Abstract
We aimed to investigate the effects of job stress on the biochemical variables of schoolteachers before and after stress reduction strategies. In 2019, 200 schoolteachers (98 males, 102 females) in the private and fully aided higher secondary schools of urban and rural areas in Vellore, India were enrolled in the study based on the principle of consecutive sampling. Questionnaires were used to investigate demographic characteristics and occupational stress-related factors. Fasting blood glucose was detected by venous plasma and a biochemical analyser with the cut-off thresholds method. Salivary cortisol was determined by the specific enzyme-linked immunosorbent assay kit method. Total cholesterol in serum was determined by the CHOD-PAP method. Serum albumin was detected by the bromocresol green dye-binding method on a Roche Modular DP analyser (Roche Diagnostics). Blood pressure was measured by aneroid sphygmomanometers. Levels of fasting blood glucose, salivary cortisol, albumin and cholesterol were lower in the high-tension group than those in the low-tension group. Those of the high-coping strategy group were higher than those of the low-coping strategy group. Systolic blood pressure and diastolic blood pressure significantly reduced among the intervention groups after administering the relaxation response programme for three and six weeks, respectively, to urban and rural school teachers. Occupational stress can lead to increased salivary cortisol, cholesterol, albumin, blood glucose and blood pressure levels. The relaxation response programme resulted in quantifiable improvements in physiological markers of stress in rural and urban schoolteachers.

Keywords: Biochemical variables. Salivary cortisol. Schoolteachers. Stress reduction.

1. Introduction

Stress may not be observed evenly or present with equal intensity throughout the academic year in teachers (Ismail and Noor 2016). The use of biomarkers (Hunter et al. 2019) during a stressful condition has gained popularity in recent decades.

Salivary biomarkers (cortisol and IgA) have been used in stress research (Sluiter et al. 2003) albumin being the major extracellular plasma protein target of oxidative stress (Momozono et al. 2020). Various studies have shown that cholesterol levels are decreased under chronic stress (Sozen and Ozer 2017). Stress can also cause fluctuations in the levels of blood sugar (Jena et al. 2016). The relaxation response might help
an individual to counteract the toxic activities of stress (Hersoug et al. 2018). The relaxation response (RR) is portrayed to diminish stress reaction (Sevinc et al. 2018).

The present study is an attempt to analyse the effectiveness of a relaxation response programme on stress among schoolteachers.

2. Material and Methods

Study Design

Participants: This study used a quantitative research method with a true experimental design (pre-test-post-test control group design).

Place and Duration of Study

The study was conducted among higher secondary schoolteachers in the Vellore district of India. The study was done from June to September 2019.

Ethical Approval and Participants’ Consent

The study was reviewed and accepted by the ethics committee of the author’s medical institution under the number (005/09/2018/IEC/SMCH, dated 7th September 2018). Study subjects were guaranteed participation on a wilful premise and that their privacy would be protected. Written informed consent was obtained from all respondents.

Inclusion and Exclusion Criteria

✓ All teachers work in private and fully aided higher secondary schools, teaching class from 10th–12th std.
✓ Teachers are between the age of 25–60 years.
✓ Both male and female teachers working in private and fully aided higher secondary schools were included.
✓ Teachers are willing to participate.
✓ Teachers who experience high and moderate levels of burnout.
✓ Teachers who experience mild and moderate stress.
✓ Teachers who have poor and average quality of life.

The exclusion criteria were the following: the individuals didn’t give consent, had a background marked by psychiatric illness, were temporary and part-time teachers, were pregnant, had an interminable illness influencing the immune system, were taking medicine with known impacts on the immune system, and presented or had a recent (prior week) history of upper respiratory tract infection (URTI), as URTI will influence the degree of IgA.

Sample Size and sampling

Experimental design

Control Group (n=100): Comprised a pre-test group and post-test group after six weeks devoid of attending the relaxation programme: Teachers from urban schools (n=50) as well as from rural schools (n=50) were selected.

