ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2020.12.04.502

Research Article

The Effect of Tomato Extract Supplementation to Interleukin-17 Serum Level in Women with Melasma

LIZA AFRILIANA[1*], LISYANI BUDIPRADIGDA[2], KIS DJAMIATUN[3]
[1]Dermatology and Venereology Department, Faculty of Medicine, Diponegoro University, Indonesia
[2]Clinical Pathology Department, Faculty of Medicine, Diponegoro University, Indonesia
[3]Biomedical Science Study Program, Faculty of Medicine, Diponegoro University, Indonesia
*Corresponding authors
Email:lizaafriliana@ymail.com
Received: 02.08.20, Revised: 14.09.20, Accepted: 10.10.20

ABSTRACT

Background: Melasma, a skin-aging-manifestation, at the face can affect the woman-quality of life. An additional-modalities is required to improve effectiveness and decrease side-effects of hydroquinone-cream, a melasma-gold-standard-treatment. Lycopene-rich-tomato-extract is considered, because it protects UV-light-induced-erythema and reduces oxidative-stress-induced-tissue-damage. IL-17-expression of melasma is higher than normal-skin. Lycopene supplementation suppresses IL-17 in cardiovascular-disease. IL-17 is not only produced by Th17-cells, but also by Treg-cells in the recent-studies.

Purpose: To determine whether tomato-extract supplementation influenced the IL-17-serum-level which accompanied by the reduced-mMASI-score, the recovered-indicator of melasma.

Methods: A true-experimental-clinic with a double-blind, randomized pre and post-test control design was performed in this study done in a dermatology-policlinic of Diponegoro-National-Hospital. There were 62 women with melasma based on consecutive-sampling, were grouped equally into treatment and control-group. They were examined for mMASI-score and IL-17-serum-level before and after-treatment. Those group received melasma-standard-therapy (hydroquinone-4%-cream and sunblock in the night and morning). Additionally, the treatment-group consumed one tomato-extract-capsule/day/patient for 12-weeks while control received a placebo. The statistical analyses used were Wilcoxon and Mann-Whitney-U test.

Results: The pre-treatment-IL-17-serum level and mMASI score between two groups was no different. The-IL-17-level of treatment-group significantly increased from pre-treatment to post-treatment (median (minimal-maximal) ng/ml, 33.3 (6.4-323) to 61.1 (13.7-380.3); $p = 0.033$). The significantly increase was not observed in control-group (47.5 (12.2-254.4) to 61.4 (8.4-417.1). A significantly-decrease-mMASI-score was found in both groups at the end of study ($p < 0.001$), however the decrease-mMASI-score was more clearly in treatment than control-group ($p < 0.001$).

Conclusion: The tomato-extract-adjuvant-therapy relates to a significantly-increase-serum-IL-17-level from pre to post-treatment accompanied with more pronounced decrease-mMASI-score in women with melasma.

Keywords: Melasma, tomato-extract, IL-17, mMASI-score.

INTRODUCTION

BACKGROUND

Skin aging is a dynamic process in human skin that causes skin changes [1]. Melasma is a manifestation of skin aging, which generally occurs in areas that are frequently exposed to UV, importantly face [2,3]. Melasma can affect the quality of life, importantly in emotional and psychological in women, which appearance is very important [4].

Melasma can occur in all genders, ages and races, but more than 90% in women [4]. Melasma generally occurs in the productive age [2]. Onset melasma between 30 until 55 years old [3]. The prevalence of melasma in Indonesia is 18,1%, while in the world varies, between 1,5% and 33,3% [4,5]. UV is the most important factors in patogenesis melasma, besides genetic and hormonal [6]. Melasma is more common in individuals who lives in areas with high UV exposure, such as Asia [7].

