

ORIGINAL RESEARCH PAPER

Effect of laughter yoga on mental symptoms and salivary cortisol levels in first-year nursing students: A randomized controlled trial

Fatma Ozlem Ozturk PhD, Lecturer  | Ayfer Tezel PhD, Professor 

Department of Nursing, Faculty of Nursing,
Ankara University, Ankara, Turkey

Correspondence

Fatma Ozlem Ozturk, Department of Nursing,
Faculty of Nursing, Ankara University, Ankara,
Turkey.

Email: fozturk@ankara.edu.tr

Abstract

Background: University students are faced with several stress factors affecting their mental health. Therefore, the first year at university is a period that calls for careful attention and research.

Aim: The aim of this study is to evaluate the effect of laughter yoga on mental symptoms and cortisol levels in nursing students.

Methods: This study is a randomized controlled study employing a pre-/post-test design with a control group. A total of 75 healthy university students were assigned to the intervention group and control group. The Brief Symptom Inventory was applied to both groups before Session 1 and after Session 8. Saliva samples were taken from the students to measure their cortisol levels before and after each session.

Results: Evaluation of the mean scores obtained from the Brief Symptom Inventory before and after the intervention showed a significant decrease in the scores between groups ($P < 0.05$). In three out of the eight sessions, there was a significant decrease in the intervention group compared with the control group regarding the mean values of pre-test and post-test salivary cortisol levels ($P < 0.05$).

Conclusion: Laughter yoga can provide an effective means to help first-year nursing students cope with stress and reduce mental symptoms.

KEYWORDS

cortisol, laughter yoga, mental symptom, nursing, student

SUMMARY STATEMENT

What is already known about this topic?

- Laughter yoga is a non-pharmacological and non-invasive method of complementary therapy.
- An ever-increasing interest in laughter yoga is observed among nurse researchers.
- There is little research on laughter yoga in nursing students and randomized controlled studies are limited.

What this paper adds?

- This study showed that laughter yoga reduced levels of anxiety, depression, negative self, somatization and hostility.
- Salivary cortisol levels significantly decreased in the intervention group in sessions after the fourth laughter yoga session.

The implications of this paper:

- Laughter yoga can provide an effective means to help first-year nursing students cope with stress and reduce mental symptoms.
- It is recommended to integrate laughter yoga into the first-year nursing education curriculum in order to alleviate the mental symptoms of students.

- Laughter clubs can be set up in nursing faculties, and laughter yoga sessions can be organized for students.

1 | INTRODUCTION

Stress factors may create a negative impact on mental health, and in consequence, mental disorders may occur. Especially the first year at university is a critical period and a new milestone as it brings along a series of developmental and environmental changes (Auerbach et al., 2018). During this period, students may encounter many social, economic, educational, environmental and social problems, experience mental, emotional and behavioural changes or be in search of identity (Auerbach et al., 2018; Zeng et al., 2019). New experiences such as living far apart from their family, getting used to a different city and adjusting to dormitory and university atmosphere may lead to stress in students (Turan et al., 2019). In addition, stepping into a profession that requires clinical practice can also be a source of stress for students (Arabacı et al., 2015).

Previous research has shown that the frequency of mental illness among university students is higher than in the general population (Stallman, 2010; Yazdani et al., 2014). In a study conducted by Auerbach et al. (2018) to examine the prevalence of common mental disorders among first-year university students in eight countries (Australia, Belgium, Germany, Mexico, Northern Ireland, South Africa, Spain and the United States), it was found that 31% of students were positive for at least one of the disorders defined as mental disorder (major depression, mania/hypomania, generalized anxiety disorder, panic disorder, alcohol use disorder and substance use disorder) in DSM-IV (Auerbach et al., 2018). Zeng et al. (2019) found that the prevalence rates of depression, anxiety and stress symptoms among nursing students in China was 28.7%, 41.7% and 20.2%, respectively. In a study by Özel et al. (2020), depression, anxiety and negative self were found to be the most common symptoms of depression among nursing students in Turkey. The high prevalence of mental symptoms among first-year nursing students has made it necessary to address this issue as an interventional research topic.

