ANALYSIS

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Tumor Necrosis Factor alpha (TNF-α), as a biomarker for disease activity among Iraqi patients with generalized vitiligo, independent on disease duration and extent of skin involvement

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ABSTRACT

Background: Vitiligo is a chronic depigmenting disorder of the skin that results from immunological distraction of functioning melanocyte. Various studies found alteration in the epidermal level of TNF-α, while only few studies determine the role of serum TNF-α in vitiligo pathogenesis. *Objectives*: To measure serum level of TNF-α among Iraqi patients with subtypes of generalized vitiligo and to correlate it with duration of presentation, activity of vitiligo and extent of skin involvement. *Material and methods*: A case control study includes 80 patients with generalized vitiligo and 40 clinically healthy control subjects, serum concentration of TNF-α was measured by Enzyme Linked Immunosorbent Assay (ELISA) technique. Patients were divided in to those presented with vitiligo ≤ 2 years and patients with> 2 years duration of vitiligo. Activity of vitiligo was assessed based on Vitiligo Disease Activity

(VIDA) score and Vitiligo Extent Score (VES) was used for measuring extent of skin surface involvements. *Results*: Statistically significant elevation of serum level of TNF-α found among patient group compared with controls (*P* value 0.01). No correlation was found between serum level of TNF-α and duration of vitiligo presentation (*P* value 0.27). Patients with active generalized vitiligo have a higher serum level of TNF-α (*P* value 0.01). Extent of skin involvement is not correlated with the serum level of TNF-α (*P* value 0.98). *Conclusion*: Our study shows that TNF-α in the serum is increased in active generalized vitiligo; hence it could be a biomarker for identifying patients with aggressive vitiligo.

Keywords: Vitiligo, Enzyme Linked Immunosorbent Assay, Vitiligo Disease Activity (VIDA) score, Vitiligo Extent Score (VES)

1. INTRODUCTION

Vitiligo is the most prevalent depigmenting disorder of the skin affecting 0.5–1% of individuals worldwide. It can develop at any age, although half of the patients have vitiligo before the age of 20 years. No difference in prevalence exists according to sex, skin type, or race (Alikhan et al., 2011). According to the distribution, pattern, extension, and number of white patches vitiligo is classified in to generalized and localized vitiligo, generalized vitiligo includes subtypes of vitiligo vulgaris, acrofacial vitiligo, vitiligo universalis, and mixed type of vitiligo. Localized vitiligo includes variants of focal, segmental, and mucosal vitiligo (Passeron and Ortonne, 2018; Faria et al., 2014; Ezzedine and Harris, 2019). Etiology of vitiligo is not fully understood; most current evidence supports an autoimmune mechanism responsible in the pathogenesis of vitiligo especially in generalized variant. Autoimmune destruction of melanocytes is mediated by innate immunity, cell- mediated and humeral immunity and with the action of cytokines (Le Poole and Luiten, 2008).

Tumor necrosis factor-alpha (TNF-α) or cachectin is the prototypic member of the TNF superfamily with diverse functions in cell differentiation, inflammation, immunity and apoptosis (Lobito et al., 2011). It is primarily secreted from activated macrophages, although it may also be secreted by other cell types including monocytes, T cells, mast cells, NK cells, keratinocytes, melanocytes, fibroblasts and neurons. TNFα is synthesized as a transmembrane precursor protein (mTNFα) with a molecular mass of 26 kDa (Tracey et al., 2008; Tam and Stępień, 2011), mTNF- is cleaved by the action of the matrix metalloproteinase known as TNFα converting enzyme (TACE) and released as soluble (sTNF-α) that circulates throughout the body and confers TNFα with its potent endocrine function far away from the site of its synthesis (Juhász et al., 2013; Horiuchi et al., 2010).

