Egypt. J. Chem. **Vol. 65,** No. 2 pp. 369 - 375 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Estimation Of Serum Levels Of Vitamin D, Calcium, Phosphorous, Matrix Metalloproteinases 1, 2 And Tissue Inhibitor Matrix Metalloproteinase -1 In Patients With Acrofacial Vitiligo During COVID-19

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Abstract

Vitiligo is the loss of functional melanocytes from epidermis. Acrofacial type occurs when depigmentation affects parts apart from the body centers as face, head, hands and feet. Vitamin D may have a role in both acrofacial vitiligo and Covid 19. The present study designed to investigate the role of serum levels of vitamin D, Calcium (Ca), phosphorous (Ph), matrix metalloproteinases 1 and 2 (MMPs 1, 2) and tissue inhibitor matrix metalloproteinase -1 **(**TIMP1) in acrofacial vitiligo during Covid-19. Activity of MMP1, MMP2 and TIMP1 calcium and phosphorous levels were measured in serum. 40 control subjects and 40 patients' suffering acrofacial vitiligo, whose ages ranged from 17-36 years with matching sex and skin phototype all were examined. Vitamin D and Ca levels showed significant decline in their levels in acrofacial vitiligo patients when compared to controls. Additionally, TIMP1 and MMP2 levels displayed significant decrease compared to control subjects, while elevation in MMP1 level was recognized. However, Phosphorous showed no significant difference between controls and cases. No significant alteration between patients and control in terms of sex & age was detected in the present study. We concluded that the selected measured parameters were suggested to participate in controlling and regulating the processes of melanocyte activity and migration in acrofacial vitiligo. These parameters were correlated to each other, since significant reduction in vitamin D & Ca was associated with the decrease in MMP2 and TIMP1 as well as significant increase in MMP1 in vitiligo patients. However, no significant differences were noticed in phosphorous levels in acrofacial vitiligo when compared to control subjects. Significant reduction in vitamin D was also correlated to Covid-19.

Keywords: Vitamin D, Ca, Ph, MMP1, MMP2, TIMP1, Acrofacial vitiligo, Covid-19

1. Introduction

Vitiligo is the most well-known disease of depigmentation, with 1% prevalence around the world. It is defined by loss of melanocytes provoking the formation of depigmented macules and patches [1]. Vitiligo is classified into segmental and non-segmental, with the latest being more usual. Non-segmental vitiligo is described as depigmentation in a symmetric, bilateral outline, and comprise generalized, universal, and acrofacial types with the latest described as depigmented macules restricted to distal extremities, fingers and periorificial areas [1]. Its pathogenesis is still unclear; Even though a compromised response to oxidative stress, autoimmunity and neurogenic constituents all participate in progression of the condition [2].

Vitamin D is a vital hormone produced in the skin [3]. Its active form 1, 25-dihydroxyvitamin D3 adjusts calcium & bone metabolism, regulates cell propagation, differentiation and activates immunoregulatory actions [3]. It influences innate and adaptive immune reactions via receptors in T & B lymphocytes, macrophages & dendritic cells [3] (Figure 1.). Multiple studies specified that 1, 25(OH) 2 D3 inhibits T lymphocytes proliferation by decreasing IL-2 transcription [4]. Vitamin D acts by binding to VDR (vitamin D receptor) forming a heterodimeric complex with retinoid X receptor, then engaged to vitamin D response elements (VDRE) in the target genes triggering their expression via interaction with additional co-regulators [5]. Further,

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Receive Date: 22 July 2021, Revise Date: 17 August 2021, Accept Date: 22 August 2021

DOI: 10.21608/EJCHEM.2021.87074.4210

vitamin D affects the cellular proliferation by modifying different processes including apoptosis, cell cycle progression & differentiation in a cell specific manner [5]. Moreover, it stimulates tyrosinase action and melanogenesis by vitamin D receptor in melanocytes [6]. Some reports have discussed the relation between vitiligo and decreased vitamin D levels [3]. In COVID-19, both pro-inflammatory and anti-inflammatory cytokines are generated. Vitamin D reduced the production of pro-inflammatory T helper (Th1) cytokines, (TNF-α, IFN-γ), and increased the expression of anti-inflammatory cytokines (Th2) [7].