Laughter yoga is a non-pharmacological and non-invasive complementary therapy method that combines unconditional laughter and breathing techniques (Strean, 2009). Laughter yoga has a positive effect on mental health and mitigates stress, depression and anxiety levels (Bennett & Lengacher, 2008; Kataria, 2011; Kaur & Walia, 2008; Kim et al., 2015; LeTexier, 2015; Shahidi et al., 2011; Yazdani et al., 2014). Rosner (2002) and Ripoll (2010) stated that laughter had many physiological effects as well. Reactions to laughter can be seen in an increase observed in breathing rate, blood oxygen saturation, endorphin level and heart rate, along with a decrease in blood pressure, salivary cortisol and muscle strain and a recovery in immune system functions and mental processes

(Fujisawa et al., 2018; Mallett, 1995; Nagendra et al., 2007; Ripoll, 2010; Rosner, 2002).

University freshmen face several stress factors that affect their mental health in their first year of education. Adapting to a difficult profession that requires knowledge and skills such as nursing and vocational courses is an important stress factor for students (Gebhart et al., 2020). Stress has been associated with smoking and substance abuse, eating disorders and suicidal behaviour (Özel et al., 2020). Students may be absent from school as a result of the stress they experience and that they can leave their nursing education without completing their education (Gebhart et al., 2020). Salivary cortisol is a possible biological marker of psychological stress (Pandey et al., 2016). For this reason, experimental studies that evaluate the successful stress coping techniques of nursing first grade students with an objective parameter such as salivary cortisol should be conducted in order to prepare for this challenging profession during the nursing education process (Arabacı et al., 2015; Gebhart et al., 2020).

Studies have evaluated the effect of laughter yoga on salivary cortisol (Fujisawa et al., 2018; Lee & Lee, 2020; Pandey et al., 2016) and on mental symptoms such as depression and anxiety (Dolgoff-Kaspar et al., 2012; Ghodsbin et al., 2014; Memarian et al., 2017; Yazdani et al., 2014). Studies evaluating the effects of laughter yoga on two variables together are needed in the literature rather than studies evaluating the effects of laughter yoga on both mental symptoms and salivary cortisol separately. This study is considered to make an important contribution to international literature as it is the first study ever to evaluate the effects of laughter yoga on both mental symptoms and salivary cortisol levels. This study was conducted with the aim of evaluating the effects of laughter yoga on the frequency of mental symptoms and salivary cortisol levels in first-year students at the nursing department.

1.1 | Hypotheses of the study

The hypotheses of the research are as follows:

- H1.1.** Laughter yoga will significantly reduce mental symptoms in the Brief Symptom Inventory in the intervention group compared with the control group.
- H1.2.** Laughter yoga will significantly reduce the mean salivary cortisol level in the intervention group at each session compared with the control group.

