Original Article

Serum Granulysin as a Possible Key Marker of Vitiligo Activity and Severity

Amany I. Mustafa, Waleed A. E. Abdel-Halim[1], Maha M. Osman[2], Shymaa M. Rezk

Departments of Dermatology, Venereology, and Andrology, Faculty of Medicine, [1]Clinical and Chemical Pathology, Faculty of Medicine, Benha University, Benha, [2]Department of Clinical and Chemical Pathology, Faculty of Medicine, Helwan Benha University, Egypt

Abstract

Background: Vitiligo is an immune-mediated, chronic skin condition that affects both the innate and adaptive immune systems. Antimicrobial peptide overexpression is one of its defining characteristics. Granulysin (GNLY), an antimicrobial peptide, may play a role in the pathogenesis of various autoimmune diseases. **Objectives:** To estimate the serum GNLY levels in vitiligo patients and to correlate those levels with the severity and activity of the disease. **Materials and Methods:** This case-control study included 60 non-segmental vitiligo patients (Group A) and a control group of 60 people who were matched for age and sex, appeared to be in good health, and were not suffering from vitiligo (Group B). The serum granulysin levels of all subjects were measured using an enzyme-linked immunosorbent assay. **Results:** When compared to the control group, vitiligo patients had significantly higher serum GNLY levels ($P = 0.001$). When compared to patients with stable disease, those with active vitiligo had significantly higher serum GNLY levels ($P = 0.008$). Additionally, there was a positive correlation between the serum GNLY levels and the vitiligo area severity index and vitiligo disease activity scores ($P = 0.004$ and <0.001, respectively). **Limitations:** Study population was relatively small. Evaluation of serum granulysin before and after treatment could have been more beneficial. **Conclusions:** Blood granulysin levels could contribute to the pathogenesis of vitiligo. A higher serum granulysin level may also be a trustworthy predictor of the severity and progression of a disease.

Keywords: *Autoimmunity, Granulysin, Vitiligo*

Introduction

Vitiligo is an immune-mediated, chronic skin condition that affects both the innate and adaptive immune systems. Despite the scientific progress in understanding the pathogenesis of disease, the cause of vitiligo is still unclear. A few theories based on evidence have been put forth to explain how melanocytes in the epidermis are damaged; however, vitiligo risk is influenced by both genetic and environmental factors. Autoimmune mechanisms provide a more comprehensive explanation for the etiopathogenesis of generalized or non-segmental (NSV) vitiligo.[1,2] Depigmentation is accompanied by an accumulation of auto-reactive CD8+T cells in the skin. The recruitment and activation of T cells may be supported by plasmacytoid dendritic cells, which are a component of the perilesional cellular infiltrate supporting the autoimmune hypothesis. Apoptosis in vitiligo melanocytes has also been shown to support this mechanism.[3]

Granulysin (GNLY) is a protein molecule found in the cytoplasm of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs), where it interacts with perforin and granzyme. It is capable of inducing apoptosis in target cells. Granulysin is also a chemotactic and proinflammatory immune effector molecule.[4-7] It induces T lymphocytes chemoattraction and inflammatory cytokines expression that affect the onset and development of vitiligo.[7] Granulysin is an essential arbitrator in a number of cutaneous inflammatory and autoimmune diseases.[8-13] However, its role in vitiligo has not yet been fully studied. In this study, we sought to assess the serum GNLY level in vitiligo patients and relate it to various clinical parameters.

Materials and Methods

Study population

Sixty vitiligo patients were included in this case-control study (Group A). A control group of 60 subjects who were matched for

How to cite this article: Mustafa AI, Abdel-Halim WA, Osman MM, Rezk SM. Serum granulysin as a possible key marker of vitiligo activity and severity. Indian Dermatol Online J 2024;15:431-6.

Received: 20-May-2023. **Revised:** 23-Jun-2023. **Accepted:** 24-Jun-2023. **Published:** 29-Apr-2024.

Address for correspondence:
Dr. Amany I. Mustafa, Department of Dermatology, Venereology, and Andrology, Faculty of Medicine, Benha University, Postal Code - 13511, Benha, Egypt. E-mail: amanyibrahim26@yahoo.com

Access this article online

Website: http://journals.lww.com/IDOJ

DOI: 10.4103/idoj.idoj_386_23

Quick Response Code:

age and sex and appeared to be in good health was also selected (Group B). All patients were chosen from the dermatology outpatient department at Benha University Hospital. The local ethics board for research involving human subjects approved the study (RC 18-12-2022). Each person was asked for their informed consent before inclusion in the study.

