis of vitiligo.

Keywords: Vitiligo, CD3+, CD4+, CD8+.

INTRODUCTION

The most prevalent depigmentation disorder is vitiligo, which has a prevalence of 0.06 to 2.28% worldwide. It is distinguished by the lack of skin color, which results from the death of melanocytes. Melanocytes are found in the skin, hair follicles, eyes, inner ear, bones, heart, and brain, among other tissues [(1)].

Melanocytes, the skin's pigment-producing cells, are the target of autoreactive CD8+ T lymphocytes in vitiligo, an autoimmune skin condition. Patients see uneven white blotches on their skin as a result. About 1% of people have vitiligo, which is gender-neutral and typically diagnosed before the age of thirty. Patients' self-esteem and quality of life are significantly impacted by vitiligo [(2)].

Like many other autoimmune diseases, vitiligo susceptibility is impacted by complex combinations between genetic, environmental, and stochastic variables. Although the exact cause of disease is unknown, either intrinsic or external cellular stress may be involved. In healthy human skin, melanocytes are found in the hair follicle and the epidermis, where they supply pigment [(3)].

Only the epidermal melanocytes are targeted for destruction in vitiligo patients, but melanocytes found in the hair follicles remain undamaged due to immune privilege mechanisms within the hair follicle. Thus, vitiligo can be reversed by suppressing the immune system and causing melanocyte precursors in the hair follicle to proliferate, migrate, and replenish lost epidermal melanocytes through a procedure known as perifollicular repigmentation [(4)].

Human vitiligo is known to be mediated by CD8+ T lymphocytes, which are both required and sufficient. Early research revealed that the quantity of HLA-A2 melanocyte-specific CD8+ T cells in vitiligo patients' blood was correlated with the severity of the condition and that these cells expressed high quantities of cutaneous lymphocyte-associated antigen, a skin homing receptor [(5)]. Additionally, vitiligo patients' isolated melanocyte-specific CD8+ T cells had the capacity to lyse melanoma cells and HLA-A2-matched peptide pulsed cells ex vivo, while non-specific CD8+ T cells lacked this capacity. When vitiligo patients' skin cells were examined via suction blistering, it was discovered that there were significantly more CD8+ T cells in active illness as opposed to stable, non-lesional, and healthy control skin [(6)]. Another study showed that when cultured ex vivo, perilesional CD8+ T cells isolated from vitiligo skin could kill melanocytes from normal pigmented skin isolated from the same patient, proving that melanocyte-specific CD8+ T cells are both necessary and sufficient for the destruction of melanocytes [(7)].

A considerable percentage of vitiligo patients have circulating autoantibodies and autoreactive CD8+ cytotoxic T-cells in their sera that are able to identify pigment cell antigens. In example, it was shown that cytotoxic T cells and autoantibodies were more prevalent in active vitiligo cases. The balance of helper/inducer and

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cytotoxic/suppressor T-cells (CD4+⁄CD8+ ratio) Most vitiligo patients have disturbed peripheral blood, which could lead to the preponderance of particular T-cell subtypes [8].

Regarding the CD4+/CD8+ ratio in vitiligo patients, several researches have produced conflicting findings. As such, there is ongoing debate on the frequency and significance of the CD4+/CD8+ ratio in these patients. Therefore this study aimed to investigate the relationship between the degree and activity of vitiligo and the CD4+/CD8+ ratio.

PATIENTS AND METHODS

This case control study was conducted at The Dermatology Department of Suez Canal University Hospital between May 2023 and June 2024. Participants were divided into: Patient group which included patients with vitiligo (n = 44) who were obtained from The Outpatient Clinic of The Dermatology department at Suez Canal University Hospital in Ismailia, whereas the control group was composed of 44 blood donors who were selected from the hospital's blood bank and seemed to be in fair health.

Inclusion criteria: i) Patients' group: Patient diagnosed with vitiligo. Patient age >18 years. Both genders. **ii) Control group:** Apparently healthy blood donors matched for age and gender to the study group.

Exclusion Criteria: Atopic dermatitis, psoriasis, insulin-dependent diabetes, autoimmune disorders other than vitiligo (such as rheumatoid arthritis), or any other dermatological condition that results in depigmentation. Additional inflammatory skin conditions or cancers. Acute or chronic infections, such as TB, hepatitis B, or hepatitis C. Using immunosuppressive medications such as methotrexate, pregnant and nursing mothers.