Experimental group (n=100): Pre-test group (urban schools=50, rural schools=50); Post-test I group (urban schools=50, rural schools=50) after receiving a stress reduction strategies intervention for three weeks.
Post-test II group (urban schools=50, rural schools=50) after receiving stress reduction strategies intervention for six weeks. Random sampling was adopted to choose 15 secondary schools among an aggregate of 30. A consecutive sampling method was employed to selected teachers.

**Statistical analysis and software used**

The data were expressed as mean ± SEM paired, and an unpaired t test and a one-way analysis of variance (ANOVA) were used for the comparison of means between the control and experimental groups. A possibility of 0.05 or less was taken as statistically significant. The analysis was done using Sigma Plot 13 (Systat software Inc., 1735 Technology, Dr#430, San Jose, CA 95110, USA).

**Stress Reduction Strategies**

The teachers selected for this study participated in a RR programme for about six weeks for at least two hours per week, relying upon class schedules, and there were consistently ten teachers in each session. **Session I** - The Nature of Stress: The meaning of stress causes and indications of stress were introduced in PowerPoint to the participants. The teachers learned about signs and symptoms, precipitating factors of stress, developed an awareness of how they could adjust to pressure, and resolved how such responses could become dysfunctional. **Session II** - RR programme: The methodology comprised the physiology of stress and the physiology of the RR. For instance, diaphragmatic breathing and cognitive rebuilding that focused on perceiving and reframing unpremeditated negative reasoning, for instance, sharing news concentrating on positive occasions (e.g., recognising and sharing something great that happened that day), were utilised to display positive psychology. **Session III** - Cognitive skills: Problem-solving training was presented in a PowerPoint presentation to the participants, which included problem-based scenarios in schools, and participants were asked to complete the problem-solving task template. **Session IV** - Affect skills: The participants were taught about anger management techniques. **Session V** - Behavioural skills: The participants were prepared in assertiveness and time management techniques. The sessions concluded with a discussion of the methods and approaches for management to minimise stress. **Session VI** - The last session deliberated the stress management techniques. The investigator gave a summary of how different techniques sway the reaction response modalities and their consequences for psychological well-being. This activity was led utilising the psycho-instructive strategy. At the end of six weeks of intervention, a post-test was carried out to both the groups (control and experimental) using the same tool with the same process. The study also collected information on demographic factors and the teacher stress inventory programme, and participants were required to submit saliva and serum samples for biomarker estimation.

**Collection of Saliva**

Participants were ahead of time prompted not to brush their teeth, eat, or drink for one hour before the saliva collection. This was to limit possible debris contamination or stimulation of saliva. They were additionally asked to wash their mouths 30 minutes before the saliva collection. Participants with oral lesions or dental bleeding disorders were excluded from the saliva collection (Yang et al. 2002). Every member was asked to latently drool (unstimulated) saliva into a sample holder. The volume of the salivation gathered was around 1–2 ml to produce an appropriate saliva flow rate. After collection, the test tubes were kept in an icebox and quickly transported to the research laboratory, and stored at −70°C until further analysis. Salivary IgA (mg/mL) and cortisol concentrations (nmol/mL) were resolved utilising the enzyme immunoassay (EIA) with the high sensitivity salivary cortisol EIA unit (ELAB science pack) separately. Salivary cortisol concentrations were accounted for accordingly as these were not influenced by the salivary flow rate since the production of cortisol for the duration of the day gives increasingly stable qualities (Ross et al. 2014).
Collection of Blood Sample

The blood sample was collected by venepuncture, from the median cubital vein in the morning by disposable syringes and a concentration of 5 ml blood was taken. The serum sample was transported to a diagnostic laboratory (Bright Care Research Centre, 32/285, Royapettah, Chennai). All of the samples were analysed by using standard methods.

Fasting Blood Sugar

For fasting blood glucose (FBG), the blood samples were collected from all of the participants after overnight fasting for a minimum of ten hours. FBG measurements were in line with national guidelines, which specify that an FBG measurement should be done using venous plasma and a biochemical analyser with cut-off thresholds depending on those recommended by the WHO. Briefly, an FBG ≥ 7.0 mmol/L (126 mg/dl) specifies diabetes mellitus (DM); a FBG of 6.1–6.9 mmol/L (110 mg/dl to less than 126 mg/dl) indicates impaired glucose tolerance; an FBG < 6.1 mmol/L (110 mg/dl) is normal (Akter et al. 2017).

