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Effects of Vitamin E Application on IFN-γ, TNF-α, IL-2 and IL-6 Levels in Elite Taekwondo Athletes

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ABSTRACT

The aim of this study was to investigate the effects of vitamin E application on secretion of cytokine in individuals who do taekwondo sport.
This research was carried out on 10 elite taekwondo athletes who were studying in Seljuk University, School of Physical Education and Sports. Their mean age; 20.67±0.24 years and mean body weight was; 65.45±1.69 kg. Every day 300 mg vitamin E (alpha tocopherol acetate) was given to the participants orally during 4 weeks, and exhaustive exercise was done until fatigue occurs before and after vitamin E treatment.
Before and following the 4 week implementation period, blood samples were taken 4 times from the participants at rest and fatigue. Interferon gamma (IFN-γ), tumour necrosis factor-alpha (TNF-α), interleukin -2 (IL-2) and interleukin -6 (IL-6) levels were determined by ELISA test kits on taken blood samples.
There were no significant differences on the levels of IFN-γ and TNF-α both before and after implementation. On the other hand, levels of serum IL-2 increased in the exercises until fatigue occurs both before and after the implementation ($P<0.05$). There was significant increase on the levels of serum IL-6 at exercise period in comparison with resting period ($P<0.05$). This increase in the levels of serum IL-6 at exercise period prior to implementation was prevented by vitamin E application.
Results indicated that; vitamin E application did not lead to significant changes on the levels of IFN-γ, TNF-α and IL-2 in individuals who practice taekwondo sport. However, Vit E blunted the increase in levels of IL-6 due to exercise.
KEY WORDS: Taekwondo, Vitamin E Application, Cytokines

INTRODUCTION

Vitamins are crucial for physical performance and regulate the metabolic functions primarily [1]. It has been reported that the long-term vitamin deficiency degrades the physical performance [2], and the study report exhibits the importance of vitamins on physical activity [2]. One of the most important vitamins which are related to physical performance is vitamin E [3]. Vitamin E, which cannot be synthesized by body and must be taken from outside is considered to be essential for humans and animals [4]. Although known as intra-cellular antioxidant substance, vitamin E also plays an important role in several physiological processes [5]. It is also indicated that vitamin E plays crucial roles on continuation of the normal functions of the male and female reproductive system, thrombocyte aggregation, mixture of high-energy phosphate, metabolism of B 12 [4]. Vitamin E also affects the immune functions. Particularly, it has been identified in recent years that it boosts the activities and the number of auxiliary t-cells [6]. It is come into prominence that vitamin E has preventive effect to the formation of free radicals caused by exercise [3].

While prescribing performance-enhancing training and diet strategies, factors which may adversely affect the health of the athletes need to be eliminated at the same time [7]. For this reason, there has been remarkable increase in the study of the relationship between exercise and immune system [8]. Regular exercises performed at moderate and mild intensities effects immune system positively [9] whereas long lasting, slow tempo exercises repress the functions of immune system [10]. Auxiliary Th- lymphocytes are divided into two parts as Th1 and Th2 according to their cytokine secretions. Th1 cells produce IFN gamma, IL-2 and TNF-α. On the other hand, Th2 produce IL-4, IL-6 and IL-10. In fact, IL-6 and IL-10 produced by Th2 inhibits the gamma interferon synthesis of Th1-cells that affects the cellular immunity negatively [11]. Slow tempo exercise increases the cytokine according as Th2 that supress to cytokine secretion of Th1 contributing to this action [12].

When the information given above is evaluated together, one can say that there was significant relationship amongst submaximal exercise, vitamin E and immune system. However, to best of our knowledge, there was limited number of research on how vitamin E application effects the cytokine secretion [13]. The present study aims to investigate the vitamin E application on secretion of cytokine in individuals who perform taekwondo sport.

METHODOLOGY

This study is on Semi-empirical method with two experimental groups. This study was carried out on 10 elite taekwondo athletes who were studying in Seljuk University, School of Physical Education and Sports. Their mean age; 20.67±0.24 years and mean body weight was; 65.45±1.69 kg.