Study variables:

1) The CD4+⁄CD8+ ratio is an independent variable.
2) Dependent variables: The disease's duration, progression, and severity.
3) Background factors: Sex and age.
4) Confounding factors: Medications that may impact vitiligo and systemic skin conditions.

Every participant had their complete medical history taken, with particular attention paid to the age at which the disease began, how it progressed, how long they were ill, and whether they developed new depigmented lesions at the locations of skin trauma or itching in previously healthy skin. Both general and local examinations, such as Wood's light examination (which shows bright bluish white when vitiligo lesions are examined), are used to determine whether trichome or confetti-like lesions are present, as has been previously documented [9].

Vitiligo area severity index (VASI): It is named after the PASI score for psoriasis. The hand units are used to calculate the proportion of vitiligo involvement. About 1% of the body's surface area is represented by one hand unit. The following percentages are used to estimate the degree of pigmentation to the closest one: 100% indicates total depigmentation, meaning there is no pigment present, 90% indicates pigment specks, 50% means that the pigmented and depigmented areas are equal, while 75% means that the depigmented area is greater than the pigmented area, 10% means that there are only a few spots of depigmentation, and 25% means that the pigmented region is larger than the depigmented area**.** The VASI for each body location is calculated by multiplying the area of vitiligo in hand units by the degree of depigmentation in each hand unit measured patch. S Everybody site [Hand Units] ´ [Residual depigmentation] = Total body VASI [10]**.**

Vitiligo disease activity score (VIDA): VIDA score [11], is a six-point rating system used to assess vitiligo activity. The foundation of a person's VIDA score is their personal viewpoint. Six stages make up the VIDA score based on patients' opinions:
+4: Activities lasting six weeks or less.
+3: Six weeks to three months of activity.
+2: Three to six months of activity.
+1: 6–12 months of activity.
0: Stable for a minimum of one year, **and −1:** Stable for a minimum of one year with spontaneous repigmentation. Less activity is indicated by lower VIDA scores.

Laboratory investigations: Every sample was gathered with written informed consent and with Suez Canal University's Ethics Committee's approval. For the first step, a fully automated 5-part differential hematology analyzer (XN1000) collected three milliliters of blood in a tube containing dipotassium ethylene diamine tetra acetic acid (k3 EDTA).

Analysis of flow cytometry: Two tubes, one unstained and the other containing a panel of conjugated monoclonal antibodies (MoAb) for memory T cells (CD3, CD4, and CD8), were labeled for each sample.

conjugated monoclonal antibodies	Description
CD3	perCP- Cy5.5 Clone (UCHT1)
CD4	FITC Clone (IM0448U)
CD8	CD8-PE Clone (IM0452U)

Protocol used for WBCs surface staining: Fill fluorescence-activated cell sorting (FACS) tubes with 100ul of whole blood, add 5µl of each mAb, and vortex

that were incubated at 40°C for 20 minutes. Following surface staining, 2 milliliters of the prepared lysing solution were added to complete the lysis process.

The lysing solution was made by adding nine volumes of DW to one volume of P-concentrated lysing solution, resulting in a dilution of 10X. Vortex and centrifuge for 5 minutes at 1500 rpm after vortexing and incubation for 10 minutes in the dark. After two rounds of washing with 2 milliliters of PBS (0.5% BSA), the pellets were centrifuged for five minutes at 1500 rpm. After removing the supernatant, the pellet was reconstituted in 300 µl of PBS and stored at 4 °C until it was acquired.

Data interpretation:

The FACS Calibur Flow Cytometer (Becton Dickinson Immunocytometry Systems, USA) was used for the flow cytometric analysis, and The Cell Quest software was used to analyze the data. At least 20,000 occurrences were obtained. Gating was completed. Based on their light scattering properties and surface staining for CD45 using monoclonal antibodies, lymphocytes were recognized and gated. Surface labeling for CD3 using monoclonal antibodies allowed for the identification of T cells. The CD4+/CD8+ ratio, the percentage of CD3+CD4+ T cells (T helper cells), and the percentage of CD3+CD8+ T cells (T cytotoxic cells) were then computed.

