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# Cumulative Solar UVA–UVB Exposure Increases Heat Shock Protein (HSP) 72 on Peripheral Blood Cutaneous Lymphocytes Antigen (CLA)+T Lymphocytes in Outdoor Workers

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*Introduction*: Solar UVA–UVB radiation is responsible for development and continuity of life in the earth, but it can endanger human health. Heat shock proteins (HSP) play an essential role in the homeostasis of the living cell when cells exposed to environmental stress. Several investigators had proved increased Hsp72 expression on keratinocytes caused by UVA–UVB exposure, but the effects in peripheral blood Cutaneous Lymphocytes Antigen (CLA)+T lymphocytes, the cells that have a significant role in skin immunity, still have not been proven. *Objective*: To investigate the effect of cumulative solar UVA–UVB exposure on Hsp72 expression on peripheral blood CLA+T lymphocytes in outdoor workers. *Method*: This cohort study had been conducted involving 70 male subjects consisting of 37 caddies of a golf course and 33 indoor workers (one was drop out) in Surabaya, on July to September, 20–45 years old, skin phototype IV/V. Doses of solar UVA–UVB in first four weeks and eight weeks of the study were measured by Viospor® blue line II dosimeter. Hsp72 expression on the peripheral blood CLA+T lymphocytes were measured at baseline, four weeks, and eight weeks by flow cytometry. *Result*: The average dose of solar UVA–UVB received over eight weeks by caddies was $12450.5 \pm 3948.8$ J/m$^2$ whereas that obtained by the indoor workers was $1794.0 \pm 1518.5$ J/m$^2$ ($p = 0.0001$). The Cumulative high dose of solar UVA–UVB exposure induced the increased of Hsp72 on peripheral blood CLA+T lymphocytes ($p = 0.0001$). *Conclusion*: The Cumulative high dose of solar UVA–UVB exposure in subjects with high outdoor activities increases Hsp72 expression on peripheral blood CLA+T lymphocytes.

**Keywords:** Solar UVA–UVB, CLA+T Lymphocytes, Hsp72, Outdoor Workers.

## 1. INTRODUCTION

Solar radiation, particularly UV radiation has both beneficial and harmful effect. The beneficial effects are for the synthesis of vitamin D and the setting of the internal clock whereas the harmful effect is being a hazard to human health due to inducing acute and chronic skin inflammation, skin cancer, premature skin aging, cells death, and can elicit adverse reactions to certain drugs.[1–3] Ultraviolet light is known as one of the sources of environmental stress can stimulate the expression of a protein known as heat shock protein (Hsp).[4] Hsp72 is a function of the formation of the body's defense against ultraviolet light exposure because of Hsp has a cytoprotective effect. It has been demonstrated that increased exposure to ultraviolet rays causes an increase in Hsp72 expression in keratinocytes.[5]

Several studies have shown that UV exposure increased Hsp72 keratinocytes expression in the skin but increased Hsp72 caused by UV exposure on peripheral blood CLA+T lymphocytes that plays a role in skin immunity are still unknown. T lymphocytes (CD3) is a member of the majority of peripheral blood mononuclear cells (PBMCs), which is about 45–70% of PBMCs. Among the T lymphocytes in the peripheral blood, there are T lymphocytes that express cutaneous lymphocyte antigen (CLA)+, which is a modified carbohydrate epitope of P-selectin glycoprotein ligand-1 (PSGL-1) can bind to E-selectin and P-selectin to facilitates T lymphocytes to penetrate the vascular endothelium leading edge skin.[6] CLA+T lymphocytes is a T lymphocyte memory that plays role in many skin disorders, including infections, inflammations, hypersensitivity, autoimmune, and even malignancies.[7–10] Many studies of inflammatory skin diseases mediated by T cells associated with CLA+T lymphocytes.

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More than 90% of T cells involved in the skin that is inflamed CLA + T lymphocytes. CLA + T lymphocytes are thought to be useful as a biomarker for peripheral cell-mediated skin disease of T lymphocytes.[11] Since CLA + T lymphocytes are often found in inflammatory skin, arises the possibility of thinking of increased Hsp72 caused by sun exposure that occurs in keratinocytes can also occur in CLA + T lymphocytes of peripheral blood that has not been studied.