The management of melasma often have difficulties. Uneffective, side effects, and recurrent often occur while melasma treatment [8]. The gold standard treatment of melasma is hydroquinone cream. Hydroquinone can inhibit the conversion of dopa to melanin by inhibiting the tyrosinase enzyme [3]. Hydroquinone often causes side effects,

such as irritation and allergic contact dermatitis, importantly in long term management. Hydroquinone could be given as the single treatment, and also be combined, double or triple treatment. Kligman's formula is the name of triple combination, there are hydroquinone with tretinoin and dexamethasone [3]. A study about effectivity of single hydroquinone therapy in melasma found the success rate was 76.9%, but 25% subjects got side effects [9]. A study by Halim E, et al (2014) shows that triple combination therapy gives faster improvement than double combination of hydroquinone and tretinoin. This study also show that double combination's therapy has more side effects and worsens more quickly when therapy is stopped [10].

In long term treatment, additional modalities is required to improve effectiveness and decrease side effects of hydroquinone cream [3,11]. One of the additional modalities is tomato extracts, which is rich of lycopene [12]. UV is one of the most important factors that induce melasma [6]. Lycopene has been proven to have a protective effect on the incidence of erythema induced by UV light [13]. Studies comparing the amount of lycopene and β-carotene in plasma and skin have shown that more skin lycopene is destroyed than β-carotene. This suggests the role of lycopene in reducing tissue damage due to oxidative stress [14].

A study found IL-17 expression in melasma lesion is higher than normal skin [15]. Study of lycopene supplementation in cardiovascular disease has been shown IL-17 suppression [16,17]. A new study found that IL-17 is not only produced by Th17 cells, but Treg can also produce IL-17 [18]. IL-17 production by Treg can increase IL-17 production and increase IL-17 serum level after intervention. This study aimed to prove that tomato extract supplementation can increase IL-17 serum level in women with melasma.

METHODS

Design and samples

The study was a true experimental clinic. It was a double-blind, randomized pre and post-test control design. It was comparing an oral tomato extract supplement (Pureclinica Pharmacy), as an additional to standart therapy of melasma (hydroquinone 4% at the night and sunblock at the daytime). Inclusion criterias were females, 30 to 55 years old, and have normal Body Mass Index (BMI). We exclude if they were pregnant or lactating patients, under supplements or medication which improve melasma, consuming alcohol or cigarettes, taking hormonal therapy and any treatment that influences melasma lesion. We had 62 subjects based on consecutive sampling. The subjects were divided into two group with 31 subjects each in the treatment and control group.

We collected characteristic data of subjects, such as age, profession, education, marital status, hormonal contraception's history, sun exposure and duration of melasma. Melasma diagnosis was done by 2 experienced dermatologist. IL-17 serum level was processed by ELISA in GAKI Laboratory, Diponegoro University.

Study procedures

A biostatistician was randomized all subjects by a randomization software. Subjects were randomized in a ratio of 1:1 to either treatment or control-group. The randomization list was sent to the independent pharmacist to be used for preparation and labelling of the study drugs. The randomization list was accessible only by the biostatistician and designated personnel directly responsible for packaging and labeling of study materials. The active supplement and placebo which were given to treatment and control-groups, respectively, were similarly packaged in identical capsules. The containers were labelled with the trial number. Adequate supply of study capsule was dispensed on day 0 and stored at room temperature. This methode could be both the patient and researcher were blinded to the capsule. Unblinding of the study occurred only after all the patients had completed the study.

Intervention

Tomato extract supplements from Pureclinica Pharmacy that were given to treatment-group. Eavh tablet was crushed into powder and put it in a capsule. The powder of placebo was filled in a capsule with the same size and colour. The active and placebo capsules were packaged in the same packaging. The capsules were distributed by a pharmacist who was uninvolved in the study assesments. Both of groups were instructed to take one capsule after each meal, for 12 weeks. All subjects were given standart therapy of melasma. They were hydroquinone 4% cream for night and sunblock for daytime use. All of subjects were educated on the need for sun protection and also informed to stop all other products or treatment for their face that can influence the study assesment.

The investigator were explained to all subjects about all of can occur in the duration of study. All of subjects were given a log book. They must write about all of occur in the duration of study, such adherence to take the supplement and cream, side effects, consuming the food or taking medication and therapy that can influence melasma. Patients were asked about the symptoms of burning, redness, pruritus and

swelling at follow up study. The telephone number of the researcher was listed in the logbook. All of subjects were measured in mMASI score and IL-17 serum level at pre and post study.

