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Effect of ultraviolet radiation on total leukocytes in postmenopausal women with vitamin D deficiency

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A number of environmental factors are involved in the immune system of postmenopausal women, one of which is vitamin D. Vitamin D deficiency is a universal health concern. Epidemiological studies report vitamin D insufficiency and deficiency are associated with poor immune functioning, which may translate to low white blood cell levels. The review adds that vitamin D deficiency is associated with increased infections and susceptibility to cancer. This has led to clinical studies to test whether increasing the levels of vitamin D increases total leukocytes which in turn improves the immune function. The purpose of this study was to investigate the effect of ultraviolet radiation on total leukocytes in postmenopausal women with vitamin D deficiency. Forty postmenopausal women who suffering from vitamin D deficiency were randomized into two groups. Group A received only vitamin D supplement (800 IU) daily for 4 weeks. Group B received ultraviolet B (UV-B) three times per week for 4 weeks with a total of 12 sessions, in addition to vitamin D supplement (800 IU) daily. Both groups were evaluated pre and post-intervention. The outcome measures were anthropometric parameters, vitamin D and total leukocytes count. There was a non-significant difference in the anthropometric parameters ($P > 0.05$) while there was a highly significant increase in total leukocytes count ($P = 0.001$) in favor of group B. This study explored that ultraviolet radiation was associated with increased serum vitamin D and total leukocytes count.

Keywords: Ultraviolet radiation, postmenopausal women, vitamin D, immune, leukocytes.

INTRODUCTION

Decline in immune function with age is viewed as a major problem for the increased risk of age-associated diseases or disabilities (Lin et al., 2016).As with aging, there is a general decline in immune function leading to immune-senescence. This decline is gender specific affecting postmenopausal women due to loss of sex hormones. They show a reduced ability to respond to pathogens or stimuli. As a result, weakened immune response and higher susceptibility to pathogenic invasion and infection (Ghosh et al., 2015).

Vitamin D is a natural regulator of human immune functions. It plays a role in endocrine system and in immunological health. It also, has a positive effect on autoimmune diseases and other immune disorders (Contil and Kempuraj, 2016). As the vitamin D receptor is expressed on immune cells (B cells, T cells and antigen presenting cells) and these immunologic cells are all capable of synthesizing the active vitamin D metabolite. Vitamin D can modulate the innate and adaptive immune responses (Aranow, 2011).

Moderate to strong inverse associations between 25(OH)D concentrations and infectious diseases, inflammation, impaired physical functioning, cardiovascular diseases, serum lipid concentrations, glucose metabolism disorders, weight gain, multiple sclerosis, mood disorders,

declining cognitive function, and all-cause mortality (Rizzoli et al., 2008).

Older people are at an increased risk of the insufficient vitamin D production in the skin due to lower sun exposure and reduced ability of the skin to synthesize vitamin D (Micić et al., 2013). Ultraviolet radiation **(**UV) irradiation of skin has immunomodulatory effects that influence the outcomes of some inflammatory, immune and infectious diseases. Immunomodulation by UV radiation may involve multiple pathways associated with the formation in UV-irradiated skin of vitamin D (Hart et al., 2011).

UVB irradiation is crucial in enabling the synthesis of vitamin D within the body, but vitamin D is also, although less commonly, obtained through diet via the ingestion of foods such as fatty fish and offal, or through the use of vitamin D supplements (Morgan et al., 2017)**.** Vitamin D is formed in the skin, by the action of UVB and UVC on 7-dehydrocholesterol. It has been understood for many years that, exposure to artificial UVR or artificial sun light is curative for vitamin D deficiencies (Svobodová et al., 2012). So, the purpose of this study was to investigate the effect of ultraviolet radiation on total leukocytes in postmenopausal women with vitamin D deficiency.