Ethical approval: The World Medical Association's (WMA) Declaration of Helsinki ethical guidelines for human subjects medical research were followed in the conduct of this study (WMA, 2014). Each patient involved in this study provided written informed permission. The Suez Canal University Ethics Committee approved this study.

Statistical analysis

The data were entered into the computer and examined using the IBM SPSS software package, version 20.0. (Armonk, NY: IBM Corp.). The Spearman coefficient, F-test (ANOVA), Mann Whitney, student t-test, Chi-square test, Kruskall Wallis test, and Spearman coefficient were all used. $P \leq 0.05$ was deemed significant.

RESULTS

The two groups differed significantly in terms of absolute lymphocytes, the patients' lymphocytes were lower than those of the control group. However, there was no discernible variation in the relative lymphocytes (Table 1).

Table (1): Comparison between the two studied groups according to Lymphocytes

	Patient (n = 44)	**Control (n = 44)**	**t**	**p**
Lymphocytes %				
Min. – Max.	19.90 – 69.60	17.70 – 72.90		
Mean ± SD.	44.69 ± 12.45	44.16 ± 13.38	0.192	0.848
Median (IQR)	43.35 (33.85 – 53.65)	45.60 (31.15 – 54.25)		
Lymphocyte (Absolute) (cell/L)				
Min. – Max.	924.1 – 4968.6	1008.9 – 6193.8		
Mean ± SD.	2899.9 ± 865.8	3454.2 ± 1413.1	2.219	**0.030***
Median (IQR)	2865.0 (2551.5 – 3316.4)	3377.7(2293.3 – 4246.3)		

IQR: **Inter quartile range**, SD: **Standard deviation, t: Student t-test,** p: p value for comparing between the two studied groups, *: Statistically significant at $p \leq 0.05$

Relative and absolute CD3+, absolute CD3+/4+, and absolute CD3+/8+ were significantly different between the two groups, with the patients having lower levels than the control group (Table 2).

Table (2): Comparison between the two studied groups according to Flow cytometry

	Patient (n = 44)	**Control (n = 44)**	**Test of Sig.**	**P**
CD3+ (%)				
Min. – Max.	25.30 – 83.80	43.20 – 87.80	t= 3.458*	**0.001***
Mean ± SD.	62.70 ± 13.36	71.30 ± 9.67		
Median (IQR)	63.80 (53.10 – 72.80)	72.50 (64.85 – 78.75)		
CD3+ (Absolute)				
Min. – Max.	370.6 – 3964.9	713.3 – 5208.7	t= 3.127*	**0.002***
Mean ± SD.	1843.6 ± 745.6	2445.9 ± 1037.6		
Median (IQR)	1749.7 (1366.8 – 2300.1)	2317.5 (1504.4 – 3068.8)		
CD3+/4+ (%)				
Min. – Max.	19.10 – 71.20	30.0 – 77.10	t= 1.975	0.051
Mean ± SD.	49.16 ± 11.56	53.92 ± 11.03		
Median (IQR)	51.65 (41.95 – 56.60)	54.55 (47.05 – 60.40)		
CD3+/4+ (Absolute)				
Min. – Max.	129.0 – 2172.8	255.4 – 3541.9	t= 3.251*	**0.002***
Mean ± SD.	930.4 ± 464.5	1346.2 ± 710.0		
Median (IQR)	869.4 (569.9 – 1310.3)	1178.2 (853.4 – 1589.7)		
CD3+/8+ (%)				
Min. – Max.	23.50 – 75.90	20.90 – 66.20	t= 1.070	0.288
Mean ± SD.	46.32 ± 10.66	43.89 ± 10.65		
Median (IQR)	45.20 (38.90 – 53.0)	43.05 (37.95 – 50.75)		
CD3+/8+(Absolute)				
Min. – Max.	190.8 – 1708.9	312.5 – 2418.1	U= 708.000*	**0.030***
Mean ± SD.	834.7 ± 355.6	1047.4 ± 454.4		
Median (IQR)	782.3 (559.7 – 999.5)	988.1 (679.1 – 1381.9)		
CD4/CD8 Ratio				
Min. – Max.	0.25 – 3.03	0.45 – 3.69	U= 812.000	0.193
Mean ± SD.	1.18 ± 0.54	1.38 ± 0.67		
Median (IQR)	1.16 (0.80 – 1.45)	1.27 (0.93 – 1.60)		

t: Student t-test, U: Mann Whitney test, p: p value for comparing the two groups under study, IQR: Interquartile range, SD: Standard deviation, and *: $p < 0.05$ indicates statistical significance.