Data analysis

The study were included by 62 subjects, but 3 of them drop out. We analyzed by protocol. We analyzed 59 subjects, they were 30 treatment-group and 29 control-group. The statistical analyses were performed using SPSS version 21. The result of mMASI score assessment by 2 dermtologists was tested by agreement test with Intraclass Corelation Coefficient (ICC). Wilcoxon signed-rank test was used to analyze differences in the changes of IL-17 serum level in each group, while Mann-Whitney U test was used to analyse differences in the changes of IL-17 serum level between pre and post study in both groups.

Ethical consideration

This study was performed according to the declaration of Helsinki and approved by the Health Research Ethics Committee Faculty of Medicine, Diponegoro University. The ethical number was 394/EC/KEPK/FK UNDIP/IX/2019. Prior to the study, the subjects were informed the purpose of this study, the intervention, the benefits and side effect that will occur during the study. The subjects were voluntary, hence, all of them had the right to withdraw from the study at any time during the study period. All subjects signed an informed consent to participate in this study.

RESULTS

Table 1. Summary characteristics of baseline variables

Baseline Variables	Treatment-group	Control-group	p-value
	(n = 30)	(n = 29)	
Age (tahun), mean (SD)	45.97 ± 5.223	47,07 ± 6.798	**0.489**
Profession, count (%)			**0.204**
- Housewife	6 (20)	8 (27.6)	
- Entrepreneur	6 (20)	5 (17.2)	
- Government employees	10 (33.3)	5 (17.2)	
- Farmer/ labor	1 (3.3)	6 (20.7)	
- Others	7 (23.3)	5 (17.2)	
Education, count (%)			**0.022**
- Elementary School	2 (6.7)	0 (0)	
- Junior High school	2 (6.7)	4 (13.8)	
- Senior High School	9 (30)	18 (62,1)	
- Academy/ University	17 (56,7)	7 (24.1)	
Marital status, count (%)			**0.174**
- Married	26 (86.7)	21 (72.4)	
- No married	2 (6.7)	2 (6.9)	
- Widow	2 (6,7)	6 (20,7)	
Hormonal contraception's history, count (%)			**0.128**
- Yes	6 (20)	11 (37.9)	
- No	24 (80)	18 (62.1)	
Sun exposure (hour), median (minimum-maximum)	1 (1-8)	2 (1-5)	**0.488**
mMASI score week 0, median (minimum-maximum)	5.25 (2.4-14.4)	6,0 (0.6-17.4)	**0.644**
IL-17 level pre, median (minimum-maximum)	33.3 (6.4-323)	47.5(12.2-254.4)	**0.161**

The statistical analyses used were Chi Square and Mann-Whitney U test.
The majority of basic characteristics are homogeneous, except education (p = 0.01).

