we investigated the effect of HT on the symptoms of earthquakerelated stress and the maintenance of its effect after a two-month follow-up (FU) period using psychological measures and salivary cortisol level.

METHODS

Participants

The participants were women aged 23–55 years who were residents of the coastal areas of Miyagi Prefecture and had experienced the Great East Japan Earthquake of March 11, 2011. We targeted the adult woman from 20 years old to 60 years old in this study. Review of the epidemiology of PTSD after Disasters reported that women are more likely than men to have PTSD after natural disasters, and low social support is associated with a higher likelihood of PTSD [16]. We focused this point. As the first step of horticultural therapy study, we performed an experimental study aimed at adult woman from 20 to 60 years old because there was no study of effect verification of horticultural therapy for a woman who lives in disaster area until now.

They were recruited through newspaper advertisements distributed in the earthquake-affected areas, to which 106 residents of the coastal areas (from Kesennuma City to Iwanuma City) responded.

These 106 applicants were screened for PTSD using a combination of the Mini-International Neuropsychiatric Interview (M.I.N.I.) [17,18] and the Clinician-Administered PTSD Scale (CAPS) [19-21]. In the CAPS, the F1/2 method was used for evaluation, with applicants regarded as symptomatic if they scored ≥1 on frequency and ≥2 on intensity. After the exclusion of 52 applicants who had no PTSD symptoms and a CAPS score of 40, 54 healthy, right-handed women participated in this study as part of our ongoing project to investigate the associations between brain structure and mental health. All participants who took part in this study also participated in our interventional studies and underwent psychological tests and MRI scans that are not described in this study but were performed together with those described in this study. All participants were diagnosed with a symptom of PTSD on the M.I.N.I., and they had one to two symptoms of all three PTSD symptom clusters, including reexperiencing the event, avoidance, and hyperarousal. The CAPS and M.I.N.I. were administered before and after the intervention. This study was approved by the Research Ethics Committee of Tohoku University Graduate School of Medicine after an ethical screening. Informed consent in writing was obtained from the experimental participants before the start of the experiment. The intervention period was from September 2011 to March 2012.

The study was a randomized, open -label assessors are blinded, crossover trial (RCT), and it was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000006170). Testers are blind to the study's hypothesis and the group membership of participants. The participants were divided into two groups, an intervention group (n = 27) and a control group (n = 27), by the permuted block method, and the intervention group underwent eight weeks of horticultural intervention followed by an eight-week FU period. The control group underwent eight weeks of stress control education, followed by eight weeks of horticultural intervention.

Although the intervention group had a follow-up period after the intervention period, participants were allowed to keep growing plants during the follow-up period because it could have been stressful or created negative emotions, if we required participants to stop their horticultural activities.

Description of interventions

Horticultural intervention (Intervention group): The HT intervention was designed in collaboration with a horticultural therapist and clinical psychologists. The intervention comprised a total of eight weekly sessions (60 min each) at a university lab and 15 minutes per day at participants' homes. The lab sessions were comprised of interactive lectures and practical training. The participants then attended six horticultural lessons, including topics such as designing a garden planter, seeding, watering, weeding, and picking flowers. Participants filled out an HT intervention session checklist after each session as a self-assessment. Participants took care of plants for 15 min per day at their convenience with horticulture kits provided by the experimenters, and recorded the completion of this task daily on forms provided by the experimenters at the intervention sessions. The participants submitted these forms to the experimenters at the HT intervention session each week.

Stress control education intervention (Control group): The SE intervention session was a 60-minute session consisting of a lecture regarding stress education, and it was managed by

of a lecture regarding stress education, and it was managed by teaching assistants who served as psychological testers. The participants in the control group attended the SE intervention sessions once each week (a total of eight lessons). The video series used in the SE intervention sessions taught participants about the human body, such as stress mechanisms, psychology, and stress management. Participants filled out an SE intervention session checklist after each session. The 2nd session and the 6th session of the HT intervention session and the SE intervention session used the same teaching aid.

Follow-up (Intervention group): At the end of the twomonth horticultural intervention, the experimental participants allocated to the intervention group entered a two-month FU period (Figure 1). During this period, they did not receive any specific instructions from the investigators and were asked to lead their normal lives.

PTSD screening: M.I.N.I. and CAPS

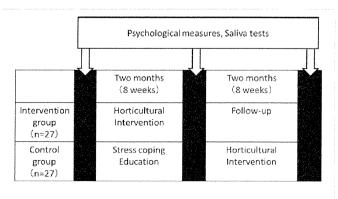


Figure 1 Intervention design. ** p <.01, * p <.05.

In this study, structured interviews were performed by six clinical psychologists and psychologists with experience in assisting earthquake victims, who underwent training before carrying out the actual interviews.