In vitiligo TNF-α initiate melanocyte apoptosis, decrease melanogenesis, inhibit melanocyte stem cell differentiation and increase melanocyte cytotoxicity (Dwivedi et al., 2013; Alghamdi et al., 2012). There is no standardized method for measuring vitiligo lesions. A quantitative parametric score, named the Vitiligo Area Scoring Index (VASI) was introduced in 2004; VASI score is derived from the Psoriasis Area and Severity Index score used in psoriasis, the score has a strong subjective component, because it involves the physician deciding both the amount of pigmentation and the area of involvement (Hamzavi et al., 2004). The Vitiligo European Task Force score (VETF) that introduced in 2007, measures the extent of vitiligo (the rule of nines), depigmentation severity grading ("staging"), and disease progression ("spreading") in five areas. With VETF score there is a moderate correlation for staging and spreading, this could be explained by that assessment of spreading and staging is based on the examination of a target lesion, the largest one in each graded body area (Taieb and Picardo, 2007).

Vitiligo Extent Score (VES) is another tool that used to measure the global extent of vitiligo, this is an objective, noninvasive method for measuring the extent of the disease and monitoring hyper- or hypopigmented skin compared with the normal skin, this score shows the important advantage of being fast to use for clinicians in everyday practice. The validated VES instrument is based on clinical pictures that mimic the natural distribution of vitiligo including 19 different body areas reflecting 6 different degrees of involvement (1%, 5%, 10%, 25%, 50%, 75%). The purpose of this tool is to select the most representing pictures from the scoring sheet. Users choose the pictures that best represent the patient's skin lesions, getting finally the total extent of the disease. If a patient's lesions differ from the user pictures can adjust the score, using different options, introducing a certain amount of subjectivity (Van Geel et al., 2016).

The evolution course of vitiligo is not easy to predict, however two states can be described, the active state and the stable state (Lahiri et al., 2004). Vitiligo Disease Activity score (VIDA) is a six-point scale used to assess vitiligo stability over time; it depends on patient's own reports of disease activity. Active vitiligo is determined by the expansion of preexisting lesionor the appearance of new lesion. VIDA Score +4 (active vitiligo 6 weeks or less); +3 (activity lasting 6 weeks to 3 months); +2 (activity lasting 3–6 months); +1 (activity lasting 6–12 months); 0 (stable lesion for 1 year or more); and –1 (stable lesion with spontaneous depigmentation for 1 year or more). A low Vitiligo disease activity score indicate less vitiligo activity (Bhor and Pande, 2006). Up to our knowledge this is the first study in Sulaymaniyah-Iraq that measure serum level of TNF- α in correlation with the assessment of duration, activity, and extent of body surface involvement among patients with generalized vitiligo.

2. MATERIALS AND METHODS

Study Design and Setting

A case-control study was conducted in Sulaymaniyah Dermatology Teaching Center – Iraq from April 2018 to December 2019. The study group comprised of 80 consecutive patients presented with subtypes of generalized vitiligo. Patients with localized vitiligo and patients with associated autoimmune diseases (autoimmune thyroiditis, pernicious anemia, diabetes mellitus, and Addison's disease) were excluded from study. Prior to the study enrollment written & oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript. Patient details (age, gender, age of onset of vitiligo, duration of presentation family history of vitiligo, history of associated diseases, etc.) were recorded in a prepared questionnaire. Diagnosis based on clinical ground, VIDA score used to assess activity of vitiligo, VES is used to determine the body surface area (BSA) involvement. Forty clinically healthy volunteers were enrolled as controls. None of the healthy individuals or their first-degree relatives had any evidence of vitiligo and any other autoimmune disease. The baseline venous blood samples (10 ml) were collected from patients and control groups under complete sterile condition, serum has been separated stored at -80°C until the time of estimation of TNFα.

Analysis of Serum Level of TNF-α

Serum levels of TNF-α in patients with generalized vitiligo and healthy controls were measured by ELISA technique using the Enzyme Immunoassay for the quantitative determination of Human Tumor Necrosis Factor α (TNF-α) kit in serum (DRG Instruments GmbH, Germany) as per manufacturers' protocols.