2. MMPs are a group of Zn-dependent proteases. The balance of epidermal melanocytes depends on adhesion protein named E-cadherin which is impaired in vitiliginous skin [8]. Disruption of melanocytes from basal layer of epidermis may result from inhibition of E-cadherin expression, or breakdown into soluble form which might be prompted by several MMPs proteases as MMP1, MMP2 and MMP-9 all are involved in remodeling and cell migration from extracellular matrix [9]. It was found that, vitamin D modulates the expression of some MMP genes in keratinocytes [10]. It regulates the expression & proteolytic activities of MMP-2 and MMP-9 via inducing the nuclear VDR expression [11]. Furthermore, MMP-9 may be an early indicator of respiratory failure in Corona vrus patients [12]. Moreover, vitamin D inhibits MMP-9 production in HaCaT cells as well as MMP-2, MMP-3, TIMP-1, TIMP-2 production in cholesteatoma keratinocytes [10]. Antioxidants (oral) can combat reactive oxygen species and boost the immune system. However, more than 90% of vitamin D which is powerful antioxidant is formed after the body is exposed to sunlight. [14, 15]. In vitiligo, Rathore et al. [16] revealed substantial improvement in CAT and SOD activity after oral antioxidants and NB-UVB phototherapy.

This article aimed to evaluate the role of serum vitamin D, calcium, phosphorous, metalloproteinases 1, 2 (MMP1 and MMP2) and TIMP-1(tissue inhibitor of metalloproteinase -1) levels in acrofacial vitiligo patients compared to control subjects in COVID pandemic.

2. Methods

A Total of 40 control (25 males and 15 females) and 40 patients (30 males and 10 females) with acrofacial vitiligo, their ages ranged from 17-36 years with matching sex and skin phototype, were examined in the Medical Excellence Centre, Dermatology outpatient clinic during the time period from January 2020 to September 2020. Patients' diagnosis was based on clinical outcomes and Wood's light assessment. Family and medical histories were registered from all patients. Controls were enlisted from family members of patients, not suffering vitiligo. Contributors have written an informed approval. Ethical Committee of National Research Centre authorized investigations, with endorsement no. 20/099. Blood samples were taken, centrifuged and stored at −80°C until analyzed. Serum Vitamin D, Calcium, Phosphorous, MMP1, MMP2 and TIMP1 levels were estimated.

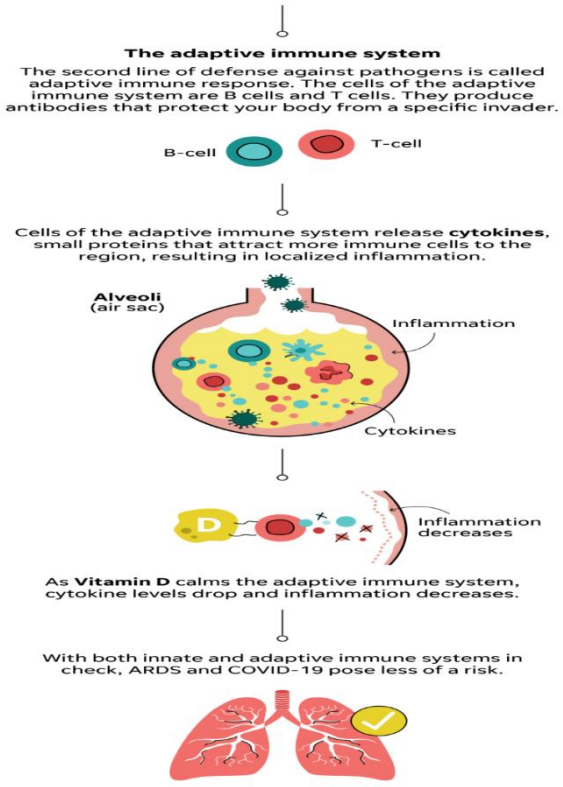

Figure 1: the role of vitamin D in innate and adaptive immune systems.

