

# The Effect of Transcendental Meditation on the Immune Response of Bali Mandara High School Students

DOI : <https://doi.org/10.22435/hsji.v13i1.4672>

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Received : March 19, 2021; Revised : November 17, 2022; Accepted : November 29, 2022

## Abstract

**Background:** Meditation has become an increasingly popular form of alternative medicine. Many studies have been conducted to assess the health benefits of meditation. In particular, Transcendental Meditation (TM) is effective in treating psychological disorders, hypertension, cardiovascular disease, and high cholesterol. It is assumed that TM can enhance the individual immune system. This study aims to determine the number of immune response cells in high school students who do transcendental meditation for a certain period of time.

**Methods:** This study has a total sample of 150 students. The study group consisted of 3 groups, namely the control group, TM group 1 (students who regularly practiced TM for 1 year), TM group 2 (students who regularly practiced TM for 2 years). Each group consists of 50 students. The control group consisted of 50 students who did not use any relaxation techniques. Total eosinophils, neutrophils, lymphocytes, monocyte, and hematocrit are counted by an automated quantitative hematology analyzer.

**Results:** The results showed high rates immune cell among the control group compared to the TM 1 and TM 2 groups. The number of immune response cells showed that the TM 2 group differed significantly from the control group and the TM 1 group in eosinophils, neutrophil, and monocyte ( $P < 0.05$ ). Even though, leukocytes, neutrophils, and lymphocytes showed a decrease although not significantly different in the TM 2 group ( $P > 0.05$ ). The correlation value shows a strong correlation between immune response cells (leukocytes, lymphocytes, eosinophils, monocyte, and hematocrit) with an increase in meditation duration.

**Conclusion:** Meditation can increase immune cells that play a role in self-protection. This study provides information that transcendental meditation has health benefits, especially in boosting the immune system.

**Keywords:** Transcendental Meditation (TM), immune respond

## Abstrak

**Latar belakang:** Meditasi telah menjadi bentuk pengobatan alternatif yang semakin populer. Banyak penelitian telah dilakukan untuk menilai manfaat meditasi pada kesehatan. Secara khusus, Meditasi Transendental (TM) telah terbukti efektif dalam mengobati gangguan psikologis, hipertensi, penyakit kardiovaskular, dan kolesterol tinggi. Hal ini diasumsikan bahwa TM dapat meningkatkan sistem imun individu. Penelitian ini bertujuan untuk mengetahui jumlah sel respon imun pada siswa SMA yang melakukan meditasi transedental pada periode waktu tertentu.

**Metode:** Penelitian ini memiliki total sampel 150 siswa. Kelompok studi terdiri dari 3 kelompok, yaitu kelompok kontrol, kelompok TM 1 (berlatih TM selama 1 tahun), dan kelompok TM 2 (berlatih TM selama 2 tahun). Tiap grup terdiri dari 50 siswa. Kelompok kontrol terdiri dari 50 siswa yang tidak menggunakan teknik relaksasi apa pun. Total eosinofil, neutrofil, limfosit, monosit dan hematokrit dihitung dengan hematologi kuantitatif otomatis.

**Hasil:** Hasil menunjukkan terdapat perbedaan bermakna di antara kelompok kontrol dibandingkan dengan kelompok TM 1 dan kelompok TM 2. Jumlah sel respon imun menunjukkan bahwa kelompok TM 2 berbeda secara signifikan dibandingkan kelompok kontrol dan kelompok TM 1 pada eosinofil, netrofil dan monosit ( $P < 0,05$ ). Hal berbeda ditunjukkan oleh leukosit, neutrofil, dan limfosit yang menurun walaupun tidak berbeda bermakna pada kelompok TM 2 ( $P > 0,05$ ). Nilai korelasi menunjukkan adanya korelasi kuat antara sel-sel respon imun (leukosit, limfosit, eosinofil, monosit, leukosit, dan hematokrit) dengan peningkatan durasi meditasi ( $p > 0,4$ ).

**Kesimpulan:** Meditasi dapat meningkatkan sel imun yang berperan dalam perlindungan diri. Studi ini memberikan informasi bahwa meditasi transendental mempunyai manfaat bagi kesehatan khususnya meningkatkan sistem kekebalan tubuh.

