Original article

Effect of PUVA on Janus Kinase/Signal Transducer and Activator of Transcription Pathway in Patients with Vitiligo

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Article Info

Abstract

The aim of the current study was to detect effect of PUVA on JAK/STAT pathway and its relationship with the pathogenesis of Vitiligo. It was a Case-Control study conducted from Abril 2019 to December 2019 in the Dermatology outpatient clinic at Beni-Suef Univeristy Hospital and included 30 patients that had Vitiligo disease (7 males and 23 females), their age ranged from 20 to 50 years, the average age was 33.73±8.7. And 30 age and sex matched healthy controls. All participants were subjected to full clinical and laboratory investigations. Skin biopsies had been taken from all studied participants (Vitiligo cases and healthy controls) to study effect of PUVA on JAK/STAT pathway

PUVA

and its relationship with the pathogenesis of Vitiligo. Tissue expression of JAK/STAT was significantly higher in Vitiligo skin lesions as compared with healthy skin biopsies taken from controls both before and even after exposure to PUVA. Tissue expression of JAK/STAT decreased significantly after exposure to PUVA in Vitiligo skin lesions. Tissue expression of JAK was significantly higher in Vitiligo skin lesions in patients with positive as compared with negative family history. There was a significant linear moderate positive correlation between tissue expression of STAT and patients' age among studied Vitiligo patients. There was a significant linear moderate negative correlation between tissue expression of STAT and duration of last new lesion among studied Vitiligo patients. There was a significant linear moderate positive correlation between tissue expression of STAT and Vitiligo disease activity (VIDA) score among studied Vitiligo patients. JAK and STAT tissue expression showed a significant linear strong positive correlation among studied population. We suggest that JAK/STAT plays an important role in the pathogenesis of the Vitiligo disease. This could also open a new era for treatment of Vitiligo disease by anti-JAK modalities.

1. Introduction:

Vitiligo is an acquired cutaneous skin and less frequently hair disease characterized by declining melanocyte function and depigmentation with an estimated prevalence of 0.5–1% in most populations [1]. Several mechanisms of melanocyte degeneration have been presented, including autoimmunity [2], autocytotoxic, metabolic mechanism [3] and impaired melanocyte migration and/or proliferation [4].

The Janus kinase (JAK) family is non-receptor protein tyrosine kinases that are expressed in many tissues. There are four JAK proteins in mammalian cells, JAK1,

JAK2, JAK3, and TYK2. The role of JAKs in cytokine signaling is evidenced by the inherited immunodeficiencies caused by mutations that block receptor-JAK interactions or the kinase activity of the JAKs [5].

JAKs bind specifically to intracellular domains of cytokine receptor signaling chains and catalyze ligand-induced phosphorylation of themselves and of intracellular tyrosine residues on the receptor, creating STAT (signal transducer and activator of transcription) docking sites [6].

STAT are inactive proteins that control the immediate responding genes. Intercellular signaling is critical for developmental regulation, growth control, and homeostasis in multicellular organisms, and STAT pathways have been found in slime molds, worms, flies, and vertebrates but are absent from fungi and plants.In mammals, there are seven STAT genes, STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6 [7].

It has been found that IFN-γ bound receptor complex recruits JAK1 and JAK2 kinases, leading to the phosphorylation and nuclear translocation of STAT, which in turn transcriptionally activates downstream IFN-γ inducible genes [8]. It is suggested that the use of the JAK 1/3 inhibitor, (tofacitinib) effectively leads to blockade of interferon gamma signaling and downstream CXCL10 expression, thus giving in rise to repigmentation Vitiligo [9].

In a previous study, it was found that the level of JAK1 was significantly higher in Vitiligo patient before and after treatment with NB-UVB 311 nm than controls. There was a statistically significant difference between the level of JAK before and after treatment with a lower JAK1 level after treatment. These findings suggest a possible role of JAK1 in the pathogenesis of Vitiligo and the possible hope of using JAK1 inhibitors as a future treatment of Vitiligo [9].

PUVA therapy is still the most common recommended treatment in the management of Vitiligo. PUVA was first reported to be a successful therapeutic approach for Vitiligo in 1948. Later, in 1976, high-intensity sources for UVA radiation were first developed for the treatment of Vitiligo.

Up to the present, numerous studies have proven the effectiveness of PUVA in treatment of various inflammatory diseases; however, the length of treatment (usually over a year), low of achieving total repigmentation of all patches, variability of response with different body sites, and

contraindication of oral photo-chemotherapy in children under 12 years of age are the major limitations of phototherapy [10].

The current study was designed with an aim to detect effect of PUVA on JAK/STAT pathway and its relationship with the pathogenesis of Vitiligo.

2. Patients And Methods :

This study was a Case-Control study conducted from Abril 2019 to December 2019 in the Dermatology outpatient clinic at Beni-Suef University Hospital, and included 30 Vitiligo patients from both sexes (7 males and 23 females) with age ranged from 20 to 50 years, the average age was, 33.73±8.7. And 30 healthy controls were taken, they were age and sex matched to the Vitiligo cases. The study was approved by the ethical committee of the Faculty of Medicine, Beni-Suef University.

2.1. Ethical Consideration:

Informed written consent was obtained from all participants before recruitment in the study, after explaining the objectives of the work. Confidentiality was guaranteed on handling the data base. Patients and controls were selected according to the following inclusion and exclusion criteria:

2.2. Inclusion criteria:

(age between 20 to 50 years old, both males and females will be included, patient before and after exposure to PUVA).

