

A Religious Life Style Centered on Gratitude may Influence on Natural Killer Cell Activity and Subjective Well-Being

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Abstract

Objectives: The aim of this pilot study is to assess the effect of religious life styles on natural killer (NK) cell activity and psychological status.

Design and Outcome Measures: Among some kinds of remarkable religious life styles, we focused on gratitude. Thirty-one religious subjects (average age, 64.5 ± 6.6 years) were recruited and instructed to recollect memories from past to present for a 1-month period by using 3 themes of Naikan therapy. Blood sampling and psychological measurements were performed pre- and post-recollection. Blood sampling was repeated after 3 and 4 months (control period).

Results: (i) A significant improvement in NK cell activity was observed after the 1-month recollection period (average, 34.3%–38.0%; $p = 0.028$), while no improvement was observed in the control period (average, 34.4%–35.7%; $p = 0.261$). (ii) A significant elevation in NK cell activity (average, 35.2%–40.6%; $p = 0.009$) and a positive affect in WHO Subjective Well-being Inventory (WHO-SUBI) (average, 42.8–44.2; $p = 0.049$) was observed in participants who reported a strong feeling of gratitude, but not in those who reported moderate or no feelings of gratitude.

Conclusion: This is the first study demonstrating the effect of gratitude in improving NK cell activity and positive affect in subjective well-being.

Keywords:

religious life style, gratitude, natural killer cell activity, subjective well-being, Naikan therapy

Introduction

Modern medicine places importance on the physical aspect of human diseases and biomedical approaches are employed as principal methods to cure diseases¹⁾. Although religion and medicine were closely associated with each other in earlier times, most present-day medical experts do not pay much heed to the influence that religion may have on medicine. On the other side, recent medicine pays much

attention to preventative medicine and some simple and convenient methods, i.e. humor and laughter, are evaluated to decrease stress and improve the quality of life (QOL)²⁻⁴⁾. From this point of view, we believe it is worth investigating whether religious life styles may have some influences on our health. Among some kinds of remarkable religious life styles, we focused on gratitude in this study. Because the importance of gratitude is emphasized in any religion, and gratitude is generally and widely considered to result in

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happiness, improve human relationships, and positively influence health. However, despite the profound impact of gratitude on health, no scientific research has been conducted thus far to examine this effect.

The concept of this study is based on the assumption that most people do not innately possess the feeling of gratitude but develop this feeling after realizing that all our lives are related to and supported by others. The 3 themes of Naikan help in understanding this concept^{5,6}. Naikan is a Japanese word that means “introspection”. Naikan as well as Morita therapy represent forms of psychotherapy developed in Japan. Naikan therapy involves the recollection of memories from past to present based on 3 themes: (i) what we have received from others, (ii) what we have offered others, and (iii) the troubles we have caused others. Naikan therapy is reported to be extremely effective in the process of self-discovery, and one of its important revelations is that our lives are supported by others. This revelation generally induces the feeling of gratitude in the minds of people⁷. However, in general, the proven positive effects of Naikan are limited to clinical case reports in the psychiatric field. Moreover, when Naikan therapy is applied to healthy people, its effectiveness is largely determined based on personal experiences and not on objective indices such as immunological function and psychological evaluation. Despite these limitations, the 3 themes of Naikan are considered to be effective in evoking the feeling of gratitude, and we believe that it is important to examine this effect.

In general, the feeling of gratitude is extremely essential for religious people, and they clearly understand its importance. Therefore, religious people were considered to be best suited to examine the effect of gratitude on health, in a similar manner as athletes are selected for examining the effects of running on health. The religious participants recollected their relationship with God from past to present for a 1-month period by using the 3 modified themes of Naikan.

Psychoneuroimmunology (PNI), which is believed to be a scientific approach for resolving a mind-body problem, has demonstrated that both physical and psychosocial stressors

may modulate immune function via the hypothalamic-pituitary-adrenal axis (HPA) and/or the autonomic nervous system (ANS)^{8,9}. In this field, natural killer (NK) cell activity is one of the popular indices used for determining the effects of various stressors on immune function^{8,10,11}, and NK cell activity is one of the important biological markers in complementary and alternative medicine (CAM)¹².

The aim of this pilot study is to assess the effect of gratitude that is one of the most remarkable religious life styles on NK cell activity and psychological status in healthy religious people with the help of questionnaires from the perspective of preventive medicine.

