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The role of alteration of the frequency of regulatory T cells and the expression of the associated cytokines in the pathogenesis of vitiligo: Meta-analysis

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Abstract:

Introduction: Vitiligo is a depigmentation disorder in the skin that occurs due to the loss of melanocytes from the epidermis. The etiology and pathogenesis of vitiligo are still unclear. Regulatory T cells (Tregs) are one of the main immune system elements which have a considerable role in disease establishment.

Aim of the study: To evaluate the role of regulatory T cells and associated cytokines in vitiligo pathogenesis.

Methods: We utilized Cochrane Library, Web of Science, Scopus, and PubMed to retrieve studies comparing vitiligo patients with healthy controls regarding the frequency of Tregs, the levels of IL-17, FOXP3, IL-10, TGF-β, and the suppressive capacity over CD4+ and CD+8. NHLB was utilized to assess the quality assessment of the included studies.

Results: 19 studies met our inclusion criteria and were included in our meta-analysis. Our analysis demonstrated that compared to healthy controls, vitiligo patients had significantly decreased Treg cells' frequency ($p < 0.001$), reduced suppressive capacity over CD4+ and CD8+ ($p < 0.001$), and decreased Tregs-associated substances including TGF-β ($p = 0.05$), IL-10 ($p = 0.004$), and FOXP3 ($p < 0.001$). However, Vitiligo cases were associated with an increased level of IL-17 ($p < 0.001$).

Conclusion: Treg cells are involved in vitiligo pathogenesis. Vitiligo patients have a significantly diminished expression and frequency of Treg cells and Treg-associated elements such as TGF-β, FOXP3, and IL-10 levels. However, IL-17 was found to be increased in vitiligo patients.

Keywords: Vitiligo; Treg cells; FOXP3; IL-17.

1. Introduction

Vitiligo is a skin depigmentation disease that occurs due to the loss of melanocytes which are the pigment-producing cells from the epidermis manifesting as amelanotic white patches and macules in skin with clear peripheral edges [1]. It is considered the most prevalent depigmented skin disorder affecting about 0.5-2% of the population worldwide [2]. Melanocytes are present in many different tissues such as hair follicles, skin, eyes, inner ear, heart, bones, and brain. Melanocytes are normally present in the basal layer of the epidermis to produce and distribute melanin in skin which is a complex process referred to as melanogenesis [3]. Despite the great advances in vitiligo understanding in recent years, the etiology and pathogenesis of vitiligo are still unclear. However, Previous research reported a considerable association between vitiligo and other autoimmune disorders including thyroid disease [4]. Thus, it is currently considered an autoimmune disease associated with metabolic and oxidative stress, including cellular detachment disorders, and environmental and genetic variables [5]. Several factors support the autoimmune nature of vitiligo such as the involvement of autoantibodies against melanocytes in about 10% of patients [6) Additionally, Previous research demonstrated that self-reactive CD8+ T lymphocytes are involved in the melanocyte destruction process by triggering anti-melanocyte auto antibodies yielding an autoimmune loss of melanocytes [7].

It is well-known that one of the primary immune system components, regulatory T cells [Tregs), are essential for maintaining peripheral self-tolerance because they inhibit autoreactive lymphocyte activation and avert its harmful effects. These cells are involved in the immune response termination after invading pathogens have been effectively eradicated [8]. Forkhead box P3 (FOXP3) represents the primary Treg mediator which that controls the production of Glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR), interleukin-10 (IL-10), and Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) which represent the main Treg's suppressive substances [9]. We believe that vitiligo pathogenesis is significantly influenced by teg cells [10]. FOXP3 expression and Tregs' function was found to be altered in patients with vitiligo [11–13]. This alteration results in a decrease of Treg's suppressive role and reducing the

synthesis of its associated immunosuppressive cytokines such as TGF-β and IL-10 yielding a shift of the balance towards autoimmunity, causing melanocytes to be destroyed and vitiligo to develop [14].

This study is a meta-analysis of the role of Treg cells in vitiligo.

2. Methods

We followed PRISMA as a guideline in performing our study [15].

2.1.Search and information

This search strategy was used till Feb 2024: (vitiligo) AND ("Tregs vitiligo" OR "regulatory T cells" OR Treg OR "Treg cells"). PubMed, Web of Science, Scopus, and Cochrane Library were used.