2.3. Exclusion criteria:

(Patients with other autoimmune diseases, patients with associated systemic or dermatological diseases and who are suffering from any infection).

2.4. All the patients and healthy controls were subjected to the following:

- Detailed history taking were obtained from all participants including: [age (years), sex, duration of Vitiligo disease (years), duration of last lesion (months) and family history of Vitiligo disease (positive and negative)].

- Vitiligo disease activity (VIDA) score is a six-point scale for evaluating Vitiligo activity. It is based on patient's own reports of disease activity.

- Tissue samples were obtained as 4-mm punch biopsies of the skin from both involved and uninvolved skin areas (matched samples) of patients with Vitiligo (two samples had been taken from the same skin leasion before and after PUVA treatment). In addition, biopsies were taken from healthy control individuals. The biopsies were taken

from the non-UV-exposed buttock region.

- **Real-time polymerase chain reaction (RT-PCR)** was performed using a Qiagen tissue extraction kit (Qiagen, USA) according to instructions of manufacture. The qPCR assay with the primer sets were optimized at the annealing temperature. The primer sequence was, With Primer sequence, STAT: Forward, ′5TGGAAGAGGCGGCAGCAGATAGC-3 and Reverse, 5-CACGGC CCCCATTCCCACAT-3. JAK: Forward, 5'- ATCCACCCAACCATGTCTTCC- 3' and Reverse,5'- ATTCCATGCCGATAGGCTCTG- 3'.β-actin Forward,5′- GGAGATTACTGCCCTGGCTCCTA-3′ and Reverse, 5′- GACTCATCGTACTCCTGCTTGCTG-3″

2.5. Statistical analysis:

The collected data were coded then entered and analyzed using the SPSS version 25 (Statistical package for social science) for windows 10. Descriptive analysis of the results in the form of percentage distribution for qualitative data and (minimum, maximum, mean and standard deviation) calculation for quantitative data. Cross tabulation and Chi Square test ($\chi2$): For comparison between categorical variables and percentage values. Student t- test: For comparison between means of two unrelated groups with a normal distribution. Paired sample t-test. For comparison of Before-and-after observations on the same subjects (Vitiligo skin Lesions JAK and STAT tissue expression before and after exposure to PUVA in Vitiligo patients). Spearman's correlation analysis was done to evaluate linear relationship between studied JAK/STAT expression and other parameters in Vitiligo patients' skin biopsies. Correlation graphs were drawn only for significant correlation which is considered significant at $P < 0.05$. Correlation is considered positive (direct correlation) when r (correlation coefficient) had a + signal and negative (inverse correlation) in case of − signal and it is considered: (Weak when r = >0 − 0.35, Moderate when r = >0.35 − 0.65; and Strong when r = > 0.65). P-values equal to or less than 0.05 were considered statistically significant. Simple graphs were used to illustrate some information.

3. Results:

Table (1) demonstrates the baseline data of the studied population, gender distribution of the cases and the controls; the Vitiligo cases; 41.2% of them were males and 53.5% were females, while the controls; 58.8% were males and 46.5% were females. The

average cases age was; 33.73±8.8 (SD) years, while average controls age was 31.13±6.4 (SD) years. The majority of the studied Vitiligo cases had no family history of the disease (22) cases (73.3%); while only 8 cases (26.7%) had a positive family history of Vitiligo. Focal Vitiligo was the most predominant among the studied Vitiligo sample; (30%), followed by Acral type in (23.3%), vulgaris was present in (16.7%), mixed type was in (13.3%), acrofacial was in (10%). All the studied Vitiligo cases had a progressive course of the disease. None of the studied Vitiligo cases had neither history of previous Vitiligo treatment or history of associated skin disorders. Vitiligo lesions were present in face among 56.7% of cases, while in hand and mouth among 63.3% of cases. Disease duration was ranged from (0.04) to (35) with a mean of 5.38 ±7.6 (SD) years of disease duration. The history of last new lesions among studied Vitiligo cases was ranged from (0.25) to (24) with a mean of 5.08 ±5.2 (SD) months. Vitiligo disease activity (VIDA) score ranged from (0) to (+4) with a mean of 2.37±1.4 (SD).

TABLE (1): BASELINE AND CLINICAL DATA OF THE STUDIED POPULATION:

		N (%)		
		Vitiligo Patients **N= 30**	**Healthy Controls** **N= 30**	**p-value***
Sex	**Male**	7 (41.2)	10 (58.8)	0.390
	Female	23 (53.5)	20 (46.5)	
Age	**Mean ±SD**	33.73 ±8.8	31.13 ±6.4	0.193
Disease Duration	**Mean ±SD**	109.90 ±87.9	NA	
Family History	**Yes**	22 (73.3)	NA	
Disease Duration (years)	**Mean ±SD**	8.09 ±**6.3**		
Last New Lesion (months)	**Mean ±SD**	5.38 ±7.6		
Vitiligo disease activity (VIDA)	**Mean ±SD**	+2.37 ± +1.4		

As demonstrated in Figure (1-a); Tissue expression of JAK was significantly higher in Vitiligo skin lesions as compared with healthy skin biopsies taken from controls; the mean JAK values (5.28 vs. 1.03) in Vitiligo skin lesions and healthy skin from