**Kata kunci:** Meditasi Transdesental (TM), respon imun

## INTRODUCTION

Transcendental Meditation (TM) is another type of stress reduction technique that individuals utilize a mantra during the process of transcendent.<sup>1</sup> TM focused on the integration of the mind and body with a sound without meaning repeated within the mind and the body will enter transcendental consciousness wherein it is calmed and quieted if the individual is successful.<sup>2,3,4</sup> This simple technique has been proven useful for several clinical and psychological conditions, even in healthy individuals. A few studies showed the TM effects on the immune system demonstrating modulation on leukocyte deoxyribonucleic acid repair and low percentage of functional lymphocyte beta-adrenergic receptor in practitioners.<sup>5</sup>

Most of the research has shown that TM may improve good outcomes and decrease for those diagnosed with cardiovascular disease, hypertension, and high cholesterol. Even, the physiological state can have positive impacts on stress-related ailments and diseases.<sup>2,3</sup> In previous studies reported modifications in the daily secretion pattern and lowered blood levels of catecholamine,  $\beta$ -endorphins and adrenocorticotropic hormone. Stress is a condition that contains a psychological and physiological response to pressure carried by someone. The response caused negative emotions and changes in balance in the body and various variations in the brain.

The emergence of negative emotions due to stress triggers mental disorders, ranging from mild to severe mental disorders. The Riskesdas data of 2013 has shown the prevalence of mental-emotional disorders as indicated by symptoms of depression and anxiety. It is shown at the age of 15 years and overreaching around 14 million people or 6% of the population of Indonesia. While the prevalence of severe mental disorders, such as schizophrenia

reaches around 400,000 people or as much as 1.7 per 1,000 populations.<sup>6</sup>

One of the major changes that occur along with the stress conditions experienced is the process of thymus involution. The thymus is an organ where T cells mature. T cells are very important as lymphocytes to kill bacteria and help other types of cells in the immune system.<sup>7</sup> The immune system on stress conditions also decreases the margination of neutrophils. Other research showed that acute stress in younger adults elicited similar changes in neutrophil function, in specifically reduced superoxide production.<sup>8,9</sup> Song and colleagues showed that subacute intra cerebrum ventricular administration of corticotropin-releasing factor for 5 days in rats reduced lymphocyte proliferation, decreased the percentage of lymphocytes, and increased the percentage of neutrophils.<sup>10</sup>

High school youth is a transition from early adolescence to late adolescence. New high school students determine the transition period, in which all reviews per period will divert doubts about the role that must be performed.<sup>11</sup> In this transition, students are vulnerable to stress because they need themselves and their new environment.

A study found that the incidence of stress in Gorontalo high school students was 62.3%. Students in grades 1 and 3 experienced the least amount of stressors with a percentage of 29.6% and 29.7%, respectively. Whereas the second-grade students most experienced mild stressors with a percentage of 22%. Research conducted by Putri in 2014 of grade XII students of the Natural Sciences (IPA) and Social Sciences (IPS) education studies in the 6th Senior High School (SMA) Denpasar found a prevalence of mild psycho social stress of 51.8%, moderate 36.9% and weight 11.3%.<sup>12</sup>

The study was conducted to look at changes in the number of immune system cell count in students who do transcendental meditation. In addition to the relationship between stress conditions and neutrophils, the immune system that tackle infection is the different types of white blood cells (eosinophil leukocytes, monocytes, lymphocytes and neutrophils). At the start of the immune response, neutrophils and macrophages are the innate immune system cells. In this study, the number of various types of white blood cells becomes a parameter as well, considering that routine blood tests on white blood cells can be a signal that the immune system is good or lacking in dealing with these infections. SMA Negeri Bali Mandara Buleleng Bali, a high school, has implemented transcendental meditation as a routine activity for students. It purposes to decrease student stress conditions and also to the best of our knowledge no previous research has been published on leukocyte, eosinophils, monocyte and hematocrite levels with this technique.

## METHODS

This study was approved by the Research and Development Unit of Udayana University, Denpasar with ethical clearance number is 095/Unwar/FKIK/EC-KEPK/II/2020 and date on Feb, 25<sup>th</sup> 2020.

