

Levels of immune cells in transcendental meditation practitioners

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ABSTRACT

Context: Relationships between mind and body have gradually become accepted. Yogic practices cause modulation of the immune system. Transcendental meditation (TM) is a specific form of mantra meditation. We reported previously different plasma levels of catecholamines and pituitary hormones in TM practitioners comparing with a control group, and patterns of the daytime secretion of these hormones different from those normally described.

Aims: The aim of the following study is to evaluate the immune system in these meditation practitioners, by determining leukocytes and lymphocytes subsets.

Methods: TM group consisted of 19 subjects who regularly practice either TM or the more advanced Sidhi-TM technique. A control group consisted of 16 healthy subjects who had not previously used any relaxation technique. Total leukocytes, granulocytes, lymphocytes and monocytes were counted by an automated quantitative hematology analyzer, whereas lymphocytes subsets were determined by flow cytometry. Samples were taken from each subject at 0900 h after an overnight fast.

Results: The results indicated that the TM group had higher values than the control group in CD3+CD4-CD8+ lymphocytes ($P < 0.05$), B lymphocytes ($P < 0.01$) and natural killer cells ($P < 0.01$), whereas CD3+CD4+CD8- lymphocytes showed low levels in meditation practitioners ($P < 0.001$). No significant differences were observed in total leukocytes, granulocytes, monocytes, total lymphocytes or CD3+ lymphocytes comparing both groups.

Conclusions: The technique of meditation studied seems to have a significant effect on immune cells, manifesting in the different circulating levels of lymphocyte subsets analyzed. The significant effect of TM on the neuroendocrine axis and its relationship with the immune system may partly explain our results.

Key words: Immune system; psychoneuroimmunology; stress; transcendental meditation; Yoga


INTRODUCTION

Relationships between mind and body have gradually become accepted. Since Ader and Cohen coined the term psychoneuroimmunology, there is a growing body of evidence on this interaction.^[1] The neuroendocrine system is considered to be the link between the immune system and the psyche. Yoga has been known as a spiritual exercise

to improve the mental state, inducing physiological changes based on these relationships. Yogic practices cause modulation of the immune system.^[2]

Transcendental meditation (TM), the main technology behind the Maharishi Vedic approach to health, is a simple, effortless and stylized form of physical and mental relaxation.^[3] This technique is one of the most widely studied. Subjects practicing TM show specific electroencephalographic and metabolic patterns, changes in hormonal concentrations, low anxiety levels and improve levels of health.^[4,5] In previous studies, our group reported modifications in the daily secretion pattern and lowered blood levels of catecholamines, β -endorphins and adrenocorticotrophic hormone. We speculated with modification of hypothalamic and hypophyseal activity due to the regular practice of meditation.^[6-8]

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A few studies have focused on the TM effects on the immune system demonstrating low percentage of functional lymphocyte beta-adrenergic receptor in practitioners and modulation on leukocyte deoxyribonucleic acid repair.^[9] To the best of our knowledge no previous research has been published on lymphocyte levels with this technique.

Based on the above information and on these latest results, the present study was undertaken with the aim of evaluating the immune system in TM practitioners, by determining leukocytes and lymphocytes subsets.

METHODS

This study was approved by the Hospital Research Ethics Committee and the procedures followed were in accordance with Helsinki Declaration.

Subjects

TM group consisted of 19 Caucasian subjects, 11 men and 8 women, aged between 18 and 40. They had practiced TM (9 subjects) or the more advanced Sidhi-TM technique (10 subjects) on a regular basis with an average period of 7 years (range 2-15).

A control group consisted of 16 healthy Caucasian subjects, 8 women and 8 men, with ages ranging from 22 to 35 and who had not previously used any relaxation technique. They were selected from among a group of individuals that came to hospital for a routine medical check-up as part of the annual medical examination carried out by the companies they worked for.

All subjects took part in the study on a voluntary basis and signed a written consent form. None of them were undergoing medical treatment, were obese or played regular sports and their jobs or social situations did not represent a high level of anxiety. All of the participants underwent a medical examination in order to rule out any pathology. This included a physical check-up, the determination of twenty biochemical parameters using a Hitachi 747 autoanalyzer and the subjects anxiety levels using the Spielberger anxiety inventory test.^[10] Concentrations of biochemical parameters were within the normal values of our laboratory, coinciding with the general literature. Both TM practitioners and the control groups followed a Mediterranean diet. The women took part in the trial during the follicular phase of their menstrual cycle.

Meditation technique

TM is a specific form of mantra meditation introduced in the mid-1950s by Maharishi Mahesh Yogi. It is a simple, natural technique practiced for 20 min morning and evening, whereas sitting comfortably with eyes closed. During technique the

thought process quietens down, reaching a state of alert hypometabolism.^[3] TM-Sidhi is an advanced meditation technique, practiced for an additional 20-30 min after TM, using aphorisms known as sutras instead of mantras.^[11]

The utilization of these techniques involves no disciplines, no change in life-style, no philosophical ideas or religious beliefs. The learning is very structured and at the same time simple, meaning its practice is not dependent on the intellectual level of the subjects.