Regarding relative CD3+, which was lower in the active group than in the control group, and absolute CD3+ and absolute CD3+/4+, which were significantly different between the active and control groups where they were lower in the active group than in the control group and lower in the non-active group than in the control group. There was no appreciable difference between active and non-active patients in terms of absolute and relative CD3+ or absolute CD3+/4+ (Table 3).

Table (3): Comparison between non active, active and control according to CD

	Non active (n = 12)	Active (n= 32)	Control (n = 44)	Test of Sig.	P
CD3+ (%)					
Min. – Max.	40.10 – 83.80	25.30 – 81.50	43.20 – 87.80	F= 6.055	**0.003***
Mean ± SD.	64.16 ± 15.83	62.15 ± 12.55	71.30 ± 9.67		
Median (IQR)	66.90 (49.15 – 78.45)	63.60 (56.70 – 68.35)	72.50 (64.85 – 78.75)		
Sig.bet.Grps	p_1=0.869,p_2=0.153,p_3=0.003*				
CD3+ (Absolute)					
Min. – Max.	370.6 – 3155.2	665.1 – 3964.9	713.3 – 5208.7	F= 4.976*	**0.009***
Mean ± SD.	1730.2 ± 784.8	1886.1 ± 738.7	2445.9 ± 1037.6		
Median (IQR)	1637.8 (1255.60 – 2341.80)	1797.7 (1428.55 – 2157.30)	2317.5 (1504.4 – 3068.8)		
Sig.bet.Grps	p_1=0.868,p_2=0.046*,p_3=0.025*				
CD3+/4+ (%)					
Min. – Max.	30.90 – 62.20	19.10 – 71.20	30.0 – 77.10	F=2.301	0.106
Mean ± SD.	46.81 ± 10.25	50.04 ± 12.04	53.92 ± 11.03		
Median (IQR)	46.25 (39.15 – 55.15)	52.80 (43.50 – 56.75)	54.55 (47.05 – 60.40)		
CD3+/4+ (Absolute)					
Min. – Max.	170.5 – 1583.7	129.0 – 2172.8	255.4 – 3541.9	F=2.485*	**0.006***
Mean ± SD.	829.2 ± 446.3	968.3 ± 472.4	1346.2 ± 710.0		
Median (IQR)	717.9 (555.65 – 1195.40)	914.6 (595.95 – 1310.30)	1178.2 (853.4 – 1589.7)		
Sig.bet.Grps	p_1=0.774,p_2=0.027*,p_3=0.022*				
CD3+/8+ (%)					
Min. – Max.	34.60 – 62.10	23.50 – 75.90	20.90 – 66.20	F=1.041	0.358
Mean ± SD.	48.86 ± 8.82	45.37 ± 11.25	43.89 ± 10.65		
Median (IQR)	48.60 (42.05 – 55.05)	43.15 (36.65 – 52.75)	43.05 (37.95 – 50.75)		
CD3+/8+(Absolute)					
Min. – Max.	190.8 – 1587.0	240.8 – 1708.9	312.5 – 2418.1	H=4.726	0.094
Mean ± SD.	825.9 ± 388.4	838.0 ± 349.0	1047.4 ± 454.4		
Median (IQR)	771.4 (594.05 – 942.85)	782.3 (559.70 – 1043.25)	988.1 (679.1 – 1381.9)		
CD4/CD8 Ratio					
Min. – Max.	0.50 – 1.80	0.25 – 3.03	0.45 – 3.69	H=3.229	0.199
Mean ± SD.	1.02 ± 0.40	1.23 ± 0.58	1.38 ± 0.67		
Median (IQR)	0.96 (0.73 – 1.32)	1.25 (0.83 – 1.53)	1.27 (0.93 – 1.60)		

IQR: Inter quartile range, **SD:** Standard deviation, **F:** F for One way ANOVA test, Pairwise comparison bet. Each 2 groups was done using Post Hoc Test (Tukey), **H:** H for Kruskal Wallis test, ***:** Statistically significant at $p \leq 0.05$

P: p value for comparing between the three studied groups. **p_1:** p value for comparing between. **Non active and active. p_2:** p value for comparing between **Non active and control, p_3:** p value for comparing between **Active and control**

In the patients' group, there was no discernible relationship between CD and the VES score (severity) (Table 4).