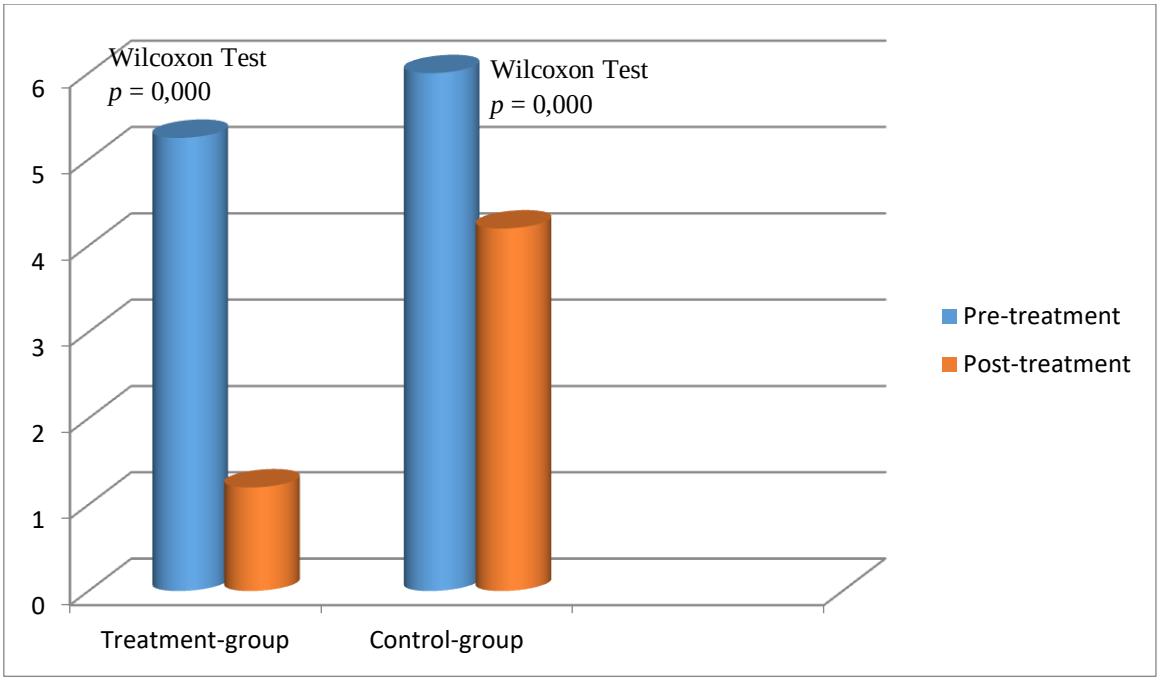

Fig.1:mMASI score pre and post study

In the treatment-group, the median of mMASI score pre-treatment was 5.25 and post-treatment was 1.2. There was a significant decrease (p = 0,000). In the control-group, the median of mMASI score pre-treatment was 6.0 and post-treatment was 4.2. There was a significant decrease (p = 0,000).

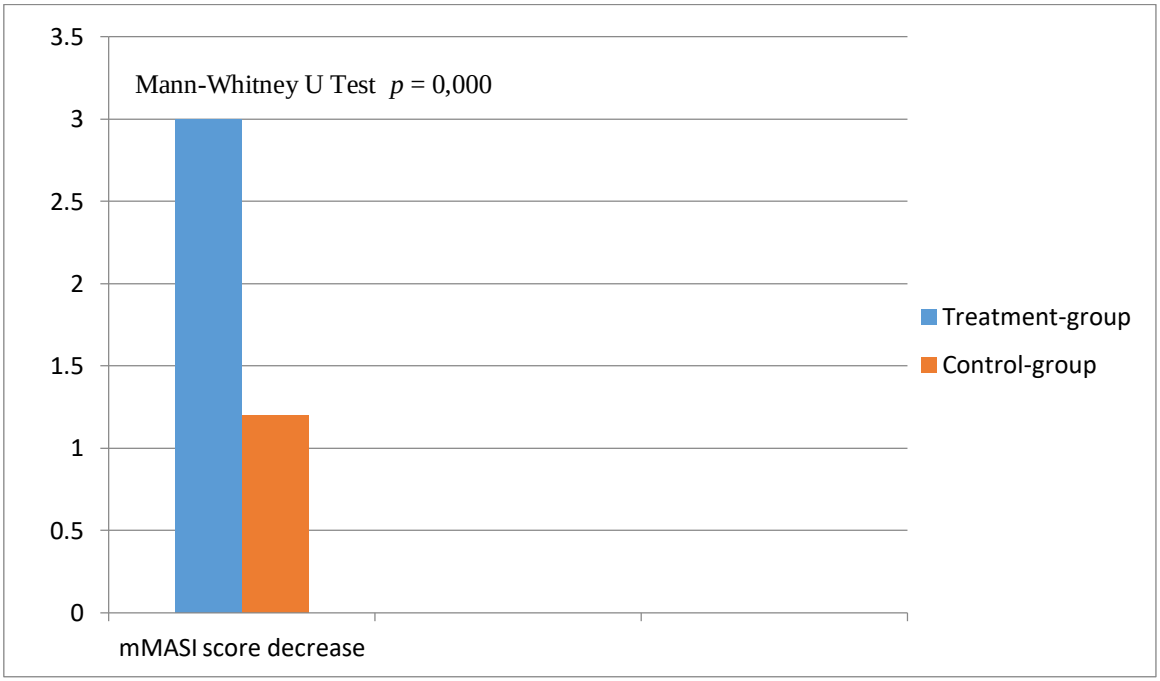

Fig.2:The different of mMASI score pre and post-study between treatment and control group

The decrease of mMASI score in the treatment-group was 3 and in the control-group was 1.2. There was a greater decrease seen in the treatment-group than the control-group and there was significant difference (p = 0.000).