Procedure

The subjects were previously familiarized with the place where the test was to be carried out, and they were asked to abstain from exercise for 2 days prior to the study. Samples were taken from each subject at 0900 h after an overnight fast. With the subject seated, a butterfly-type cannula was inserted into the antecubital vein and connected to a drip system, ensuring a free flow. After waiting 15 min for the stress produced by the cannulation to subside, a blood sample was collected in a Vacutainer tube with anticoagulant ethylenediaminetetraacetic acid (K3) after discarding the first 5 ml. The same physician was in charge of obtaining and manipulating the blood samples.

Blood sample analyses

Number of total leukocytes, granulocytes, lymphocytes and monocytes were counted by a Sysmex K-1000 automated quantitative hematology analyzer (Sysmex Corporation). Lymphocytes subsets were determined by flow cytometry in a FACSort (Becton Dickinson).

Were used monoclonal antibodies conjugated with phycoerythrin and fluorescein isothiocyanate to determine lymphocyte surface antigens. CD3 was used to identify T-cells, CD4 for helper T-cells, CD8 for cytotoxic T-cells, CD20 for B-cells, CD16 and CD56 for natural killer (NK) cells. A total volume of 50 μ l of blood was mixed with 10 μ l of monoclonal antibody and incubated for 20 min at room temperature. Lysing solution was added, centrifuged and the cell pellet re-suspended in phosphate-buffered saline.

Statistical analyses

Through the application of the statistical program Statistical Graphics System (STATGRAF, Statistical Graphics Corporations, Manugistic, USA), data were analyzed using the Mann-Whitney U test for unpaired data. $P < 0.05$ were considered to be statistically significant.

RESULTS

Table 1 shows state and trait anxiety levels for both study groups. No significant differences can be seen, with both

groups inside the ranges of normality defined for the Spanish population.

Table 2 shows mean values \pm standard error. of immune cells levels for TM and control groups. Variables concentrations were within the normal values of our laboratory, coinciding with the general literature. The results indicated that the TM group had higher values than the control group in CD3+CD4–CD8+ lymphocytes ($P < 0.05$), B lymphocytes ($P < 0.01$) and NK cells ($P < 0.01$), whereas CD3+CD4+CD8– lymphocytes showed low levels in meditation practitioners ($P < 0.001$). No significant differences were observed in total leukocytes, granulocytes, monocytes, total lymphocytes or CD3+ lymphocytes comparing both groups.

There was no statistically significant difference in age between both groups.

DISCUSSION

The present study found significant differences in blood levels of lymphocyte subsets comparing TM practitioners and control group. These results add to those previously published by our group suggesting an effect of meditation on psychoneuroendocrine axis.

Ader and Cohen coined the term psychoneuroimmunology, defined as the study of the interactions between psychological factors, the central nervous system and immune function as modulated by the neuroendocrine system.^[1] There is a reciprocal relationship between mind and body. Yoga is considered to induce physiological changes based on this relationship.

The autonomic nervous system and the neuroendocrine system link the brain and the immune system.

Table 1: Anxiety levels in both groups

	TM group	Control group	Significance
State	14.7 \pm 4.5	23.7 \pm 4.2	NS
Trait	36.2 \pm 8.1	37.7 \pm 7.1	NS

Mean values \pm SE together with the statistical significance differences. NS=Not significant; TM=Transcendental meditation; SE=Standard error

Table 2: Immune cells levels in both groups

Variable (cell/ μ l)	TM group	Control group	Significance
Leukocytes	5323.5 \pm 202.5	5158.7 \pm 166.1	NS
Granulocytes	3062.7 \pm 142.6	2955.8 \pm 121.3	NS
Monocytes	434.6 \pm 24.8	408.6 \pm 36.3	NS
Lymphocytes	1841.4 \pm 85.1	1801.1 \pm 56.5	NS
CD3+lymphocytes	1338.4 \pm 82.6	1438.1 \pm 44.6	NS
CD3+CD4+CD8–cells	725.7 \pm 61.9	902.6 \pm 34.2	$P < 0.001$
CD3+CD4–CD8+cells	630.9 \pm 40.0	525.8 \pm 25.4	$P < 0.05$
B lymphocytes	273.1 \pm 32.8	180.0 \pm 10.7	$P < 0.01$
NK cells	203.5 \pm 28.5	121.2 \pm 11.0	$P < 0.01$

Mean values \pm SE together with the statistical significance differences. NS=Not significant; TM=Transcendental meditation; SE=Standard error; NK=Natural killer