Subjects and Methods

Subjects

The present study included 31 healthy male and female participants (4 males and 27 females) with an average age of 64.5 ± 6.6 years (age, 49–78 years). Volunteers were prescreened based on inclusion and exclusion criteria prior to enrollment in the study. Those with depression symptoms, sleeping difficulties, and unexplained weight changes and those on immunosuppressive medications, antidepressants, and herbal supplements were excluded from the study¹³. Of the 31 participants, 1 participant who failed to provide blood samples at all the 4 sampling instances was excluded from the analysis of NK cell activity. All the subjects were religious and belonged to a religious organization that was based on Shintoism. Further, all the subjects provided written informed consent. The experimental procedure was approved by the Institutional Review Board of MOA Health Science Foundation and was conducted in accordance with the policies and principles of the Declaration of Helsinki.

Recollection by using the 3 themes of Naikan

Naikan is a form of directed meditation practiced in Japan and other countries and is reported to elicit positive effects on a number of neurotic, psychosomatic, and delinquency disorders⁵. It aims at reconstructing an

individual's view of his past in order to modify his present attitude and behavior. In Naikan therapy, the past is explored based on 3 themes: (i) what we have received from others, (ii) what we have offered others, and (iii) the troubles we have caused others. In this study, we adopted these themes and modified them for the religious subjects as follows: (i) what we have received from God (e.g., a happy life free of any severe diseases.), (ii) what we have offered God (e.g., thanking God everyday and trying to love our fellow beings), and (iii) the troubles we have caused God (e.g., losing one's temper against God's will). The method used in this study is not formal Naikan therapy but a modified method that could be easily practiced by the healthy religious participants.

Prior to the start of this experiment, the participants were lectured on how to recollect memories based on the 3 themes. Each participant was given a notebook and was instructed to record his/her recollections at the end of each day. In brief, they were required to recollect 1 theme per day from past to present; for example, what they had received from God during their first 3 years as members of the religious organization. Hence, if a person had been a member of the organization for 30 years and he/she recollected memories based on 1 of the 3 themes per day for every 3 years of his/her past life, then he/she would complete the recollection process in 30 days.

Immunological measurements

NK cell activity was determined at an effector-to-target ratio of 20:1¹⁴⁾ by using a standard chromium release assay, as described previously. The target cells were ⁵¹Cr-labeled human myeloid K562 cells. The measurements were performed at SRL Inc. (Tokyo, Japan).

Psychological measurements

The participants completed 2 psychological questionnaires: WHO Subjective Well-being Inventory (WHO-SUBI) and Profiles of Mood States-Brief (POMS-Brief). WHO-SUBI is a scale for measuring the subjective health status, and it employs a self-entry-type questionnaire developed by

Nagpal and Sell¹⁵⁾. It comprises 41 items; each item is answered on a 3-point scale. These items are divided into those that elicit a positive affect, i.e., indices of psychological health status (19 items) and those that elicit a negative affect, i.e., indices of non-well-being (21 items). The mood states of the participants prior to and after this intervention were evaluated using POMS-Brief. POMS-Brief comprises 30 items, and the obtained scores can indicate psychological distress symptoms such as depression, lack-of-vigor, aggression-hostility, fatigue, tension-anxiety, confusion, and total mood disturbance¹⁶⁾. The questionnaires with incomplete answers were excluded from the analysis.

Experimental procedure

The protocol of the experiment is schematically represented in Fig. 1. All blood samplings were performed between 9:30 A.M. and 11:30 A.M. due to the circadian rhythm of NK cell activity. After blood sampling, the psychological state of the subjects was assessed using WHO-SUBI and POMS-Brief.

The recollection process was conducted for 1 month. After 2 weeks, a trained staff member interviewed the subjects for approximately 20 min each and inquired if they faced any difficulties during recollection and regarding their feelings in relation to the trial. At the end of the 1-month recollection period, the tests, including blood sampling and psychological measurements, were repeated, and data was obtained. Moreover, the participants responded to the following 3 questions on a 3-point scale: (i) Were you eager to recollect the memories? (ii) Was this practice helpful in strengthening your religious faith? (iii) Did this practice leave you with a sense of gratitude? Blood sampling was repeated for these participants after 3 and 4 months.