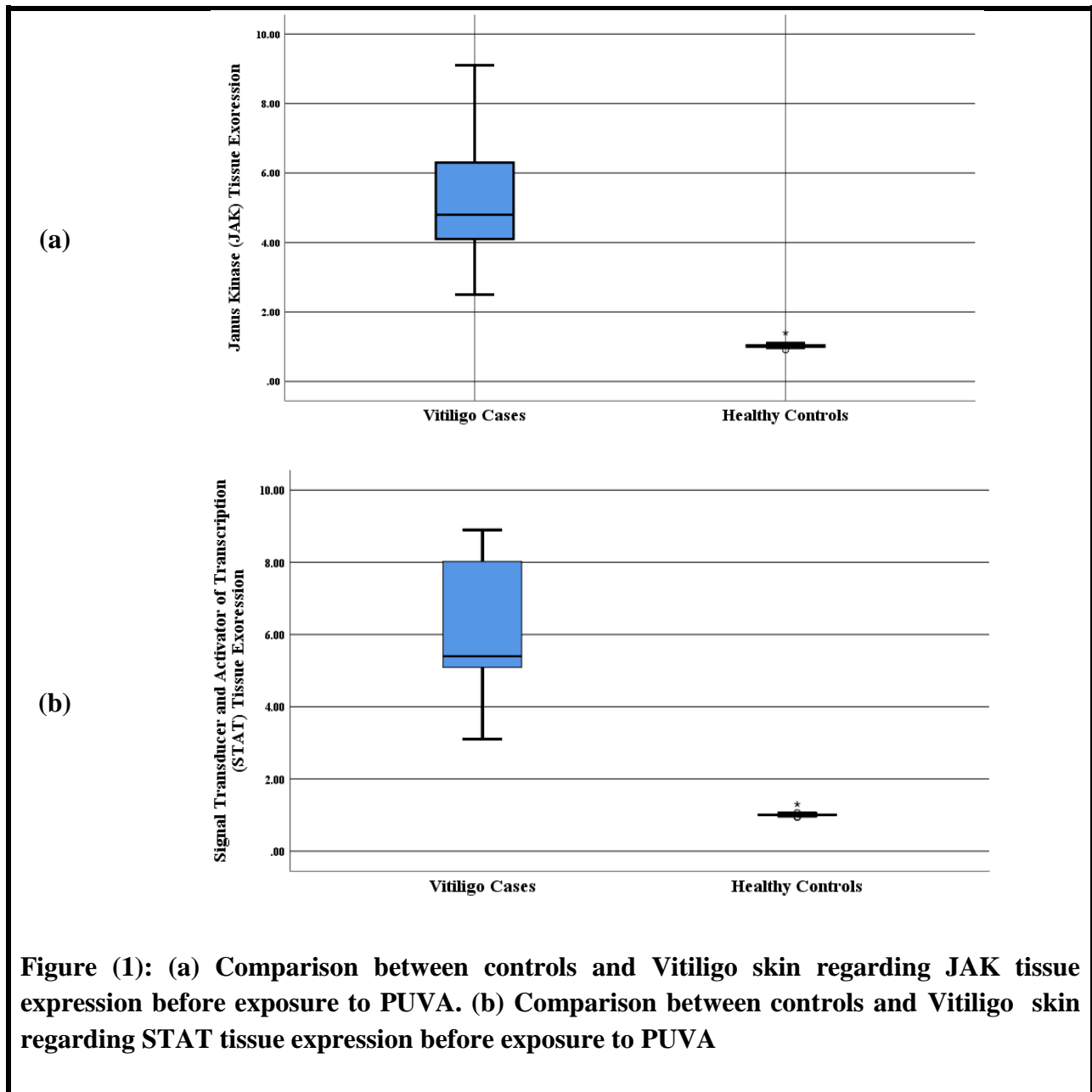controls respectively with a statistically significant p-value< 0.001. In Figure (1-b), tissue expression of STAT was significantly higher in Vitiligo skin lesions as compared with healthy skin biopsies taken from controls; the mean STAT values (5.92 vs. 1.01) in Vitiligo skin lesions and healthy skin from controls respectively with a statistically significant p-value< 0.001.
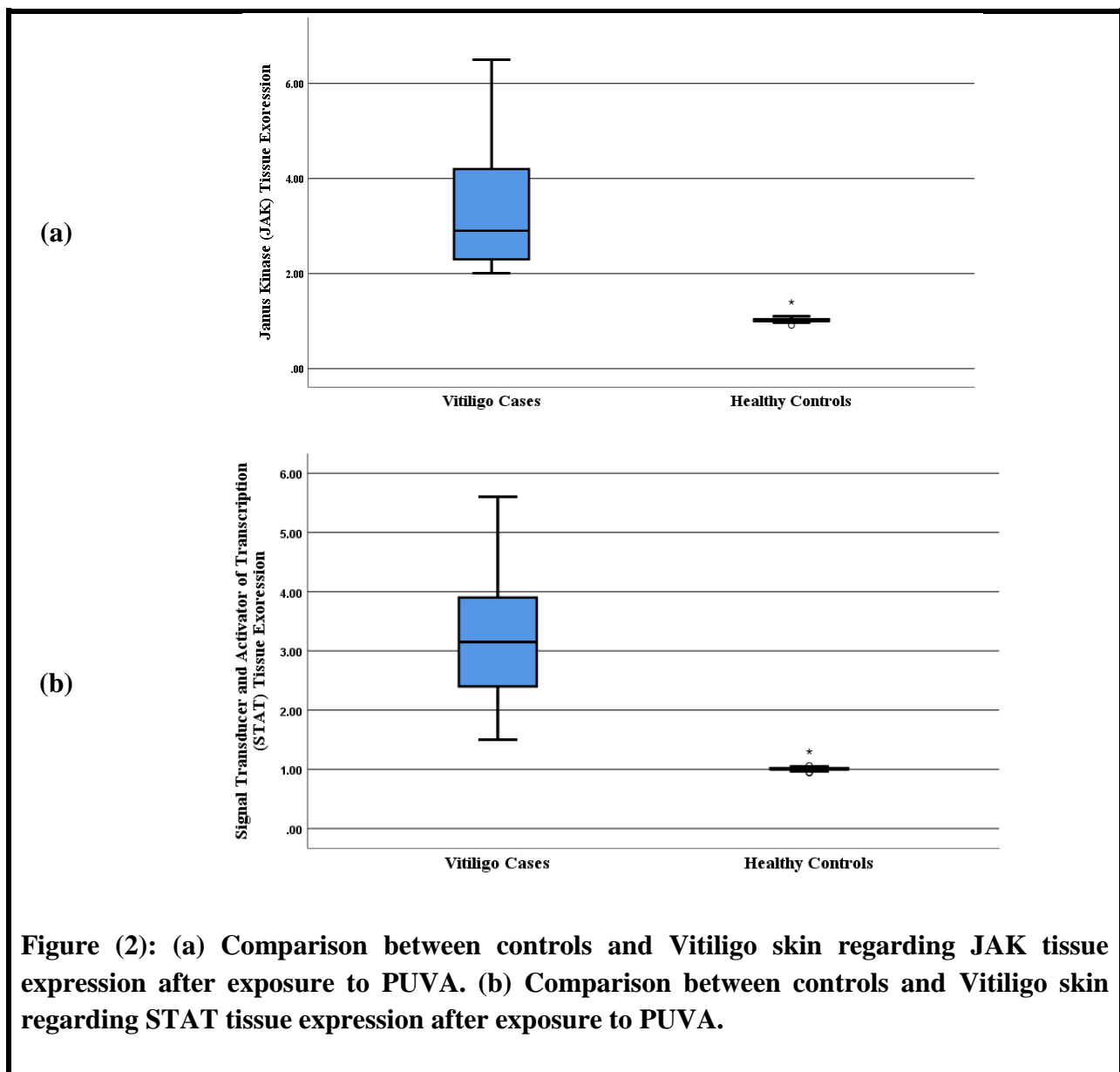