Adrenocorticotrophic hormone (ACTH) was one of the first neuropeptides shown to bind to receptors on leukocytes and modulate immune responses. Lymphoid organs receive extensive sympathetic innervation. Norepinephrine is released from the sympathetic nerve terminals in these organs, and the immune cells express adrenoceptors.^[12] Catecholamines modulate human NK cells circulation and function by adrenergic mechanisms. Percentage of NK cells in peripheral blood in normal subjects is negatively correlated to plasma epinephrine levels.^[13]

Through the neuroendocrine system, stressful events affect the absolute and relative number of lymphocyte subsets, including B-cells and NK cells. Significant declines in NK cell numbers and activity have been observed during periods of high stress. The level of secretory immunoglobulin A measured in saliva is down regulated during periods of chronic stress. Depression is associated with alterations in immune function with a decrease in CD4+ and CD8+ T-cells.^[14]

A number of researchers have shown that stress-reducing interventions and methods of meditation can improve immune functions. Our results partially coincide with previous studies. Authors found NK cells and lymphocytes elevation in women with breast cancer following massage therapy. Mindfulness-based stress reduction increase NK cell activity and number. A group of cancer patients practicing Sudarshan Kriya and Pranayam increased NK cells with no effect on T-cell subsets.^[15]

Kamei *et al.*^[16] in their study examined changes in brain rhythms and NK cell activity during Yoga exercises. They found a correlation between the frontal alpha wave activation and the increase in NK activity. TM-Sidhi practice has been characterized by higher frontal alpha1 and beta1 amplitudes in electroencephalographic research.^[4]

In relation to T lymphocyte cells, we found in our study a disparity of values with high levels of CD3+CD4–CD8+ cells and lower levels of CD3+CD4+CD8– cells in TM group compared with the control group. We rule out a possible relationship between concentrations of CD3+CD4+CD8– cells and presence of pathology, since their values were within the normal values of our laboratory, coinciding with the general literature.

It is interesting to note that there is a negative correlation between cortisol and CD4+ cells levels and that circadian rhythms in circulating T-cell subpopulations is controlled by glucocorticoids via the hypothalamo-pituitary-adrenal system and catecholamines via the sympathetic nervous system.^[17] In this context, various studies have reported changes in hormone levels during practice of TM technique

and with its regular use, It has been demonstrated to decrease the percentage of β -adrenergic receptors in the functional high affinity state in practitioners of meditation.^[9] A study done by Tooley *et al.*^[18] showed significantly higher plasma melatonin levels in meditators practicing TM-Sidhi considering that it might be one avenue through which the claim health promoting effects of meditation occur. Melatonin has been shown to be involved in the regulation of both cellular and humoral immunity and stimulates the production of NK cells.

We reported previously different plasma levels of catecholamines (norepinephrine and epinephrine) and pituitary hormones (ACTH and β -endorphin) in TM practitioners comparing with a control group. We also described patterns of the daytime secretion of ACTH and catecholamines differing from those normally described.^[6-8] These results could partly explain our findings.

In this regard, other authors have described very similar results on short-term psychiatric group intervention in patients with malignant melanoma, in which significant increases in NK cells, and a small decrease in the percent of CD4 cells were found.^[19] Nuamtanung *et al.*^[20] found an increase in the number of CD8+ lymphocytes and NK cells after 4 months of meditation, with not significant modification in CD4+ cells.

However, not all studies of the immune effects of interventions find increases in immune function or similar results to ours. Different authors found no changes in numbers of any of lymphocyte subtypes with mindfulness-based stress reduction meditation program and both elevation and no changes in T helper cells after Qigong training.^[21] This disparity of results can be explained by methodological differences or the durations of the meditation. On the other hand, different relaxation techniques do not seem to have a similar physiological effect.^[22]

On the basis of these researches, it is reasonable to think that the differences in blood levels of lymphocyte subsets found in TM group may be due to modulation of neuroendocrine axis activity through regular meditation. On the fact that anxiety levels were similar in both groups, it could be considered that the cause of different immune cells levels found in the TM group with regard to the control group could be due to a modulation effect as a result of the relaxation technique and not to different levels of anxiety.

Our results would partly explain previous research supporting the effectiveness of TM practiced for preventing disease, lower medical utilization and expenditures, since NK cells play a role in tumor prevention and serve an

early defense against intracellular infections. However, we cannot rule out that different levels in lymphocyte subset would have occurred due to external environmental factors. On the other hand, a small sample size is the major limitation of our paper. Further studies with a greater number of subjects are needed to confirm these findings.

CONCLUSION

The technique of meditation studied seems to have a significant effect on immune cells. This effect is manifest in the different circulating levels of lymphocyte subsets analyzed. The significant effect of TM on the neuroendocrine axis and his relationship with the immune system may partly explain our results.

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