2.1 Inclusion criteria

Male and female vitiligo patients aging 17-36 years old. The disease is stable for 1 year. Patients not suffering from other autoimmune diseases in the last 6 months. Moreover; patients were receiving neither hormonal therapy, nor ultraviolet therapy and psoralen nor any topical or systemic treatments in the last 6 months.

2.2 Exclusion criteria

Patients with hepato-renal, thyroid disorders, orthopedic problems (e.g. osteoporosis), and neurological disorders were excluded. Also, first degree relatives were excluded from any specimen as vitiligo has a hereditary factor in the pathogenesis. Besides, those taking drugs comprising vitamin D or calcium, or therapy for vitiligo in the preceding 3 months as well as post-menopausal, pregnant and lactating women were also omitted. Moreover,

patients utilizing oral contraceptives, antipsychotics, methyldopa and sunblocks were avoided.

2.3. Preparation of blood samples

Blood samples were taken from patients and controls and divided into two aliquots and immediately centrifuged at 5000 rpm for 10 min at 4°C. Serum was alienated into 8 micro tubes and was separately stored at −80°C until estimation of serum vitamin D, total Ca, Ph, MMP1 MMP2 and TIMP1.

2.4 Assessment of MMP1, MMP2 and TIMP1

The serum activity of human MPP1, MMP2 and TIMP1was measured by ELISA kits according to instruction of manufacturing.

2.5 Assessment of Calcium and Phosphorous

An Olympus AU400 automatic analyzer (Olympus Corporation, Tokyo, Japan) was used to measure total Ca and Ph levels in serum.

2.6 Assessment of 25-OH vitamin D

Serum 25-OH vitamin D was evaluated by High Performance Liquid Chromatography (HPLC).

2.7 HPLC Procedure

400 µl of serum (control, calibrator and sample) was dispensed into tubes, thereafter, 400 µl each of the extraction and precipitation reagents were added. The tubes were vortex-mixed and supernatant was taken after centrifugation. Supernatant was put in tubes, capped and placed in the auto-sampler unit of the HPLC system. The software calculated retention time for peak identification and peak height ratio for quantification.

2.8 Statistical Analysis

Data analyzed by SPSS statistical package version 22. Excel computer program was used to tabulate the results, and represent it graphically. For the quantitative variables which are normally distributed, independent t-test used to declare the significant difference between two independent groups at $p<0.05$.

3. Results

Altogether, 55 males (30 patients [75%] and 25 [62.5%] control) and 25 females (10 patients [25%] and 15 [37.5] control) were incorporated in this study. Mean ages of patient and control groups were 26.90 ±4.60 and 30.50 ±3.33 years, correspondingly. No significant alteration between patients and controls in terms of sex and ages. Mean age at vitiligo macules onset was 10.94 ±3.80 years. Duration of lesions extended from 2 to 10 years. 1 patient informed positive family history.

Present results (Table 1 and 2) showed significant difference in all measured parameters (except Ph) between patients as compared to control, since there is a significant reduction in vitamin D and total Ca as well as TIMP1 and MMP2 levels but significant increase in MMP1 levels in patients compared to controls. No significant variation was detected in Ph levels concerning patients and control groups. Gender showed no significant difference within control or cases groups.

Table 1: Comparison between case and control in levels of Vit. D, Ca and Ph

Parameter	Gender	Case	Control	T-value	P-value
		Mean ± S.D.	Mean ± S.D.		
Vitamin D	Male	15.59 ± 8.22	30 ± 8	6.55	0.001 *
	Female	16.22 ± 7	27 ± 5.88	4.16	0.001 *
Calcium	Male	6.4 ± 3	10.2 ± 9.4	2.09	0.021 *
	Female	6 ± 4.9	10.4 ± 0.85	3.44	0.001 *
phosphorus	Male	5.55 ± 5.37	5.9 ± 3.85	0.27	0.400
	Female	6.10 ± 0.7	6.22 ± 1.7	0.21	0.420