Psychological measures

The following questionnaires were administered three times (pre-intervention, post-intervention, and FU): (a) the World Health Organization Quality of Life 26 instrument questionnaire (WHO-QOL26) [22], (b) the Center for Epidemiologic Studies Depression Scale (CESD) [23,24], (c) the General Health Questionnaire 30 (GHQ30) [25,26], (d) the Positive and Negative Affect Schedule (PANAS) [27,28], and (e) the Posttraumatic Growth Inventory (PTGI) [29,30]. We used the Japanese version of these psychological measures.

Saliva sampling

We collected saliva samples from participants to measure the salivary cortisol levels. Distressing psychological stimuli are associated with an increased cortisol level [1]. In consideration of the participants' circadian cortisol rhythms, we collected all saliva samples at 4:00 p.m. on weekdays both before and after the intervention. We selected 4:00 p.m. because humans are less affected by circadian cortisol rhythms at this time of day [31]. Participants were asked to refrain from drinking, eating [32], and exercising [33] for two hours before saliva sampling.

Measurement of salivary cortisol

To assess physiological stress, we used the same technique to measure salivary cortisol as described in a previous study [2]. Saliva samples were collected using the salivette apparatus (Sarstedt, Nümbrecht, Germany). We stored the supernatant solutions in airtight containers at -80° C and measured salivary cortisol using the solutions. We measured salivary cortisol with a semi-microcolumn high-performance liquid chromatography (HPLC) system (Shiseido, Tokyo).

ANALYTICAL METHODS

The psychological and salivary data were analyzed using the PASW statistical software package (ver. 18 for Windows; SPSS Inc., Chicago, IL, USA). Demographic and clinical data were subjected to the one-way analyses of variance. The one-way analyses of covariance were conducted with the differences between the pre- and post-intervention scores included as dependent variables and pretest scores as covariates of each psychological measurement. Because our primary endpoint of interest was the beneficial effect of intervention training, testretest changes were compared between the intervention and control groups using one-tailed tests (p < 0.05), in the same manner as in previous studies [34].

Moreover, changes in the intervention group were confirmed by the analysis of variance of psychological measure scores and salivary cortisol level at three points (pre-intervention, post-intervention, and FU) using a mixed model. Changes in each measure over time in the intervention and control groups were then compared by means of analysis of variance using repeated measures.

RESULTS

A. Comparison of an intervention group with a control group (Pre vs. Post)

Psychological measures: The demographic and clinical data for the study participants are given in (Table 1). The participants age and Clinician-Administered PTSD Scale (CAPS) scores did not differ significantly between the intervention group and control group. Comparisons of the psychological changes before and after the intervention between the two groups are shown in (Table 2). The intervention group had a significant decrease in the postintervention CAPS score (F[1,51] = 13.526, p < 0.001). The PTSD symptoms reduced more in the intervention group than in the control group. The intervention group also showed a significantly larger increase in the post-intervention Posttraumatic Growth Inventory (PTGI)-J total scores [F(1,51) = 4.315, p < 0.05](Figure 2). The PTGI-J total score was significantly higher in the intervention group than in the control group, and the PTGI-J score improved more in the intervention group compared with the control group. Moreover, the intervention group showed a significantly larger pre- to post-intervention increase in the Positive and Negative Affect Schedule-Positive Affects (PANAS-PA) scores (F[1,51] = 5.66, p < 0.05). The PANAS-PA score was significantly higher in the intervention group compared to the control group, and the PA increased in the intervention group compared with the control group.

Salivary cortisol level: Comparisons of salivary cortisol level pre- and post-intervention are summarized in (Table 2). The intervention group had a significantly larger pre- to post-intervention decrease in salivary cortisol (F[2, 52] = 14.077, p = 0.001), indicating that stress was more effectively reduced in the intervention group compared with the control group.

B. Annual changes of an intervention group (The effectiveness of HT intervention)

Psychological measures: Changes in the various psychological measures of the intervention group are shown in (Table 3). Analysis of CAPS scores showed that the main effect of time (pre-intervention, post-intervention, and FU) was significant [F(2,52) = 459.12, p < 0.001]. Bonferroni's multiple comparison showed a significant decrease in the post-intervention score compared with the pre-intervention score (p < 0.001), with the FU score also significantly lower than the pre-intervention score (p < 0.001) and post-intervention score (p < 0.005), confirming that this effect was maintained (Figure 3).

The main effect of time was significant for PANAS-PA score (F[2,52] = 8.40, p < 0.001). Multiple comparisons showed that the post-intervention score was significantly lower than the pre-intervention score (p < 0.05), and that the FU score was also

Table 1: Baseline demographic and clinical data of the participants.

	Interventi	on group	Control group			
Factor	Mean	SD	Mean	SD	pa	
Age (years)	42.48	9.72	44.22	7.78	0.884	
CAPS score	31.52	6.5	31.25	6.47	0.471	

One-way analysis of variance.