Table (4): Correlation between VES score (severity) % and CD in patients group (n= 44)

	VES score (severity) %	
	r_s	**p**
CD3+ (%)	0.028	0.859
CD3+ (Absolute)	0.180	0.244
CD3+/4+ (%)	0.153	0.323
CD3+/4+ (Absolute)	0.181	0.239
CD3+/8+ (%)	-0.140	0.364
CD3+/8+(Absolute)	0.074	0.633
CD4/CD8 Ratio	0.141	0.361

r_s: Spearman coefficient

There was no significant correlation between CD4/CD8 Ratio and different parameters in patients group (Table 5).

Table (5): Correlation between CD4/CD8 Ratio and different parameters in patients group (n= 44)

	CD4/CD8 Ratio	
	r_s	p
Age (years)	0.100	0.517
Age of onset (years)	0.060	0.700
Duration (years)	-0.005	0.973
VIDA score	-0.058	0.707
VASI score	0.195	0.205
VES score (severity) %	0.150	0.330
Hb (gm/dl)	0.189	0.219
HCT (%)	0.119	0.440
RBCs	-0.288	0.058
WBCs (cell/L)	-0.274	0.072
Platelets (x109/L)	-0.271	0.075
Neutrophil (%)	0.053	0.735
Neutrophil (Absolute) (cell/L)	0.190	0.217
MID %	-0.126	0.417
MID (Absolute) (cell/L)	-0.010	0.950
Lymphocytes %	-0.144	0.351
Lymphocyte (Absolute) (cell/L)	-0.012	0.940

r_s: Spearman coefficient.

There was a substantial positive correlation between CD3+/8+ (Absolute) and WBCs, Lymphocytes %, and Lymphocyte (Absolute) with significant differences in differentiating patients from control (Table 6).

Table (6): Correlation between CD3+/8+ (Absolute) and different parameters in patients group (n= 44)

	CD3+/8+ (Absolute)	
	r_s	p
Age (years)	-0.114	0.460
Age of onset (years)	-0.058	0.707
Duration (years)	-0.015	0.922
VIDA score	-0.053	0.735
VASI score	0.149	0.334
VES score (severity) %	0.074	0.633
Hb (gm/dl)	0.00	1.000
HCT (%)	0.044	0.779
RBCs	0.127	0.410
WBCs (cell/L)	0.412*	0.005*
Platelets (x109/L)	0.020	0.896
Neutrophil (%)	-0.291	0.055
Neutrophil (Absolute) (cell/L)	0.099	0.521
MID %	-0.201	0.191
MID (Absolute) (cell/L)	0.081	0.599
Lymphocytes %	0.361*	0.016*
Lymphocyte (Absolute) (cell/L)	0.665*	<0.001*

r_s: Spearman coefficient**, *:** Statistically significant at $p \leq 0.05$.

There was no relation between CD4/CD8 ratio and different parameters in the patients' group except family history that CD4/CD8 ratio was significantly lower in patients with a positive family history than in patients with a negative family history (Table 7).

Table (7): Relation between CD4/CD8 Ratio and different parameters in patients group (n= 44)