Figure (1): (a) Comparison between controls and Vitiligo skin regarding JAK tissue expression before exposure to PUVA. (b) Comparison between controls and Vitiligo skin regarding STAT tissue expression before exposure to PUVA

Figure (2-a) demonstrates that tissue expression of JAK was still significantly higher in Vitiligo skin lesions as compared with healthy skin from controls even after exposure to PUVA; the mean JAK tissue expression values (3.40 vs. 1.03) in Vitiligo skin lesions and healthy skin respectively with a statistically significant p-value< 0.001. Figure (s-b) illustrates that tissue expression of STAT was still significantly higher in Vitiligo skin lesions as compared with healthy skin from controls even after exposure to PUVA; the mean STAT tissue expression values (3.28 vs. 1.01) in Vitiligo skin lesions and healthy skin respectively with a statistically significant p-value< 0.001.

Figure (2): (a) Comparison between controls and Vitiligo skin regarding JAK tissue expression after exposure to PUVA. (b) Comparison between controls and Vitiligo skin regarding STAT tissue expression after exposure to PUVA.

Figure (3-a) showed that tissue expression of JAK decreased significantly after exposure to PUVA in Vitiligo skin lesions; the mean tissue expression values (5.28 vs. 4.40) before and after exposure to PUVA in Vitiligo skin lesions respectively with a statistically significant p-value< 0.001.