Several studies leading to systemic effects due to exposure to ultraviolet light has been performed, but the mechanism of increased Hsp72 expression particularly on peripheral blood CLA + T lymphocytes to due to ultraviolet light exposure is not fully known. The increased expression of Hsp72 in keratinocytes due to exposure to ultraviolet light raises questions about the possibility of exposure to ultraviolet light can also affect the increased expression of Hsp72 on peripheral blood CLA + T lymphocytes. An increase of Hsp 72 in the cell indicates that the cell is experiencing environmental stress which can lead to a decrease or alteration in function. CLA + T lymphocytes plays a role in many skin disorders, including infections, inflammations, hypersensitivity reactions, autoimmune diseases, and even malignancies.[7–10] If solar UVA–UVB could be proven to cause an increase of Hsp72 in CLA + T lymphocytes, it is a dangerous condition and necessary to do prevention, especially in outdoors workers.

This study aimed to investigate the effect of cumulative solar UVA–UVB exposure on increased Hsp72 expression of peripheral blood CLA + T lymphocytes in outdoor workers.

## 2. METHODS

The method of study was cohort study conducted from July to September 2013. Doses of solar UVA–UVB radiation at 28 days (4 weeks) and 56 days (8 weeks) were measured. Hsp72 expression of peripheral blood CLA + T lymphocytes performed at the beginning of study, after 28 days (4 weeks), and after 56 days (8 weeks).

The observational group of study were the caddies who are related with their work predicted to receive many solar UVA–UVB exposure. The control group was indoor workers who are related to their activities predicted to receive little solar UVA–UVB exposure. Sample formula for this study with cohort design was

$$N = \left( \left\{ \frac{Z1-a}{2}\sqrt{2}P2(1-P2)+Z1 \right. \right.$$
$$\left. \left. -\beta\sqrt{P1(1-P1)+P2(1-P2)}2 \right) \right/ ((P1-P2)2)$$

Correction factor: $1/(1-f)$; $n$ = minimum sample size; $Z1-\alpha/2$ = normal raw distribution value (table $Z$) at $\alpha 0.05 = 1.96$; $Z1-\beta$ = normal raw distribution value (table $Z$) at $\beta 0.95 = 0.842$; $P1$ = estimated probability of outcome (+) population 1 (70%); $P2$ = estimated probability of outcome (+) population 2 (30%) (Ichihasi, 2003)[1]; $P = (P1+P2)/2 = 50\%$; $\alpha = 0.05$; $\beta = 95\%$; $f$ = the proportion of missing or resigned observation units.

From the formula of the sample size, the calculation of sample size for each group is 28. In the cohort study, to anticipate the loss of the observation unit because of resigned or dropped out, this study used the correction factor $1/(1-f)$, where $f$ is the proportion of observation units lost or resigned or dropped out. Taking into account the drop out cases by 10% of the sample size of each group are $n = n/(1-0.1) = 28/(0.9) = 31.1$. Thus, the minimal sample size required in this study for each group was 32 subjects.

Inclusion criteria of the observational group (caddy) were men, age 20 to 45 years, healthy, working as a caddy at least six months and maximum two years, the skin phototype IV/V according to the Fitzpatrick's classification, and wearing short-sleeved shirts when working. Exclusion criteria for the observation group (caddy) were having a skin disorders (atopic dermatitis, inflammations, infections), suffering from chronic illness and/or immunosuppressed disorders, using topical medications including sun-protective drugs, taking medications that affect solar UVA–UVB rays exposure, taking drugs that cause immunosuppression, receiving light treatment (either for treatment, cosmetics, or related to his work). Inclusion criteria for control groups (indoor worker) were men, age between 20 to 45 years, healthy, skin phototype IV/V according to Fitzpatrick's classification, wear long sleeve or jacket when in outdoor. Exclusion control group (indoor workers) were having a skin disorders (atopic dermatitis, inflammations, infections), suffering from chronic illness, suffer from immunosuppressed disorders, using topical medications including sun-protective drugs, taking medications that affect solar UVA–UVB rays exposure, taking drugs that cause immunosuppression, receiving light treatments (either for treatment or cosmetics), doing outdoor activities in the last 6 months.

The selection of research subjects, initially, would be done by a simple random sampling. Ultimately, this study used total sampling because after collecting the caddies at "Yani Golf Course" Surabaya, there were 81 caddies registered and the caddies who meet the inclusion criteria as many as 37 caddies. Finally, the total sample of 37 caddies was taken. For the control group, 33 indoor workers were obtained. To determine the control group was used a matching system considering the age and skin phototype of the observational group (caddy).