Statistical Analysis

Continuous variables were presented as mean, standard deviation and median. The results were analyzed statistically using independent t test and Analysis of Variance (ANOVA) test (P value ≤ .05) was assumed as significant in the statistical tests). All the calculations were performed using Statistical Package for the Social Sciences from IBM (SPSS), version 24.

3. RESULTS

Demographic and Clinical characteristics of patients with generalized vitiligo

The mean age of vitiligo patients and controls were 28.8 ± 12.4 years and 33.0 ± 12.7 years, respectively, the age of the patient varied from 7 to 50 years and in healthy control from 9 to 49 years. Female patients with subtypes of generalized vitiligo were 57 (71.2%) and 23(28.8%) were males compared to 18(45%) female with 22(55%) male among control group. Fitzpatrick's skin phototypes IV (light brown skin) constitute most of the patients in the current study (53.0%). Vitiligo vulgaris was the most frequent variant of generalized vitiligo accounts for 71(88.8%) patients followed by acrofacial vitiligo 5 (6.35%). Cosmetic appearance was the most frequent chief complaint of enrolled patients in this study account for 67 (83.7%) of patients. Patients were divided in to those presented with vitiligo ≤ 2 years and patients with > 2 years duration of vitiligo. Positive family history for vitiligo among first-degree relatives of patients was 22.5% and 20% of patients had positive family history of vitiligo among their second-degree relatives.

Based on VIDA score active vitiligo was determined either by expansion of preexisting vitiligo lesions reported in 24 patients (30%) or the appearance of new lesions recorded in 56 patients (70%), VIDA score of 1 was the most frequent grading of vitiligo activity reported in 35 patients (43.8%). A lowest VIDA score that is -1 was recorded in 7 patients only (8.8%) and highest score which is 4 is recorded in 9 patients (11.3%). Associated Leukotrichia reported among 24 patients (30.0%) premature hair greying is found 15 (18.8%) of patients with generalized vitiligo. Positive Koebner phenomenon was found in 22 patients (27.5%). For the first time in Sulaymaniyah Iraq we used Vitiligo Extent Score (VES) for determination of extent of skin surface involvement in patients with generalized vitiligo and according to our results we classify patients to those with VES to less than 2, 2-10, 10.1-20 and more than 20. Majority of our patients (38.8%) record VES less than 2 and 8 patients (10.0%) had VES more than 20. Table 1 shows demographic and clinical characteristics of patients with generalized vitiligo.

Serum Level of TNF-α in Patients and Controls

In the current study serum concentration was measured for patients with generalized vitiligo and clinically healthy controls using ELISA technique, TNF-α concentration was significantly higher among patient group compared to age and sex matched healthy controls (*p*-value 0.01) (Table 2 and Figure 1).

Table 1 Demographic and clinical characteristics of patents with generalized vitiligo

Variables	Frequency	Percentage
Fitzpatrick's skin phototypes		
II (Fair skin blue eyes)	11	1.3
III (Darker white skin)	20	25.0
IV (light brown skin	43	53.0
V (Brown skin)	16	20.0
Clinical variants of vitiligo		
Vitiligo vulgaris	71	88.75
Acrofacial vitiligo	5	6.3
Vitiligo universalis	3	3.8
Mixed vitiligo	1	1.25
Chief complaint		
Cosmetic appearance	67	83.8
Cosmetic appearance and pruritus	10	12.5
Pruritus	2	2.5
Sun burn	1	1.3
Duration of presentation		
Vitiligo > 2 years	56	70.0
Vitiligo ≤ 2 years	24	30.0
Family history of vitiligo		
Positive for first degree relative	18	22.5
Positive for second degree relative	16	20.0
Grades of VIDA score		
+ 4 Activity of 6 weeks or less duration	9	11.3
+3 Activity of 6 weeks -3 months	4	5.0
+2 Activity of 3-6 months	14	17.5
+1 Activity of 6-12 months	35	43.8
0 Stable for 1 year or more	11	13.8
-1 Stable with spontaneous re-pigmentation for 1 year or more	7	8.8
Vitiligo Extent Score		
< 2	31	38.8
2-10	30	37.5
10.1.20	11	13.7
>20	8	10.0

Table 2 Serum concentration of (TNF-α) of patients and control groups

TNF-α concentration	Patients (n= 80)	Control (n= 40)	*p* value
Mean ±SD (pg/mL)	12.92 ± 14.40	6.43 ± 4.24	0.01*

*P value< 0.05, Significant; SD; standard deviation;

pg/mL; picogram/milliliter concentration of TNF-α in the serum.

