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Original Article

Correlation between transepidermal water loss with proinflammatory cytokines in non-aging and aging group

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Abstract

Background Aging is a complex process and makes progressive loss of functional capacity of organ such as skin. Transepidermal water loss represents changes in skin permeability which happen in aging. There is an imbalance of immune system in aging. It can cause condition called inflammaging. Proinflammatory cytokines such as IL-6 and TNF-α were elevated in skin aging.

Purpose: This study aims to find correlation between TEWL and proinflammatory cytokines in non-aging and aging.

Methods This research is an analytic observational study with cross sectional design. The IL-6 and TNF-α level were measured by using sandwich ELISA technique. TEWL was measured by Tewameter.

Results The mean value of IL-6 and TNF-α in both non-aging and aging group showed no significant difference. The TEWL in aging and non-aging group showed no statistically significant difference with IL-6 and TNF-α.

Conclusion There was no significant relationship between TEWL and proinflammatory cytokines in non-aging and aging group.

Key words
Aging, TEWL, IL-6, TNF-α.

Introduction

Aging is an unavoidable and complex process characterized by progressive loss of functional capacity of organ such as skin. This biological phenomenon happens overlapping between internal (genetic, hormonal) and environmental (ultraviolet, pollution) factors.[1-4] Recently, people in all the world aged 60 years or over are increasing rapidly. This phenomenon will spend high costs of medical care for the elderly.[5] In aging skin, there are changes in skin barrier permeability and these changes can be measured with transepidermal water loss (TEWL). TEWL refers to the total amount of water loss through the skin and generally accepted as skin parameter for skin barrier function of stratum corneum.[6-8] It varies with gender, body site and pigment types.[8]

The immune system of aging or known as immunosenescence decreases, with major implications for health and survival.[9,10] Changes in skin barrier function can lead to a sustained increase in cutaneous inflammation and moreover it could finally become systemic inflammation into a condition which is called inflammaging.[8] Inflammaging has become widely used to describe inflammatory process of aging and is characterized by low-grade, chronic

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pro-inflammatory state. During this process, the balance of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are elevated.[11,12] Interleukin-6 are commonly found in middle aged population than TNF-α which is more found in age 80 years or above.[11] Previous study showed that TEWL value varied with aging. The correlation between TEWL and proinflammatory cytokines had not been studied yet, so this study wanted to find correlation between TEWL and proinflammatory cytokines.

Methods

This research is an analytic observational study with cross sectional group design. It was conducted at our hospital and was approved by the hospital's ethics committee. Thirty-eight healthy volunteers aged 25-44 years (non-aging) and 45-65 years (aging) were enrolled for this study. Exclusion criteria were: 1- Subjects had any chronic diseases such as diabetes, liver and renal dysfunction or cancer, 2- Pregnant women, 3- Those who consumed any medication within three months before this research was conducted. All the subjects gave their written informed consent to participate this study. Anthropometric measurements including weight (in kg) and height (in cm) were measured in all subjects. Body mass index (BMI) was calculated using the formula: weight (kg)/height^2 (m^2). The assessment of TEWL was done in right forearm (extensor area) volunteers. TEWL of forearm was measured using the Tewameter® TM300. The temperature in the room was 20°C and the relative humidity was 50%. The subjects were acclimatized in the test room 20 minutes before measuring TEWL variables. For interpretation of results, readings were adjusted with the Research and Development Department Courage & Khazaka Electronics GmbH, Koln, Germany namely: under (<)8 grams/ hour/ m^2= decreased; Between 8-15 grams/ hour/ m^2=normal; above (>)15 grams/ hour/ m^2=increase. Before the test, all the instruments were calibrated to ensure the accuracy of the instrument. IL-6 and TNF-α level measurements were assessed by sandwich ELISA technique in Biochemistry laboratory, Faculty of Medicine, Brawijaya University.

All statistical analyses were performed using *Statistical Package for the Social Sciences* (SPSS) data format version 20.0 (SPSS, Inc., Chicago, Illinois). The main outcome measures in this study were IL-6 and TNF-α levels. The statistical test used was the normality test with the *Shapiro-Wilk* test because the sample number was smaller than 30 per group and the data were normally distributed (p> 0.05). Spearman's correlation test was also used to analyze correlation between TEWL and proinflammatory cytokines (IL-6 and TNF-α).

Results

The characteristics of the sociodemographic data show that the number of non-aging and aging group are the same. The mean value of IL-6 in non-aging group was higher than aging group but it was not significantly different. On the contrary, the mean value of TNF-α in non-aging group was lower than aging group but it was also not significantly different **(Table 1)**. This study consisted of more women (60.5%) than men (39.5%). Most TEWL values were increased (68.4%) which then followed by normal (23.7%) and decreased (7.9%) values. The mean value of IL-6 in decreased value of TEWL was higher than increased and normal value of TEWL but it was not significantly different. On the contrary, the mean value of TNF-α in normal value of TEWL was lower than increased and decreased value of TEWL but it was not significantly different.