Figure (3-b) showed that tissue expression of STAT decreased significantly after exposure to PUVA in Vitiligo skin lesions; the mean tissue expression values (5.92 vs. 3.28) before and after exposure to PUVA in Vitiligo skin lesions respectively with a statistically significant p-value< 0.001.
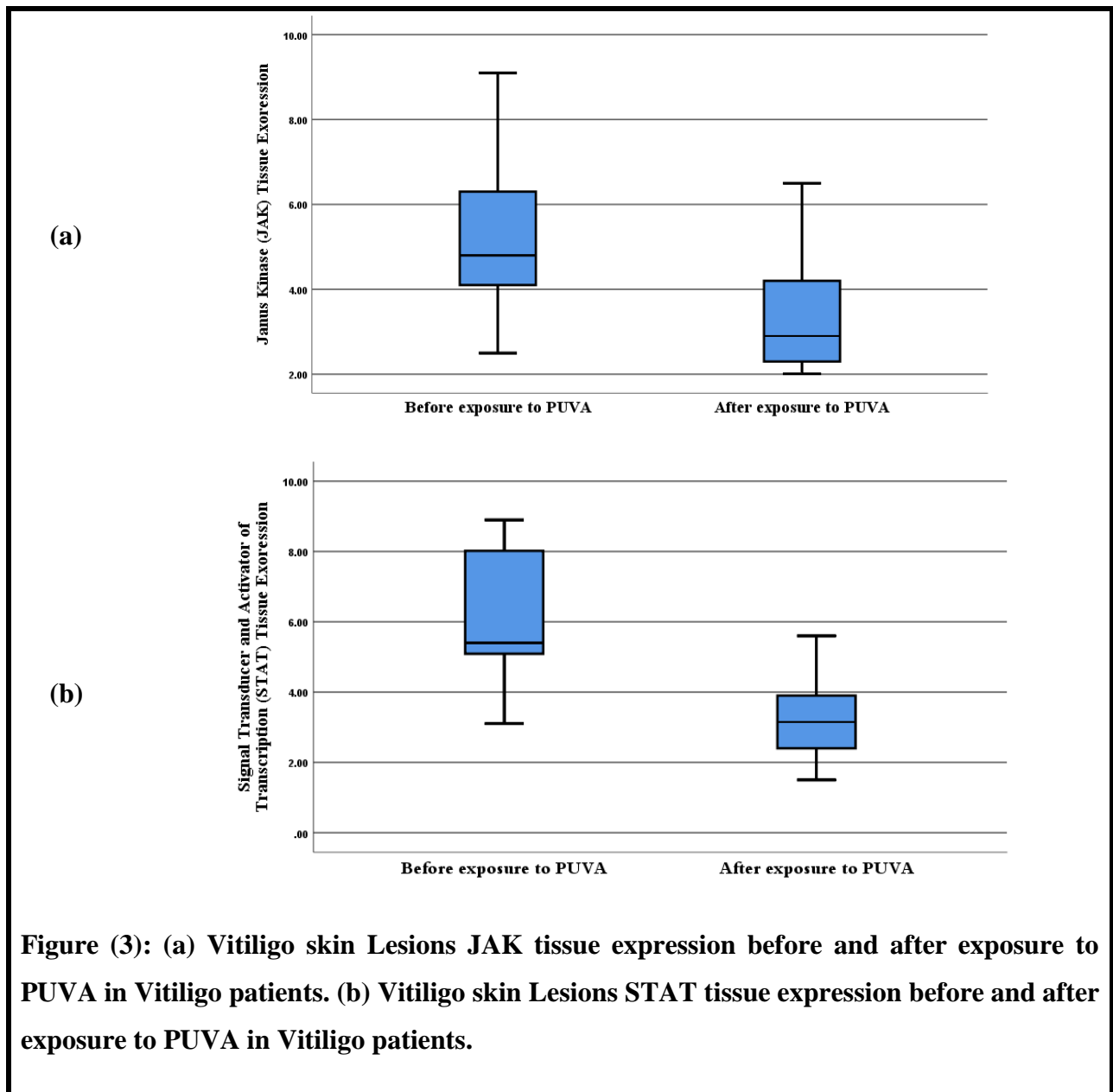

Figure (3): (a) Vitiligo skin Lesions JAK tissue expression before and after exposure to PUVA in Vitiligo patients. (b) Vitiligo skin Lesions STAT tissue expression before and after exposure to PUVA in Vitiligo patients.

Tissue expression of JAK/STAT was slightly higher in female Vitiligo skin lesions as compared with males; however no statistically significant difference was detected in relation between gender and tissue expression of JAK/STAT (p-value= 0.778, 343 respectively). Tissue expression of JAK was significantly higher in Vitiligo skin lesions in patients with positive as compared with negative family history; with a statistically significant (p-value= 0.011); however no statistically significant

difference was detected regarding STAT tissue expression and family history; (p-value= 0.137).

Figure (4) demonstrates a significant linear moderate positive correlation between tissue expression of STAT and patients' age among studied Vitiligo patients; (r= 0.423, p=0.020). However; no detected significant linear correlation between tissue expression of JAK and patients' age in studied Vitiligo patients; (p-value >0.05).
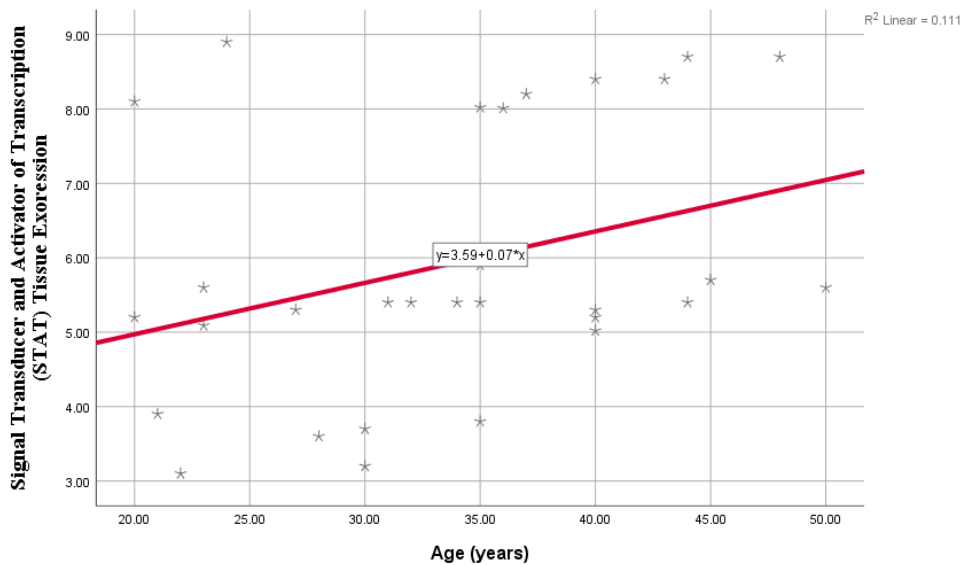

Figure (4): Correlation between JAK/STAT tissue expression and Patients' Age in studied Vitiligo patients