HT, horticultural therapy; SE, stress education; SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale.

Table 2: Psychological measures pre- and post-intervention.

	Intervention group			Control group			pa		
	Pre		Post		Pre		Post		
Measurements	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
CAPS score	31.5	6,5	10.0	7.1	31.3	6.5	16.1	9.3	<0.001
WHO-QOL26 total score	52.5	5.8	53.9	6.5	52.3	6.3	51.0	5.9	0.297
CES-D score	13.4	7.1	11.8	7.4	14.6	6.9	12.5	5.2	0.934
GHQ score	7.1	5.1	4.4	4.2	6.8	5.7	5.0	4.7	0.248
PANAS positive affect	20.5	6.4	23.3	7.4	23.6	7.8	21.0	7.2	0.011
PANAS negative affect	18.9	7.7	15.1	6.0	21.8	7.1	18.5	6.4	0.071
PTGI total score	66.6	18.1	72,3	15.7	68.4	18.3	66,5	17.9	0.022
Salivary cortisol level	8.3	6.4	3.0	4.9	7.5	7.8	11.9	11.6	0.001

*One-way analyses of covariance with pre-post differences in psychological measures as dependent variables and pre-intervention scores as covariates (one-tailed).

HT, horticultural therapy; SE, stress education; SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale; WHO-QOL26, World Health
Organization Quality of Life 26; CES-D, Center for Epidemiologic Studies Depression Scale; GHQ, General Health Questionnaire; PANAS, Positive and Negative Affect Schedule;
PTGI, Posttraumatic Growth Inventory.

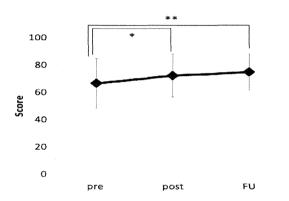


Figure 2 The change in PTGI-J score of the intervention group. *** p < .001, * p < .05.

Table 3: Psychological changes of Intervention group (Pre, Post, and FU).

	D ₁		re Po		FI	IT.
Measurements	Mean	SD	Mean	SD	Mean	SD
CAPS score	31.5	6.5	10.0	7.1	8.7	6.2
WHO-QOL26 total score	52.5	5.8	53.9	6.5	54.2	6.3
CES-D score	13.4	7.1	11.8	7.4	12.0	6.8
GHQ score	7.1	5.1	4.4	4.2	4.3	4.3
PANAS positive affect	20.5	6.4	23.3	7,4	24.2	6.4
PANAS negative affect	18.9	7.7	15.1	6.0	15.2	5.9
PTGI total score	66.6	18.1	72.3	15.7	74.9	13.4
Salivary cortisol level	8.3	6.4	3.0	4.9	2.0	3.2

HT, horticultural therapy; SD, standard deviation; CAPS, Clinician-Administered Post-Traumatic Stress Disorder Scale; WHO-QOL26, World Health Organization Quality of Life 26; CES-D, Center for Epidemiologic Studies Depression Scale; GHQ, General Health Questionnaire; PANAS, Positive and Negative Affect Schedule; PTGI, Posttraumatic Growth Inventory.

significantly lower than the pre-intervention score (p < 0.01) and post-intervention score (p < 0.01), confirming that this effect was maintained (Figure 4). The main effect of time was significant for total PTGI score (F[2,52] = 7.54, p < 0.001). Bonferroni's

multiple comparison showed that the post-intervention score was significantly higher than the pre-intervention score (p < 0.05). The FU score was also significantly higher than the pre-intervention score (p < 0.01), confirming that this effect was maintained.

Salivary cortisol level:Changes in salivary cortisol level

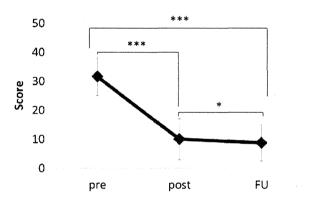


Figure 3 The change in CAPS score of the intervention group. ** p <.01, * p <.05.

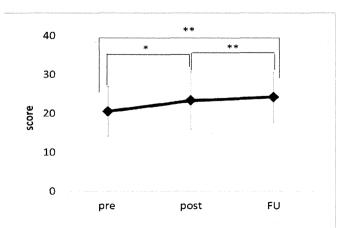


Figure 4 The change in PANAS-PA score of the intervention group. *** p <.001, * p < .05.

in the intervention group are shown in (Table 3) and (Figure 5). The main effect of time was significant for salivary cortisol (F[2,52] = 25.88, p < 0.001). Multiple comparisons showed that the post-intervention score was significantly lower than the preintervention score (p < 0.001), and the FU score was significantly lower than the pre-intervention score (p < 0.001).