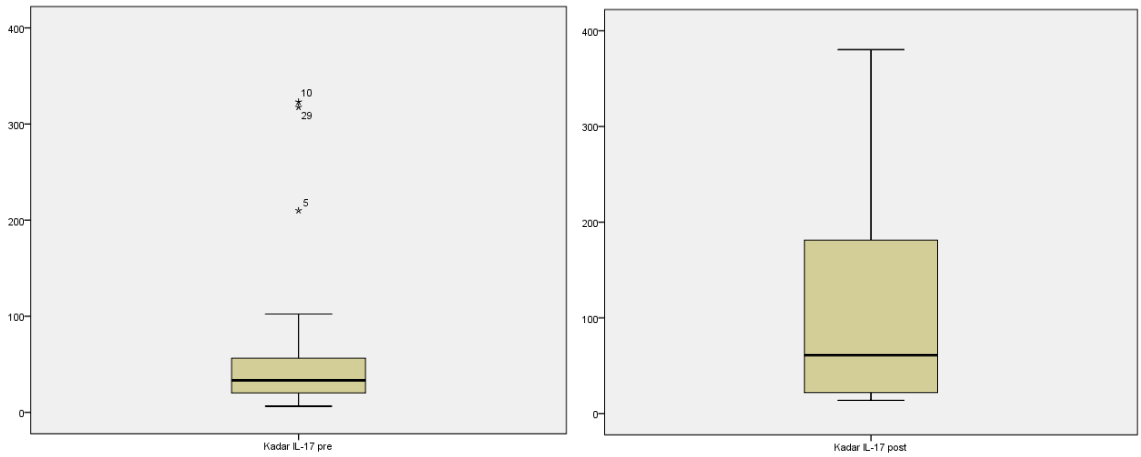

Fig.3:The box plot of IL-17 serum level's treatment-group

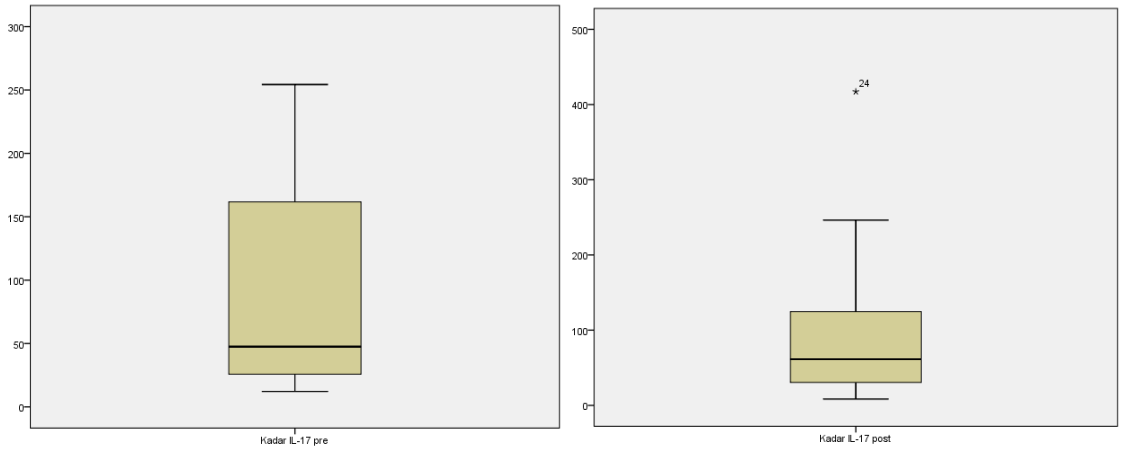

Fig.4:The box plot of IL-17 serum level's control-group

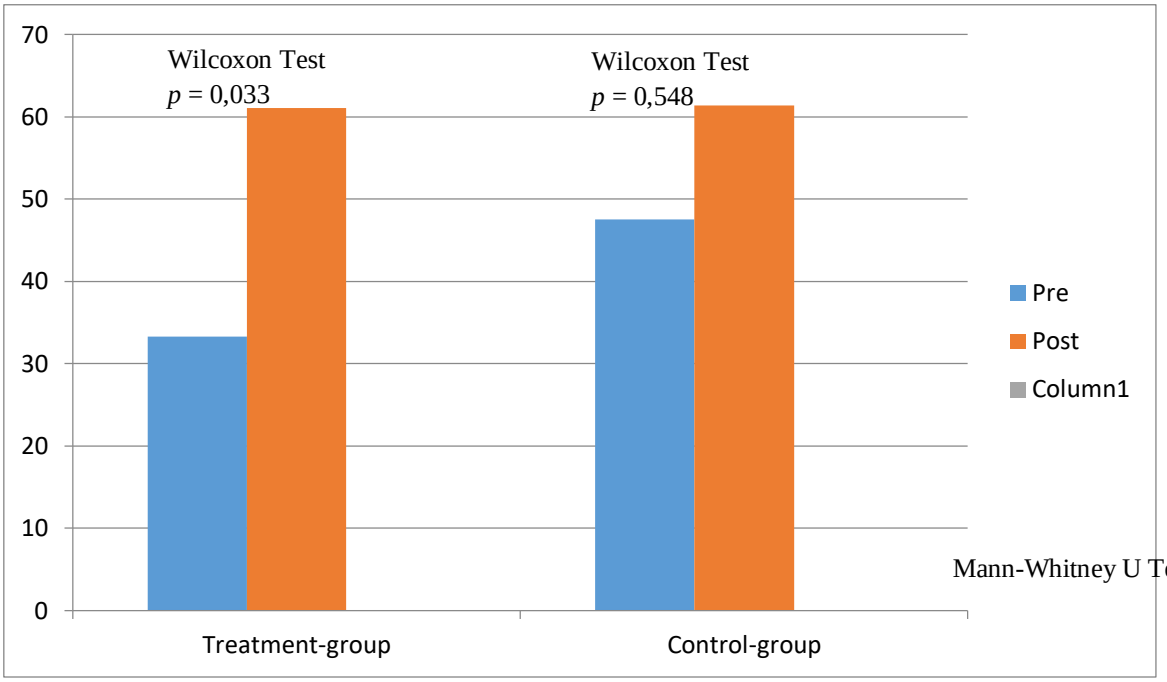

Fig.5:IL-17 Serum Level in treatment and control-group

The increase of IL-17 serum level in the treatment-group was greater than the control-group. There was a significant increase of IL-17 serum level pre and post-treatment in the

treatment-group and no significant in the control-group.

Table 2. IL-17 Serum Level pre and post-study

IL-17 Level (ng/ml)	Treatment-Group (n=31)		Control-Group (n=31)		p*
	Mean ± SD	Median (Minimum-Maximum)	Mean ± SD	Median (Minimum-Maximum)	
• Pre	61.32 ± 80,81	33.3 (6.4-323)	89.34 ± 82.31	47.5 (12.2-254.4)	0.161
• Post	107.78 ± 101.61	61.1 (13.7-380.3)	95.11 ± 91.21	61.4 (8.4-417.1)	0.940
p**	0.033		0,548		

*The result of Mann-Whitney Test**The Result of Wilcoxon Signed Ranks Test

The median IL-17 serum level pre-treatment between both of the groups was homogen (p = 0.161). There was also unsignificant difference at post-treatment between both of the groups. (p = 0.940). The median IL-17 level in treatment-group at pre-treatment was 33.3 (6.4-323) and post-treatment was 61.1 (13.7-380.3). It was significant increase (p = 0,033). The median IL-17 serum level in control-group at pre-treatment was 47.5 (12.2-254.4) and post treatment was 61.4 (8.4-417.1). It was unsignificant increase.