Figure (5) demonstrates a significant linear moderate negative correlation between tissue expression of STAT and duration of last new lesion among studied Vitiligo

patients; (r= -0.561, p=0.001). However; no detected significant linear correlation between tissue expression of JAK and last lesion duration in studied Vitiligo patients; (p-value >0.05).
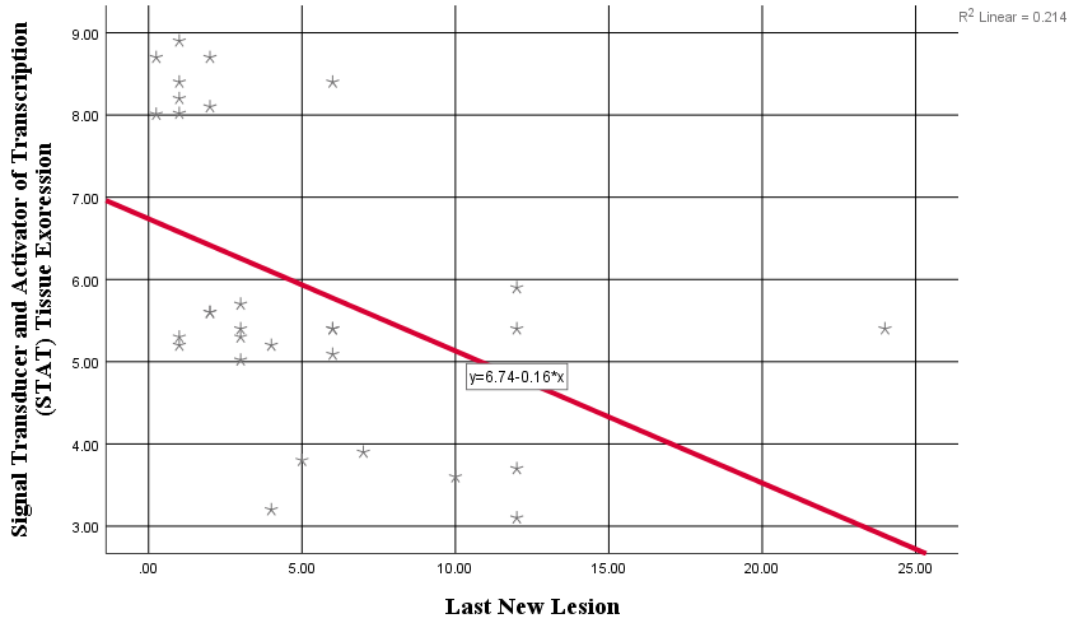
Figure (5): Correlation between JAK/STAT tissue expression and History of Last New Lesion in studied Vitiligo patients

Figure (6) demonstrates a significant linear moderate positive correlation between tissue expression of STAT and Vitiligo disease activity (VIDA) score among studied Vitiligo patients; (r= 0.547, p=0.002). However; no detected significant linear correlation between tissue expression of JAK and Vitiligo disease activity (VIDA)

score in studied Vitiligo patients; (p-value >0.05).

Figure (7) demonstrates a significant linear strong positive correlation between tissue expression of JAK and tissue expression of STAT among studied population; (r= 0.762, p< 0.001).
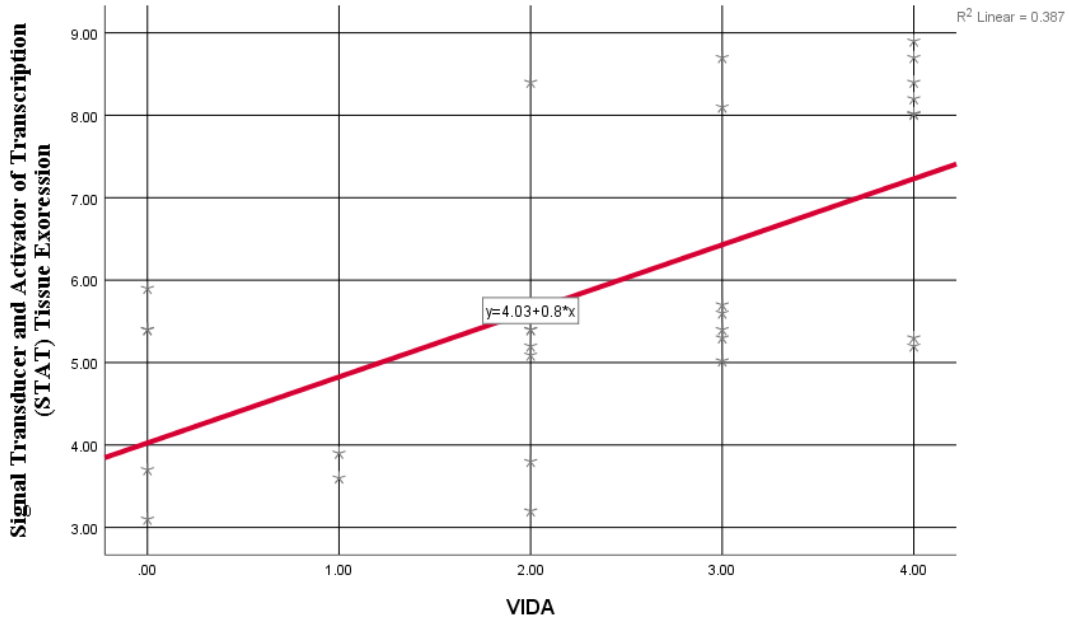
Figure (6): Correlation between JAK/STAT tissue expression and Vitiligo disease activity (VIDA) in studied Vitiligo patients
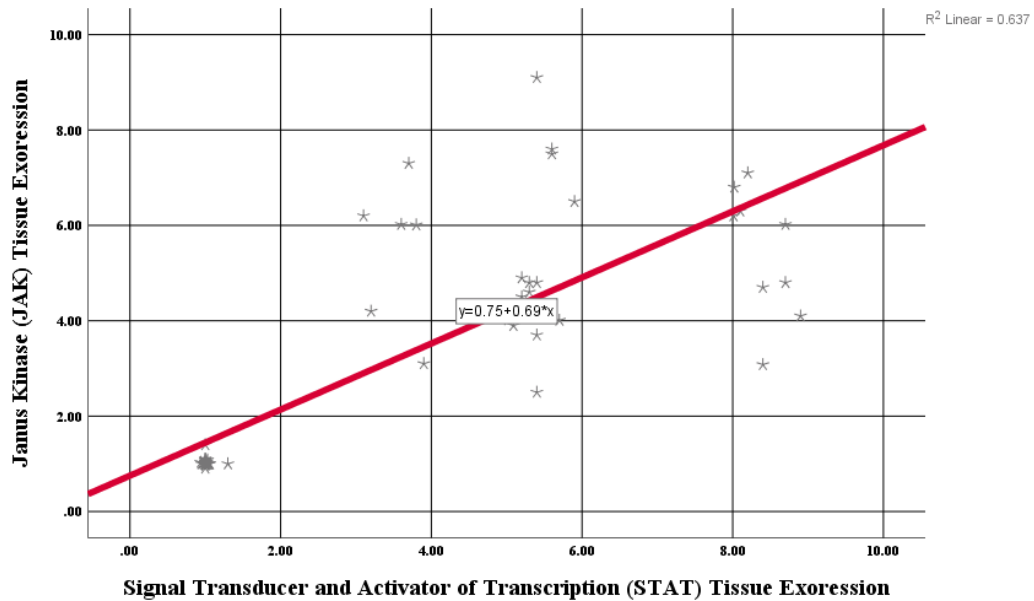