Selection and eligibility criteria

At first, the authors screened the studies' titles and abstracts to reach out to relevant studies. The selected studies underwent full-text screening according to our inclusion criteria to select the final included studies.

Inclusion criteria

We included the studies which compared vitiligo patients with healthy controls regarding the frequency of Tregs, the expression of FOXP3, IL-10, TGF-β, IL-17 and the suppressive capacity over CD4+ and CD+8.

Exclusion criteria

We excluded single-arm studies, studies older than 2015, and studies which did not measure our selection outcomes.

2.2.Study design

We included observational studies and excluded secondary research such as meta-analyses, and review articles.

Data extraction

We retrieved data from the eligible articles. we extracted the baseline data of the included participants and general information of the included studies such as the disease status. We also extracted data from our selected outcomes, including the frequency of Tregs, the levels of IL-17, FOXP3, IL-10, TGF-β, and the suppressive capacity over CD4+ and CD+8. The risk of bias required data to be extracted.

Quality Assessment

We included observational articles in our meta-analysis. Thus, the risk of bias in

these articles was measured using the National Heart, Lung, and Blood Institute (NHLB) quality assessment tool [16].

2.3.Statistical methods

All extracted outcomes were continuous. Under the inverse variance analysis method, we utilized Review Manager 5.4.1 to analyze them using standardized mean difference (SMD) and 95% confidence intervals (CIs). The random effect analysis model was utilized in all outcomes. The I2 and the p-value measured the inconsistency among the articles. The outcome becomes heterogeneous if $p < 0.1$ or I2 > 50%. We conducted a subgroup analysis in the majority of outcomes [17].

3. Results

The PRISMA figure shows our search **(Figure 1)**. We included 19 studies which met our inclusion criteria [11,12,25–33,13,18–24]. We studied a total of 1305 vitiligo patients and 1286 healthy controls.

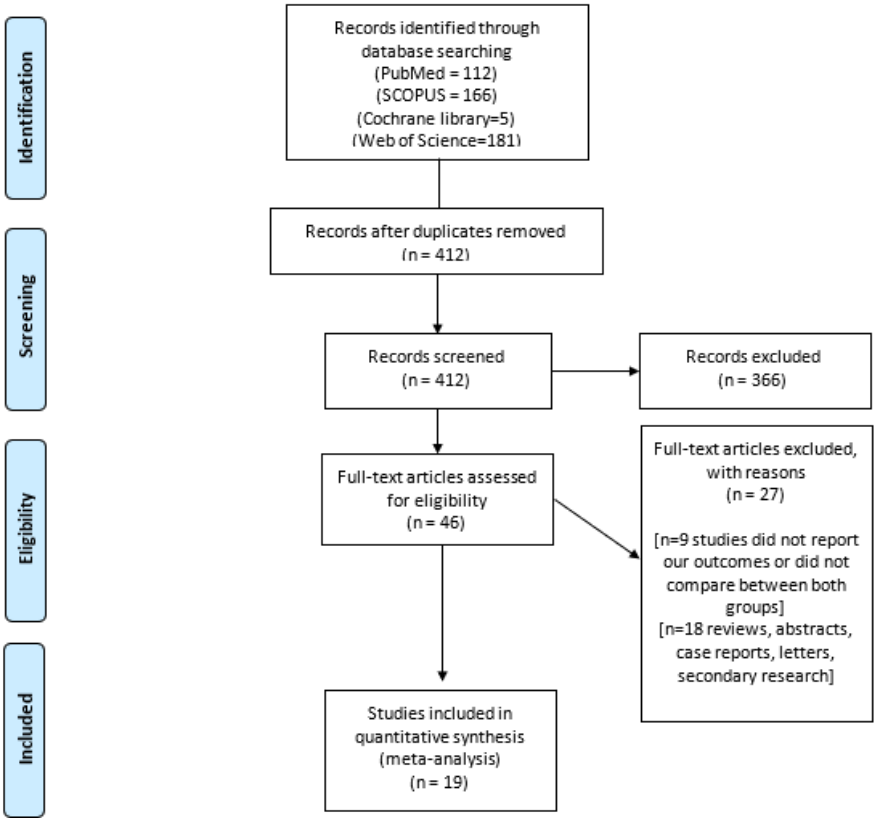

Figure 1: shows the PRISMA flow diagram.