	N	CD4/CD8 Ratio		Test of Sig	p
		Mean ± SD.	Median (Min. – Max.)		
Sex					
Male	**25**	1.16 ± 0.55	1.04 (0.47 – 3.03)	U= 224.000	0.749
Female	**19**	1.20 ± 0.53	1.22 (0.25 – 2.33)		
Family history					
No	**34**	1.26 ± 0.57	1.28 (0.25 – 3.03)	U= 96.000*	0.038*
Yes	**10**	0.90 ± 0.27	0.89 (0.61 – 1.30)		
Chronic illness					
No	**36**	1.14 ± 0.45	1.16 (0.25 – 2.33)	U= 134.000	0.777
Yes	**8**	1.35 ± 0.85	1.21 (0.47 – 3.03)		
Pattern of vitiligo					
Generalized	**37**	1.21 ± 0.56	1.21 (0.25 – 3.03)	U= 99.000	0.343
Acrofacial	**7**	0.99 ± 0.34	0.98 (0.47 – 1.41)		
Sign of activity					
0	**15**	1.11 ± 0.51	0.93 (0.25 – 1.80)	H= 1.748	0.782
1	**10**	1.40 ± 0.59	1.28 (0.98 – 3.03)		
2	**1#**	1.22			
3	**7**	1.11 ± 0.52	1.04 (0.53 – 1.89)		
4	**11**	1.09 ± 0.58	1.0 (0.40 – 2.33)		
Sign of improvement					
0	**21**	1.10 ± 0.61	1.02 (0.25 – 3.03)	H= 2.694	0.610
1	**5**	1.22 ± 0.35	1.11 (0.89 – 1.79)		
2	**7**	1.32 ± 0.58	1.41 (0.40 – 2.33)		
3	**10**	1.18 ± 0.48	1.26 (0.53 – 1.89)		
4	**1#**	1.53			
Treatment					
No	**44**	1.18 ± 0.54	1.16 (0.25 – 3.03)	–	–
Yes	**0**	–	–		
VIDA score					
0 Stable at least for 1 year	**12**	1.02 ± 0.40	0.96 (0.50 – 1.80)	H= 5.417	0.247
+1 Activity of 6 to 12 months	**7**	1.14 ± 0.43	1.30 (0.47 – 1.60)		
+2 Activity of 3 to 6 months	**6**	1.07 ± 0.49	1.0 (0.40 – 1.89)		
+3 Activity of 6 weeks to 3 months	**13**	1.48 ± 0.65	1.30 (0.64 – 3.03)		
+4 Activity of 6 weeks or less period	**6**	0.97 ± 0.53	0.98 (0.25 – 1.69)		

SD: Standard deviation **U:** Mann Whitney test **H:** H for Kruskall Wallis test **P:** p value for comparing between different categories, ***:** Statistically significant at $p \leq 0.05$, **#:** Excluded from the comparing due to small number of cases (n = 1).

CD3+/4+ (Absolute) had the best AUC (0.688) at a cut off ≤ 1126.7 with sensitivity 70.45% and specificity 59.09%, with significant difference in discriminating patients from control, CD3+/8+ (Absolute) had AUC (0.634) at a cut off ≤ 894.1 with sensitivity 68.18% and specificity 63.64%, with significant difference in discriminating patients from control (Table 8 & figure 1).

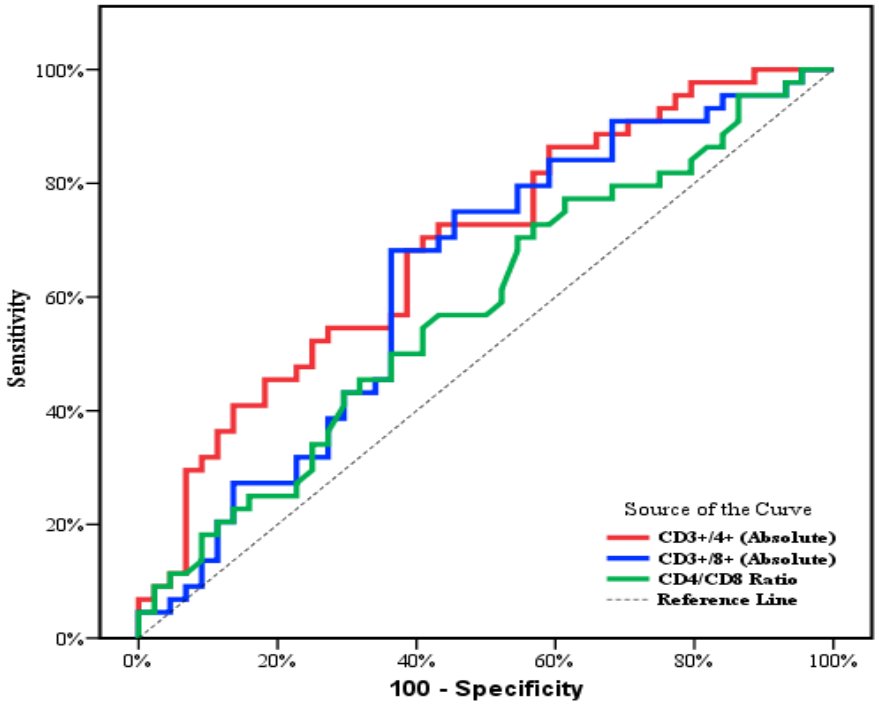

Figure (1): ROC curve for CD to discriminate patients (n = 44) from control (n = 44).