Every day 300 mg vitamin E (alpha tocopherol acetate) was given to the participants orally during 4 weeks and exhaustive exercise was done until fatigue occurs before and after vitamin E treatment. The research protocol was approved by Ethics Committee of Seljuk University, School of Physical Education and Sports, The athletes were asked to complete a consent form.

Vitamin E Implementation

Every day 300 mg vitamin E (alpha tocopherol acetate) was given to the participants orally during 4 weeks as tablets at 10.00 o'clock

Taekwondo Exercise

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Every day, during 4 days, taekwondo exercise was done. Starting by minutes general warm-up and then they were taken to hand drills as one by one. Athletes applied all taekwondo techniques at maximum load till fatigue occurs. This was repeated as 3 sets and finished by cool down exercises

Exhaustion Exercise (Bruce Protocol)

Prior and following to the 4 week vitamin E implementation, athletes were performed exhaustion exercise until fatigue occurs. Bruce Protocol (Cosmed T150 treadmill) which is mostly used exhaustion test among clinical exercise tests performed as the both incline and the pace increased in every 3 minutes was used until the moment that participants cannot continue [14].

Blood Sampling From the Participants

As it rest and fatigue blood samples were taken at 09.00 o'clock in the morning (on an empty stomach) from forearm vein (5ml.) 4 times prior and following to 4 week implementation period. Serums of the taken blood samples separated by centrifuging at 3000 rpm for 10 minutes and then stored at - 80 oC until the analysis.

Biochemical Analyses

IFN-γ (LOT:121101/A) "IU/ml", IL-2 (LOT:122301) "U/ml", IL-6 (LOT:124802) "pg/ml", and TNF-α (LOT:121902/A) "pg/ml" parameters on the blood samples of which their serums were separated and measured by using colorimetric kits (DIA Source ELISA Test, Belgium).

Statistics

Findings' statistical evaluation was performed by SPSS 16.0 (Statistical Package for Windows). All parameters' mean and standard deviation were calculated. Analysis of Variance (ANOVA) was used on repetitive measurements. Least Significant Difference (LSD) test was used to detect the difference based on which timing. Statistical significance threshold was set to $P<0.05$.

RESULTS

There was no significant difference on participants' serum levels of IFN-γ and TNF-α both prior to implementation (rest-exercise) and following the implementation (rest-exercise) measurements (Table 1).

On the other hand, there was an increase in serum levels of IL-2 ($p<0.05$, Table-1) at the exercise performed by subjects both before and after. Serum levels of IL-2 were not different at rest following vitamin E implementation in comparison with the rest period prior to implementation. Nevertheless, Serum levels of IL-2 were not different at fatigue period following vitamin E implementation in comparison with the fatigue period prior to implementation.

There was significant increase in serum levels of IL-6 at exercise period relatively rest period ($P<0.05$), However, this increase in IL-6 levels emerged prior to implementation was prevented by vitamin E application (Table 1).

Table1: Evaluation of Serum Levels of IFN-γ, TNF-α, IL-2 and IL-6

Measurement Timing		N	IFN-γ Levels (IU/ml)	TNF-α Levels (pg/ml)	IL-2 Levels (U/ml)	IL-6 Levels (pg/ml)
Before vitamin E implementation	Rest	10	0.20 ± 0.18	4.64 ± 1.59	0.53 ± 0.16*	16.98 ± 3.33*
	Fatigue	10	0.19 ± 0.13	5.45 ± 2.37	0.67 ± 0.14*	23.47 ± 5.06*
Following to vitamin E Implementation	Rest	10	0.13 ± 0.02	4.66 ± 1.34	0.40 ± 0.14*	16.99 ± 2.75*
	Fatigue	10	0.18 ± 0.40	3.94 ± 1.56	0.62 ± 0,13*	18.88 ± 1.88*

* Means statistically significant ($P<0.05$).