Table 1 shows the demographic data of the involved participants.

Table 1: The demographic characteristics of included participants.

Study ID	Sample Size		Age(years)		Age of onset (years)		Gender (Male/Female)	
	V	C	V	C	V	C	V	C
Abdallah 2018	55	30	29.40 ±13.15	37.07 ±8.12	-	-	21/24	7/23
Ala 2015	130	150	27.6 ±6.9	26.9 ±5.6	23.3 ±7.0	-	76/54	85/65
Aly 2017	30	40	30.23 ±13.58	35.38 ±11.81	-	-	9/21	19/21
Bhardwaj 2020	30	30	33.03 ±9.76	27.86 ±5.5	20.8 ±10.05	-	18/12	23/7
George 2023	42	42	39.10 ±12.32	39.10 ±12.32	28.79 ±14.22	-	22/20	22/20
Ghanem 2017	38	40	34.7 ±16.5	32.4 ±13.1	-	-	12/26	16/24
Giri 2020a	48	45	36.5 ±17.8	22 ±6.07	23.46 ±16.97	-	22/26	21/24
Giri 2020b	55	45	35.04 ±14.24	22.76 ±3.54	22.69 ±12.01	-	25/30	26/19
Giri 2021	419	429	37.62 ±19.43	32.76 ±3.54	23.69 ±12.01	-	175/244	288/157
Hegab 2015	80	60	27.25 ±14.32	33.9 ±9.5	-	-	36/44	36/24
Huo 2021	15	15	-	-	-	-	-	-
Kalaiselvi 2019	80	80	40.05 ±14.68	38.26 ±11.62	35.46 ±15.63	-	52/28	51/29
Kidir 2017	30	30	41.03 ±14.77	40.06 ±12.87	34.06 ±14.72	-	13/17	13/17
Osman 2015	42	43	42 ±5	41 ±8	-	-	11/31	11/32
Shah 2023	25	20	35.67 ±5.26	27.80 ±4.45	21.36 ±3.93	-	15/10	10/10
Sohafy 2021	40	40	32.8 ±8.3	30.7 ±9.5	26.5 ±8.3	-	17/23	20/20
Tembhre 2015	50	51	28.4 ±9.7	30.7 ±6.9	-	-	33/17	35/16
Zhang 2018	51	51	36.35 ±14.35	35.55 ±14.28	-	-	34/17	34/17
Zhou 2015	45	45	44.5 ±12.25	42.75 ±12	-	-	15/30	15/30

Data are presented as mean ±standard deviation. V: vitiligo; C: Control.

Table 2 shows the disease characteristics including the disease duration, status, VASI score, and family history of vitiligo.

Table 2: The disease characteristics including the duration, status, VASI score, and family history of vitiligo.

Study ID	Sample Size		Age(years)		Age of onset (years)		Gender	
	V	C	V	C	V	C	V	C
Abdallah 2018	8.03 ±7.18	-	30 (54.5)	25 (45.5)	-	-	-	-
Ala 2015	4.2 ±3.1	-	83 (63.8)	47 (36.2)	-	-	52 (40)	-
Aly 2017	5.83 ±5.96	-	12 (40)	18 (60)	-	-	4 (13)	-
Bhardwaj 2020	6.2 ±5.07	-	-	-	-	-	7 (23.3)	-
George 2023	-	-	26 (61.9)	16 (38.1)	1372.9 ±2983.19	-	9 (21.4)	-
Ghanem 2017	11.8 ±8.83	-	31 (81.6)	7 (18.4)	-	-	10 (26.3)	-
Giri 2020a	13.10 ±12.51	-	27 (56.25)	21 (43.75)	-	-	-	-
Giri 2020b	5.35 ±7.51	-	44 (80)	11 (20)	61.91 ±23.87	-	15 (27.27)	-
Giri 2021	6.35 ±7.51	-	200 (47.77)	219 (52.23)	-	-	72 (17.18)	-
Hegab 2015	5.34 ±4.81	-	-	-	42.3 ±26.7	-	-	-
Huo 2021	-	-	-	-	-	-	-	-
Kalaiselvi 2019	4.49 ±6.9	-	64 (80)	16 (20)	-	-	-	-
Kidir 2017	6.98 ±8.67	-	-	-	-	-	14 (46.6)	-
Osman 2015	12	-	-	-	-	-	-	-
Shah 2023	11.66 ±10.54	-	15 (60)	10 (40)	25.42 ±28.56	-	3 (16)	-
Sohafy 2021	-	-	-	-	6.7 ±2.2	-	8 (20)	-
Tembhre 2015	0.85 ±0.82	-	-	-	-	-	-	-
Zhang 2018	10.77 ±10.89	-	31 (60.78)	20 (39.22)	-	-	-	-
Zhou 2015	20.75 ±10.66	-	-	-	-	-	19 (42.2)	-

