EFFECTS OF SMILING ON NURSING STUDENT STRESS AND CORRELATES WITH IMMUNE FUNCTION

by

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Abstract

Stress is linked to many different pathological disorders in various body systems and can trigger inflammation which in turn correlates with certain disease states (Yaribeygi et al., 2017). Interleukin-6 (IL-6) is a pro-inflammatory cytokine that triggers immune response to help the body return to homeostasis by affecting different types of cells. The presence of IL-6 is increased during periods of environmental stress (Tanaka et al., 2014). This study examined the effects of a web-based smiling exercise on stress levels in nursing students. Additionally, it explored the correlation between stress and IL-6. Participants (n=14) were evenly divided into a control group and a smiling group. The smiling group completed the intervention prior to their vital sign checkoff, a stressful event for nursing students. Each participant completed stress visual analog scales pre-checkoff, during checkoff and post-checkoff and provided a saliva sample for IL-6 analysis. For pre-checkoff and during checkoff, the smiling group had a lower average reported stress level. However, the difference between the groups was not statistically significant ($p>0.05$). Twelve participants had elevated IL-6 levels and there was moderate correlation between reported stress levels during checkoff and IL-6 levels. Further research needs to be done with a larger sample size to determine if the IL-6 levels and correlation are significant and to further explore the intervention. These study findings highlight the importance of focusing on the health and wellness of nursing students.

Introduction

Stress is linked to many different pathological disorders in various body systems and can trigger inflammation which in turn correlates with certain disease states (Yaribeygi et al., 2017). Chronic inflammation from an immune system that is being constantly activated can occur in different diseases, like infections, diabetes, cancer, autoimmune diseases, and cardiovascular disease (Seiler et al., 2019). IL-6 is a pro-inflammatory cytokine that triggers immune response to help the body return to homeostasis by affecting different types of cells. The presence of IL-6 is increased during periods of environmental stress (Tanaka et al., 2014).

If a simple task such as smiling can help reduce stress, individuals can incorporate smiling exercises into their daily routine or before demanding tasks. Similarly, knowing the relationship between stress and the immune system can help individuals know more about the systemic effects of stress. The link between smiling and stress needs to be addressed due to the negative consequences that come with long-term stress.

The purpose of this feasibility pilot study is to determine if a web-based smiling exercise can be an effective strategy in reducing stress in nursing students. A primary objective is to validate the use of a smiling application by measuring the ability of this tool to reduce short term stress levels in nursing students at the time of a high-stakes skills check-off. Additionally, we will describe IL-6 levels in nursing students and explore the relationship between IL-6 and stress.

Review of Literature

Stress and Immunity

The relationship between stress and immunity is a contemporary topic that has emerged in research in the past few years. Researchers have found that there is a change in the immune status of an individual's body while they are experiencing stress (Kamezaki et al., 2012; Pilger et

al., 2014). One study by Kamezaki et al. (2012) demonstrated an increase in proinflammatory cytokines, Th2 cytokines, and beta-nerve growth factor during stress, with peak levels occurring immediately after the stressful event occurred. Similarly, Pilger et al., (2014) discovered that stress occurring during a public performance resulted in a 20% increase in plasma myeloperoxidase, a 27% increase in IL-6 and a 44% increase in salivary cortisol. Plasma myeloperoxidase and IL-6 are both pro-inflammatory markers, meaning there is a positive correlation between inflammation and stress (Pilger et al., 2014). While both of these studies resulted in positive relationships between immune levels and perceived stress levels, more research needs to be done to look at the effects of interventions in a variety of populations.

Smiling and Stress

Smiling has been found to have a positive impact on an individual's well-being due to the increase in dopamine and serotonin levels that result from the action (Nettle et al., 2005). However, there has been a significant gap in literature addressing this potential correlation between smiling and stress levels. One of the few studies on smiling and stress highlights how smiling can lower heart rate levels in individuals who are recovering from a stressful activity (Kraft & Pressman, 2012). In the randomized control trial, 170 participants held chopsticks in their mouths in different positions to make different smiling faces during a stressful task of completing a tracing exercise or submerging their hands in ice water. Some participants also activated their zygomaticus major and orbicularis oculi muscles to have an active, aware smile. Kraft & Pressman (2012) found that standard smilers who activated their zygomaticus major muscle around their mouth had the greatest decrease in heart rate after stressful activities. Participants with chopsticks in a position to make their face mimic smiling also had a decrease, implying that not activating facial muscles and having a "fake" smile may also be used in

stressful situations but will not have as much of an impact as sincere smiling, with participants keeping their mouth and eye muscles activated. (Kraft & Pressman, 2012). This poses the question regarding the effect of smiling to decrease stress during a real-life stressful event.