Table (8): Diagnostic performance for CD to discriminate patients (n = 44) from control (n = 44)

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
CD3+/4+ (Absolute)	0.688	0.002*	0.578 – 0.798	≤1126.7	70.45	59.09	63.3	66.7
CD3+/8+ (Absolute)	0.634	0.030*	0.517 – 0.752	≤894.1	68.18	63.64	65.2	66.7
CD4/CD8 Ratio	0.581	0.193	0.461 – 0.700	≤1.26	56.82	50.0	53.2	53.7

AUC: Area Under a Curve, **p value:** Probability value, **CI:** Confidence Intervals, **NPV:** Negative predictive value, **PPV:** Positive predictive value, ***:** Statistically significant at p ≤ 0.05

DISCUSSION

Melanocytes from the epidermis are gradually and specifically destroyed in vitiligo, an acquired cutaneous condition. Its pathophysiology is closely linked to autoimmunity. The imbalance of peripheral blood lymphocytes, mostly cytotoxic T cells (CD8+ cells) and helper T cells (CD4+ cells), is associated with the death of melanocytes. Higher CD8+ and decreased CD4+ counts are linked to the evolution of vitiligo, changing the CD4+:CD8+ ratio [12].

By determining the CD4+/CD8+ ratio in vitiligo patients and controls, evaluating the relationship between the CD4+/CD8+ ratio and the activity and severity of vitiligo. The current study sought to better understand the pathophysiology of vitiligo and assist in its therapy by investigating the association between the activity and severity of the illness and the levels of various T-cell subsets. The results of the current study demonstrated that the absolute number of lymphocytes in the two groups differed significantly, with the patients having fewer than the control group. However, there was no discernible variation in the proportion of lymphocytes. However, **Nigam *et al.*** [13] noted in their research that all vitiligo patients had normal absolute and relative lymphocyte subtype counts. Conversely, **Kanani *et al.*** [14] showed that the mean absolute count of T-lymphocyte populations in vitiligo patients and controls was nearly equal. Additionally, vitiligo patients' total lymphocyte counts were much higher than those of controls, which supports the results of prior research showing that cellular immunity plays a role in the development of the disease. More research on other peripheral lymphocyte subtypes, like B-lymphocytes and natural killer cells, is advised in order to provide more accurate correlative biomarkers for assessing the progression of vitiligo. It is also preferable to carry out studies on the corresponding T-cells in the skin and circulation, as this will be essential to comprehending the pathophysiology of the condition.

According to the current study's flow cytometry results, there was a significant difference between the control and vitiligo groups. Patients had lower levels of relative and absolute CD3+, absolute CD3+/4+, and absolute CD3+/8+ than the control group. However, in terms of CD3+/4+, CD3+/8+, and CD4/CD8 ratio, there were no statistically significant differences between the two groups. **Kanani *et al.*** [14] demonstrated that the mean absolute count of CD3+CD4+ and CD3+CD8+ T-lymphocyte populations was comparable between patient and control groups, even within patient categories. Between patients and controls, the CD4/CD8 ratio mean was higher than one and did not differ significantly (p-value=0.74). The CD4+:CD8+ ratio, on the other hand, decreased statistically significantly, as demonstrated by **Kaur *et al.*** [12]**.** When CD8+ counts increased and CD4+ counts declined, the CD4+:CD8+ ratio decreased in 57.5% of vitiligo patients. They therefore came to the conclusion that cellular immunity may contribute to the pathophysiology of vitiligo by destroying melanocytes. Also, **Dwivedi *et al.*** [15] in 82 vitiligo patients and 50 controls had their CD4(+)/CD8(+) ratio and Tregs measured using flow cytometry. They showed that, in comparison to controls, the CD4(+)/CD8(+) ratio dramatically dropped in patients ($p = 0.001$). In addition, **Pichler *et al.*** [16] discovered that the CD4+/CD8+ ratio was higher in vitiligo patients. However, their results might have been impacted because 40% of their patients also had autoimmune thyroiditis. Furthermore, it has been discovered that autologous melanocyte death is triggered by CD8+ T lymphocytes from vitiligo perilesional margins that release IL6 and IL13 [17]**.** The frequency of melanocyte-specific CD8+ T cells in the total T-cell pool was directly correlated with disease activity, indicating that these cells may have played a pathogenetic role in vitiligo [18]**.**