Student's *t*-test was used for statistical analysis, and a two-sided *p* < 0.05 was considered statistically significant. All the statistical analyses were performed using SPSS (version 12.0).

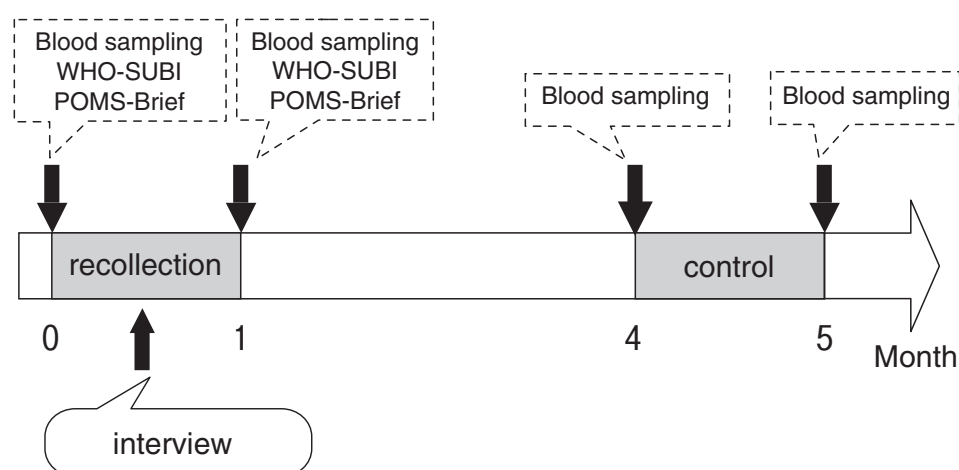


Figure 1. The protocol of the experiment.

Table 1. The distribution of NK cell activity at baseline.

NK cell activity	male	female
Less than 10%		0 (0%)
10-19%		5 (19%)
20-29%		7 (27%)
30-39%		11 (42%)
40-49%		1 (4%)
50-59%		2 (8%)
60-69%	4 (100%)	0 (0%)
Total	4(100%)	26(100%)

Table 2. Self-rated evaluation of 3 questions after a 1-month recollection period.

	eagerness	religion	gratitude
strongly	12	16	22
moderately	15	11	8
never	4	4	1
Total	31	31	31

Results

The distribution of NK cell activity at baseline is shown in Table 1. All the male participants ($n = 4$) exhibited higher NK cell activity (62%–66%) than the female participants; this finding has also been reported by other researchers¹⁷.

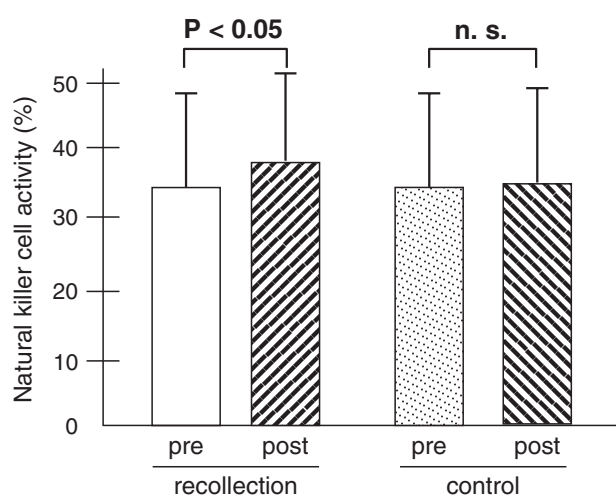


Figure 2. The pre- and post-recollection scores of NK cell activity ($N=30$). A significant improvement in NK cell activity was observed (average, $34.3\% \pm 15.2\%$ – $38.0\% \pm 14.6\%$; $p = 0.028$) after the 1-month recollection period. During 1-month interval (control), the average NK cell activity of all the participants changed from $34.4\% \pm 13.1\%$ to $35.7\% \pm 13.3\%$, and the paired t -test revealed no statistically significant difference ($p = 0.261$).

After the 1-month recollection period, the participants were asked 3 questions as described above. An evaluation of their responses is shown in Table 2; it indicated that this practice helped strengthen their religious faith and increase their sense of gratitude.