### *Subjects Technical of TM*

TM group consisted 150 single healthy students at SMA Negeri Bali Mandara, Buleleng, Bali from July to December 2020. All students in this high school completed a questionnaire about their health status, such as physical and mental conditions, and also signed a written consent form. None of them were undergoing medical treatment. Only students with good condition and have no allergies who join in this study as sample. The students have done the TM technique for 20 minutes twice a day before and after attending the lesson. TM technique is an effortless, systematic procedure and sitting comfortably with eyes closed.<sup>13</sup>

The mind effortlessly settles down through increasingly quiet levels of thought until it naturally reaches its quietest state, beyond the finest level of thinking during the technique. This process is known transcending. During this process of transcending, individuals utilize a mantra, meaningless sounds repeated within the mind. This mantra purposes to quiet their thoughts until their mind reaches the silent state of transcendental consciousness. When mental

activity is transcended, a state of inner silence, called transcendental consciousness or pure consciousness is experienced. Therefore, the participants have transcendental consciousness when the correct initial conditions are set during TM practice.<sup>14</sup>

Participants must meet inclusion criteria of (a) students who have been doing transcendental meditation for several months, one year and two years, (b) have a normal blood pressure range based on American Heart Association definitions of hypertension and hypotension, (c) have no history of systemic disease, hypertension, hypotension, hypoglycemia, and coronary heart disease.

The participant who had recent symptoms of illness were excluded from this study, so no participants were receiving any therapy or drugs during this experiment.

### *Procedure*

This study used a comparative cross-sectional study design as the research method. Several cell subtype of white blood cell (WBC) count are an inexpensive. WBC can be simple biomarker of systemic inflammations, these component are monocytes, lymphocytes, basophils, eosinophils, and basophils. All WBC components play various role in the host immunity and inflammatory response. 2 ml blood samples were taken and collected from students of SMA Negeri Bali Mandara (tenth grade as control group or TM0 group, eleventh as TM1 group, and twelfth grade as TM2 group) by clinicians who are the research team taking the blood of students at school. Tenth (TM0) grade as a beginner's meditation group, eleventh grade is a group that has been doing meditation for a year (TM1) and, senior or twelfth grade (TM2) as a group that has been doing meditation for two years. The students had filled out an informed consent explaining the purpose of the study and the risks that might be encountered during and after sampling. Sample size by using single proportion formula is 18 sample, but we tried to take as much as possible in, so we used 50 students per group in this study.<sup>15</sup>

$$\begin{aligned}
 N &= \frac{(Z\alpha)^2 p.q}{d^2} \\
 &= \frac{(1,96)^2 (0,25) (0,75)}{(0,2)^2} \\
 &= 18
 \end{aligned}$$

Blood sampling was conducted at SMA Negeri Bali Mandara and the results were analyzed at the Faculty of Medicine and Health Sciences, Warmadewa University.

Then, blood sample calculation of the number of immune cell (Eosinophil, Neutrophil, Monocyte, Leukocyte, Lymphocyte and Hematocrit) was carried out using the flow cytometry method (Prodia Clinical Laboratories).

#### Statistical analyses

Through the application of the statistical program SPSS 22, data were analyzed using the Anova One Way test for unpaired data.  $P < 0.05$  were considered

to be statistically significant. The correlation between TM and immune response counted too with Pearson correlation.

#### RESULTS

The characteristics of the sample in this study were the students of SMA Negeri Bali Mandara which were grouped by education level. SMA Negeri Bali Mandara has applied transcendental meditation to every student from the first level of education at the high school. The grouping in this study was in the form of the first group of 50 first-year students who had just meditated, the second group was 50 second-year students who had been meditating for one year, and the third group was a third-year student of 50 students who had meditated for 2 years.