DISCUSSION

In the treatment-group, there was a significant decrease of mMASI score between pre and post-study, while in the control-group was no significant. There was a greater decrease seen in the treatment-group than the control-group and there was significant difference. The median of IL-17 serum level significant increase in treatment-group. The aim of this study to prove that tomato extract supplementation can increase IL-17 serum level in women with melasma. This study proven that tomato extract supplement can increase IL-17 serum level in melasma patients.

The etiopathogenesis of melasma is very complex and not yet fully known [8]. Oxidative stress and inflammation play a major role in the development of melasma [19]. UV, genetic and hormonal are the most important factors in patogenesis melasma [6]. UV is one of the most important factors that induce melasma [6].

Supplementation of tomato extract is important in tissue that is inflammed, prevents the formation and fight of free radicals, so it plays a positive effect to overcome the signs of aging, such as melasma [10]. The main composition of tomato extract is lycopene [5]. Lycopene is a powerful antioxidant. It plays a role in neutralizing free radicals and reducing tissue damage due to oxidative stress. Lycopene also has anti-inflammatory effects [20].

Lycopene has been shown to have a protective effect on the incidence of UV-induced erythema [6]. A study comparing the amount of lycopene and β-carotene in plasma and skin showed more skin lycopene destroyed than β-carotene. This shows the role of lycopene in reducing tissue damage due to oxidative stress [14].

Role of IL-17 has been known in psoriasis, but research by Arambula, et al showed a role for IL-17 in the pathogenesis of melasma [15]. IL-17 can induce the production of chemokines and other cytokines in various cells and will attract neutrophils to the site of inflammation [21]. IL -17 interacted synergistically with TNF-α to induce increased expression of innate inflammatory mediators [22]. IL-17 enhances the TNF effect by increasing the expression of the TNF 2 receptor (TNFR2). TNF-α, IL-1β, TGF-β and IL-6 can enhance Th17 differentiation. IL-17 cannot inhibit Th1 or Th2 differentiation, so Th1 and Th2 usually dominate Th17 cells. TGF-β can stimulate Th17 differentiation by suppressing the production of IFN-γ and IL-4 inhibitor cytokines. TGF-β together with IL-6 can induce the expression of the transcription factor RORγt, which is a regulator of Th17 [23]. A new literature found that IL-17 is not only produced by Th17 cells, but Treg can also produce IL-17 [18]. IL-17 production by Treg can increase IL-17 production and increase IL-17 serum level after intervention. Treg can also produce TNF-α. The TNF-α produced will attach to the cell membrane of TNF-α (mTNF) and bind to TNFR2, which will induce tissue repair. Tissue repair induction can improve in the degree of melasma severity and decrease in the mMASI score.

CONCLUSIONS

Overall of these finding indicate that there was significant increase of IL-17 serum level in treatment-group, between pre and post treatment. In conclusion, this study shows that tomato extract supplement can increase IL-17

serum level in melasma patients. So, tomato extract supplement potentially has an additional role in melasma treatment, although further larger-scale and longer studies are required to prove its effectiveness over topicals alone.

Acknowledgements
The author would like to acknowledge Mrs Maria for her assistance in the statistical analyses.