DISCUSSION

The purpose of this study was to verify the reduction in the symptoms of earthquake-related stress in women who live in disaster areas using HT intervention, and to investigate the effect of HT on the symptoms of earthquake-related stress and the maintenance of its effect after a two-month follow-up period. The present study revealed that HT affected the psychological changes and salivary cortisol level in women with earthquake-related stress. These results are consistent with our hypothesis that HT may help women with earthquake-related stress improve their mental and physical functioning affected due to the traumatic experience.

Compared to the intervention group and control group, the intervention group showed improved CAPS scores, indicating that HT reduced PTSD symptoms. This finding extends the previous findings of the effect of HT on severe PTSD in males, by showing its efficacy with women with earthquake-related stress. In addition, the intervention group showed improved PTGI-J total scores and PANAS-PA scores, and reduced salivary cortisol levels after intervention compared with the control group, indicating that HT reduced stress levels. Findings related to salivary stress marker are consistent with previous research [15,35]. Previous studies reported that HT intervention for patients with depression improves mental health indices including the PANAS scores [36]. We believe that the CAPS and PANAS-PA scores of intervention group improved through stress reduction and elevated mood brought about by the HT [10-12]. About the result of PTGI, a study of PTG process suggests that people suffer emotional pain due to disruptions of their personal growth resulting from traumatic experiences [37]. However, people use PTG to cope in diverse ways, such as remembering their status before the event, referring to their own personality characteristics, relying on the support of others, and self-

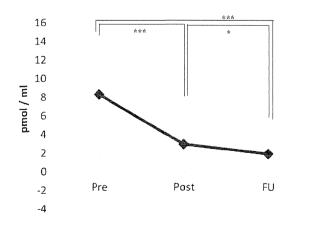


Figure 5 The change in salivary cortisol level of the intervention group. *** p <.001. * p <.05.

disclosing their own experiences with the negative event [37,38]. Horticulture activity involves instinctive and creative action and leads to improvement of humanity [39]. PTG and horticulture share a key feature. We suggest that people feel their own growth overlap with the growth process of the plant while cultivating the plant. This could be considered a psychological effect because horticulture work offers fulfillment, pleasure, challenges, and a sense of accomplishment.

The salivary cortisol levels decreased after HT in this study. Cortisol is considered an indicator of psychological and physiological stress and can be used to examine the pathophysiology of PTSD [40]. Previous studies reported that people with severe PTSD due to the Hanshin-Awaji earthquake had significantly higher cortisol levels than unaffected individuals [3]; salivary cortisol levels were significantly decreased and the PANAS-PA scores were fully restored after horticultural activity [15]. We think that the improvement of the earthquakerelated stress by HT was reflected in the endocrine system, specifically in salivary cortisol. In addition, the maintenance of these post-intervention improvements at FU after two months was confirmed from the results of the psychological measures (CAPS, PANAS-PA, and PTGI-J) and salivary cortisol levels after follow-up. These results indicate that horticultural intervention has an effect on earthquake-related stress, and that this effect is sustained for a certain period, i.e., two to four months.

The generally recommended treatments for PTSD that have demonstrated validity are cognitive behavioral therapy or eye movement desensitization and reprocessing (EMDR). These may also be accompanied by drug therapy in some cases. Art therapy, music therapy, and other complementary therapies have also been reported as effective. However, there is still little scientific evidence of the effectiveness of these methods for earthquake-related stress. The results of our present study suggest the possibility of HT as an effective intervention against the earthquake-related stress.

There are some limitations. The major limitation of this study was the small sample size. In the results of analysis of variance (ANOVA) for sensitivity, the effect size of this study was 0.39 and power was 0.8, and αerr prob was 0.05. Therefore, a possible future direction would be to replicate and extend the results of current study with larger sample and a lighter (more casually controlled) trial design. Second limitation is the study participants were all women participants. Therefore, it is difficult to say whether a similar effect would be observed in male participants, if horticultural interventions were provided for men. We believe that further investigation is required on this issue. Third, although our study showed that coming into contact with plants through horticultural activities had a beneficial effect on earthquake-related stress, we are not able to consider which horticultural activities (e.g., touching the ground, planting a seed and a seedling) produce the strongest benefit. We intend to carry out further studies to explore this limitation immediately. The final limitation is the verification of the difference in the effect of the horticultural therapy by interpersonal relations (communication), such as group intervention and individual intervention. We are experimenting about this problem now. In the near future, we will report the results of this research.

In conclusion, the present study suggested that, the present study suggested that it has been improved earthquake-related stress of women who live in disaster area by our HT intervention for two months from the result of a CAPS score, PANAS PA score, PTGI-J score, and a salivary cortisol level. Additionally, it suggested that these effects may sustain after intervention from the result of a psychological measures and a salivary cortisol level. We believe horticultural therapy may be able to suggest the possibility is one of the effective interventions for earthquake-related stress.

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