2 | METHODS

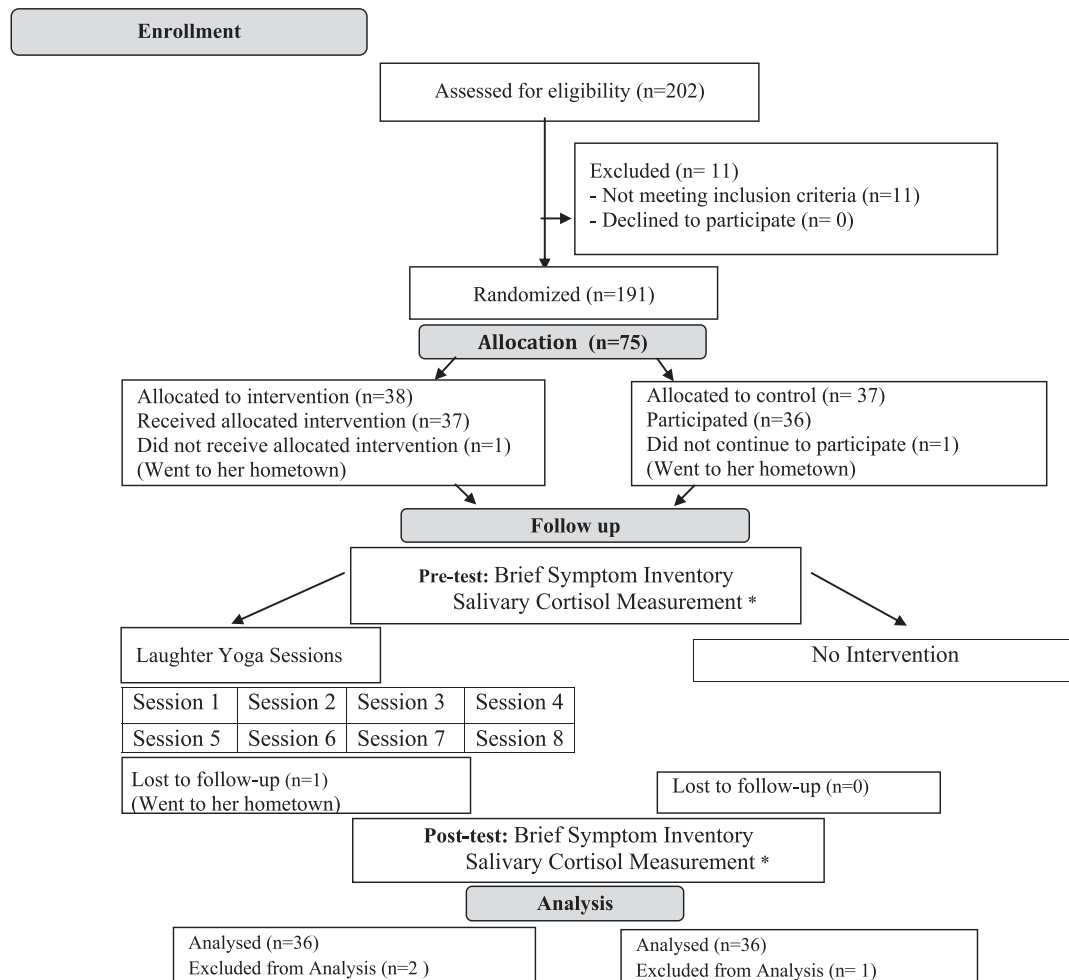
The present paper is a randomized and controlled study performed with an experimental design that involved pre-test, post-test and a control group.

2.1 | Population and sample

The study's population consisted of 202 students in their first year at a State University's Nursing Department during the academic year 2017–2018. Subsequent to a descriptive features question form applied to the whole population; 191 students met the inclusion criteria and thus were included in the study. A power analysis was performed for the sample size. With 0.80 effect size, 0.90 power and 0.05 margin of error, the sample size required for each group to determine whether the difference between the two groups' mean values was different from 0 was calculated to be 34. Hence, taking into consideration of possible dropouts, the study's sample was formed of 75 students comprising 38 students in the intervention group and 37 students in the control group (Figure 1).

2.2 | Randomization

To form the intervention group and control group, random selection was made via <https://www.random.org>, an actual random number selector website, from among the 191 students studying at the Nursing Department of a State University, who met the inclusion criteria, starting from the student listed in line one. Selection was continued until the required sample size was reached. Thirty-eight students were included in the intervention group, and 37 students in the control group. However, one student each from the intervention group and control group did not participate in the study at all; both students did neither fill out the Brief Symptom Inventory (BSI), nor did they give any saliva samples for salivary cortisol measurement. One student from the intervention group participated only in Sessions 1, 2, 4 and 5, but did not attend Sessions 3, 6, 7 and 8, and thus dropped out of the



*Saliva samples for cortisol testing were taken from each student in the intervention and control group before and after each laughter yoga session.

FIGURE 1 Consort diagram

study. During the course of research, two students from the intervention group and one student from the control group decided to abandon the study. As a result, research was completed with 72 students. Descriptive features of the students participating in research are shown in Table 1.

2.3 | Inclusion criteria

A first-year student in good standing at the Nursing Department of the relevant State University, no health problems and not on medication.

2.4 | Exclusion criteria

Any condition in which laughter yoga must be avoided (e.g. history of abdominal surgery in the last 3 months, being on regular medication, uncontrollable hypertension, glaucoma, hernia and epilepsy).