Data are presented as mean ±standard deviation or number (%) and n= number. V: vitiligo; C: Control.

The average risk of bias score of our included studies according to the National Heart, Lung, and Blood Institute (NHLB) quality assessment tool was 10.3 out of 14 [16].

We analyzed 658 patients from seven studies [13,19–22,25,26] which reported this outcome. Our analysis showed a considerable decrease by -1.7 SMD in Treg cells' frequency among patients SMD=-1.70 [-2.52, -0.87], (p <0.001)). We also observed heterogeneity among studies (p <0.01); I^2 = 95% **(Figure 2)**.

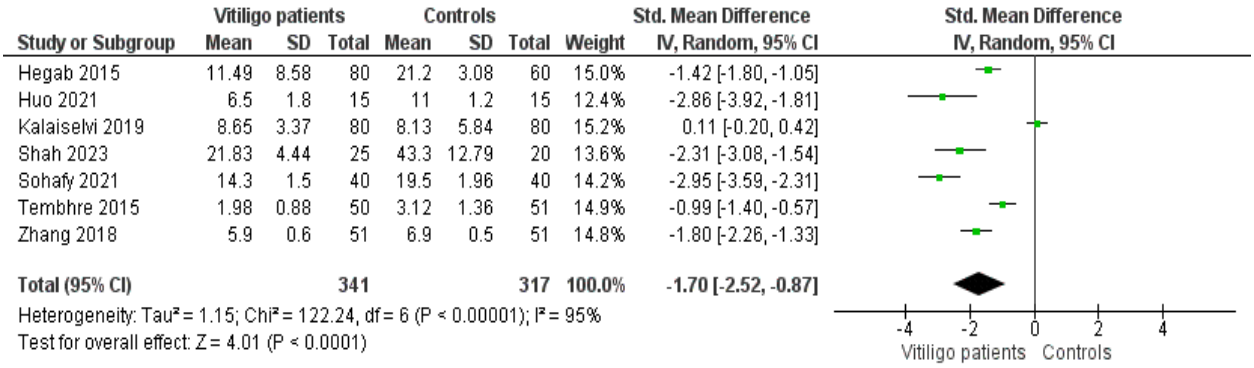

Figure 2: The forest plot of the frequency of Tregs.

We investigated the ability of Treg cells to inhibit CD4+ and CD8+ independently by conducting a subgroup analysis. Regarding the ability of Tregs to inhibit CD4+, two studies investigated this outcome [22,27]. We found that the Treg's suppressive capacity over CD4+ in vitiligo patients and controls was similar (SMD= -8.18 [-17.09, 0.75], (p =0.07) **(Figure 3a)**. Concerning the ability of Tregs to inhibit CD8+ cells, the analysis of three studies (19,22,27) demonstrated a considerable diminish in the overall suppressive capacity over CD8+ by (SMD= -3.43 [-4.62, -2.23], (p <0.001)) **(Figure 3b)**. The overall analysis of both subgroups also demonstrated a substantial decline in the ability of Tregs to control CD4+ and CD8+ patients compared to healthy controls by (SMD=-4.61 [-6.02, -3.21], (p <0.01)) **(Figure 3)**.