Vitiligo patients who were newly diagnosed or had discontinued treatment (topical or systemic) for 4 weeks before the study were recruited and underwent Wood's lamp examination. Patients with segmental vitiligo, those with infectious, inflammatory, or other autoimmune diseases, those who were taking any systemic medications or receiving phototherapy that might affect vitiligo, pregnant women, and lactating mothers were excluded from the study.

All studied patients provided a detailed history. The Taïeb and Picardo[14] classification was used to classify the studied patients, and the vitiligo area severity index (VASI) score was used to assess disease severity.[15] To assess disease activity, the vitiligo disease activity (VIDA) score was used.[16]

Laboratory investigations

Five milliliters of fresh venous blood were collected from vitiligo patients and controls under complete aseptic conditions by venepuncture on the plain tube for serum separation. The collected samples were left to clot at room temperature for 30 min and were then centrifuged at 1860 g for 10 min. The separated serum samples were kept frozen at -20°C. Serum GNLY was assessed using an enzyme-linked immunosorbent assay kit for human granulysin (human granulysin ELISA kit; Sun Red Biotechnology Company Catalogue No. 201-12-0355).

Statistical analysis

Data was analyzed by SPSS version 25 (IBM Inc., Chicago, IL, USA). Parametric quantitative variables were presented as mean and standard deviation (SD) and compared using Student's *t*-test. Non-parametric quantitative variables were presented as median, minimum, and maximum and compared using Mann–Whitney test for comparison between two groups or Kruskal–Wallis test for comparison between three groups. Qualitative variables were presented as frequency and percentage (%). The receiver operating characteristic (ROC) curve was used to determine the serum GNLY cutoff values with the highest sensitivity and specificity for predicting disease activity. Spearman's correlation analysis was performed to assess the strength of the association between two quantitative variables. A *P* value of 0.05 was considered significant.

Results

The mean age of the studied vitiligo patients was 41.1 ± 14.7 years. Twenty of the study patients (33.33%) were males, while 40 (66.67%) were females. The control group's mean age was 39.6 ± 12.8 years, and in regard to sex, 18 (30%) were males while 42 (70%) were females. The basic clinical and laboratory data of the studied patients are presented in Table 1. When compared to the control group, the vitiligo group had significantly higher levels of serum GNLY ($P < 0.001$) [Table 1 and Figure 1].

Receiver operating characteristic (ROC) analysis revealed that serum granulysin shows 80% sensitivity and 90% specificity in diagnosing vitiligo and 88.9% sensitivity and 66.7% specificity in differentiating active from stable vitiligo at the best cutoff value (41.5 ng/ml) [Table 2, Figures 2 and 3].

When compared to patients with stable vitiligo, those with active vitiligo had significantly higher serum GNLY levels ($P = 0.008$). Nonetheless, no significant relation or correlation was discovered between serum GNLY levels and other clinical characteristics in vitiligo patients ($P > 0.05$ for each) [Table 3 and Figure 4]. Serum GNLY levels correlated significantly with VASI and VIDA scores ($P = 0.004$ and <0.001, respectively) [Table 4].

Discussion

The pathogenesis of vitiligo has been attributed to a number of mechanisms with the leading theory being the autoimmunity theory.[17] A cytolytic protein, granulysin, is produced by activated CTLs. Granulysin levels in the serum accurately indicate the activity of cell-mediated cytotoxic immunity. Consequently, patients with other autoimmune diseases were found to have high serum granulysin levels.[8-12]

In the current study, when compared to the control group, the vitiligo group had significantly higher serum GNLY levels ($P < 0.001$). Cytotoxic CD8+T cells and secreted cytokines are found to be high in vitiligo patients and play a vital role in vitiligo pathogenesis by destroying

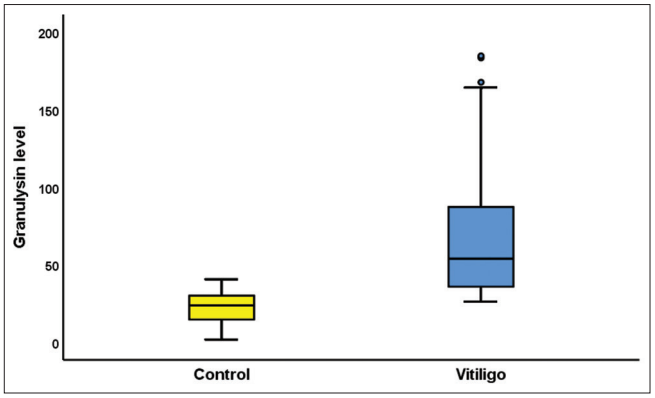

Figure 1: Box plot for serum granulysin level in control and vitiligo groups. The centerline of the box denotes the median value; the extremes of the box are the interquartile range; and the bars are the upper and lower limits of 95% of the data. The circles represent outlying data