Analysis using One-way ANOVA showed that there were no significant differences in mean IL- and mean TNF-α between groups (p value>0.05).

Table 1 Subject distribution based on age.

Age (years)	*N*	*%*	*Mean IL-6 ± SD*	*p*	*Mean TNF-α ± SD*	*p*
Non-aging (25-44)	19	50.0	20.058 ± 10.215	0.775	2.489 ± 1.356	0.744
Aging (45-65)	19	50.0	19. 294 ± 5.467		2.622 ± 1.130	
Total	38	100				

Table 2 Subject distribution based on TEWL.

TEWL	*N*	*%*	*Mean IL-6 ± SD*	*p*	*Mean TNF-α ± SD*	*p*
Decreased	3	7.9	23.347 ± 4.722		2.390 ± 0.330	
Normal	9	23.7	16.610 ± 2.826	0.365	2.388 ± 0.958	0.859
Increased	26	68.4	20.314 ± 9.332		2.633 ± 1.388	
Total	38	100				

Spearman Correlation Test was used to prove:

1. The correlation between age and proinflammatory cytokines

In the output results, the IL-6 correlation coefficients in non-aging and aging group are 0.114 and 0.021 respectively. This means that the correlation between age with IL-6 in both groups are very weak. The IL-6 correlation coefficient in non-aging group is negative, which means that the older subject, the lower IL-6 or vice versa. Meanwhile the IL-6 correlation coefficient in aging group is positive which means that the older subject, the higher IL-6 or vice versa. In addition, the Sig (2-tailed) value of IL-6 in non-aging and aging group were not significant because p> 0.05. The TNF-α correlation coefficients in non-aging and aging group are 0.004 and 0.201 respectively. This means that the correlation between age with TNF-α in non-aging group is weaker than aging group. The TNF-α correlation coefficient in both groups are positive which means that the older subject, the higher TNF-α or vice versa. In addition, the Sig (2-tailed) value of TNF-α in non-aging and aging group were not significant (p> 0.05).

2. The correlation between TEWL and proinflammatory cytokines

In the output results, the IL-6 correlation coefficients of TEWL in non-aging and aging group are -0.261 and 0.365, respectively. This means that the correlation between TEWL with IL-6 in both groups are weak. The IL-6 correlation coefficient in non-aging group is negative, which means that the higher TEWL, the lower IL-6 or vice versa. Meanwhile the IL-6 correlation coefficient in aging group is positive which means that the higher TEWL, the higher IL-6 or vice versa. In addition, the Sig (2-tailed) value of IL-6 in non-aging and aging group were not significant (p> 0.05). The TNF-α correlation coefficients of TEWL in non-aging and aging group are -0.198 and 0.125, respectively. This means that the correlation between age of TEWL with TNF-α in both groups are weak. The TNF-α correlation coefficient in non-aging group is negative, which means that the higher TEWL, the lower TNF-α or vice versa. Meanwhile the TNF-α correlation coefficient in aging group is positive which means that the higher TEWL, the higher TNF-α or vice versa. In addition, the Sig (2-tailed) value of TNF-α in non-aging and aging group were not significant (p>0.05).

Discussion

To our knowledge, this is the first study that find correlation between proinflammatory cytokines and TEWL in non-aging and aging subjects. As compared to aging group, the non-aging group had higher IL-6 but not significantly different. It also had lower TNF-α than in non-aging group but not significantly different. In accordance

with our result, Kim *et al*.[13] found that there were no significant association of IL-6 and TNF-α levels with age. On the contrary, study of Wu *et al.*[14] and Ye *et al*.[15] proved that both IL-6 and TNF-α were significantly elevated in chronologically aged than young subjects. Lumentut *et al*.[16] found that TNF-α level in age 64-74 years and >90 years were higher than age 75-90 years.

Our results showed that there were weak correlations between TEWL and proinflammatory cytokines both IL-6 and TNF-α in non-aging and aging group. TEWL in aging group seems to be similar or slight decreased compared with non-aging group. It would be possible because TEWL values vary with environmental factor and internal factor like body metabolism and sweat gland activity. In aging there is disruption of epidermal permeability barrier which can increase expression levels of cutaneous cytokines and inflammatory infiltration in the skin. These may lead to systemic inflammation and increase proinflammatory cytokines level.[8] Based on our results, the subject in non-aging and aging group did not have any skin disease like atopic dermatitis, seborrheic dermatitis or psoriasis. It means that inflammaging process was minimal and that might make systemic inflammation minimal too. A larger sample size also should be used in the future so that it will be able to provide a more accurate depiction of the relationships involved. It is better to evaluate cutaneous inflammatory cell to know the inflammaging process.

Conclusion

Based on our results, we have shown proinflammatory cytokines changes in aging and non-aging groups. IL-6 was elevated in non-aging group and TNF-α was elevated in aging group. We also measured TEWL and find correlation between TEWL and proinflammatory cytokines. There was no significant relationship between TEWL and proinflammatory cytokines in non-aging and aging group.

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