Table 1. Characteristic of Research Subject

Characteristics	Group of TM		
	TM0	TM1	TM2
<b>Sample size (N)</b>	50	50	50
<b>Grade of students</b>			-
First grade	50	-	-
Second grade	-	50	-
<b>Time of TM (year)</b>			
First grade	0	-	-
Second grade	-	1	-
Third grade	-	-	2
Third grade	-	-	50
<b>Gender</b>			
Male	25	25	26
Female	25	25	24
Object	blood	blood	blood
<b>Health status</b>			
C Allergy	none	none	none
Infectious disease	none	none	none
Mental health	none	none	none
<b>Immune system cell</b>			
Eosinophil	1.58(1.29)	0.86(1.71)	0.58(0.97)
Neutrophils	65.34(9.35)	65.48(10.63)	69.02(6.94)
Monocyte	2.94(1.59)	4.60(1.52)	3.98(1.53)
Leukocytes	7.55(1.58)	7.50(1.46)	6.95(1.32)
Lymphocytes	30.14(8.81)	29.06(9.92)	26.42(6.64)
Hematocrit	39.22(2.82)	41.97(4.63)	42.19(3.79)

The general characteristics of the research subjects were male and female students who underwent transcendental meditation successively. The students were not experiencing a decline in their health condition when the students' blood sample was taken. Immune system cells calculated in this study include eosinophil, neutrophil, monocyte, lymphocyte, and leukocyte. Meditation may influence psychological mechanisms. They are also shown to influence of immune system directly. These immune responses were the reaction of cells and fluids towards the presence of an entity. It was typically not recognized as a constituent of body. The ability of immune response to tackle infectious diseases was thought to be influenced by the psychological status. Tackling infections is the job of different types of white blood cell. Early in an immune response, the most important of these are the innate immune system cells, which are the first at the scene of an environmental breach.<sup>16,17</sup> Hematocrit was calculated too. The distribution can calculate the probability of any one particular

observation in the sample space, or the likelihood that observation has a value that is less than (or greater than) a point of interest and to decide the post hoc ANOVA.

The distribution of leukocytes and lymphocytes did not differ between each group. Unlike the case with the distribution of hematocrit, data showed median differences compared to other groups. The distribution of immune system cells and hematocrit showed relevance with ANOVA and its correlation.

The total eosinophil, neutrophil, and monocyte were statistically significant differences between groups ( $P < 0.005$ ) (table 2). Games-Howell Post Hoc from eosinophil significantly differs between TM0 and TM1 group ( $F = 0.72$ ) and TM0 with TM2 group ( $F = 2.0$ ). Whereas, Games-Howell Post Hoc from monocyte significantly differ between TM0 group with TM1 group ( $F = -1.66$ ) and TM2 group ( $F = -1.04$ ).

Table 2. Immune Cells Level in 3 Groups

Variable (cell/ $\mu$ l)	TM0 (no meditation)	TM1 (1 year meditation)	TM2 (2 year meditation)	Significance (p)
Eosinophil	1.58 $\pm$ 1.83	0.86 $\pm$ 1.71	0.58 $\pm$ 0.97	0.001
Neutrophil	65.34 $\pm$ 1.32	65.48 $\pm$ 1.50	69.02 $\pm$ 0.98	0.003
Monocyte	2.94 $\pm$ 0.22	4.60 $\pm$ 0.21	3.98 $\pm$ 0.21	0.000
Leukocyte	7.55 $\pm$ 0.22	7.50 $\pm$ 0.20	6.95 $\pm$ 0.18	NS
Lymphocyte	30.14 $\pm$ 1.24	29.06 $\pm$ 1.40	26.42 $\pm$ 0.93	NS
Hematocrit	39.22 $\pm$ 0.39	41.97 $\pm$ 0.65	42.19 $\pm$ 0.53	NS

Mean values $\pm$ SE together with the statistical significance differences. NS=Not significant; TM=Transcendental meditation; SE=Standard error,  $p < 0.005$ =significant

Table 3. TM0 correlation immune cells level with duration of meditation (year)

Variable (cell/ $\mu$ l)	TM1			TM2		
	n	r	p	n	r	p
Eosinophil	50	-0.05	0.70	50	0.13	0.43
neutrophil	50	0.09	0.50	50	-0.10	0.48
monocyte	50	0.03	0.82	50	-0.26	0.06
leucocyte	50	-0.77	0.59	50	0.06	0.06
lymphocyte	50	0.43	0.43	50	-0.15	0.28
hematocrit	50	0.00	0.00	50	0.33	0.01