2.5 | Data collection

2.5.1 | Data collection forms and tools

Data were collected through Descriptive Questionnaire Form, BSI and saliva sampling.

The Descriptive Questionnaire Form contains questions on age, gender, high school, income level, family structure, number of siblings, residence, health status, drug usage and surgical history in the last 3 months.

BSI developed by Derogatis and Savitz (1999) is a tool to validly and reliably measure mental indications and psychological problems in students (α : 0.95). The scale's Turkish validity and reliability research was performed by Şahin and Durak (1994) (α : 0.95). The Turkish version of BSI contains 53 items divided into five sub-dimensions, namely, 'hostility', 'somatization', 'depression', 'anxiety' and 'negative self'. The respondents' answers to each question are scored from 0 to 4, that is, 0 for *not at all*, 1 for *a*

TABLE 1 Descriptive features of the students

Variables	Intervention (n = 36)		Control (n = 36)		Total (n = 72)		Test value and significance
	Number (n)	Per cent (%)	Number (n)	Per cent (%)	Number (n)	Per cent (%)	
Gender							
Female	28	77.8	29	80.6	57	79.2	$\chi^2 = 0.084$
Male	8	22.2	7	19.4	15	20.8	$P = 0.999$
High school							
Science high school	3	8.3	5	13.9	8	11.1	$\chi^2 = 2.813$
Anatolian high school	21	58.3	23	63.9	44	61.1	$P = 0.576$
Vocational school of Health	2	5.6	0	0	2	2.8	
Other	10	27.8	8	22.2	18	25	
Family structure							
Nuclear family	28	77.8	32	88.9	60	83.3	$\chi^2 = 3.539$
Extended family	8	22.2	3	8.3	11	15.3	$P = 0.189$
Single-parent family	0	0	1	2.8	1	1.4	
The number of siblings							
One sibling	1	2.8	1	2.8	2	2.8	$\chi^2 = 0.265$
Two siblings	10	27.8	12	33.3	22	30.6	$P = 0.987$
Three and more	25	69.4	23	63.9	48	66.7	
Income status							
Income more than Outcome	3	8.3	4	11.1	7	9.7	$\chi^2 = 0.334$
Equal income and outcome	20	55.6	21	58.3	41	56.9	$P = 0.885$
Income less than outcome	13	36.1	11	30.6	24	33.3	
Residence							
Home	11	30.6	15	41.7	26	36.1	$\chi^2 = 4.144$
Dormitory	24	66.7	17	47.2	41	56.9	$P = 0.268$
With relatives	1	2.8	2	5.6	3	4.2	
Other	0	0	2	5.6	2	2.8	

little bit, 2 for moderately, 3 for quite a bit and 4 for extremely, which are then summed up to calculate the total score. The higher the score, the more frequent are the respondent's symptoms. In this study, Cronbach's alpha reliability coefficient of BSI was found to be 0.92.

Saliva samples were collected from the intervention group before and after each laughter yoga session at 7:55 and 8:40, respectively. The control group's saliva samples were taken at the same time as the intervention group. The students were instructed to soak the cotton balls handed over to them in salivary cortisol tubes with their saliva for at least 1 min and then insert them back into the tube. Samples of both groups were centrifuged for 2 min at 1000 rpm. Measurement was carried out with a full-automatic Roche Elecsys 2010 device using a cortisol kit according to electrochemiluminescence immunoassay (ECLIA) method.

2.6 | Intervention: Laughter yoga

The corresponding author implementing the intervention is holder of an International Laughter Yoga Leader Certificate, and the intervention was implemented by the researcher herself. Laughter yoga was applied to the intervention group in the activity class. The intervention group took eight sessions of laughter yoga, that is, two sessions per week for 4 weeks (Table 2). Yazdani et al.'s (2014) research was taken as reference to determine the number of sessions to be applied in this study. Each laughter yoga session lasted about 40–45 min.

2.6.1 | Parts of a laughter yoga session

Part 1: Deep breathing exercises (5–10 min)

By lifting the arms up, a breath is taken as deeply as possible. After deep inhalation, breath is held for 4–5 s and then exhaled slowly and rhythmically after arms are brought to normal position. In any deep breath exercise technique, exhalation time should be longer than inhalation time.