A significant improvement in NK cell activity was observed (average, $34.3\% \pm 15.2\%$ – $38.0\% \pm 14.6\%$; $p =$

0.028) after the 1-month recollection period (Fig. 2). In this experiment, the participants practiced recollection for only 1 month. Further, blood sampling was repeated after 3 and 4 months. During this 1-month interval, the average NK cell activity of all the participants changed from $34.4\% \pm 13.1\%$ to $35.7\% \pm 13.3\%$, and the paired *t*-test revealed no statistically significant difference ($p = 0.261$).

It has been reported that individuals who have relatively high NK cell activities at the start of therapy may not experience significant improvement after the therapy that enhances NK cell activity; this is known as the ceiling effect¹⁸⁾ or regression toward the mean. The same effect is observed in individuals with relatively low NK cell activity scores. The measurement of NK cell activity, which was performed at SRL Inc., revealed a reference value ranging from 18% to 40%. At the start of this study, 4 males and 2 females (6 participants) demonstrated high NK cell activity (>50%) and 3 females demonstrated low NK cell activity (<18%), while the remaining 21 females demonstrated

moderate NK cell activity ranging from 19% to 41%. Thus, in order to precisely clarify the effect of recollection on immune function, we excluded the NK cell activity scores of the 6 participants with high NK cell activity (>50%) and the 3 participants with low NK cell activity (<18%) and analyzed the scores of the remaining participants. As shown in Fig. 3, the average scores of the participants with moderate NK cell activity (19%–41%) changed from $29.7\% \pm 6.22\%$ to $35.5\% \pm 9.79\%$, and the paired *t*-test revealed a higher statistically significant difference in the NK cell activity scores of these participants ($p = 0.005$) as compared to those of all the other participants. In contrast, during the 1-month interval in which the participants did not practice recollection, the average NK cell activity changed from $32.1\% \pm 9.46\%$ to $32.7\% \pm 8.19\%$, and no statistically significant difference in NK cell activity was observed ($p = 0.644$). Fig. 3 illustrates an additional important observation indicating that the NK cell activity was maintained even 3 and 4 months post-recollection.

Fig. 4 show the relationship between the NK cell activity scores and the self-rated degree of gratitude. A significant elevation was observed ($p = 0.009$) in participants who reported a strong feeling of gratitude after recollection ($n = 22$). However, no significant change was observed ($p = 0.765$) in those who reported a moderate feeling of gratitude ($n = 7$), while it decreased from 31% to 29% in those who reported no feeling of gratitude.

In addition to the immunological measurement of NK cell activity, we obtained scores for the following 2 parameters: subjective health status (WHO-SUBI) and mood states (POMS-Brief). When the questionnaires of all the participants were analyzed, we did not observe any significant changes in their responses after recollection in WHO-SUBI (Tables 3A) and POMS-Brief (data not shown). However, when the questionnaires of participants who reported a strong feeling of gratitude after recollection were analyzed, we observed a significant change in the responses to positive affect in WHO-SUBI after recollection ($p = 0.049$; Table 3B), while POMS-Brief revealed no significant changes (data not shown).

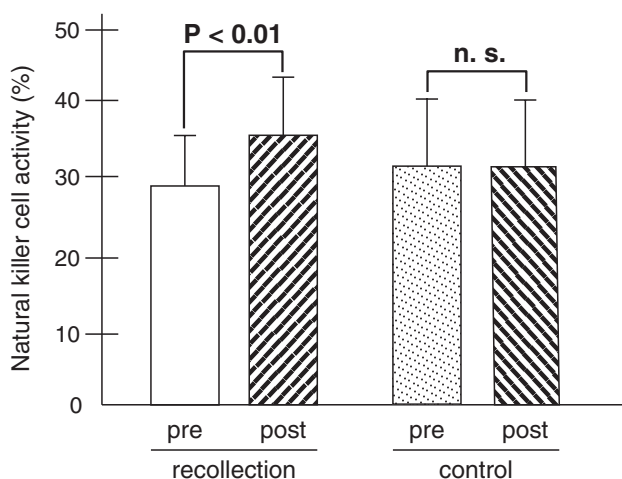


Figure 3. The pre- and post-recollection scores of NK cell activity in the participants whose NK cell activity at baseline was from 19% to 41% (N=21). The average scores of the participants with moderate NK cell activity (19%–41%) changed from $29.7\% \pm 6.22\%$ to $35.5\% \pm 9.79\%$, and the paired *t*-test revealed a higher statistically significant difference in the NK cell activity scores of these participants ($p = 0.005$) as compared to those of all the other participants. In contrast, during the 1-month interval in which the participants did not practice recollection, the average NK cell activity changed from $32.1\% \pm 9.46\%$ to $32.7\% \pm 8.19\%$, and no statistically significant difference in NK cell activity was observed ($p = 0.644$).