Correlation of serum TNF-α with duration of vitiligo (VIDA score and VES score)

Mean ± SD of TNF-α serum concentration for 24 patients with duration of vitiligo ≤ 2 years was 15.62 ± 18.99(pg/mL) and for 56 patients with duration of vitiligo>2 years was 11.76 ± 11.93(pg/mL), negative correlation was found between serum level of TNF-α and duration of generalized vitiligo (*P* value 0.27). In instant study correlation of serum TNF-α concentration with the activity of generalized vitiligo based on VIDA score found that patients with active generalized vitiligo have significantly higher TNF-α levels (*P* value 0.01) (Table 3, Figure 2).

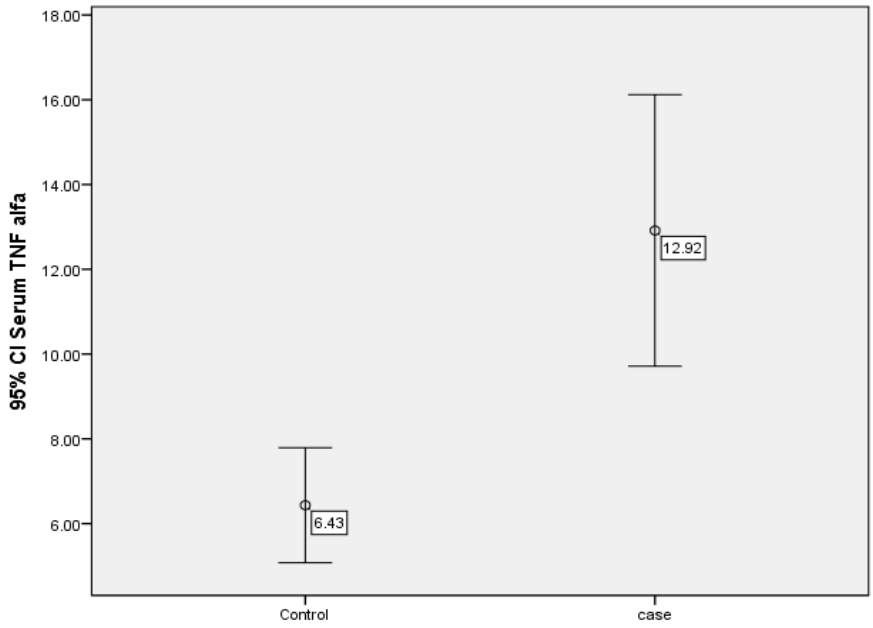

Figure 1 Error-bar showing the difference in the serum level of TNF-α between patients and controls.