Part 2: Warm-up exercises (10 min)

Hands are kept parallel to each other, followed by clapping the hands with the fingertips and palms facing each other. Thus, the acupuncture points on both hands are stimulated, causing the individual's energy level to increase. Clapping is performed with a rhythmic pattern of 1–2, 1–2–3 to increase energy level and create synchrony across the group. The rhythmic patterns is accompanied by the mantra 'ho-ho, ha-ha-ha' chanted aloud.

Part 3: Childlike playfulness (10 min)

Childlike playfulness is used to help laugh for no reason just like a child. With the arms raised towards the sky, the body takes the form of a 'Y', and the mantra 'very good, very good, yeah' is chanted.

Part 4: Laughter exercises (15 min)

Laughter is encouraged by various laughter exercises, which in this study included elevator, hot soup, national lottery, strawberry milk, greeting, lion, laughter lotion, orchestra, aloha and motorcycle laughter.

2.7 | Application

- **Pre-test:** BSI was applied to the students in the intervention group and control group before the first session. Before each laughter yoga session, saliva samples were taken from students of both the intervention and control group for salivary cortisol level measurement.
- **Control group:** No intervention was applied to the control group. Saliva samples of the control group were taken simultaneously with samples from the intervention group before and after each laughter yoga session.
- **Post-test:** After completion of all laughter yoga sessions, BSI was applied to the students of both the intervention and control group. After each session, saliva samples were taken from both groups for salivary cortisol level measurement.

TABLE 2 Laughter yoga program

Sessions	Intervention group (n = 38) ^a	Control group (n = 37) ^a	Dates	Hours
Session 1	37	36	21 November 2017	08.00–08.40
Session 2	37	36	15 November 2017	08.00–08.40
Session 3	36	36	20 November 2017	08.00–08.40
Session 4	37	36	21 November 2017	08.00–08.40
Session 5	37	36	27 November 2017	08.00–08.40
Session 6	36	36	28 November 2017	08.00–08.40
Session 7	36	36	4 December 2017	08.00–08.40
Session 8	36	36	5 December 2017	08.00–08.40

^aInformation about the students who left the study is shown in the consort diagram.

2.8 | Data analysis and evaluation methods

Before statistical analysis, data were examined using the Shapiro–Wilk test for normality and Levene test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as ‘mean ± standard error of the mean’. Fisher's exact test was used for data with two nominal variables. All data were analysed using the MIXED procedure of SPSS (V14.01; SPSS Inc., Chicago, IL, USA). The effect of group, time and their interaction on anxiety, depression, negative self, somatization, hostility and salivary cortisol were analysed through the following model with repeated measurements:

$$Y_{ijk} = \mu + G_i + T_j + (G \times T)_{ij} + e_{ijk}$$

wherein Y_{ijk} refers to dependent variable; μ overall mean; G_i effect of the group (i = individuals in treatment and control group); T_j effect of time (j = before and after treatment); $(G \times T)$ interaction between group i and day of sampling j ; and e_{ijk} residual error.

Fisher's exact test was used to compare two categorical variables. Individuals within group were assessed as a random effect, whereas group, time and their interaction were assessed as a fixed effect. $P < 0.05$ was considered as significant in all analyses. In order to avoid partiality in the evaluation of data, analysis of data recorded on the database was performed by a statistician independent from the researcher.

2.9 | Ethical considerations

Permission was obtained from the institution and ethical committee (date: 25/09/2017, number: 15-981-17) for this research. The participating students were provided with information about the study, and their informed consent was obtained. Salivary samples were taken from the students on an empty stomach, and the students had to wait on an empty stomach for the purpose of research. Therefore, each student was given a breakfast package by the researcher, not only as an expression of ethical behaviour but to encourage participants to stick with this research.

Trial registration number: Clinical Trials.gov NCT03814265.

3 | RESULTS

79.2% of the students participating in this study were females, 61.1% had graduated from an Anatolian high school, 83.3% of their families were a nuclear family, 66.7% had more than three siblings, 56.9% reported that their family's household income was equal to their expenditure, and 56.9% were staying in a dormitory. As regards these characteristics, the groups were homogeneous (Table 1).