Table 1: Comparison of basic data between studied groups

Variable	Control (*n*=60)		Vitiligo (*n*=60)		*P*
Age (years) Mean±SD	39.6±12.8		41.1±14.7		0.721
Sex					
Males	18	30%	20	33.3%	0.804
Females	42	70%	40	66.7%	
Age of onset (years)	-		32.5 (16–67)		-
Duration (years)	-		11.5 (0.25–84)		-
Positive family history	-		8 (26.7%)		-
Course					
Stationary	-		24 (40.0%)		
Progressive			36 (60.0%)		-
Site					
Head and neck	-		36 (60%)		-
Upper limb	-		24 (40.0%)		
Lower limb	-		20 (33.3%)		
Trunk	-		14 (23.3%)		
Clinical types					
Focal	-		24 (40%)		-
Vulgaris	-		16 (26.7%)		
Acrofacial	-		20 (33.3%)		
VASI	-				-
Mean±SD (Range)			9.17±1.95 (0.2–42)		
VIDA	-				-
Mean±SD (Range)			1.17±0.17 (0–3)		
Serum granulysin level (ng/ml) Median (Range)	23.9 (2-40.8)		54.1 (26.4–184.5)		<0.001

VASI - Vitiligo area severity index; VIDA - Vitiligo disease activity score. Mann–Whitney test was used for the comparison of numerical parameters. Numerical data are expressed as mean and SD, compared by *t*-test; categorical data are expressed as number and percentage, compared by Chi-square test

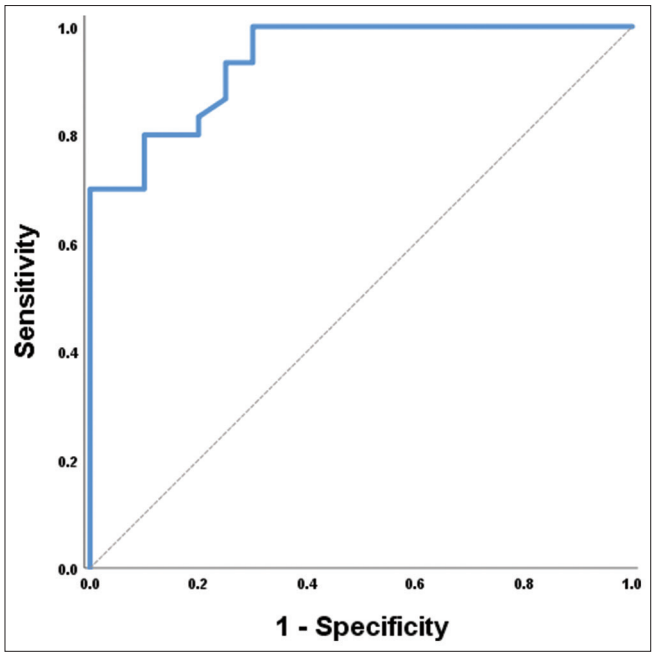

Figure 2: ROC curve of serum granulysin level for discrimination between vitiligo cases and control groups