Significance (p) > 0.4



Based on table 3, eosinophil, neutrophil, monocytes and leucocyte of TM0 have strong correlation ( $p > 0.4$ ) with TM1. This was different from the lymphocyte and hematocrit TM0 with TM1 showed no correlation ( $p = 0.003$ ). Leukocytes have a strong negative (inversely proportional) correlation ( $r$  value is close to  $-1$ ). The direction of this correlation indicates that the longer the meditation, the lower the eosinophils, although in TM2 leukocytes show the opposite direction (positive/unidirectional) but the direction of this correlation tends to be weak. Different things are shown in eosinophils, neutrophils, monocytes, lymphocytes and hematocrit which have a positive or negative correlation direction, but this correlation direction is very weak.

Eosinophil and neutrophil of TM0 has strong correlation with TM2 after two-year (2 year) meditation ( $p > 0.43$ ). However, it was different from the correlation between monocyte, leucocyte, lymphocyte, and hematocrit TM0 with TM2, it showed no correlation ( $p < 0.4$ ).

The duration of meditation affects the increase in the value of the cell's immune system in the body, it showed eosinophil, neutrophil, monocyte, leucocyte, and lymphocyte elevated after a year of meditation (TM1 group). After two-year meditation (TM2 group) showed a decrease in the leucocyte and lymphocyte. No significant difference was seen in neutrophils between the control group and others.

## DISCUSSIONS

Transcendental meditation is a systematic technique taught by specially trained instructors and it is a simple way that elicits an innate physiological response and requires no particular changes in lifestyle or adherence to a belief system.<sup>4</sup> Recent evidence suggests a bidirectional communication between the gut microbiota and the central nervous system.<sup>18</sup> A few studies have reported 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. Transcending is also correlated with alpha wave EEG coherence and synchrony. Additionally, two meta-analyses have found that the TM technique was more effective than other methods of meditation and relaxation for individuals with high levels of anxiety.<sup>19,20,21,22</sup>

The fluctuated regulation of immune function may be seen as one of the adaptive mechanism regulated by stress hormones. Many studies have reported chronic stress impairs immune system response as well as triggering inflammation and the reduction of stress associated with meditative practices can undo many of the physiological reactions leading to inflammation and vice versa. One of the major changes that occur along with the stress conditions experienced is the process of the thymus involution. The thymus is an organ in which T cells are mature. T cells are very important as lymphocytes to kill bacteria and help other types of cells in the immune system.<sup>2</sup>

In addition to WBC, in this study the hematocrit value was also calculated. Hematocrit is the level of red blood cells in the blood. Red blood cell levels that are too low or too high can be a sign that you are suffering from certain diseases, such as anemia or dehydration.

It has been well-established that psychological stress and depression impair anti-viral immune responses and activate innate immunity or markers of inflammation via effector pathways, such as the sympathetic nervous system and the hypothalamus-pituitary-adrenal (HPA) axis. Meditation, including more integrative, mindfulness-based, stress-reduction programs, has also been shown to regulate emotional and affective responses to stress and therefore may influence the immune system even in the absence of physical activity.<sup>23,24,25,26</sup>

Several studies have shown the ability of meditation to increase natural-killer (NK) cells and B-lymphocytes. NK cells are white blood cells that target and kill stressed or abnormal cells, playing a role in tumor prevention and serve as an initial defense against viral infections. A decrease in these cells may result in then the development or progression of different forms of cancer acute and chronic viral infections and various autoimmune diseases<sup>27</sup>. The hypothalamic-pituitary-adrenal axis that release of neuropeptide such as corticotropin-releasing factor hormone was triggered by stressful conditions. These stress hormones have been modulated immune function with decrease lymphocyte in blood compared with normal. Song and colleagues showed that corticotropin-releasing-factor in rats as animal testing reduced lymphocyte proliferation, decreased the percentage of lymphocyte and increased the percentage of neutrophils, compared with normal rats<sup>28</sup>. It showed there was no significance different on leucocyte and monocyte counts of academic stress, but relative and absolute

neutrophil counts were significantly higher in the stress samples than normal, also the stress participants as sample had a lower lymphocyte count.<sup>29</sup>

Based on that, it can be assumed that the WBC component can also be a reference in determining the immune response of students who have done meditation. In addition to the relatively inexpensive WBC examination, this examination has also become a common blood test in diagnosing an individual's health status. Concerning the preliminary study, this study showed the increase of lymphocyte and leukocyte from the control group (TM0) that have no experience on routine meditation to a year meditation (TM1). Nevertheless, the lymphocyte value was decreased on two-year meditation (TM2).