3.1 | Pre-test and post-test BSI means of students in the intervention group and control group

The students' BSI mean scores in the intervention group and the control group before and after the laughter sessions are shown in Table 3. Evaluation of the mean scores obtained in BSI sub-dimensions (i.e. anxiety, depression, negative self, somatization and hostility) before and after the intervention showed a significant decrease in the scores of the intervention group compared with the control group ($P < 0.05$).

3.2 | Pre-test and post-test salivary cortisol values of students in the intervention group and control group

The values obtained from pre-test and post-test salivary cortisol measurements in the intervention group and control group are shown in the Table 4. It was observed that, in all sessions, the mean cortisol levels measured in students of the intervention group before laughter yoga had decreased after laughter yoga. Comparison of the salivary cortisol levels in the intervention group and control group before and after the intervention revealed that the decrease in the intervention group's salivary cortisol levels compared with the control group was significantly higher in Sessions 5, 7 and 8 ($P < 0.05$).

4 | DISCUSSION

The current study was sought to examine the effect of laughter yoga on mental symptoms and cortisol in first-year nursing students. The hypotheses of this research were that laughter yoga would significantly reduce both mental symptoms and salivary cortisol levels. In the intervention group, laughter yoga significantly reduced mental symptoms but without any significant reduction in salivary cortisol levels in each session, which means that H1.1 was accepted, but H1.2 was rejected.

4.1 | Effect of laughter yoga on mental symptoms of students

Bahari and Lorica (2019) stated that laughter yoga had positive effects on mental health. In this study, laughter yoga was seen to create a significant effect on the intervention group's mean score in the BSI sub-dimensions anxiety and depression compared with the control group (see Table 3). Previous studies have shown that laughter yoga has positive effects on individuals' anxiety level (Dolgooff-Kaspar et al., 2012; Ghodsbini et al., 2014; Memarian et al., 2017). There are also studies showing that laughter yoga has positive effects on individuals' depression levels (Ko & Youn, 2011; Shahidi et al., 2011). Yet in another study, it was stated that laughter yoga did not change

TABLE 3 Pre-test–post-test Brief Symptom Inventory (BSI) mean scores of the students

BSI		Mean scores of BSI scale		Time	
		Intervention group (n = 36) mean (SD)	Control group (n = 36) mean (SD)	P	Time*group
Sub-dimensions	Anxiety (pre-test)	1.08 (0.47)	1.02 (0.61)	<0.001	0.004
	Anxiety (post-test)	0.67 (0.50)	0.84 (0.58)		
	Depression (pre-test)	1.34 (0.57)	1.33 (0.69)	<0.001	0.001
	Depression (post-test)	0.89 (0.55)	1.25 (0.57)		
	Negative self (pre-test)	1.05 (0.61)	0.98 (0.63)	<0.001	0.006
	Negative self (post-test)	0.72 (0.55)	0.93 (0.59)		
	Somatisation (pre-test)	0.78 (0.46)	0.65 (0.53)	0.003	0.001
	Somatisation (post-test)	0.51 (0.39)	0.70 (0.49)		
	Hostility (pre-test)	1.12 (0.55)	1.35 (0.71)	0.002	0.001
	Hostility (post-test)	0.75 (0.56)	1.36 (0.66)		

Abbreviation: SD, standard deviation.