Figure (7): Correlation between JAK and STAT tissue expression in studied Population

4. Discussion:

Vitiligo is the most common de-pigmenting disorder, with an estimated prevalence of 0.5% to 1% in most populations [11]. It is an acquired chronic skin disease characterized by the development of well-circumscribed, white, non-scaly macules and patches due to the destruction of melanocytes in the skin, hair, or both [1].

Janus kinases (JAKs) are a group of non-receptor intracellular tyrosine kinases (TYKs) that could modify cytokine-mediated signals through the JAK signal transducer and activator of transcription (STAT) pathway [6].

The JAK-STAT pathway is critical for the downstream signaling of inflammatory cytokines and growth hormones [12]. JAKs are tyrosine kinases that are associated with the cytoplasmic domain of type I and type II chemokine receptors, with ligands such as IFN-γ. Extracellular binding of cytokines activates their receptors, inducing apposition of JAKs and self-activation by auto-phosphorylation. Activated JAKs bind STATs, which undergo JAK-mediated phosphorylation leading to STAT dimerization, translocation to the nucleus,

DNA binding, and regulation of gene expression [13].

Studies have reported that multiple cytokines such as IFN-γ [14], tumor necrosis factor-alpha [15], and chemokine (C-C motif) ligand 22 (CCL-22) are differently expressed in the vitiliginous skin and serum of patients than controls, proving their roles in Vitiligo [16].

It has been found that IFN-γ bound receptor complex recruits JAK1 and JAK2 kinases, leading to the phosphorylation and nuclear translocation of STAT, which in turn transcriptionally activates downstream IFN-γ inducible genes [17].

It is suggested that the use of the JAK 1/3 inhibitor effectively leads to blockade of interferon gamma signaling and downstream CXCL10 expression, thus giving rise to re-pigmentation in Vitiligo [18].

In the current study, we attempted to detect effect of PUVA on JAK/STAT pathway and its relationship with the pathogenesis of Vitiligo; the study included 30 Vitiligo patients from both sexes, all presented to dermatology department at Beni-Suef University hospital. The Vitiligo patients were 7 males and 23 females patients, their age ranged from 20 to 50 years, the average

age was; 33.73±8, together with 30 healthy controls were taken, they were age and sex matched to the Vitiligo cases with no statistically significant difference between the cases and the control groups regarding sex (p-value> 0.05).

The majority of the studied Vitiligo cases had no family history of the disease (22) cases (73.3%); while only 8 cases (26.7%) had a positive family history of Vitiligo. As regard Vitiligo types; focal Vitiligo was the most predominant among the studied Vitiligo sample; (30%), followed by Acral type in (23.3%), vulgaris was present in (16.7%), mixed type was in (13.3%), acrofacial was in (10%).

All the studied Vitiligo cases had a progressive course of the disease. None of the studied Vitiligo cases had neither history of previous Vitiligo treatment nor history of associated skin disorders. Vitiligo lesions were present in face among 56.7% of cases. Vitiligo lesions were present in hand and mouth among 63.3% of cases. The history of last new lesions among studied Vitiligo cases was ranged from (0.25) to (24) with a mean of 5.08 ±5.2 (SD) months. Vitiligo disease activity (VIDA) score ranged from (0) to (+4) with a mean of 2.37±1.4 (SD).