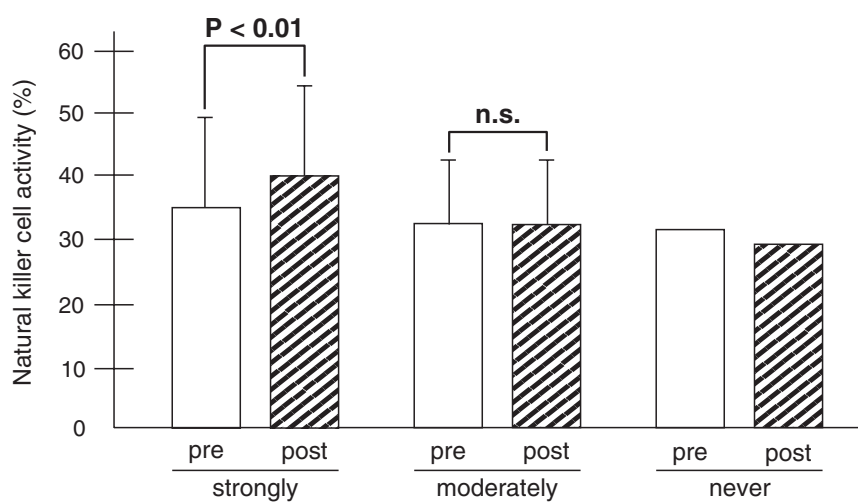


Figure 4. The relationship between the score of NK cell activity and the self-rated degree of gratitude. A significant elevation was observed ($p = 0.009$) in participants who reported a strong feeling of gratitude after recollection ($n = 22$). However, no significant change was observed ($p = 0.765$) in those who reported a moderate feeling of gratitude ($n = 7$), while it decreased from 31% to 29% in those who reported no feeling of gratitude.

Table 3A. The pre- and post-recollection scores of WHO-SUBI in all participants (N = 28).

	Pre-recollection		Post-recollection		P-value
	average	S.D.	average	S.D.	
Positive affect	42.9	5.09	43.4	5.76	0.390
Negative affect	54.4	4.22	54.1	4.76	0.556

Table 3B. The pre- and post-recollection scores of WHO-SUBI in participants who reported a strong feeling of gratitude after recollection (N = 21).

	Pre-recollection		Post-recollection		P-value
	average	S.D.	average	S.D.	
Positive affect	42.8	5.49	44.2	5.21	0.049
Negative affect	53.9	4.54	53.3	5.02	0.376

Discussion

This is a pilot study aimed at assessing the effect of gratitude that is one of the most remarkable religious life styles on NK cell activity and psychological status.

We can assume that the importance of gratitude is recognized by all religions, races, and cultures. However, no scientific research on the effect of gratitude on health

has been performed thus far. In this study, we have provided data to substantiate this finding: (i) A significant improvement in NK cell activity was observed after the 1-month recollection period (average, 34.3%–38.0%; $p = 0.028$) but not after the control period (average, 34.4%–35.7%; $p = 0.261$). (ii) This improvement in NK cell activity attributed to gratitude was observed more clearly in participants who demonstrated moderate NK cell activity

(19%–41%) at the baseline. (iii) A significant elevation in NK cell activity (average, 35.2%–40.6%; $p = 0.009$) and a positive affect in SUBI (average, 42.8–44.2; $p = 0.049$) was observed in participants who reported a strong feeling of gratitude but not in those who reported a moderate or no feelings of gratitude. Thus, this is the first evidence to show the effect of gratitude in improving NK cell activity as well as positive affect.

In psychoneuroimmunology (PNI), NK cell activity has been widely used to examine the effect of psychological as well as physical stressors on immune function. However, the effects of negative psychological stressors such as bereavement, divorce, and examinations on immune function were primarily investigated and proved to suppress NK cell activity^{19,20}. In contrast, few medical investigations have been conducted to examine the effect of positive psychological events on health. Laughter and gratitude are positive psychological events, and previous medical studies on laughter reporting that positive psychological events elevate NK cell activity have been important for guiding investigations.