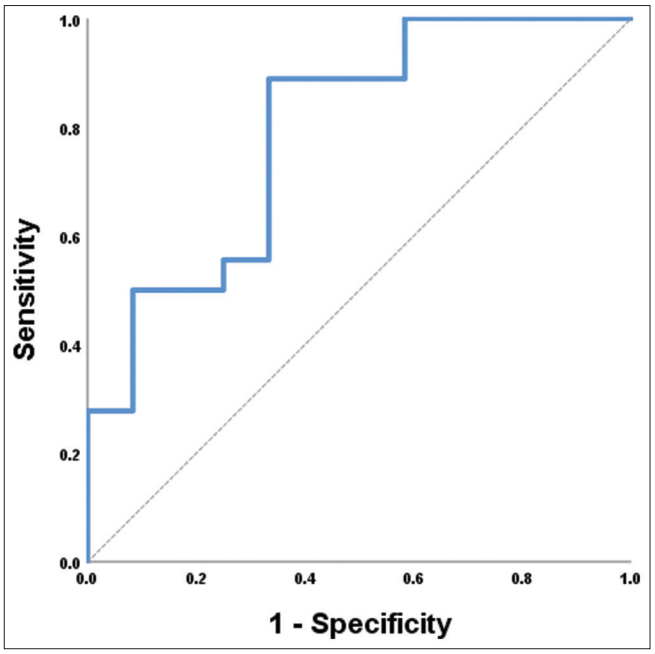

Figure 3: ROC curve of serum granulysin level for prediction of active vitiligo

melanocytes.[18] Hogg *et al*.[19] mentioned that IL-15 and IL-21 induce GNLY expression by peripheral blood CTLs. Also, IL-15 and IL-21 were detected to be high in the serum of vitiligo patients and play crucial roles in vitiligo etiopathogenesis, as IL-15 is crucial for the natural

Table 2: Diagnostic performance of serum granulysin in vitiligo

No. 120	Cutoff value (ng/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	AUC
Patients vs controls	35.3	80.0	90.0	92.3	75.0	84	0.939
Active vs stable	41.5	88.90	66.70	80.0	80.0	80.0	0.792

AUC - Area under ROC (receiver operating characteristic) curve; PPV - Positive predictive value; NPV- Negative predictive value

Table 3: Comparison of serum granulysin level according to the studied parameters in vitiligo patients

Variable	Serum granulysin level (ng/ml)			*P*
	Median	Minimum	Maximum	
Sex				
Males	50.8	26.40	110.80	0.235
Females	56.9	30.00	184.50	
Clinical data				
Course based on history				
Progressive	77.0	30.00	184.50	0.220
Stationary	49.0	26.40	164.10	
Clinical type				
Focal	48.2	30.70	184.50	0.986
Vulgaris	60.1	29.30	164.10	
Acrofacial	130.00	57.00	190.00	
Activity based on VIDA				
Stable	36.6	26.40	110.80	0.008*
Active	76.9	32.40	184.50	

Mann–Whitney test was used for the comparison of numerical parameters between two groups, while Kruskal–Wallis was used for the comparison of numerical parameters between more than two groups. VIDA - Vitiligo disease activity score; *Significant *P*

Table 4: Correlations between the level of serum granulysin (GNLY) and different tested variables in the vitiligo patients

Variable	GNLY level	
	Correlation coefficient (*r*)	*P*
Age	0.055	0.828
Age of onset	-0.254	0.310
Duration	0.342	0.304
VASI	0.641	0.004*
VIDA	0.684	<0.001*

r - Spearman's correlation coefficient. *Significant *P*

killer (NK) cells, neutrophils, and dendritic cells survival and maturation.[20] Granulysin participates in various immune responses with other granular components such as perforin and granzyme.[6,21]

The significant elevation in serum granulysin in vitiligo patients compared to control subjects, as well as its high sensitivity (80%) and specificity (90%) in distinguishing between patients and healthy controls at a cutoff point of 35.3 ng/mL, contributes to a better understanding of vitiligo pathogenesis. Furthermore, its significant elevation in patients with active vitiligo compared to those with stable

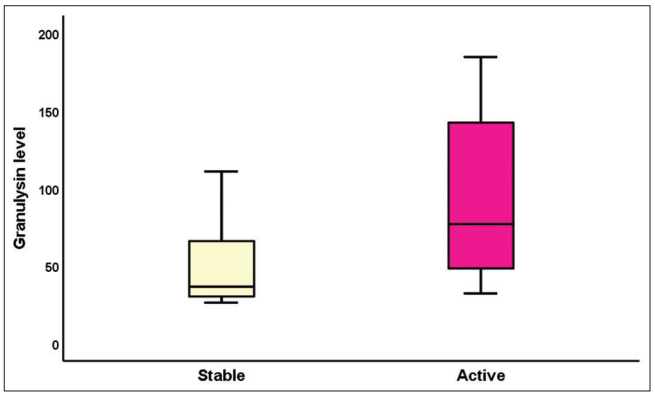

Figure 4: Box plot for serum granulysin level in active and stable vitiligo cases. The centerline of box denotes the median value; the extremes of the box are the interquartile range; and the bars are the upper and lower limits of 95% of the data

lesions, combined with its high sensitivity (88.9%) and specificity (66.7%) in differentiating active from stable vitiligo at a cutoff value of 41.5 ng/mL, suggests that serum granulysin is a promising marker to predict vitiligo progression.