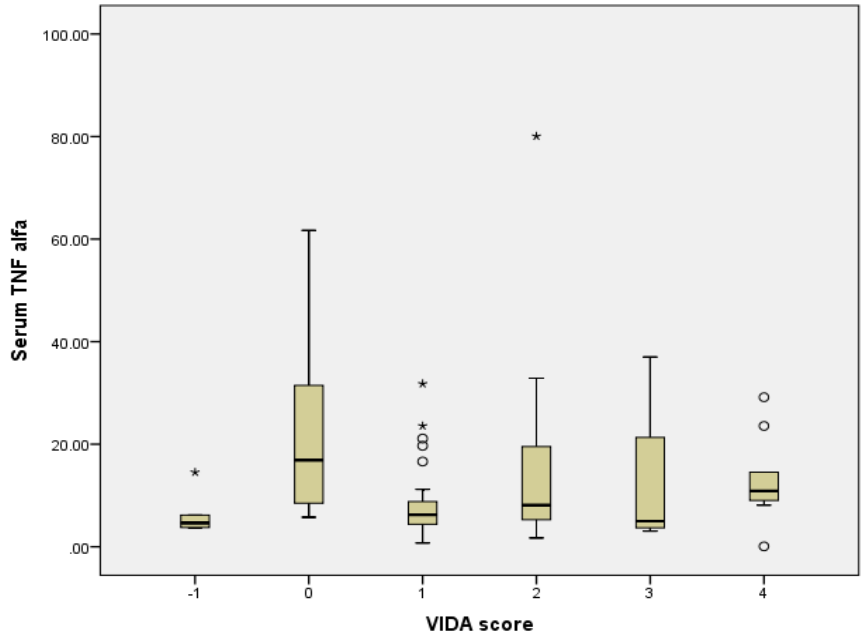

Figure 2 Box plot shows correlation of serum level of TNF-α with VIDA score.

Table 3 Correlation of serum level of TNF-α with the activity of generalized vitiligo based on VIDA score.

TNF-α/ VIDA Score	VIDA Score						Total (n = 80)	*P*-value
	-1 (n = 7)	0 (n =11)	1 (n = 35)	2 (n = 14)	3 (n = 4)	4 (n = 9)		
TNF-α concentration Mean ± SD(pg/mL)	6.10 (4.63)	22.98 (16.91)	8.27 (6.22)	19.98 (23.42)	12.51 (4.99)	13.20 (10.89)	13.20 (7.17)	0.01

*Significant considered as *P* value<0.5.
SD; Standard Deviation
pg/mL; pictogram /milliliter, concentration of TNF-α in the serum.
n; number of patients and controls, VIDA score (-1,0,1,2,3 and 4).

Skin surface involvement estimated based on VES score, negative correlation was found between the four groups of VES score with serum TNF-α concentration (*p* value 0.98) (Table 4, Figure 3).

Table 4 Correlation of serum level of TNF-α with the body surface involvement based on VES score.

TNF-α/ VES score	VES groups				Total (n = 80)	*P* value
	< 2 (n = 31)	2 – 10 (n = 30)	10.1 - 20 (n = 11)	> 20 (n = 8)		
TNF-α concentration Mean ± SD (pg/mL)	12.37 16.90	13.77 14.20	12.79 10.97	12.02 10.12	12.92 14.40	0.98 *

*Significant considered as P <0.5.

SD; Standard Deviation

pg/mL; pictogram /milliliter, concentration of TNF-α in the serum.