* Means a significant difference by using independent student T-test at $p < 0.05$. Data are expressed as Mean ± S.D. S.D.: Standard deviation

Table 2: Comparison between case and control in levels of MMP-1, MMP-2 and TIMP-1

Parameter	Gender	Case	Control	T-value	P-value
		Mean ± S.D.	Mean ± S.D.		
MMP-1	Male	1747 ± 180.84	1390 ± 250.5	6.13	0.001 *
	Female	1700 ± 316.6	1348.89 ± 154	3.71	0.001 *
MMP-2	Male	157.77 ± 49.32	250.67 ± 58.9	6.37	0.001 *
	Female	163 ± 26	255.8 ± 46.44	5.72	0.001 *
TIMP-1	Male	700 ± 180.84	925.76 ± 250	3.88	0.001 *
	Female	679 ± 142.2	980 ± 116.1	5.81	0.001 *

* There is a significant difference by using independent student T-test at $p < 0.05$. Data are expressed as Mean ± S.D. S.D.: Standard deviation.

4. Discussion

The current results indicated marked decrease in vitamin D level in vitiligo patients in when compared to control, which is also indicated by Silverberg et al. (25-hydroxyvitamin D level <15 ng/mL [17]. Also, vitamin D affects melanocytes and keratinocytes by increasing the action of tyrosinase and melanogenesis [18] that may add a role in repigmentation. In addition to immunomodulatory impacts, vitamin D showed inhibition in interleukin-6 ,-8 (IL- 6), IL-8 and tumor necrosis factor (TNF- α) [19].

Further support by Finamor et al. [20], declared the association of long-term treatment of vitamin D in the repigmentation of vitiligo lesion. Several researches declared reduced levels of vitamin D in several autoimmune disorders, however, it is still vague whether vitamin D is a chief cause or outcome of autoimmune disorders [21]. In agreement with the present study, Karagün et al. [3] revealed declined serum vitamin D levels in the vitiligo patients rather than controls. Patients with autoimmune disease reported low serum vitamin D and Ca levels [22] as shown in the present research (acrofacial vitiligo patients).

Vitamin D3 down regulates the production of inflammatory cytokines, such as TNF-alpha and IL6, but increases inhibitory cytokines, as a result, adequate levels of vitamin D might reduce the incidence of cytokine storm, which can occur in COVID-19 [23].

Current outcomes are in accordance with AlGhamdi et al. [6] who clarified the importance of vitamin D in regulating Ca2+ homeostasis for pigmentation. The default in calcium transport has been revealed in vitiligo keratinocytes and melanocytes. Lower intracellular Ca2+ increases the level of reduced thioredoxin which will inhibit the activity of tyrosinase thus, inhibiting the synthesis of melanin. Calcipotriol participates in Ca2+ regulation through 1, 25-dihydroxyvitamin D3 receptors on melanocytes. Furthermore, El-Sayed et al. [24] pronounced that higher extracellular calcium levels causes production of superoxide radicals that inhibit tyrosinase enzyme. This may be clarified on the basis of the melanogenesis started by oxidation of L-tyrosine to dopaquinone (DQ) by tyrosinase (TYR). The resulting quinone aids as a substrate for the synthesis of eumelanin and pheomelanin [25].

Bergqvist and Ezzedine [2] clarified that, oxidative stress activates transient receptor potential cation channel subfamily M part 2 & subsequently assists mitochondrial dependent apoptosis of melanocytes by increasing calcium inflow [26]. The critical decrease in Ca 2+ level in acrofacial vitiligo patients in the current outcomes might be a main cause of oxidative stress. Mitochondria vigorously creates ROS causing disturbance in the potential of mitochondrial transmembrane and electron transport chain leading to an elevation in the activity of mitochondrial malate dehydrogenase and membrane lipid constituents' modification resulting in disruption in the membrane receptors function, electron transfer and production of ATP in mitochondria [27-29]. This in turn influence the level of Ph production, though in this study, insignificant change was noted in the Ph level in acrofacial vitiligo relative to control subjects.