Roemer (2014) also found smiling resulted in increased positive affect and higher levels of positive emotion in a randomized control trial. Similarly, Smiling Meditation was used to determine changes in emotions of individuals. Smiling Meditation includes a five-minute period of practicing mindfulness, deep breathing, and focusing on how adding smiling a few minutes into meditating makes the participant feel. After just one week of practicing this type of meditation, participants reported improved mindfulness, gratitude, and compassion (Strasser, 2018). While the emotions that resulted from both studies may be contributing factors to decreasing stress, they do not directly show the impacts that smiling has on stress levels (Roemer, 2014; Strasser, 2018). There is also a gap in the relationship between smiling and spontaneous stress instead of a stressful situation that is specifically created.

Methods

Study Design, Setting, and Participants

In this pilot feasibility study, we used a nonrandomized control group pretest-posttest design comparing self-reported stress levels of students based on their exposure to a web-based smiling exercise (the intervention)**.** The study took place on the day of vital signs check-off on the Texas Christian University campus in the Bass Building outside of the Health Professions Learning Center. Vital signs check-off is a timed skills demonstration during which students are evaluated by their instructor for the accuracy of taking temperature, respirations, pulse, and blood pressure measurements.

Participation took no more than 25 minutes per participant. The study participants were nursing students who were in their Sophomore II semester and enrolled in a required course, Health Assessment Lab, within the Harris College of Nursing at TCU in the fall of 2021. Inclusion criteria included nursing students enrolled in the Health Assessment lab who were at least 18 years old. There were no exclusion criteria. Participants were randomly divided into the control group and intervention based on their lab section and assigned study ID numbers using a random number generator. The groups were divided into equal sample sizes. Dividing into groups by lab section reduced the risk of participants smiling in the intervention group influencing the control group.

Recruitment of potential participants occurred in person during lecture time in the companion course, Health Assessment Concepts. Leaflets were also distributed by email and in the classroom when the student investigator spoke to the class. No student in any section of Health Assessment Lab was required to participate in this study and their grades in the course were not dependent on their willingness to participate in this study.

Intervention

The intervention used in this study was a smiling exercise created by Wayne Martin, a licensed clinical social worker and psychophysiologist (Martin, n.d). Participants watched a video that contains 20 clips of different individuals smiling. Participants were instructed to mimic the smiles that they watch in the video. The intervention takes less than two minutes and can be easily accessed on any smartphone or computer.

Measurements

Stress was measured using a visual analog scale (0-100) to measure their level of short-term stress (Yeung & Wong, 2019). The visual analog scale is an easily administered scale

that is routinely used in research to evaluate different perceptions of a topic or feeling that the subjects may have (Yeung & Wong, 2019). Research has shown that visual analog scales are efficient in assessing stress in a specific group and they accurately measure what they are intended to (Lesage et al., 2012). Salivary IL-6 was measured using the ELISA method following the protocol outlined by Salimetrics (Salimetrics, 2019).

Procedures

Participants signed up for a time slot for check off in their Health Assessment Lab. After signing up for the study, participants were instructed by the student investigator to arrive 10 minutes before their Health Assessment Lab checkoff time in order to allow adequate time to complete the first part of the study. When arriving to the data collection area before checkoff, participants picked up a packet containing a pre-labeled saliva cryovial labeled with a study ID number and a collection label. Participants then accessed the Qualtrics study survey link provided and entered their study ID number. All participants then completed the stress scale on the survey. The Qualtrics link could be accessed on mobile phones or computers. After this scale was completed, and before proceeding to skills check-off, participants in the intervention group accessed the online smiling intervention and followed the prompts from the web-based smiling exercise (Martin, n.d).

After checkoff, both groups completed the stress scale again regarding current stress and recall of stress during the checkoff for comparison. Additionally, all participants provided a saliva sample. They were notified before their check off date that they should avoid foods with high sugar, acidity, or caffeine immediately before the sample collection. Samples were collected by the passive drool method into cryovials. After the saliva was collected, participants placed their cryovials labeled with their study ID number, date, and time in a plastic bag and then into a

smaller cooler. The cooler was transported to the lab by one of the investigators where the individual samples were stored in a -80 C freezer in the TCU Harris Research lab on the TCU campus until they were processed using the Salimetrics HS IL-6 Salivary Kit. This lab is directed by Dr. Dennis Cheek, who assisted in the analysis of the samples. At completion of data analysis, saliva samples were destroyed according to TCU Biological Samples disposal protocol.