MATERIALS AND METHODS

Participants:

Forty postmenopausal women who were suffering from vitamin D deficiency (Total serum 25(OH) D ranged from 12 - < 30 ng/ml. were selected from the outpatient clinic, faculty of physical therapy, Cairo University.Their age ranged from 55 to 65 years and their body mass index ranged from 25 to 35 kg/m^2.They were medically stable. The postmenopausal women were excluded from this study if they had any neurological, cardiovascular disorders or metabolic disease or been received hormonal replacement therapy or any drug known to affect the immune function. Also, postmenopausal women who had a history of photosensitivity, a history of active skin disease or any history of skin cancer were excluded from this study.

The study was approved by Research Ethical Committee, Faculty of Physical Therapy, Cairo University. The study protocol was explained to all postmenopausal women, who had signed an informed consent form. The postmenopausal women were randomly assigned to one of 2 groups using computer generated random numbers. Allocation was concealed in sequentially numbered opaque envelopes. Group A consisted of 20 postmenopausal women who received only vitamin D supplement (800 IU) daily for 4 weeks. Group B received ultraviolet B (UV-B) three times per week for 4 weeks with a total of 12 sessions, in addition to vitamin D supplement (800 IU) daily.

Outcome measures:

Anthropometric measures:

Weight-Height scale was used to measure the weight and height of each postmenopausal woman before starting the study and after the end of the interventional program. Then, BMI was calculated according to the following formula:
BMI = weight (kg) / height square (m)^2

Blood collection and analysis:

A blood sample was taken from each subject in both groups (A and B) before and after the treatment at to estimate blood leukocytes' level. This was done two times, before starting the study and after the end of the study. Each woman was asked to lie in half lying position, with well supported back and arms. The antecubital area was cleaned with alcohol. Blood sample was drawn from the antecubital vein from all women by disposable sterile syringe by venipuncture to determine the blood leukocytes' level.

3. Interventions:

Vitamin D supplementation (for both groups):

All postmenopausal women received vitamin D supplementation (800 IU) daily for 4 weeks.

Ultraviolet device (for only group B):

Ultraviolet arc lamp, it was low-pressure mercury discharge tube with a phosphor coating on inside. The particular wavelength and the amount of each emitted light will depend on the composition of the phosphor used (mixtures of phosphates, borates, and silicates). This gives a considerable UVA and UVB output but no UVC. Radiation spectrum: 280- 320nm. Type: 4004/2n-verreét quartz was used for treating postmenopausal women in the group (B). Before starting the treatment, a full explanation was given to each postmenopausal woman about what was going to be done either in group (A) or in group (B).

postmenopausal women in group (B) were treated by ultraviolet through the following technique:

Before application of UV, the physical therapist determined the individual postmenopausal woman's sensitivity to UV by using dose response assessment which is graded according to the individual's erythema response and is categorized as:

Suberythemal dose (SED): No change in skin redness occurs in the 24 hours following UV exposure.

Minimal erythemal dose (MED) or E1: The smallest dose producing erythema within 8 hours after exposure that disappears within 24 hours after exposure.

Second-degree erythema (E2): Definite redness with some mild desquamation appears within 6 hours after exposure and lasts for 1 to 3 days. E1=MED X 2.5

Third-degree erythema (E3): Intense erythema with edema, peeling, and pigmentation appears within 2 hours or less after treatment and is like severe sunburn. E3= MED X 5.

Fourth-degree erythema (E4): Erythema with severe blistering, peeling, and exudation. E4= MED X 10.

In general, treatment time is selected as a proportion of the MED of UV which was determined through following steps: Physical therapist asked postmenopausal woman to wear safety glasses (goggles) to protect her eyes from UV. Then, asked the woman to remove all clothing and wash abdominal area. After explaining the procedures to the woman and asking her not to look to the lamp because of possibility of damage the eyes. Therapist took a piece of cardboard approximately 4 by 20 cm and cut three different shape holes 2 by 2 cm in it.