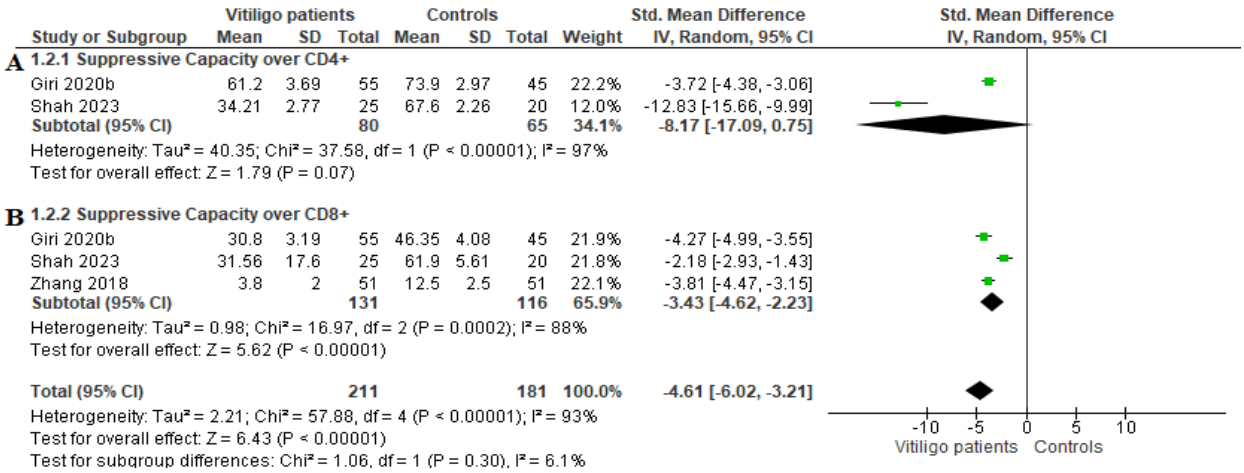

Figure 3: Forest plot for Tregs cells' ability to suppress CD4+ (A) and CD8+ (B).

FOXP3 is considered one of the most important substances for assessing the Treg cell's frequency and suppressive capacity. Five studies measured the FOXP3 expression level in serum [11–13,25,27]. We observed that FOXP3 was considerably decreased in patients with vitiligo (SMD = -7.98 [-11.15, -4.81], (p <0.001)) **(Figure 4a)**. However, the analysis of two studies [24,29] that assessed the FOXP3 expression level in skin showed no considerable variation between healthy controls and vitiligo patients (SMD= -9.51 [-23.23, 4.20], (p =0.17)) **(Figure 4b)**. The combined analysis of both subgroups showed an overall decrease of FOXP3 expression in vitiligo patients by (SMD = -8.32 [-11.08, -5.56], (p <0.01)) **(Figure 4)**.

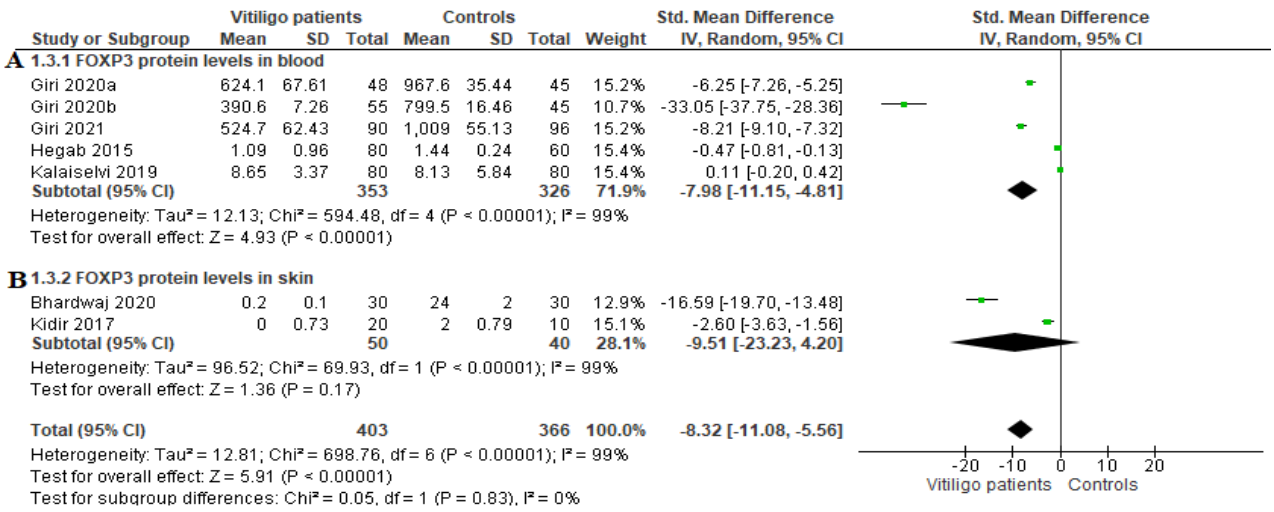

Figure 4: illustrates the forest plots of FOXP3 levels in serum blood (A) and skin (B).