Human Subjects Protection

Participants had the opportunity to read the consent document and have all their questions answered before they enrolled in the study. Consent was obtained prior to completing study procedures. Participation in the study did not affect the grade the participants received in their Health Assessment Lab course. Participation was voluntary, and participants were able to withdraw at any time by notifying the investigators. Before and after checkoff, participants completed all study procedures behind a privacy divider in order to reduce any embarrassment that they may have felt.

Participants had a chance to win a gift card. A random number generator was used to determine the winners prior to data collection. There were four $100 Amazon gift cards as prizes for the raffle and participants were aware of the odds of winning the prize before they agreed to the experiment.

Participants were assigned a study ID number. An excel document linking the participants names and study ID number is password protected and kept in TCU Box. Only the researchers have access to this document. Participants were able to access the stress scale on their mobile phone or computer through Qualtrics, and Qualtrics results are password-protected. Results of the IL-6 levels were recorded for each study ID in a separate password protected excel file owned by the PI.

Analysis Plan

Saliva samples were analyzed in the TCU Nursing Research lab using the ELISA method following the protocol outlined by Salimetrics (Salmetrics, 2019). All samples were brought to room temperature and mixed in the centrifuge. Wash buffer was prepared from a combination of wash buffer concentrate and room temperature deionized water. Serial dilutions of the IL-6 standard were prepared in polypropylene microcentrifuge tubes. 300 µl of Assay Diluent was pipetted into each tube. The standards were serially diluted using a new pipette tip between each dilution. The concentrations of the standards were 100pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL and 1.56 pg/mL. Saliva samples were diluted five times in in IL-6 Sample Diluent using 60 µl saliva and 240 µl sample diluent. 100 µl of standards, controls, and diluted saliva samples were pipetted into their designated wells. 100 µl of IL-6 Assay Diluent was pipetted into two wells as the zero standard. The plate was mixed on a plate rotator continuously at 500 rpm for one hour. The plate was washed four times with 300 µl of wash buffer in each well. After the plate was washed, it was blotted thoroughly on paper towels. The antibody conjugate was diluted by adding 24 µl of antibody conjugate and 12 mL of IL-6 Assay Diluent. 100 µl of this solution was added to each well with a pipette. The plate was mixed on a plate rotator continuously at 500 rpm for 2 hours. The plate was washed four times with 300 µl of wash buffer in each well. 100 µl of TMB Substrate Solution was added to each well. The plate was mixed on a plate rotator at 500 rpm for 5 minutes and then was incubated in the dark for 15 minutes. 50 µl of stop solution was pipetted into each well. The plate was mixed on a plate rotator at 500 rpm for three minutes. All wells appeared yellow. The solution was read in a plate reader at 450 nm within 10 minutes of adding the stop solution. Data were analyzed by running bivariate and descriptive analysis through Statistical Package for the Social Sciences (SPSS) version 25 (IBM, 2017).

Results

Out of 109 students recruited, there were a total of 14 participants who consented to the study. The response rate was 12.8%. Seven participants were in the control group and seven participants were in the experimental (smiling) group. This was deemed an adequate sample size since this is a pilot feasibility study.

The highest possible score for the visual analog stress scale was 100 and the lowest was 0. There were a range of self-reported stress scores (see Figure 1). For the experimental group, before check-off the minimum stress level was 10 and the maximum was 80 (*M*=60.7, SD=23.92). During check-off, the minimum self-reported stress score was 10 and the maximum was 87 (*M*=57.29, SD=25.92). After check-off, the minimum stress level was 0 and the maximum was 50 (*M*=23.71, SD=18.50). For the control group, before check-off the minimum stress level was 28 and the maximum was 92 (*M*=61.57, SD=20.53). During check-off, the minimum stress level was 23 and the maximum was 100 (*M*=64.00, SD=31.49). After check-off, the minimum stress level was 0 and the maximum was 45 (*M*=17.86, SD=17.16).