Then placed this cardboard on the abdominal area and draped the area around the cardboard so that the surrounding skin was not exposed to the radiation. The lamp set up at 60 to 80 cm away from and perpendicular to the abdominal area and turned on the lamp. The arc lamp took 5 to10 minutes warming up to reach full power before turning it toward the woman. Once the lamp had reached full power, the UV beam directed directly toward the abdominal area and started the timer. The three holes were exposed to UV at one time for 30 seconds, then covered 2 holes and leave one for 30 seconds then after 30 seconds covered one hole and leave the other for another 30 seconds.

According to this protocol, the first hole was exposed for 90 seconds, the second for 60 seconds, the third for 30 seconds. The therapist asked the woman to record the response of the area for the 24 hours following exposure. The part that showed mild reddening of the skin within 8 hours that disappeared within 24 hours was treated with the MED. In this study the desired dose was E_1.

After determining the MED for each woman, the session of UV was applied as follows:

The postmenopausal woman was instructed to remove clothing from the lower abdomen and lay in supine lying position to allow the maximum exposure of the part being treated and to avoid undue exposure of other parts.

A thick blanket was used to cover rest of the body which did not need exposure. Eyes need to be protected by goggles to avoid exposure.

The therapist adjusted the ultraviolet Arc lamp to be in a perpendicular position above the lower abdominal area by about 60- 80 cm. The lamp turned on and adjusted the timer according the detected MED for each woman.

When the treatment time was completed, switched off the lamp and turned it away from the postmenopausal woman.

Any observable response to the treatment was documented.

Treatment session was given three sessions per week up to complete a month.

Statistical analysis

All statistical measures were performed through the Statistical Package for Social Studies (SPSS) version 23 for windows. The current test involved two independent variables. The first one was the (tested group); between subjects' factor which had two levels (Group A receiving vitamin D supplementation (800 IU) & Group B receiving ultraviolet B (UV-B) three times per week for 4 weeks with a total of 12 sessions, in addition to vitamin D supplement (800 IU) daily). The second one was the (training periods); within subject factor which had two levels (pre-treatment and post treatment). In addition, this test involved two tested dependent variables (Leukocytes and vitamin D). Preliminary assumption checking revealed that data was normally distributed for all dependent variables, as assessed by Shapiro-Wilk test ($p > 0.05$); there were no univariate or multivariate outliers, as assessed by boxplot and Mahalanobis distance ($p > 0.05$), respectively; there were linear relationships, as assessed by

scatterplot; no multi collinearity. There was homogeneity of variances ($p > 0.05$) and co-variances ($p > 0.05$), as assessed by Levene's test of homogeneity of variances and Box's M test, respectively. Accordingly, 2×2 mixed design MANOVA was used to compare the tested variables of interest at different tested groups and training periods. The MANOVAs were conducted with the initial alpha level set at 0.05.

RESULTS

Baseline and demographic data

As indicated by the independent t test, there were no statistically significant differences ($P>0.05$) between subjects in both groups concerning age, body mass, height and BMI (Table 1).

Statistical analysis using mixed design MANOVA analyzed thirty patients assigned into two equal groups. It revealed that there were significant within subject ($F= 91.357$, $p = 0.0001$), treatment*time ($F = 74.668$, $p = 0.0001$) and between subject effects ($F = 26.429$, $p = 0.0001$). Table (2) present descriptive statistic and multiple pairwise comparison tests (Post hoc tests) for the leukocytes and vitamin D. In the same context, the multiple pair wise comparison tests revealed that there was significant increase ($p <0.05$) in leukocytes and vitamin D in the post treatment condition compared with the pre-treatment one in group B with no significant differences in leukocytes and vitamin D ($p > 0.05$) in the post treatment condition compared with the pre treatment one in group A. Regarding between subject effects multiple pair wise comparisons revealed that there was significant increase ($p < 0.05$) in leukocytes and vitamin D in group B compared with group A,.