However, eosinophil, neutrophil, and monocyte showed a significant difference between the TM0 with TM1 and TM2 group. It is relevant with the result of previous studies, that showed there was a significant increase in neutrophils and no significant change in monocytes compared to normal under stress conditions, whereas in the results of participants using TM techniques, the longer the meditation duration there was a significant decrease in eosinophils, stable neutrophil counts and a significant increase in monocytes. This illustrates the transcendent meditation can release a negative vibration then it made the process of those maturation cells goes well. As previously reported that chronic stress can suppress immune function. The maturation cells of immunological responses to stress have been clinically related to increased susceptibility to infections. One pathway through which this occurs is the inflammatory response. In brief, immune cells produce inflammatory cytokines—proteins that regulate the immune response to injury and infection—which have either pro-inflammatory or anti-inflammatory effects.<sup>30,31</sup>

Pearson Correlation analysis shows that there is a strong correlation between eosinophil, neutrophil, leukocyte, lymphocyte, and monocyte with the duration of meditation (Table 3). The table shows the correlation of TM0 with TM1 and TM2 in various WBC components with each significance value. Similarly, the table shows the correlation of WBC components between TM1 and TM2. Mostly the correlation shows a positive correlation. It means the increase of each immune cell related to another cell. However, eosinophil, monocyte, and hematocrit showed significantly difference correlation between TM0 group with TM1 group and TM2 group. This

illustrate the transcendent meditation can release a negative vibration then it made the process of those maturation cell goes well. As previously reported that chronic stress can be suppresses immune function. One pathway through which this occurs is the inflammatory response. In brief, immune cells produce inflammatory cytokines—proteins that regulate the immune response to injury and infection—which have either pro-inflammatory or anti-inflammatory effects.<sup>31</sup>

It means the increase of each immune cell-related to another cell. A major finding of this study is that the increase of immune system cells (WBC) may be attributable to an increase in neutrophil counts. A systematic review demonstrated the possibility that some other causes that can be a source of stress for students are grouped into four factors.<sup>32</sup> These factors are relationship factors (adapting to new people), environmental factors (home or school environment), academic factors (facing the many tasks assigned), and personal factors. This condition in students can lead to less than optimal academic performance. Students experience difficulty concentrating, productivity and creativity decline, reduced readiness to face exams which results in unsatisfactory results, sometimes disrupt sleep quality, and have an impact on physical health.<sup>33,34,35</sup> It may negatively impact immune responses (such as WBC components activity).

Limitations on this study include the number of secondary parameters such as characteristics of respondents, blood pressure, quality of sleep and activity, even psychological survey to make this study complete. The increase of immune system cells in this study did not statistically significant because the data did not separate by sex and also the number of samples is too small, a small sample size is the major limitation of our paper. Many previous studies showed the differences white blood cells produce in men and women.

## CONCLUSION

Transcendental meditation (TM) seems to have a significant effect on immune cells. TM was proposed to be effective and efficient as an effort to improve body immune response was strengthening psychological balance in context of psychological well-being. The mechanism of increase and decrease of immune cells after TM as routine activity is the inflammatory cytokines, it explains our result and suggest the path of its mechanism to be further studies.

## Acknowledgment

The authors thank to faculty of medicine and health sciences, Warmadewa University as fund provider and also thank to SMA Negeri Bali Mandara, Jalan Raya Sanih, Buleleng, Bali. Acknowledge the people or organization(s) that have technically supported this work.

## Funding Statement

This research did not receive any specific grant funding agencies in the public, commercial, or not-for-profit sectors.

## Conflict of Interest

All authors declare no conflict of interest.

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