Four studies analyzed IL-10 levels in blood [11,19,27,31]. The overall standardized mean difference showed considerable IL-10 levels decline in patients (SMD= -5.48 [-9.20, -1.75], (*p* =0.004)) **(Figure 5a)**. Only Kidir et al 2017 [24] measured IL-10 levels in the skin and also showed a decrease in its level in vitiligo patients by – 0.63 SMD **(Figure 5b)**. The total analysis of all studies demonstrated lower levels of IL-10 in vitiligo patients than in healthy controls (SMD= -4.45 [-6.91, -1.99], (*p* =0.004) **(Figure 5)**.

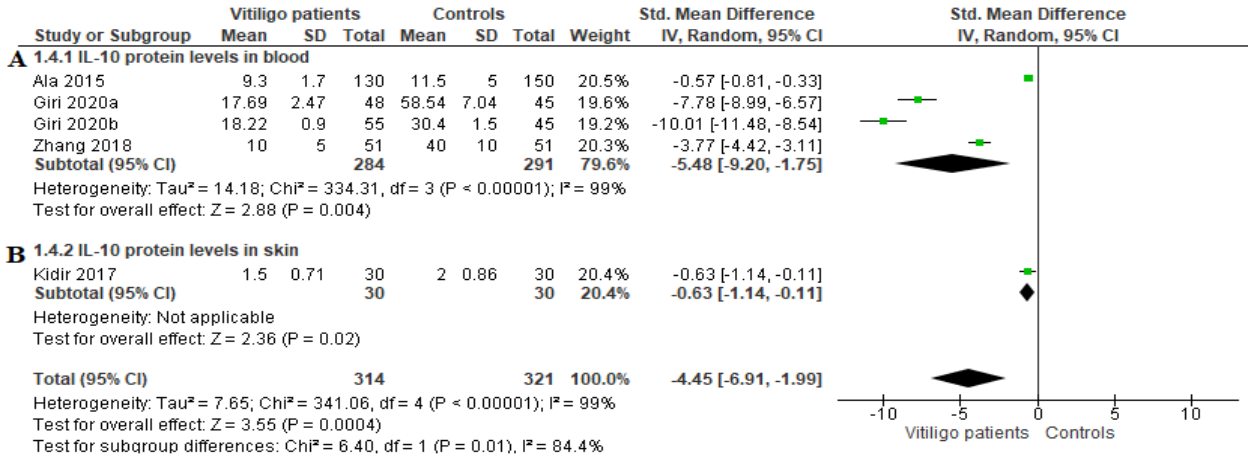

Figure 5: The forest plots of IL-10 protein levels in serum (A) and skin (B).

Eight studies [18, 21, 23, 26, 29, 30, 32, 33] measured IL-17 levels. The overall standardized mean difference showed a considerable rise in IL-17 levels in patients compared to controls (SMD= 2.92 [1.65, 4.18], (*p* <0.001)) which proves its involvement in the pathogenesis of the disease. The combined analysis was heterogeneous (*p* <0.001); I^2 = 97% **(Figure 6)**.