The current results are associated with significant reduction in MMP2, but increase in the levels of MMP1 in acrofacial vitiligo. In consideration to our results, It is assumed that IFN-γ and TNF-α formed by resident memory T cells in vitiligo skin, might participate in melanocyte disruption. In addition to type 1 cytokines, IL-17& IL-6 are produced in blood or skin of vitiligo patients and elevate MMP1 and MMP-9[30]. IL-1β inhibited MMP-2, MMP-9, TIMP-1 and TIMP-2 production and and vitamin D3 blocked IL-1β inhibition thus the decrease in Vitamin D3 resulted in subsequent decrease in MMP-2 and TIMP-1 in patients suffering acrofacial vitiligo [31]. Although new studies stated that MMP-9 is used as a predictor of Covid 19[12]. Conversely, another gelatinase, MMP-2, was diminished in the sera of vitiligo patients and its formation was reduced by IFN-γ and TNF-α. MMP-2 resulted in migration of melanocyte precursors to their ideal epidermal replenishment [32]. MMP-2 decrease, in addition to elevation in MMP-9 and MMP-1 formation, affecting steadiness of epidermal melanocytes and their replenishment from melanocyte reservoir, resulting in depigmentation in vitiligo. As per the current outcomes, Kumar et al. [33] showed that both MMP-2 and MMP-9 activity was significantly decreased in vitiligo patients. This significant decrease probably diminished migration of melanoblasts from hair follicles or movement of melanocytes from borders of vitiligo lesions into clinically depigmented epidermis necessary for repigmentation of vitiliginous skin.

The current results indicated significant decrease of TIMP1 in vitiligo patients when compared to control. TIMP-1 has been connected to extracellular matrix (ECM) degradation and excessive ECM deposition relies upon the harmony among MMPs and TIMPs. While MMPs degrade the ECM, TIMP suppress MMPs, accordingly preferring deposition of ECM [31]. In this respects Kumar et al. [33] clarified that regulation function of MMPs is inhibited by different TIMPs and transcriptional regulation by TIMP-1 displayed insignificant reduction in expression of mRNA of TIMP-1 between vitiligo patients compared with control.

5. Conclusion

Vitiligo is a complex disorder with multiple factors resulting in its pathogenesis. Though preliminary

outcomes are associated with significant reduction in vitamin D, Ca in the sera of vitiligo patients with marked reduction in MMP2 and TIMP1 while significant increase in MMP1 in vitiligo. However, no significant differences were observed in Ph levels in acrofacial vitiligo compared to control subjects. The marked decline in vitamin D level has been associated with an increase in inflammatory cytokines which increase the risk of coronavirus. No significant difference was detected in all measured parameters in vitiligo patients in terms of sex and gender.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Calcium (Ca)
Phosphorous (Ph)
Matrix Metalloproteinases 1 and 2 (MMPs -1 and -2)
Tissue Inhibitor Matrix Metalloproteinase -1 **(**TIMP1)
Catalase (CAT)
Superoxide Dismutase (SOD)
Interleukin-6,-8 (IL- 6), IL-8
Tumor necrosis factor (TNF- α)
Inflammatory T helper (Th1) cytokines
Viitamin D receptor (VDR)
Vitamin D response elements (VDRE)

Declarations

Ethics approval and consent to participate

Ethical Committee of National Research Centre authorized investigations, with endorsement no. 20/099.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

Funding

No funding was received for this research

Authors' contributions

Paper work for ethics committee approval and blood samples were collected from patients and relative control subjects by Dr. Sherief Mahdy in the Centre of Excellence, Dermatology outpatient Clinic, National Research Centre, Dokki, Egypt. While, Biochemical analysis is carried out by prof. Eman Refaat Youness and Dr. Nadia M. Ahmed in Medical Biochemical lab , Medical Division, National Research Centre , Egypt. Data collecting, statistical analysis and writing, revision of paper were performed by Prof. Hanan F. Aly, Department of Therapeutic Chemistry, National Research Centre, Egypt. All authors have read and approved the manuscript.

Acknowledgements

Authors thank all participants

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