The results of the current investigation demonstrated that the active, non-active, and control groups' flow cytometry results differed significantly from one another. In particular, the non-active group had lower absolute CD3+ and absolute CD3+/4+ than the control group, and the active group had lower relative CD3+. Conversely, there was no appreciable difference between active and non-active patients in terms of absolute and relative CD3+ or CD3+/4+. Similarly, **Kanani *et al.*** [14] showed that all of the parameters they examined (total lymphocytes, CD3 CD4, CD8, and CD4/CD8 ratio) were linked to negligible differences between the active and non-active vitiligo groups, however they did not compare these parameters between the active and control groups. **Nigam's study,** [13] studied fifty patients with non-segmental vitiligo. The CD4+:CD8+ ratio also deviated from its usual value, meaning that it was less than 1, since the mean CD4+ and CD8+ T-cell count was out of range. Compared to stable vitiligo cases, the mean absolute CD4+ and CD8+ counts were substantially reduced in patients with active vitiligo. Nevertheless, the CD4+:CD8+ ratio did not differ statistically significantly between these two groups. Similarly, **Grimes *et al.*** [19] and **Halder *et al.*** [20] have documented a significant decline in the CD4+/CD8+ ratio in vitiligo patients, accompanied by a rise in the CD8+ T-cell population and a decline in the CD4+ T-cell population. **Pichler *et al.*** [16] discovered that 40% of their patients also had autoimmune thyroiditis, which may have influenced their results even though they observed that vitiligo patients had a higher CD4+/CD8+ ratio.

Together with clinical data and standard vitiligo research, **Nigam *et al.*** [13] came to the conclusion that estimating cell surface markers in patients with vitiligo provided further diagnostic value. Vitiligo may be diagnosed by estimating the quantity of CD molecules (CD3+, CD4+, and CD8+) and the CD4+:CD8+ ratio

particularly in cases of early vitiligo with diagnostic challenges or facial lesions where biopsy is impractical for aesthetic reasons. To support our findings, larger sample studies are required. Patients with vitiligo may experience progressive skin depigmentation as a result of an inability to suppress a continuing immunological response to self-antigens. By actively suppressing self-reactive T-cell activation and proliferation, natural Tregs contribute significantly to the maintenance of peripheral tolerance in vivo [(21)]. Also, **Lili *et al.*** [(22)] demonstrated that GV (50 patients/20 controls) the death of melanocytes was because the diminished quantity and compromised function of natural Tregs are unable to regulate the widespread activation of CD8+ T-cells.

The current study demonstrated that there was significant positive correlation between CD3+/8+ (%) as well as RBCs. With the exception of family history, which was considerably lower in patients with positive family histories than in those with negative ones, there was no correlation found between the CD4/CD8 ratio and any of the other characteristics in the patient group.

In terms of CD's ability to distinguish patients from control, the current study showed that CD3+/4+ (absolute) had the best AUC (0.688) at a cutoff ≤ 1126.7 with sensitivity 70.45% and specificity 59.09% and that there was a significant difference in the ability to distinguish patients from control. Additionally, CD3+/8+ (absolute) had an AUC (0.634) at a cutoff ≤ 894.1 with sensitivity of 68.18% and specificity of 63.64%.

LIMITATIONS

The current investigation was the dearth of active vitiligo patients with recent start, which is often linked to active autoimmune processes that could alter flow cytometry parameters. Most vitiligo patients have disturbed peripheral blood T-cells, which may result in a preponderance of T-cell subtypes in the intracutaneous site of autoimmune melanocyte loss [(16)].

CONCLUSION

This study showed that the pathophysiology of vitiligo may involve CD3+, CD4+, and CD8+.

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