REFERENCES

1. Stojiljkovic D, Pavlovic D, Arsic I. Oxidative stress, skin aging and antioxidant therapy. Acta Fac Med Naiss. 2014; 31 (4): 207-17.
2. Lapeere H, Boone B, de Schepper S, Verhaeghe E, Gele MV. Ongenae K, et al. Hypomelanosis and Hypermelanosis. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, editors. Fitzpatrick's Dermatology in General Medicine, 8th ed. New York: McGraw-Hill; 2012: p819.
3. Bagherani N, Gianfaldoni S, Smoller B. An overview of melasma. J Pigmen Disorder, hybrid open acces journal. 2015; 2: 10.
4. Sarkar R, Arora P, Garg VK, Sonthalia S, Gokhale N. Indian Derm Online J. 2014; 5(4): 426-35.
5. Melyawati, Suseno LS, Bernadette, Legiawati L. MDVI. 2014; 41(3): 133-8.
6. Harumi O, Goh CL. The effect of Melasma on the quality of life in a sample of women living in Singapore. J Clin Aesthet Dermatol. 2016, 9(1): 21-4.
7. Sarkar R, Puri P, Jain RK, Singh A, Desai A. Melasma in men: a clinical, aetiological and histological study. J Eur Acad Dermatol Venereol. 2010; 24: 768-72.
8. Guarneri F. Etiopathogenesis of Melasma. J Pigment Disorder. 2014; S1 (003): 1-2.
9. Haddal AL, Matos LF, Brunstein F, Ferreira LM, Silva A, Costa D, et al. A clinical, prospective, randomized, double blind trial comparing skin whitening complex with hydroquinone vs. placebo in the treatment of melasma. Int J Dermatol 2003; 42: 153-6.
10. Halim E, Triestianawati W, Nilasari H, Legiawati L, Novianto E, Indriatmi W. Perbandingan efektivitas dan keamanan terapi krim kombinasi asam retinoat 0,05%, hidrokuinon 4% dan flusinolon asetonid 0,01% dengan krim kombinasi asam retinoat 0,05% dan hidrokuinon 4% untuk terapi melasma pada orang Indonesia. MDVI 2014; 41 (2): 60-5.
11. Zaleski L, Fabi SG, Goldman MP. Treatment of melasma and the use of intense pulsed light: a review. J Drugs Dermatol. 2012; 11: 1316-20.
12. Rosita N, Haryadi DM, Erawati T, Nanda RP, Soeratri W. Photostability study on character and antioxidant activity of tomato extract (Solanum lycopersicum l.) in Nanostructured Lipid Carrier (NLC) and conventional creame. Int J Drug Deliv Tech. 2017; 7(1): 71-4.
13. Rizwan M, Rodriguez-Blanco I, Harbottle A, Birch-Machin M.A, Watson R.E.B and L.E. Rhodes. Tomato paste rich in lycopene protects against cutaneous photodamage in humans in vivo: A randomized controlled trial. British J Dermatol. 2011 (164): 154–62.
14. Schagen SK, Zampeli VA, Makrantonaki E, Zouboulis CC. Discovering the link between nutrition and skin aging. Dermato-Endocrinology. 2012; 4 (3): 298-307.
15. Arambula AR, Alvarez BT, Garcia DC, Ahumada CF, Cazares PC. CD4, IL-17, and COX-2 are associated with subclinical inflammation in malar melasma. Am J Dermatopathol. 2015; 37: 10.
16. Mills LM, Wilson H, Frank T. Abstract 425: Lycopene Modulates T Lymphocyte Function by Modulating Th1 Responses and Treg Lymphocyte Population. Arterioscler, Thromb, Vasc Biol. 2015; 35: A425.
17. Campano CGA, Ward F, Mois R, Thies F. Lycopene inhibits mitogen-activated lymphocyte activity by modulating T helper type 1 and type 17 activities. Proc Nutr Soc. 2017; 76 OCE2: E32.
18. Jung Min Kyung, Kwak Jeong-EUn, Shin Eui-Cheol. IL-17A-Producing Foxp3+ regulatory T cells and human diseases. Immune Network. 2017; 276.
19. Yaar M, Gilchrest BA. Aging of skin. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, editors. Fitzpatrick's Dermatology in General Medicine, 8th ed. New York: McGraw-Hill; 2012: p1213-26.
20. Sheth Vaneeta M, Pandya Amit G. Melasma: A comprehensive update part 1. J Am Acad. 2011; 65 (4): 689–97.
21. Speeckaert R, Lambert J, Grine L, Van Gele M, De Schepper S, Van Geel N. The many faces of interleukin-17 in inflammatory skin diseases. Br J Dermatol. 2016; 175(5): 892-901.
22. Beringer A, Noack M, Miossec P. IL-17 in chronic inflammation: from discovery to targeting. Trends Mol Med. 2016; 1099: 12
23. Shabgah AG, Fattahi E, Shahneh FZ. Interleukin-17 in human inflammatory diseases. Postep Derm Alergol. 2014; XXXI (4): 256-61.