VioSpor® blue line II type dosimeter, which has the ability to detect UVA–UVB sun rays were used to measure solar UVA–UVB radiation received by subjects. VioSpor® applied on lower right hand during the first 28 days (4 weeks) and 56 days (4 weeks). VioSpor® were analyzed by BioSense, Laboratory for Biosensory Systems, Postfach Bornheim, Germany, Postfach 5161, D-53318 Bornheim, Germany.

Peripheral blood mononuclear cells (PBMCs) examination was conducted in Animal Physiology Laboratorium, Mathematics and Science Faculty, Brawijaya University, Malang. Peripheral blood CLA + T lymphocytes obtained by examination of peripheral blood mononuclear cells (PBMC) using PE anti-human CD3 (BioLegend) and FITC anti-human Cutaneous Lymphocyte Antigen (CLA)-HECA-452 (BioLegend). Hsp72 is an expression of heat shock protein with 72 kilodaltons molecular weight. In this study, expression of Hsp72 in peripheral blood CLA + T lymphocytes was assessed by flow cytometry examination using Rabbit Anti-Hsp70 (72), PE-Cy5 Conjugated (Bioss) and was conducted at the Biology Laboratorium, Mathematics and Science Faculty, Brawijaya University, Malang. The study had been approved by Ethical Committee of Research, Research and Development Institution, Universitas Airlangga, Surabaya.

The homogeneity test was conducted using independent samples t-test. Kolmogorov-Smirnov statistical test was used to examine the distribution of data. To prove the different dose of solar

UVA–UVB exposure received by outdoor workers (caddy) and indoor workers as well as differences of Hsp72 in peripheral blood CLA + T lymphocytes were conducted using *t*-test. To examine the effect of solar UVA–UVB exposure on Hsp72 expression in peripheral blood CLA + T lymphocytes was used Pearson correlation test. The statistical test of this study used the value of $\alpha$ 0.05 and 95% confidence interval with significance level $p \leq 0.05$.

## 3. RESULT

Independent sample t-test showed that the subjects in this study were homogenous in age ($p = 0.905$). Kolmogorov-Smirnov test showed that the data of this study showed a normal distribution.

Measurement results of solar UVA–UVB doses of the control group (indoor workers) from 32 VioSpor® dosimeters there were three dosimeters that showed zero result, so it was considered as missing data, thus only 29 dosimeter data was analyzed. Dosimeter results of three subjects that showed the zero result meant under exposure to solar UVA–UVB. The exploration of data from the three subjects found that the cause of underexposure was the subjects did daily activities by car and almost never exposed to direct sun while working/doing daily activities. Besides that, the subjects often forgot to wear dosimeter.

The results of this study showed that there was a significant difference in doses of solar UVA–UVB received by observation group (caddy) and control group (indoor workers) during the study ($p = 0.0001$). Average doses of solar UVA–UVB received over 28 days (4 weeks) by observation group (caddy) was of $6226.2 \pm 1974.0$ J/m$^2$ while the observation group (indoor workers) was $897.1 \pm 759.3$ J/m$^2$. The average doses of solar UVA–UVB received over 56 days (8 weeks) by observation group (caddy) was $12450.5 \pm 3948.8$ J/m$^2$ whereas the observation group (indoor workers) was $1793.9 \pm 1518.5$ J/m$^2$ (Fig. 1).

Figure 2 showed an example of flow cytometry examination of Hsp72 expression in peripheral blood CLA + T lymphocytes (UR) from one of subject (UR). Figure 3 showed the median of Hsp72 expression on peripheral blood CLA + T lymphocytes at initial of study, after 28 days (4 weeks), and after 56 days (8 weeks). There was increased of Hsp72 expression from initial of study to 28 days (4 weeks) and 56 days (8 weeks) in the observation group (caddies). Contrast to the control group