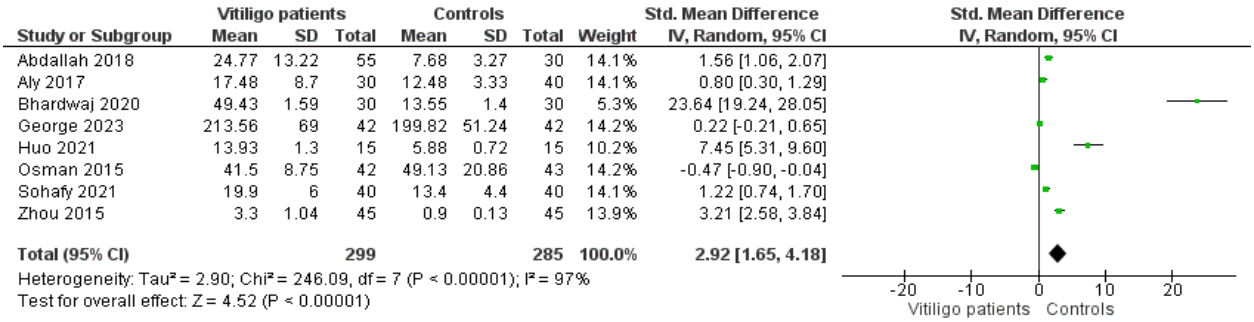

Figure 6: The forest plots of IL-17 levels.

Six studies [11, 18, 19, 23, 27, 34] assessed the TGF-β level in blood showing a decline in its level in patients compared to healthy individuals (SMD= -1.90 [-3.50, -0.30], *p* =0.02) **(Figure 7a)**.

Only two studies [24, 34] assessed TGF-β in the skin. The overall standardized mean difference showed no difference between patients and healthy individuals (SMD= 0.47 [-0.52, 1.47], (*p* =0.35)) **(Figure 7b)**. The combined analysis of both subgroups yielded an overall considerable decline of TGF-β level in patients by SMD= -1.28 [-2.53, -0.02] **(Figure 7)**.

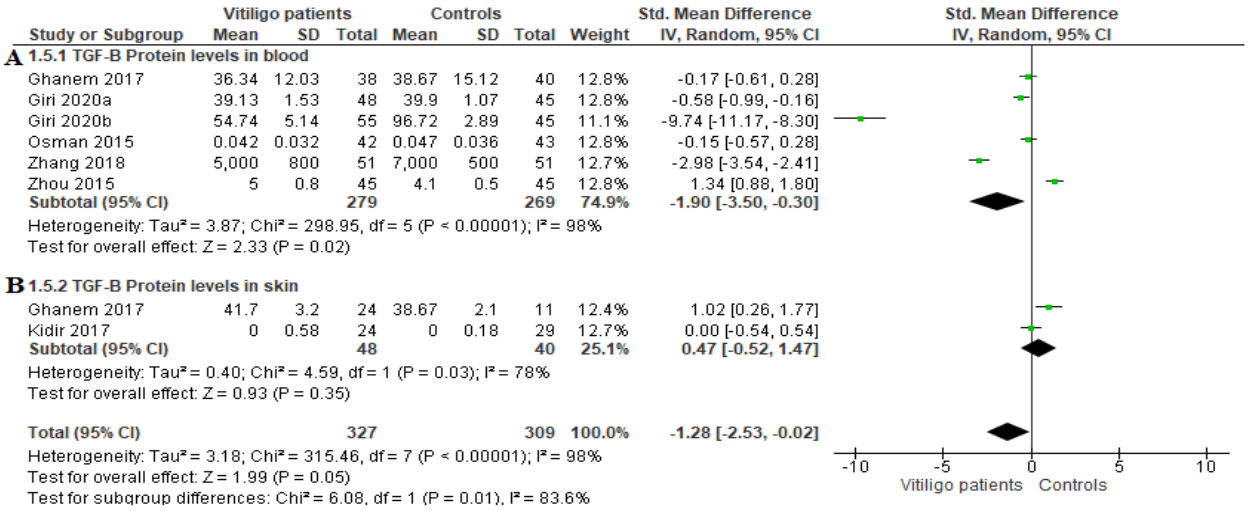

Figure 7: TGF-β protein levels in serum (A) and skin (B) forest plots.