DISCUSSION

There were no significant differences between serum levels of IFN-γ and TNF-α at the measurements performed at rest and fatigue in both prior and following the implementation. On the other hand, serum levels of IL-2 increased at exercise performed till fatigue occurs in both prior and following the implementation. Findings of the studies in which were focused on the relationship between exercise and cytokine are contradictory. It was identified that 32 week exercise program do not affect the TNF-α levels [15]. It has been indicated that acute aerobic exercise do not affect the t-cells' cytokine activity in humans [16], nevertheless combined strength exercises do not change the IFN-γ, TNF-α levels in humans with diabetes type 2 [17]. 12 week swimming exercise resulted in an increase in the levels of IFN-γ and TNF-α in mouse [18], resulting in increased concentrations of neutrophils, lymphocytes and monocytes. The increased lymphocyte concentration is caused by recruitment of all lymphocyte subsets (NK, T and B cells). Strenuous exercise, but not moderate exercise, is followed by decreased concentrations of lymphocytes and impaired cellular-mediated immunity.2 Increased levels of adrenalin, and to a lesser degree noradrenalin, are believed to be the main responsible factors for recruitment of lymphocytes during acute exercise. Catecholamines, together with growth hormones, may mediate the acute effects on neutrophils, whereas cortisol exerts its effect within a time lag of at least 2 h.3. Similarly exercise increased the secretion of IFN-γ in mice [19]. It was showed that an intense physical activity can degrade the immune responses [20], moreover swimming exercise composed of 8 week, 6 days in a week and 150 minutes long for each session supress the IFN-γ and IL-2 [12]. Exercise can have both positive and negative effects on immune function and susceptibility to minor illnesses. The relationship between exercise and susceptibility to infection, and during exercise, exposure to airborne pathogens is increased due to the higher rate and depth of breathing. Results of the studies related to exercise and cytokine relationship presented above are not consistent. These differences among reports may be caused by exercise, time-intensity, participants and differences in various applications. In the present study, IFN-γ and TNF-α level of the subjects were not affected by both exercise and the 4 week vitamin E implementation. This finding is coherent with the findings of Marques et.al. [15] and Touvra et.al. [17] In which cytokines are not affected by exercises. However, in our study both exercise and 4 week vitamin E implementation did not affect the levels of IFN-γ and TNF-α. Parallel to our research; Silva et.al. Indicated that vitamin E implementation did not affect the levels of TNF-α [21]. In the

present study which was worked with elite athletes IL-2 levels increased both before the implementation and exercise after the 4 week vitamin application until fatigue occurs. Period after the application of vitamin E which increased fatigue IL-2 levels were not different from the period prior to application of fatigue. This showed that increase in IL-2 levels is more likely the results of exercise than vitamin E implementation. Results of the study conducted by Lu et al. in which 8 week swimming exercise increase the IL-2 levels in rats [12] is strongly support our findings.

In our study, IFN-gamma, IL-2 and TNF-α cytokines which were secreted by Th1 cells is one of the indicators of immunity [11]. Vitamin E implementation does not affect these cytokines; however IL-6 levels secreted by Th2 effect immune functions negatively and it increased significantly at the exercise performed till fatigue prior to vitamin E application [11]. It was already shown in several studies that levels of IL-6 increases [11, 22, and 23]. But, the main findings of the present research is not only the levels of IL-6 was increased significantly at the exercise performed until fatigue occurs, but also suppression of it following to 4 week vitamin E implementation. Yfanti et al. [24] revealed that increase in IL-6 concentration with acute exercise can be prevented by vitamin E application. Davison et.al [25] and Vincent et al. [26] were reported that increased IL-6 levels at exercise cause oxidative stress by supressing the cellular immunity and the vitamin E application prevents the these negativities by supressing the increase in IL-6 levels. Similar findings have been reported by some researchers [27,28]. Increase in IL-6 levels at exercise till fatigue occurs and vitamin E application supresses it is consistent with the findings presented above.

CONCLUSION

1. Serum levels of IFN-γ and TNF-α were affected from both exercise and Vitamin E application in athletes.
2. Serum IL-2 levels increased at exercise independently from vitamin E application.
3. IL-6 levels increased significantly at exercise were supressed by vitamin E implementation.

Findings of this study show that vitamin E implementation does not make significant change on the levels of IFN-γ, TNF-α and IL-2 in individuals who practice taekwondo sport, on the other hand vitamin E application suppress the increased IL-6 levels at exercise which cause degradation in cellular immune functions.

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