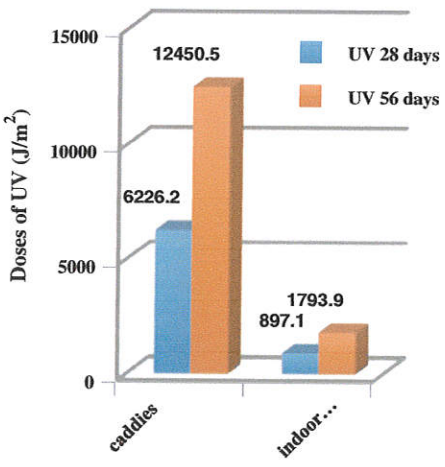
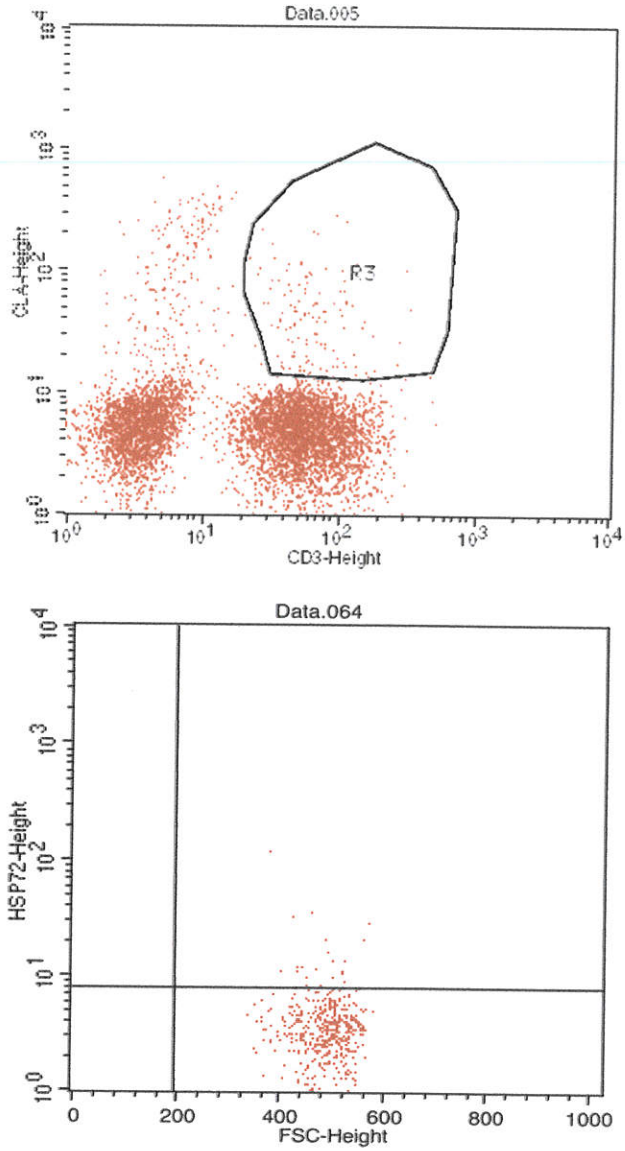



**Fig. 1.** The median dose of solar UVA–UVB exposure received by subjects in 28 days and 56 days.




| Quad | Events | % Gated | % Total |
|---|---|---|---|
| UL | 0 | 0.00 | 0.00 |
| UR | 22 | 4.93 | 0.09 |
| LL | 0 | 0.00 | 0.00 |
| LR | 424 | 95.07 | 1.66 |

**Fig. 2.** The example of flow cytometry examination of Hsp72 expression on peripheral blood CLA + T lymphocytes.

(indoor workers); there was a decline in expression of Hsp72 after 28 days (4 weeks) then increased of Hsp72 expression after 56 days (8 weeks).

In the observation group (caddies), there was a significant increase of Hsp72 expression from initial of study to 28 days (4 weeks) with a significance value of $p = 0.0001$, as well as after 28 days (4 weeks) to 56 days (8 weeks) with a significance value of $p = 0.001$. If assessed from baseline to 56 days (8 weeks) found a significantly increased expression of Hsp72 in

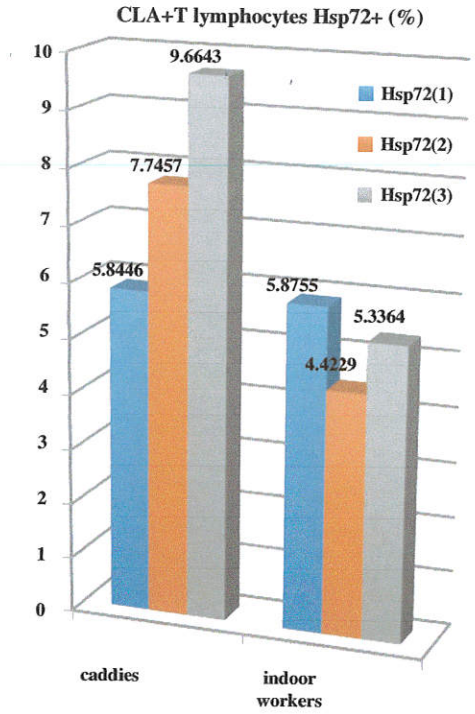



**Fig. 3.** The median of Hsp72 peripheral blood CLA + T lymphocytes at beginning of the study–Hsp72(1), after 28 days/4 weeks–Hsp72(2), and after 56 days/8 weeks–Hsp72(3) in the observation group (caddies) and the control group (indoor workers).