4. Discussion

Vitiligo is a complex skin disease that occurs because of the destruction of melanocytes manifesting as milky white macules that vary in size and form, enlarging peripherally with time [35]. It is considered an inflammatory and autoimmune disorder characterized by increased expression of specific inflammatory cytokines and decreased expression of other molecules which result in loss of pigment cells in the skin [36]. Although vitiligo is a prevalent disorder affecting about 0.5% to 2% of the population worldwide [5], the exact pathogenesis of this condition is still not clear. However, it has been suggested that autoimmunity has a crucial role in disease establishment. The melanocytes may be destroyed by cell-mediated immunity or humoral immunity evidenced by the existence of circulating melanocyte autoantibodies and autoreactive cytotoxic (CD8+) T cells [10]. Tregs are one of the primary components of the immune system that is essential for maintaining peripheral self-tolerance because they inhibit autoreactive lymphocyte activation and prevent harmful effects from occurring [8]. We conducted our meta-analysis to study the pathogenesis of vitiligo and evaluate the role of Tregs in disease progression by investigating the frequency of Treg cells, the expression of IL-10, IL-17, FOXP3, TGF-β in addition to studying the Tregs' ability to suppress CD4+ and CD8+ in vitiligo patients compared to healthy controls.

Previous studies reported a rise in both CD4+ and CD8+ T cells in skin lesions especially in active patients. The uncontrolled activation of CD8+ T cells may destroy melanocytes yielding white areas in vitiligo patients [37]. Tregs suppress auto-reactive CD8+ which limits the progression of vitiligo [7]. Our analysis demonstrated a significant reduction of the suppressive capacity of Tregs over CD8+ proliferation by -3.43 SMD in vitiligo patients which proves autoimmune theory and these results are consistent with previous studies [19]. Lili et al assess the CD8+ Cytotoxic T cell activation in vitiligo patients. They found that CD8+ cytotoxic lymphocytes were significantly increased in participants with progressive generalized vitiligo which also proves its involvement in the disease pathogenesis [38]. However, we found no variation between patients with vitiligo and controls regarding the Tregs' suppressive role over CD4+. Our analysis showed that patients have a significantly lower frequency of Treg cells than controls. this finding is strongly supported by previous studies that also reported a decline in Tregs levels among vitiligo patients [10]. However, other studies reported neither significant alteration nor elevation of Tregs in vitiligo patients in comparison to healthy individuals which is in contrast to our results [39]. This variation may be due to the variations in the antibody clones used in flow cytometry studies which may potentially explain variations in the frequency of Tregs or even the difference in Treg cells' characterizations [40]. Additionally, these studies explain the elevation of Treg cells by immune system activation trying to suppress the disease. FOXP3 represent the key element of Tregs which regulates its function and the production of Tregs-associated suppressive mediators [41]. Our analysis revealed a considerable decrease in the FOXP3 expression in the serum of vitiligo patients which contributed to the progression of the disease due to loss of its suppressive function. These results are consistent with earlier studies which investigated the role of FOXP3 alteration in vitiligo pathogenesis [27,29]. Furthermore, we found a reduced expression of FOXP3 in the skin of vitiligo patients by - 9.51 SMD compared to healthy individuals with no statistically significant difference.

TGF-β and IL-10 are Treg-associated suppressive cytokines which regulate the immune response and maintain the production of Treg cells. FOXP3 is responsible for controlling the expression of these cytokines. Our analysis showed a

significant decrease in TGF-β and IL-10 levels in the serum of vitiligo patients which is in line with previous evidence [31]. However, TGF-β expression levels were similar in the skin of both vitiligo patients and healthy controls. Previous studies found that IL-17, a pro-inflammatory cytokine, may contribute to the pathophysiology of vitiligo and its expression increases in patients with vitiligo [30]. This was supported by our results which showed a significant increase in IL-17 in patients compared to healthy individuals. Sohafy et al reported a significant reduction of IL-17 levels after treatment of vitiligo patients compared to pretreatment levels [21].

We conducted a subgroup analysis to properly estimate the FOXP3, IL-10, and TGF-β levels in serum and skin. However, the main limitations of our study are the small sample size and the heterogeneity among data. Moreover, we did not consider the different types of vitiligo and the different estimation methods for each element in our analysis.

5. Conclusion

Treg cells, IL-17, and FOXP3 expression have an important role in vitiligo pathogenesis. The impairment of Treg's function was associated with disease establishment and progression. Moreover, Vitiligo patients have a significantly diminished expression and frequency of Treg cells and Treg-associated elements such as FOXP3, TGF-β, and IL-10 levels. However, IL-17 is significantly increased in vitiligo patients. Further large-scale studies are needed to confirm the role of Tregs in vitiligo pathogenesis.

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Conflicts of Interest: All authors declare they have no conflicts of interest.

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