The present study does not explain the mechanism by which gratitude elevates NK cell activity; however, the possible mechanism has been explained by some previous studies. It is well known that negative stressors can affect the HPA and ANS causing an increase in the concentrations of cortisol, epinephrine, and norepinephrine and resulting in a decrease in NK cell activity^{9, 21, 22}. Hence, it might be postulated that gratitude attenuates the magnitude of daily stressful events by altering the cognitive response to these events and by maintaining the concentrations of these stress hormones at a lower level. However, the stress hormone levels were not measured in our pilot study; thus, the mechanism of elevation of NK cell activity by gratitude remains speculative.

In addition to the measurement of NK cell activity, the participants completed 2 psychological questionnaires: WHO-SUBI and POMS-Brief. An improvement in positive affect, i.e., indices of psychological health status, was observed using WHO-SUBI in participants who reported a

strong feeling of gratitude (average, 42.8–44.2; $p = 0.049$); however, no significant changes were observed in the responses to these questionnaires after recollection in any of the participants. It is evident that the WHO-SUBI scores were only moderately elevated; however, it should be considered that the baseline scores were considerably high, and therefore, the effect of recollection was not clearly observable. For example, the positive affect score at baseline was 42.8 in this study, and a Japanese manual on WHO-SUBI states that scores above 42 are regarded as high²³.

The evaluation of positive as well as negative affects is considered important because positive affect can increase resistance to stressful events²⁴. Moreover, it has been shown that SUBI is not only strongly associated with a variety of well-being indicators but is also a strong predictor of mortality in longitudinal studies^{25, 26}. Thus, it is extremely important to clarify whether gratitude increases resilience to psychological stress and to investigate whether gratitude contributes to prolonged life expectancy by using a greater number of participants.

No significant changes in the POMS-Brief scores were noted. The most probable reason for this finding was that the participants were instructed to complete the POMS-Brief questionnaire based on their present state of mind and not on their general state of mind during the previous 1 week as indicated by the POMS and POMS-Brief manuals¹⁶. This was done because the questionnaires were also used to measure the mood changes induced by relatively short-term interventions, such as watching a comic video for a few hours, etc². Our staff was accustomed to using POMS-Brief for short-term interventions and instructed the participants accordingly. Therefore, the POMS-Brief scores presented here are not likely to reflect the actual pre- and post-recollection scores.

The methodological features of this study are as follows: (i) the 3 themes used in Naikan were adopted and (ii) the participants were religious people. We believe that this methodological strategy was successful because the importance of gratitude can be appreciated by accepting

that our lives are supported by others and/or God and by considering that religious people are accustomed to the feeling of gratitude. We believe that the negative effects of religion on mental health must not be ignored²⁷⁾; however, from the viewpoint of developing a strong feeling of gratitude, the possible effects of religion on health are important and promising²⁷⁻²⁹⁾.

Naikan is a form of directed meditation founded by Yoshimoto Ishin (1916–1988), and this therapy as well as Morita therapy is now regarded as forms of representative psychotherapy founded in Japan^{5, 7)}. In the psychiatric field, Naikan is reported to be effective not only as a form of psychotherapy but also as a method of reforming criminal offenders and resolving issues such as bullying at school, which in turn results in problems like the victims of bullying refusing to attend school³⁰⁾. Thus, this method can be applied to a wide range of people with varying health statuses. It helps us understand that our lives are linked to those of others, that we are supported by others, and that we receive considerable kindness from others without acknowledging or appreciating it or extending our gratitude to them. The process of self-exploration by using the 3 themes of Naikan can help us develop trust in others, become more responsible, behave more constructively, and develop a sense of gratitude.

Most studies on the effectiveness of Naikan reported thus far have been based on clinical cases and personal experiences, and the quality of evidence has not been satisfactory. No studies have examined whether Naikan can improve immunological function.