Since granulysin is stable in serum and has a longer half-life, measuring its levels is thought to be helpful for tracking *in vivo* cell-mediated cytotoxic immune responses.[22] Although there are conflicting reports, it has been suggested that apoptotic mechanisms involving the perforin/granzyme B system are responsible for the persistence and severity of some autoimmune diseases in cell-mediated cytotoxicity.[23,24] In vitiligo, chemokine dysregulation results in leukocyte recruitment and the development of melanocyte-specific adaptive immunity.[8,25,26]

Serum GNLY level showed a significant positive correlation with both VASI and VIDA scores ($P = 0.004$ and <0.001, respectively). According to our knowledge, this study is the first to examine the association between serum GNLY levels and vitiligo based on investigating its levels in other autoimmune and inflammatory diseases. Autoimmune factors have been strongly linked to NSV pathogenesis, and studies have mainly concentrated on CD8+CTLs and shown how crucial is a role they play in vitiligo's melanocyte destruction.[27] NSV patients' peripheral blood mononuclear cells and perilesional skin showed significantly higher levels of CD8+CTLs that produce interferon, granzyme B, and perforin.[28,29] The cytolytic granule protein, granulysin, collaborates with perforin and induces melanocytes apoptosis.[6] Granulysin stimulates T lymphocytes chemoattraction and the expression of several inflammatory cytokines that have an impact on the onset and progression of vitiligo.[7,30]

Limitations

The relatively small study population was the current work constraint, so it would be important to conduct a larger-scale study. Further studies evaluating serum GNLY before and after treatment could be more beneficial in assessing the relationship between GNLY, disease pathogenesis, and therapeutic efficacy because samples were not collected both before and after stopping therapy. Further research is advised because our study did not evaluate the GNLY-secreting NK cells in the perilesional/lesional skin of vitiligo patients.

Conclusions

The study's findings imply that vitiligo may be affected by granulysin levels in the serum, and it could also be employed as a predictor of its severity and activity. The clinical relevance of serum granulysin levels in vitiligo has not been extensively studied. To define the function of serum granulysin as a novel marker for vitiligo prognosis, more research is necessary.