TABLE 4 Pre-test–post-test salivary cortisol values of the students

Sessions		Salivary cortisol values		Time	
		Intervention group (n = 36) mean (SD)	Control group (n = 36) mean (SD)	P	Time*group
Session 1	Pre-test	0.700 (0.29)	0.640 (0.29)	0.041	0.340
	Post-test	0.592 (0.25)	0.600 (0.33)		
Session 2	Pre-test	0.631 (0.22)	0.612 (0.30)	0.145	0.071
	Post-test	0.518 (0.19)	0.624 (0.28)		
Session 3	Pre-test	0.629 ± 0.24	0.629 ± 0.33	0.034	0.358
	Post-test	0.503 (0.26)	0.578 (0.34)		
Session 4	Pre-test	0.640 (0.29)	0.546 (0.25)	0.233	0.576
	Post-test	0.569 (0.26)	0.520 (0.22)		
Session 5	Pre-test	0.684 (0.36)	0.571 (0.35)	0.520	0.002
	Post-test	0.550 (0.29)	0.659 (0.39)		
Session 6	Pre-test	0.578 (0.25)	0.538 (0.28)	0.012	0.118
	Post-test	0.442 (0.27)	0.506 (0.29)		
Session 7	Pre-test	0.596 (0.25)	0.570 (0.27)	0.530	0.002
	Post-test	0.462 (0.17)	0.660 (0.33)		
Session 8	Pre-test	0.543 (0.22)	0.591 (0.31)	0.026	0.012
	Post-test	0.410 (0.21)	0.599 (0.29)		

Abbreviation: SD, standard deviation.

the individuals' depression level (Ghodsbin et al., 2014). Similarly, there are studies reporting that laughter yoga significantly reduced individuals' anxiety and depression levels (Kim et al., 2015; Yazdani et al., 2014; Yu & Kim, 2009). Zhao et al.'s (2019) meta-analysis study stated that laughter yoga significantly reduced depression and anxiety. The results of most of the above studies have shown that laughter yoga provides a significant reduction in anxiety and depression levels, which is supportive of the results obtained in our study. Arabacı et al. (2015) concluded in their study that nursing students had more anxiety before clinical practice. In the nursing faculty where this study was conducted, nursing students go into clinical practice in the second semester of the first year of their education. The current

study was conducted in the first semester in the first year of nursing education, and it was determined that the intervention had a positive effect on students' anxiety levels. Laughter yoga can be considered as a non-invasive and non-pharmacological initiative that students can adapt and exercise in their daily lives to decrease both anxiety and depression.

In this study, it was determined that laughter yoga had a significant positive effect on negative self in the intervention group compared with the control group (see Table 3). Because negative self involves negative thoughts and considerations related to a person's own self, it can also be referred to as negative self-respect or self-esteem. Kim et al. (2015) stated that laughter yoga significantly

increased self-esteem in cancer patients. De Francisco et al. (2019) found that laughter yoga increased self-esteem in individuals with substance use disorder. The results of this research are similar those mentioned above. In his book *Laugh for No Reason*, Kataria (2011) draws attention to the fact that laughter yoga increases the individual's self-confidence and self-esteem. Hence, laughter yoga can be considered as an effective means to reduce negative self-symptoms in first-year nursing students.

In this study, it was also determined that laughter yoga significantly decreased the somatization level in the intervention group compared with the control group (see Table 3). Lee and Sohn (2010) stated that laughter yoga provided a significant decrease in somatization levels in people staying in a shelter, with results similar to those obtained in this study. In a study conducted on 1404 university students, Bilgin et al. (2020) found that 16% of their students had high levels of somatization. It is stated that somatization is an expression of mental problems and psychosocial stress through physical symptoms and that somatization is common in women (Lipowski, 1988). The results of this study show that this initiative can be used to decrease somatization levels in nursing students, who are mostly women.

The present study also showed that laughter yoga significantly reduced the level of hostility in the intervention group compared with the control group (see Table 3). Hostility can be described as thoughts, feelings and actions characterized by the effects of anger. Because hostility involves anger, a study evaluating the effect of laughter yoga on anger was included in this part of the study. Lee and Sohn (2010) stated that laughter yoga provided a significant reduction in anger level among people staying in a shelter and is an effective nursing intervention that can be used to reduce anger. In a study conducted in Japan, laughter yoga was found to be effective in reducing hostility levels (Tanaka et al., 2018). It is thought that laughter yoga can be used to reduce the level of hostility in students.