It was found that tissue expression of JAK was significantly higher in Vitiligo skin lesions as compared with healthy skin biopsies taken from controls; with a statistically significant p-value< 0.001 (the mean JAK values (5.28 vs. 1.03) in Vitiligo skin lesions and healthy skin from controls respectively). Tissue expression of STAT was significantly higher in Vitiligo skin lesions as compared with healthy skin biopsies taken from controls; the mean STAT values (5.92 vs. 1.01) in Vitiligo skin lesions and healthy skin from controls respectively with a statistically significant p-value< 0.001. We also reported that; tissue expression of JAK was still significantly higher in Vitiligo skin lesions as compared with healthy skin from controls even after exposure to PUVA; the mean JAK tissue expression values (3.40 vs. 1.03) in Vitiligo skin lesions and healthy skin respectively with a statistically significant p-value< 0.001, also; tissue expression of STAT was still significantly higher in Vitiligo skin lesions as compared with healthy skin from controls even after exposure to PUVA; the mean STAT tissue expression values (3.28 vs. 1.01) in Vitiligo skin lesions and healthy skin respectively with a statistically significant p-value< 0.001. Those findings

suggest a possible role of JAK/STAT in the pathogenesis of Vitiligo.

Consistent with those findings; a prospective, case-control comparative study designed to assess and compare the expression of JAK expression in the affected skin of Vitiligo and psoriatic patients in comparison to normal healthy controls; reported that the level of JAK before treatment ranged from 7.5 to 16.8 with a mean of 11.98 ± 2.62, which was significantly higher than the level in healthy controls (ranged from 0.95 to 1.03 with a mean of 1.01 ± 0.06). The level of JAK1 after treatment ranged from (4.02 to 9.1) with a mean of (6.37 ± 1.73). Upon comparing between the levels of JAK1 in Vitiligo patients before and after treatment, a statistically significant decrease was found ($p = 0.005$). There was a considerable decrease in the mean level of JAK1 of − 5.60 [9].

Opposite to those findings; a study designed to evaluate role of JAK1 and STAT3 in Vitiligo reported that the expression of JAK1 in the epidermis and dermal adnexa showed no significant differences between patients and controls; however; there were significant differences between the groups regarding STAT3 expression in lesional than

normal skin (P=0.02), [19]. This discrepancy in results could be attributed to different techniques used and small sample size of patients and controls. Future studies with a larger scale number of patients are highly recommended to prove or contradict those findings.

In the present study; tissue expression of JAK decreased significantly after exposure to PUVA in Vitiligo skin lesions with a statistically significant p-value< 0.001, also; tissue expression of STAT decreased significantly after exposure to PUVA in Vitiligo skin lesions with a statistically significant p-value< 0.001.

No prior studies were found to prove or contradict our results; however; one study compared the level of JAK1 in Vitiligo patients before and after treatment using NB-UVB 311 nm, they found a statistically significant difference between the level of JAK before and after treatment with a lower JAK level after treatment [9]. These findings suggest a possible hope of using JAK inhibitors as a future treatment of Vitiligo.

We found that Tofacitinib citrate, (an oral Janus kinase 1/3 inhibitor) was tried in a case of generalized Vitiligo and has been reported to result in significant re-

pigmentation that could support the present study findings [18]. Additionally, oral ruxolitinib (JAK1/2 inhibitor), in one case report, was observed to cause rapid skin re-pigmentation in a patient with coexistent Vitiligo and alopecia areata which also supports our findings [20].

In this current study; tissue expression of JAK/STAT was slightly higher in female Vitiligo skin lesions as compared with males; however this level had non-statistically significant difference. Comparable to this finding; *Nada et al.* reported non-statistically significant association between gender and JAK expression in Vitiligo patients, but on the other hand JAK level showed significant higher values in female psoriatic patients in comparison to male ones [9]. JAKs play an important role in adipose tissue development [21] and females usually have higher fat content as compared to males, which could be a probable explanation for this finding [22]. However, proving or denying this observation requires conducting more studies on larger numbers of population.

Tissue expression of JAK in the current study was significantly higher in Vitiligo skin lesions in patients with positive as compared with negative family history; with

a statistically significant (p-value= 0.011). This was in accordance with the reported significant relationships between JAK1 expression and family history of patients [19]. It had been found that three single-nucleotide polymorphisms (rs310230, rs310236, and rs310241) in JAK1 were associated with susceptibility to Vogt–Koyanagi–Harada syndrome, which is a rare presentation of Vitiligo.

In the present study; non-significant correlation was detected between JAK and patients' age or duration of last new lesion. This was similar to *Nada et al.* study no significant correlation between the JAK1 level in relation to disease duration, disease extent, severity, VASI change or VASI score before and after treatment [9].

In the current study; there was a significant linear moderate positive correlation between tissue expression of STAT and Vitiligo disease activity (VIDA) score among studied Vitiligo patients; (r= 0.547, p=0.002). This was opposite to *Samaka et al.,* study who reported; there was no significant relationship between STAT3 expression and VIDA score [19].

In the current study, there was a significant linear strong positive correlation between

tissue expression of JAK and tissue expression of STAT among studied population; (r= 0.762, p<0.001). These findings are supported by *Samaka et al.,* study results; there was a positive moderate correlation between lesional expression of JAK1 and lesional expression of STAT3 in Vitiligo skin (r=0.52, P=0.004); [19]. Those results suggest a role of JAK1 and STAT3 in the pathogenesis of Vitiligo upon activation of the JAK–STAT pathway. Further studies are recommended to assess this correlation.

Limitations of the study: (the small number of participated patients (cases and controls), it was a short term study).

5. Conclusion:

The overexpression of JAK/STAT in the skin of patients with active Vitiligo, particularly in lesional skin, highlights their pivotal role in the pathogenesis of Vitiligo. Considering the prominently stronger JAK3 expression, selective JAK3 targeting may be a novel promising therapeutic approach for Vitiligo.

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