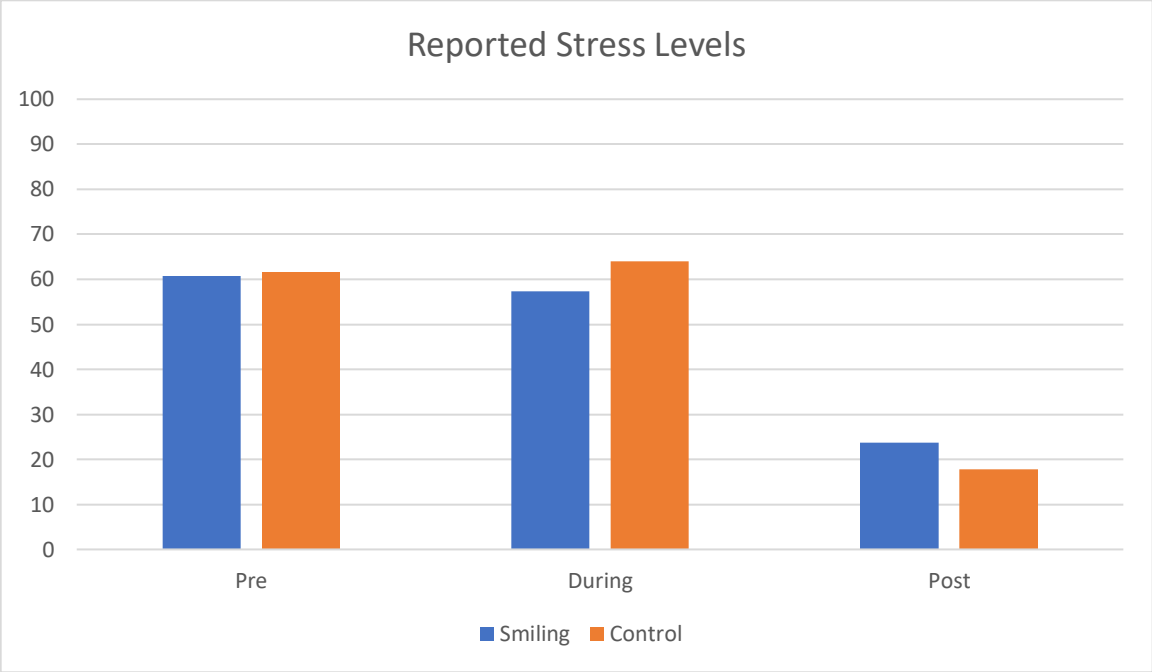

Figure 1: Reported stress levels results from visual analog scale

An independent-samples t-test was conducted to compare the experimental and control groups. There was no significant difference between groups for pre, during, and post checkoff stress levels ($p>0.05$). Refer to table 1 for additional findings.

Table 1

Reported Stress Levels Descriptive Statistics

		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Pre-checkoff	Equal variances not assumed	-.072	11.731	.944	-.85714	11.91552	-26.88511	25.17083
During checkoff	Equal variances not assumed	-.436	11.574	.671	-6.71429	15.41600	-40.44044	27.01187
Post-checkoff	Equal variances not assumed	.614	11.933	.551	5.85714	9.53725	-14.93572	26.65001

IL-6 analysis was completed in the TCU Harris Research Lab using the ELISA method kits obtained from Salimetrics (Salimetrics, 2019). For the control group, the minimum IL-6 level was 5.81 and the maximum was 56.82 (M=22.93, SD=16.43). For the smiling group, the minimum IL-6 level was 0 and the maximum was 47.25 (M=16.86, SD=19.19). Refer to Table 2 and Figure 2 for findings.

Table 2

IL-6 Level Results in pg/ml for Smiling and Control Groups

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Control	7	5.81	56.82	22.9300	16.42864
Smiling	7	.00	47.25	16.8629	19.18724