Acknowledgments

We are extremely grateful to all patients and volunteers who took part in this study and the research team who collected the data.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Xu M, Liu Y, Liu Y, Li X, Chen G, Dong W, *et al*. Genetic polymorphisms of GZMB and vitiligo: A genetic association study based on Chinese Han population. Sci Rep 2018;8:13001.
2. Lepe V, Moncada B, Castanedo-Cazares JP, Torres-Alvarez MB, Ortiz CA, Torres-Rubalcava AB. A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol 2003;139:581-5.
3. Wu J, Zhou M, Wan Y, Xu A. CD8+T cells from vitiligo perilesional margins induce autologous melanocyte apoptosis. Mol Med Rep 2013;7:237-41.
4. Krensky AM and Clayberger C. Granulysin: A novel host defense molecule. Am J Transplant 2005;5:1789-92.
5. Pena SV, Hanson DA, Carr BA, Goralski TJ, Krensky AM. Processing, subcellular localization, and function of 519 (granulysin), a human late T cell activation molecule with homology to small, lytic, granule proteins. J Immunol 1997;158:2680-8.
6. Kaspar AA, Okada S, Kumar J, Poulain FR, Drouvalakis KA, Kelekar A, *et al*. A distinct pathway of cell-mediated apoptosis initiated by granulysin. J Immunol 2001;167:350-6.
7. Deng A, Chen S, Li Q, Lyu SC, Clayberger C, Krensky AM. Granulysin, a cytolytic molecule, is also a chemoattractant and proinflammatory activator. J Immunol 2005;174:5243-8.
8. Oono T, Morizane S, Yamasaki O, Shirafuji Y, Huh WK, Akiyama H, *et al*. Involvement of granulysin-producing T cells in the development of superficial microbial folliculitis. Br J Dermatol 2004;150:904-9.
9. Morizane S, Suzuki D, Tsuji K, Oono T, Iwatsuki K. The role of CD4 and CD8 cytotoxic T lymphocytes in the formation of viral vesicles. Br J Dermatol 2005;153:981-6.
10. Raychaudhuri SP, Jiang WY, Raychaudhuri SK, Krensky AM. Lesional T cells and dermal dendrocytes in psoriasis plaque express increased levels of granulysin. J Am Acad Dermatol 2004;51:1006-8.
11. Ammar M, Mokni M, Boubaker S, El Gaied A, Ben Osman A, Louzir H. Involvement of granzyme B and granulysin in the cytotoxic response in lichen planus. J Cutan Pathol 2008;35:630-4.
12. Ono S, Otsuka A, Yamamoto Y, Kataoka TR, Koyanagi I, Miyachi Y, *et al*. Serum granulysin as a possible key marker of the activity of alopecia areata. J Dermatol Sci 2014;73:74-9.
13. Chung WH, Hung SI, Yang JY, Su SC, Huang SP, Wei CY, *et al*. Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis. Nat Med 2008;14:1343-50.
14. Taïeb A, Picardo M. The definition and assessment of vitiligo: A consensus report of the Vitiligo European Task Force. Pigment Cell Res 2007;20:27-35.
15. Kawakami T, Hashimoto T. Disease severity indexes and treatment evaluation criteria in vitiligo. Dermatol Res Pract 2011;2011:750342.
16. Bhor U, Pande S. Scoring systems in dermatolog. Indian J Dermatol Venereol Leprol 2006;72:315-21.
17. Rashighi M, Harris JE. Vitiligo pathogenesis and emerging treatments. Dermatol Clin 2017;35:257-65.
18. Atwa MA, Ali SMM, Youssef N, Mahmoud Marie RE-S. Elevated serum level of interleukin-15 in vitiligo patients and its correlation with disease severity but not activity. J Cosmet Dermatol 2021;20:2640-4.
19. Hogg AE, Bowick GC, Herzog NK, Cloyd MW, Endsley JJ. Induction of granulysin in $CD8^{+}$T cells by IL-21 and IL-15 is suppressed by human immunodeficiency virus-1. J Leukoc Biol 2009;86:1191-203.
20. Zhou L, Shi YL, Li K, Hamzavi I, Gao TW, Huggins RH, *et al*. Increased circulating Th17 cells and elevated serum levels of TGF-beta and IL-21 are correlated with human non-segmental vitiligo development. Pigment Cell Melanoma Res 2015;28:324-9.
21. Riding RL, Harris JE. The role of memory $CD8^{+}$T cells in vitiligo. J Immunol 2019;203:11-9.
22. Suda G, Yamamoto Y, Nagasaka A, Furuya K, Kudo M, Chuganji Y, *et al*. Serum granulysin levels as a predictor of serious telaprevir-induced dermatological reactions. Hepatol Res 2015;45:837-45.
23. Ogawa K, Takamori Y, Suzuki K, Nagasawa M, Takano S, Kasahara Y, *et al*. Granulysin in human serum as a marker of cell-mediated immunity. Eur J Immunol 2003;33:1925-33.
24. Bodemer C, Peuchmaur M, Fraitaig S, Chatenoud L, Brousse N, De Prost Y. Role of cytotoxic T cells in chronic alopecia areata. J Invest Dermatol 2000;114:112-6.
25. Kim MO, Suh HS, Brosnan CF, Lee SC. Regulation of RANTES/CCL5 expression in human astrocytes by interleukin-1 and interferon-beta. J Neurochem 2004;90:297-308.
26. Komatani H, Sugita Y, Arakawa F, Ohshima K, Shigemori M. Expression of CXCL12 on pseudopalisading cells and proliferating microvessels in glioblastomas: An accelerated growth factor in glioblastomas. Int J Oncol 2009;34:665-72.

27. Birol A, Kisa U, KurtipekGS, Kara F, Kocak M, Erkek E, *et al*. Increased tumor necrosis factor alpha (TNF-alpha) and interleukin 1 alpha (IL1-alpha) levels in the lesional skin of patients with nonsegmental vitiligo. Int J Dermatol 2006;45:992-3.
28. Lang KS, Caroli CC, Muhm A, Wernet D, Moris A, Schittek B, *et al*. HLA-A2 restricted, melanocyte-specific CD8(+) T lymphocytes detected in vitiligo patients are related to disease activity and are predominantly directed against MelanA/MART1. J Invest Dermatol 2001;116:891-7.
29. Lili Y, Yi W, Ji Y, Yue S, Weimin S, Ming L. Global activation of $CD8^+$ cytotoxic T lymphocytes correlates with an impairment in regulatory T cells in patients with generalized vitiligo. PLoS One 2012;7:e37513.
30. Custurone P, Di Bartolomeo L, Irrera N, Borgia F, Altavilla D, Bitto A, *et al*. Role of cytokines in vitiligo: Pathogenesis and possible targets for old and new treatments. Int J Mol Sci 2021;22:11429.