Table (1): Demographic characteristics of both groups:

	Group A	Group B	Comparison	
	Mean ± SD	Mean ± SD	t-value	P-value
Age (years)	59.3±3.34	58.85±3.03	0.446	0.658
Body mass (Kg)	71.25±4.9	75.15±5.90	-2.271	0.055
Height (cm)	159.25±5.4	160.55±5.44	-0.758	0.453
BMI (kg/m^2)	28.11±1.79	29.21±2.71	-1.507	0.14

Table (2): Descriptive statistic and multiple pairwise comparison tests (Post hoc tests) for the leukocytes and vitamin D for both groups at different measuring periods.

	Group A		Group B	
Variables	**Pre**	**Post**	**Pre**	**Post**
Leukocytes	3940(343.97)	4025(343.16)	3895(387.26)	5030 (1222)
Vitamin D	15.49 (3.15)	16.2 (3.13)	17.55 (3.33)	31.61 (6.08)

Within groups (Pre Vs. post)		
p-value	**Leukocytes**	**Vitamin D**
Group A	0.624	0.358
Group B	0.0001*	0.0001*
p-value	Leukocytes	Vitamin D
Pre treatment	0.7	0.052
Post treatment	0.001*	0.0001*

*The mean difference is significant at the alpha level ($p< 0.05$).

DISCUSSION

The aim of this study was to investigate the effect of ultraviolet radiation on total leukocytes in postmenopausal women with vitamin D deficiency. The finding of the current study that there was a non-significant difference in the anthropometric parameter, while there was a highly significant increase in total leukocytes count in favor of group B.This study conducted a physiotherapy intervention (ultraviolet therapy device) owing to its low cost, ease of use and that may lead to improve immunity and quality of life in postmenopausal women and decrease the need of higher dose of vitamin D supplementation with side effects which may affect postmenopausal women.Blood leukocyte count and composition provide an indicator of the inflammatory and immune status of an individual. Leukocytes play various roles in inflammation and immunity. Neutrophils and monocytes are key players in innate immune system, with neutrophils being the first-responders of inflammatory cells to migrate towards the site of inflammation at the acute phase. Lymphocytes play central roles in adaptive immune system, with T lymphocytes being instrumental to cell-mediated immune response whilst B lymphocytes being responsible for production of antibodies in humoral immunity. Eosinophils and basophils are involved in allergy (Chen et al., 2016).

Richards et al.,[11]reported that a longer Leukocyte telomere length (LTL) is associated with increased serum vitamin D concentrations in women. The positive association between LTL and vitamin D concentrations is independent of age and many other covariates. Vitamin D exerts immunomodulatory effects that may attenuate LTL attrition rate.

There are several mechanisms that may explain the association between LTL and vitamin D concentrations. Vitamin D decreases the mediators of systemic inflammation, such as interleukin-2 and tumor necrosis factor-α. Vitamin D receptors are ubiquitously expressed in T and B lymphocytes, natural killer cells, and monocytes, and through the down-regulation of cytokines and other pro inflammatory factors, vitamin D exerts profound anti-inflammatory and anti-proliferative actions, which would affect the turnover rate of leukocytes. It follows that vitamin D would reduce the rate of LTL attrition.

In addition, previous study revealed that there is association between vitamin D deficiency and leukopenia in systemic lupus erythematosus (SLE) patients (Simioni et al., 2016).All previous studies examined the effect of vitamin D supplementation on the immune system and none of these studies analyzed the effect of ultraviolet irradiation. Therefore, this study aimed to investigate the effect of ultraviolet on total leukocytes in postmenopausal women. This study may expand the role of physiotherapy in the field of the women's health. Although the current study provides objective data with statistically significant differences, there may be some limitations that include the short duration of follow up. So, further studies are needed to examine the long term effect of ultraviolet irradiation on total leukocytes in postmenopausal women.

CONCLUSION

Ultraviolet irradiation may have positive effects on immunity through increasing its total leukocytes counts.

CONFLICT OF INTEREST

Authors declare no potential conflicts of interests.

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AUTHOR CONTRIBUTIONS

MA proposed the research idea and design, performed the practical part, and helped in writing the manuscript. MA, SM, AM and HM designed the experiment, performed the statistics, writing and reviewing the manuscript. All authors read and approved the final version.

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