4.2 | Effect of laughter yoga on salivary cortisol level of students

Changes in the students' state of mind are reported to affect their cortisol level (Guyton et al., 2007). It was seen that the students' mean cortisol values in the intervention group decreased after laughter yoga at each session. However, in the comparison between groups, the decrease in cortisol level did not reach a significant level in every session. Comparison of the salivary cortisol levels in the intervention group and control group before and after the intervention revealed that the decrease in the intervention group's salivary cortisol levels compared with the control group was significantly higher in Sessions 5, 7 and 8 ($P < 0.05$) (see Table 4). In other words, it was determined that salivary cortisol levels decreased significantly in the intervention group compared with the control group in the sessions after the fourth laughing yoga session, which suggests that the effect of the intervention on salivary cortisol started after the fourth session. Tanaka et al. (2018) found that laughter yoga significantly reduced cortisol levels after the fourth session. The fact that onset of significant

decrease in cortisol values occurred after the fourth session suggests that the students might have learned the laughter yoga intervention over time, got used to it and participated in the sessions more eagerly. The decrease in cortisol level may have reached a significance level with the group's increasing enthusiasm to do laughter yoga.

Studies have shown that laughter yoga can help to significantly decrease cortisol, which is a biological marker of stress. In a study conducted with nursing students in Korea, it was found that laughter yoga significantly reduced salivary cortisol in the intervention group (Lee & Lee, 2020). In a study by Chang et al. (2013) on seventh-grade adolescents in Thailand, they found that laughter yoga provides a significant decrease in cortisol levels. Berk et al. (2008) stated that laughter yoga provides a decrease in cortisol levels in healthy adult men, whereas Pandey et al. (2016) found that laughter yoga significantly decreased salivary cortisol in medical students. Furthermore, Nagendra et al. (2007) found that laughter yoga significantly reduced cortisol levels in office staff, whereas Fujisawa et al. (2018) found that laughter yoga significantly reduced salivary cortisol in university students. Similar results were obtained in the present study to those in the literature. Apart from these, there are also studies that do not support the results of the present study. For instance, Heo et al. (2016) stated that laughter yoga did not have a significant effect on serum cortisol level in haemodialysis patients. Likewise, Cha and Hong (2013) noted that laughter yoga did not affect cortisol levels in middle-aged women. Cortisol levels may not be affected in these two studies depending on the sample size and the frequency of the intervention.

4.3 | Limitations of the research

This research is limited to first-year nursing students participating in this study. Hence, the results can only be generalized for those nursing students that were included in this research. Yet, it is not possible to control the environmental factors such as time, personal habits or social relations affecting the nursing students' state of mind. The students' psychological symptoms and mental problems addressed in this study are limited to those measured by BSI. Cortisol is a stress-dependent variable, but it is not possible to control personal stress factors. According to the circadian rhythm of cortisol, cortisol is at its highest after waking up in the morning and tends to drop during the day. The circadian rhythm of the participants may limit the effect of the intervention. However, because this rhythm is valid for all participants, the impact of the intervention can be observed, nevertheless.

5 | CONCLUSION

Laughter yoga was applied to the intervention group twice a week for a total of eight times. It was found that laughter yoga reduced mental symptoms (anxiety, depression, negative self, somatization and hostility) and that salivary cortisol levels decreased significantly in the intervention group compared with the control group starting from the fourth session onwards. It was seen that laughing yoga sessions

should be scheduled for more than four session to achieve significant cortisol reduction. Based on results obtained in this research, laughter yoga can be applied to first-year nursing students to help them cope with stress. Therefore, it is recommendable to incorporate laughter yoga into curricula of first-year nursing education to alleviate the students' mental symptoms and expand its use. It is recommended that further randomized controlled experimental studies analysing the physiological and psychological effects of laughter yoga be performed with objective and subjective variables.

DISCLOSURE/CONFLICT OF INTEREST

The cost of salivary cortisol tubes (about 1200 pcs), cortisol kit (about 13 box) and the breakfast package for students (about 75 students) was paid by the corresponding author. All authors report no conflict of interest related to this manuscript.

AUTHORSHIP STATEMENT

FOO, AT were designed the study. FOO was responsible for collecting the data, implementing laughter yoga and drafting the manuscript. A statistician made the data analysis. AT critically reviewed the manuscript. All authors revised and approved the manuscript.

ORCID

Fatma Ozlem Ozturk  <https://orcid.org/0000-0003-1942-6637>

Ayfer Tezel  <https://orcid.org/0000-0001-6370-883X>

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