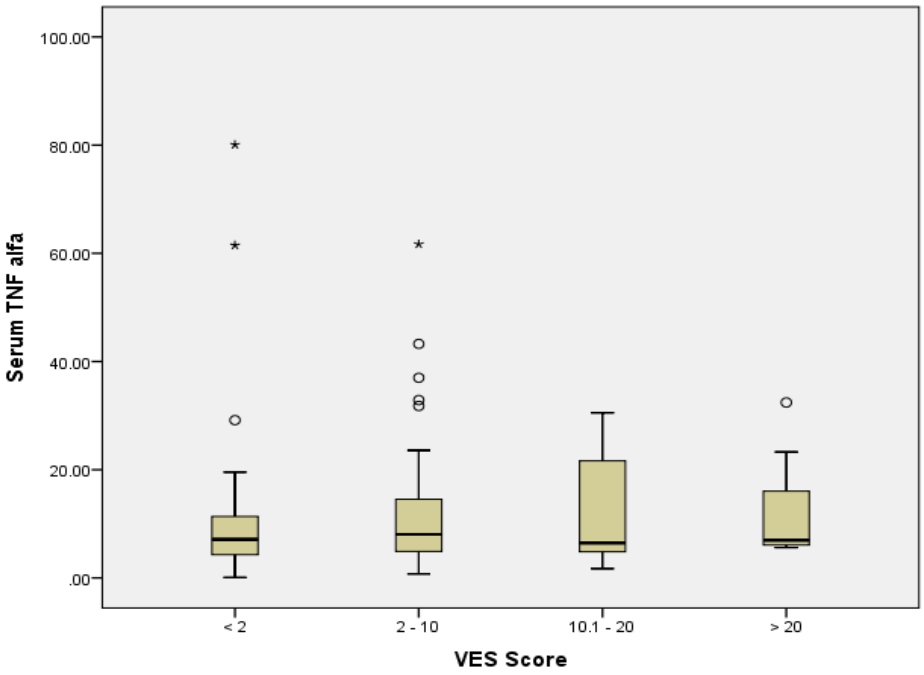

Figure 3 Box plot- correlation of serum level of TNF-α with VES score

4. DISCUSSION

In vitiligo melanocyte function, including proliferation, differentiation and immunologic susceptibility to cytotoxicity can be altered by TNF-α (Morelli and Norris, 1993). Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) are overexpressed in melanocytes from vitiligo lesions, and cytokines such as TNF-α can induce their expression on the surface of epidermal melanocytes (Zhang et al., 2011), this pathway could influence melanocyte target recognition by T-cells and mediate immunologic cytotoxic damage. TNF-αcan inhibit melanogenesis by decreasing the intracellular levels of tyrosinase and tyrosinase-related protein 1, an abundant melanosomal glycoprotein involved in both melanogenesis and prevention of melanocyte death (Norris, 1990; Martínez-Esparza et al., 1998).

In our study significant elevation of serum TNF-α concentration has been found among patients with generalized vitiligo, same result also found in earlier studies by Sushama et al., (2019) and Singh et al., (2012), although in the later study the result was not significant statistically. Mean ± SD of TNF-α serum concentration for 24 patients with duration of vitiligo ≤2 years was 15.62 ± 18.99 (pg/mL) and for 56 patients with duration of vitiligo > 2 years Mean ± SD of TNF-α serum concentration was 11.76 ± 11.93(pg/mL). Negative correlation was found between serum level of TNF-α and duration of generalized vitiligo (*P* value 0.27), for the first-time

negative correlation of serum level of TNF-α with duration of vitiligo das been estimated by Sushama et al., (2019) similar to our result.

In the current study activity of vitiligo determined based on VIDA score, in 56 patients (70%) there was appearance of new white macule of vitiligo, in the remainder 24 patients (30%) enlargement of preexisting vitiligo lesion was a marker as activity over variable periods of time as graded in VIDA score, we found that patients with active generalized vitiligo have significantly higher TNF-α levels, similar result reported by Laddha et al., (2012) and Aydingoz et al., (2015). Negative correlation of serum level of TNF-α with and extent of skin involvement is found in our study, similar to a study by Laddha et al., (2012) but contrary to a study by Sushama et al., (2019) that found elevated serum level of TNF-α among both patient groups with localized and generalized skin surface involvements.

5. CONCLUSION

Serum TNF-α concentration is increased in patients with generalized vitiligo and patients with active disease have higher level than those with stable vitiligo, these findings allow better understanding the role of TNF-α in the pathogenesis of generalized vitiligo and TNF-α could be the future promising cytokine target for biological therapies in patients with progressive vitiligo.

Ethical approval

All procedures performed in this study was approved by the Research Ethics Committee of the College of Medicine, University of Sulaimani (Ethical approval no.3, 20.12.2018)

Abbreviations

TNF-α - Tumor Necrosis Factor Alpha; VIDA - Vitiligo Disease Activity; VES - Vitiligo Extent Score; SD - Standard Deviation; ANOVA - Analysis of Variance.

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Author Contributions

All authors read and approved the final manuscript

1. Conception, data collection, analysis, drafting and writing the article.
2. Conception, design, critical review and final approval of the article.
3. Conception, design, critical review and final approval of the article.
4. Conception, ELISA supervision, critical review and final approval of the article.

Conflict of interest

There are no conflicts of interests

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Data and materials Availability

All data associated with this study are present in the paper.

Peer-review

External peer-review was done through double-blind method.

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