The formal method of intensive Naikan therapy is rather strict; it is practiced as follows. The subject sits on a cushion and relaxes in a comfortable position. He is surrounded by folding screens to enable him to perform intense introspection. Activities such as watching television, listening to the radio, reading books, and talking to others are prohibited during intensive Naikan therapy. The subject strictly practices Naikan from 6:00 or 7:00 AM to 9:00 PM for 1 week. Intensive Naikan may be effective in curing mental disorders but it is difficult to practice it in our daily life.

Therefore, a variety of modified Naikan are practiced in some facilities for each purpose. Anyway, I believe that the 3 themes of Naikan are effective tools for improving the levels of gratitude, and the modified method used in this study may be one of the suitable tools for further investigations of the effect of gratitude on health, particularly among the religious participants.

Laughter and gratitude affect health in both similar and distinct manners. Their effects are similar in that they both may elevate NK cell activity and positively influence health without the use of any materials/medicines or financial investments. Thus, for improving the health status, it is safer to use laughter and gratitude rather than materials/medicines used for the cure and/or prevention of disorders because the latter are associated with several side effects. The differences in the effects of laughter and gratitude may be the time of onset and duration of the effect. Based on the character of laughter and gratitude, we believe that the positive effects of gratitude will take longer to appear and will last longer than those of laughter. As mentioned in our results, the NK cell activity was maintained even 3 and 4 months post-recollection (Fig. 3). In general, the feeling of gratitude is developed by accepting that our lives are related to and supported by others. In contrast, laughter is induced more easily and rapidly; for example, on watching comedy television programs. According to previous reports, the effect of laughter is rapid and short-lived³¹⁾. Therefore, the combination of laughter (short-term effect) and gratitude (long-term effect) will be an extremely effective tool for the cure and prevention of disorders and for health promotion.

Thus far, the importance of NK cell activity has been discussed in PNI and CAM, and the results presented by Imai et al. strongly support the positive effect of NK cell activity on health. They demonstrated that moderate and high cytotoxic activity of peripheral blood lymphocytes is associated with reduced cancer risk, whereas low activity is associated with increased cancer risk; these findings suggested the role of the natural immunological host defense mechanism against cancer¹⁷⁾. These results

suggested that an elevation in NK cell activity may prevent the initiation of cancer. Thus, further medical research on the effect of gratitude on health may reveal its possible application in cancer prevention.

We recognize that this pilot study has several limitations: (i) the participant population was small, (ii) most participants were female, (iii) a crossover design study might have been more preferable, and (iv) the psychological test POMS-Brief was not conducted in keeping with the instructions provided the manual. Especially, referring to a crossover design study, we have to admit the methodological insufficiency in this research. Despite these limitations, this pilot study has provided the first evidence of the effect of gratitude on immune function as well as positive affect in WHO-SUBI.

The effect of religious life styles on health has not been investigated by present medical research dominated by biomedicine. In this study, we focused on gratitude because it seems that gratitude always lies in the center of religious life styles. Moreover, in order to precisely assess the effect of gratitude on health, we used 3 themes of Naikan therapy. Considering the safety and cost effectiveness of increasing the level of gratitude by using the modified Naikan method, the effect of gratitude on health will be extremely promising for the cure and prevention of disorders and for health promotion. This study may serve as a stepping stone for future research on the effect of religious life styles on health.

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感謝を中心とする宗教的ライフスタイルはナチュラルキラー細胞活性と主観的健康感を高める可能性がある

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抄 録

目的：宗教では、感謝の大切さが重視されているが、感謝が健康に与える影響を医学的に明らかにする研究は殆んど見られない。今回の研究の目的は、感謝がナチュラルキラー（NK）細胞活性や心理状態に与える影響を明らかにする事である。

方法：宗教団体に属している31名の参加者をボランティアで募り、内観三問に従って、1ヶ月間神仏と自分の関係を回想してもらった。採血と心理テストは回想前後で行った。採血と心理テストはその後3および4ヶ月後にも行った（コントロール期間）。

結果：1ヶ月の回想により、NK細胞活性の増加が認められた（34.3%→38.0%; $p = 0.028$ ）。この増加はコントロール期間では認められなかった。参加者のうち、感謝の気持ちが非常に高まったと答えた群ではNK細胞活性の増加と主観的健康感の上昇が認められた。

結論：感謝がNK細胞活性と主観的健康感を高める可能性が示された。

キーワード

宗教的ライフスタイル、感謝、ナチュラルキラー細胞活性、主観的健康感、内観療法

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