the observation groups (caddies) with a significance value of $p = 0.0001$. In the control group (indoor workers), Hsp72 expression from baseline to 28 days (4 weeks) did not significantly increase (even decrease) with the value of significance $p = 0.057$. Similarly, there was no significant increase in Hsp72 after 28 days (4 weeks) to 56 days (8 weeks) with a significance value of $p = 0.098$. If assessed from baseline to 56 days (8 weeks), there was no significant expression increase of Hsp72 with a significance value of $p = 0.626$ (Fig. 4).

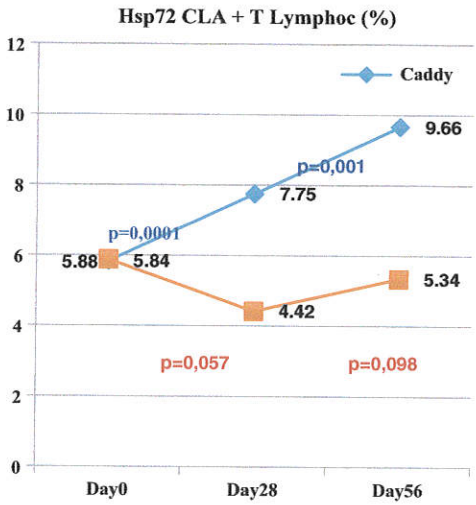



**Fig. 4.** The changes of Hsp72 in peripheral blood CLA+T lymphocytes during the study in the observation group (caddies) and control groups (indoor workers).

Pearson correlation test of this study showed that after 28 days (4 weeks), there was a significant correlation between solar UVA–UVB dose and Hsp72 expression ($p = 0.001$). After 56 days (8 weeks), the results showed that there was more a significant correlation between solar UVA–UVB dose and Hsp72 expression ($p = 0.0001$).

The result of study showed that after 28 days (4 weeks) even more after 56 days (8 weeks) of solar UVA–UVB exposure in outdoor workers (caddy) influenced increased Hsp72 expression on peripheral blood CLA + T lymphocytes.

## 4. DISCUSSION

This study showed that there was significant difference in solar UVA–UVB doses received by observation group (caddy) and control group (indoor workers) during 56 days (8 weeks) of the study ($p = 0.0001$). This results are consistent with a research in Surabaya which proved that there was a significant difference between the solar doses received by the caddies ($12080.6 \pm 8553.9$ J/m$^2$) and students ($4315.1 \pm 1988.1$ J/m$^2$) for one month on July-August, 2001.[12]

A study comparing doses of UV in February 1997 in Toowoomba (27.5°S; 151.9°E) and Brisbane (27.4°S; 153.1°E) in Southeast Queensland, Australia received by outdoor workers, school children, and home workers had been done. The median UVB (280–320 nm) dose received in Toowoomba was 10–16% greater than Brisbane at 7:00 a.m. to 17:00 Australian Eastern Standard Time (EST), and the median dose of UV received by all subjects of study over 2 days (without distinguishing the work) was 2 MED (minimally erythema doses), but a median dose of UV received by outdoor workers in Toowoomba for 2 days was 6 MED. Outdoor workers in Toowoomba received doses of SUV 33% greater than outdoor workers in Brisbane. It showed that outdoor workers received solar UVA–UVB exposure much larger than indoor workers.[13]

Study of two groups of outdoor workers, gardeners and guards of beach in Valencia, Spain in June to July 2008 for 4 and 6 days showed that the average UV dose received by the gardeners was $4{:}13 \pm 0.60$ SED/day (1 SED = 100 J/m$^2$) while those received by guards was $11{:}43 \pm 2{:}15$ SED/day. Average exposure ratio (ER) relative to ambient received by gardeners was $0{:}09 \pm 0{:}01$ and guards of beach $0{:}27 \pm 0.05$ level. ER is the ratio between the personal dose associated with certain anatomical side horizontal plane during the time of exposure. Guards of beach received larger UVER, although both groups received exposure to UV excess than the recommended ambient.[14]