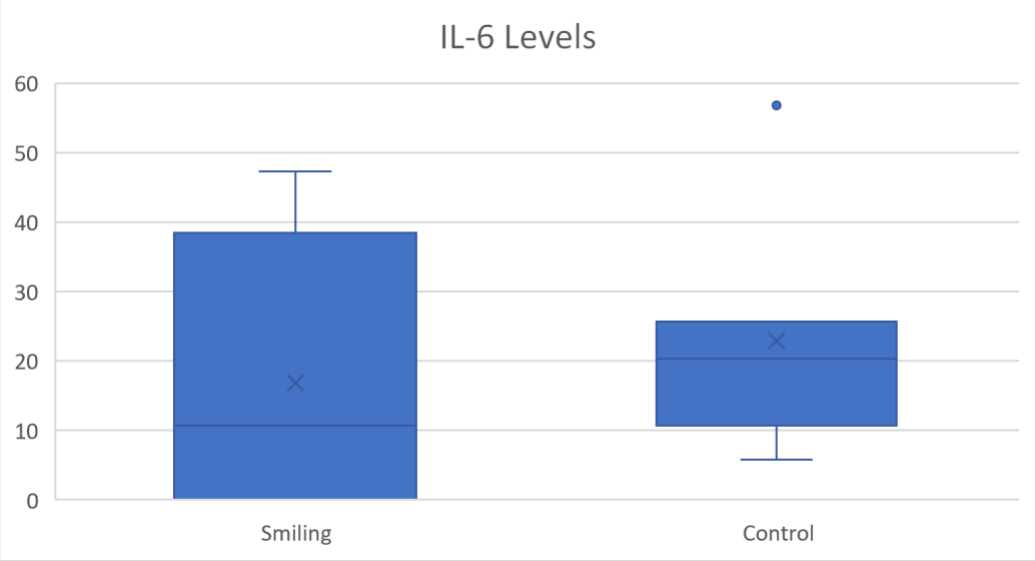

Figure 2: IL-6 levels for smiling and control groups

Correlation analysis revealed a small correlation between self-reported stress and IL-6 levels pre-checkoff (R=0.26); and moderate correlations between self-reported stress and IL-6 during and post-checkoff (R=0.42; 0.45).

Discussion

This pilot study was successfully completed and was able to be done in a timely manner. The process could be repeated in the future with a larger sample size to gain more insight into how effective this intervention is. While the average stress level for pre-checkoff and during checkoff was lower in the smiling group, the difference between the groups was not statistically significant. Even though the results were not statistically significant, they still are clinically

relevant. Smiling is a low stakes intervention that can be quickly used in stressful situations. As discussed earlier, smiling causes an increase in dopamine and serotonin levels, resulting in an improved mood, which could contribute to a decrease in stress (Nettle et al., 2005). If the intervention is helpful in reducing self-reported stress levels even by a small amount, it can be used in the future. There may be a few explanations for why the differences in stress levels were not statistically significant. A main reason is the small sample size due to it being a pilot feasibility study. Another factor that could impact the results is each individual student's perception of stress. Some students may have different standards for what they consider a moderately stressful experience than others.

The average IL-6 levels in the smiling group were also lower than the control group. However, the difference was not statistically significant. In a healthy individual, it is normal for IL-6 levels to be undetectable (Salimetrics, 2019). In the control group, there was one outlier with a substantially higher IL-6 level than the other participants, as seen in Figure 2. Without the outlier, the average IL-6 level for the control group would be 17.27 instead of 22.92. This would make the difference in IL-6 between the two groups even less statistically significant.

In this study, all participants except for two had detectable IL-6 levels. There are multiple possibilities that may explain why a majority of this sample had elevated IL-6 levels. As seen through the stress responses from the visual analog scale, nursing students experience significant stress related to their required schoolwork. The positive correlations between self-reported stress and IL-6 in the sample illustrate this relationship. Constant stress can impact the immune response pathway, leading to higher levels of inflammation in their body. Additionally, this checkoff took place during a surge of COVID-19 with the delta variant. If any of the participants

had recently contracted or been exposed to COVID-19, they may have had an unusual amount of inflammation and IL-6 in their body at that time.

Conclusion

This study is one of the first to examine the relationship between stress, IL-6, and smiling as an intervention. While there was a difference in stress levels, the data showed that the difference between the smiling and control groups was not statistically significant ($p>0.05$). Nursing students in both the intervention and control group had elevated salivary IL-6 levels, posing questions for future research looking into possible explanations for this finding.

Nursing Implications

If this study is repeated in the future, vital signs, specifically heart rate and blood pressure, could be added as another indicator of stress. This checkoff was the vital sign checkoff, so the students will already be taking those measures during checkoff. Additionally, taking vital signs pre-checkoff, during, and post checkoff would not add a significant amount of time to the study. One other aspect of the study that could be added is including a brief health history about the participants. This may give insight into why certain participants have elevated IL-6, including the presence of conditions that increases their inflammation.

Although the differences in the stress levels between the smiling and control groups were not statistically significant, the smiling group had lower mean stress levels pre-checkoff and during checkoff. This indicates that the smiling exercise could be beneficial to help reduce stress in nursing students. This exercise could be recommended by nursing faculty working with students under stress.

Elevated IL-6 levels in the group suggest the need for increased attention to physical well-being in nursing students. This holistic approach to nursing education is necessary,

especially during times of widespread diseases such as a pandemic. The role of the nurse as an advocate for well-being begins during the pre-licensure educational phase and should involve self-care. Overall, this study highlights the importance in addressing nursing student stress and creating strategies to help combat this issue. A smiling exercise to reduce stress related to high-stakes skills check-offs or exams could be one of many tools used to help improve the overall wellbeing of nursing students.

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