Study of five cyclists for 4 days in Valencia, Spain in the summer of 2008 and winter of 2009. The results showed that the UV dose received by cyclists in the summer more than the winter, but the UV dose received at the hot and cold season remained higher than that recommended for ambient and recreation workers.[15] Another study in Valencia, Spain conducted in July 2010 to evaluate UV dose received by an outdoor construction workers when they work for 5 days. Received a median dose of 6.11 SED/day (1 SED = 100 J/m$^2$). It concluded that outdoor workers are receiving UV 13.9% greater than the total daily ambient erythemal ultraviolet radiation (UVER) are recommended for outdoor workers as well as for recreational purposes so that the possibility of receiving the adverse effects of UVA–UVB sun including immunosuppression is greater in outdoor workers.[16]

This study proved that exposure to solar UVA–UVB cause increased Hsp72 expression. Increased Hsp72 expression have been seen in 28 days (4 weeks) and more increased in 56 days (8 weeks). Heat shock proteins (HSP) are a group of proteins which expression is increased when cells are exposed to stressors, including ultraviolet light.[17–19] Previous studies that have been conducted prove that after given exposure to UVA and UVB, Hsp72 could be detected in all cell extracts by immunoblotting examination with maximum expression at a dose of 40 J/cm$^2$ UVA–UVB within 8–12 hours after exposure.[17] This report supports the results of this study that demonstrate an increase in Hsp72 in CLA + T lymphocytes of peripheral blood after exposure to solar UVA–UVB for 4 weeks increased even after 8 weeks.

Heat shock protein (HSP) is one of the defense systems of all living things. These proteins act as molecular chaperones to assist refolding of proteins that undergo misfolding and assisting the determination of conformation/shape corresponding protein and the prevention of unwanted protein aggregation, also directs elimination when proteins undergo irreversible damage.[20] Other studies on melanocytes which are given to prove that exposure to UVB UVB exposure on melanocytes leads to increased expression of Hsp70 of melanocytes.[21]

Study using cultured epidermal keratinocyte damage due to exposure to UVB rays can be prevented by Hsp72 expression through increased IKB-$\alpha$ which is an Nfk-B inhibitor, but it also decreases proinflammatory cytokines and chemokines. This suggests that the protective activity of Hsp72 is through anti-apoptotic, anti-inflammatory, and anti-damage DNA.[5] One study proved that there was evidenced that UVA1-induced impacts and clinical consequences of UVA1 exposure such as photo-aging, photo-immunosuppression, and cancer. Molecular events support the contribution of UVA1 to long-term harmful consequences of UV exposure and underline the need of an adequate UVA1 photoprotection.[22]

This study proved that exposure to solar UVA–UVB exposure can cause damage on peripheral blood CLA + T lymphocytes affecting the increased Hsp72 expression in peripheral blood CLA + T lymphocytes. Hsp72 has cytoprotective properties. The increased Hsp72 expression on peripheral blood CLA + T lymphocytes serves protection to the peripheral blood CLA + T lymphocytes from the damage caused by solar UVA–UVB exposure.

The limitation of this study, ideally, the study proving the effects of UVA–UVB exposure to Hsp72 expression in CLA + T lymphocyte was performed by exposing UVA–UVB radiation to the subjects compared to control group not given UVA–UVB radiation. However, with respect to ethical research, it can not be done so that the study is conducted by observation and evaluate the subject which is expected in daily activities receive solar UVA–UVB exposure with a larger dose compare to the control group which is in daily activities receive solar UVA–UVB exposure in a small dose. In this study, caddy selected as the observation group, as it relates to its work, is expected during the study (July to September 2013), caddy group received greater solar UVA–UVB exposure than the control group (indoor workers).

## 5. CONCLUSION

This study concluded that the cumulative exposure of solar UVA–UVB radiation leads to increasing Hsp72 expression of peripheral blood CLA + T lymphocytes. It indicates that cumulative solar UVA–UVB exposure known as one of the sources of environmental stress causing increased Hsp72 in peripheral blood CLA + T lymphocytes. Hsp72 has a function on the formation of the body's defense against ultraviolet light exposure because of Hsp has a cytoprotective effect.

So that it is necessary to protect against high dose of solar UVA–UVB exposure in people with a lot of outdoor activities. It is also necessary to guard against the occurrence of adverse effects of solar exposure. It requires furthermore studies on the effects of solar UVA–UVB exposure on Hsp72 expression to determine the cut-off of solar UVA–UVB dose causing negative effects because in low dose solar UVA–UVB radiation has benefit effects on human life.

Finally, indebted to caddies and indoor workers as the subjects of this study for their participation to prove the effect of cumulative exposure of solar UVA–UVB radiation, so unthinkable efforts to prevent the negative effects of solar UVA–UVB exposure in high doses.

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