Salivary Cortisol Measurement

Salivary cortisol concentration was determined using a specific enzyme-linked immunosorbent assay kit for cortisol following the manufacturer’s instructions. To avoid the fluctuations on the concentration of cortisol due to circadian rhythms, the saliva sample was collected between the hours of 10:00 and 12:00 (Hawley et al. 2016).

Serum Albumin and Cholesterol Levels

Serum albumin levels were measured conferring the bromocresol green dye-binding method on a Roche Modular DP analyser (Roche Diagnostics) (Delanghe et al. 2018). Cholesterol levels were measured with the CHOD-PAP method (Barzehkar et al. 2016).

Blood Pressure Measurement

Blood pressure (BP) measurement was done by a common protocol modified from the WHO stepwise approach. Aneroid sphygmomanometers with suitably sized cuffs were used. BP was measured twice, five minutes apart, with participants in a sitting position after five minutes of rest, in one visit. A third measurement was performed if the difference between the first two was over 10mmHg for systolic or diastolic BP. The average of the second and the third BP measurements was used for the analyses. In addition, the participants were advised to avoid smoking, consuming coffee or tea and performing any exercise for at least 30 minutes before measuring their BP. The Aneroid BP machine was calibrated against a mercury BP machine weekly (Lamego et al. 2017).

3. Results

In the present study, it has been found that the effect of stress increased FBS and was reduced after the relaxation response programme. When the body is stressed, the adrenal glands activate the discharge of glucose stored in various organs, which often elevated the levels of glucose in the blood stream. Fasting blood sugar levels in the post-test groups were significantly reduced when compared with the pre-test groups (Figure 1).

A reading of 130 to 159 mg/dL cholesterol is borderline high, while 160 to 189 mg/dL is high. The present study showed compelling evidence that the level of stress caused an increase in cholesterol. Urban and rural post-experimental groups showed a reduction in the level of cholesterol when compared with the pre-test groups. Statistically significant differences were not found between urban and rural experimental groups (Figure 2).
THEPHILAH, C, ARUNA, S. and VIJAYARAGAHAVAN, R.

Figure 1. Effect of stress reduction strategies on fasting blood sugar (FBS) levels in urban and rural schoolteachers.
(Note: The values are expressed as mean ± SEM (n = 50 each). The t and P values are by paired t test. The F and P values are calculated by a one-way RM ANOVA; *p<0.05 is significantly different from the respective pre-test. The respective pre-test and post-test2 are analysed by unpaired t test.)

Figure 2. Effect of stress reduction strategies on blood cholesterol levels among urban and rural schoolteachers.

The level of salivary cortisol was different for the experimental and control groups. Urban and rural experimental groups showed significant reduction in cortisol levels among the post-test 1 and 2 groups as compared with the pre-test group. The sample taken after the procedure, as well as the sample taken after the end of the task, showed a significant decrease for the experimental groups (Figure 3).

No significant difference was observed in the pre-and post-test groups of rural schoolteachers. The urban experimental post-test group showed a slight reduction in the level of albumin as compared with the pre-test group (Figure 4).

A reaction to stress affects blood pressure. A slight increase in the systolic and diastolic blood pressure of urban and rural populations was noted among the control post-test group. SBP and DBP significantly reduced among the experimental groups both after giving the stress relaxation programme for three and six weeks in urban and rural schoolteachers. No significant differences were observed between the rural and urban experimental group (Figures 5 and 6).
Effects of stress reduction strategies of breathing technique relaxation programme and positive psychology on biochemical variable

**Figure 3.** Effect of stress reduction strategies on salivary cortisol levels among urban and rural schoolteachers.

**Figure 4.** Effect of stress reduction strategies on albumin levels among urban and rural schoolteachers.

**Figure 5.** Effect of stress reduction strategies on systolic blood pressure (SBP) among urban and rural schoolteachers.
4. Discussion

Stress is a condition in which homeostasis in the system is altered regarding several internal and external stressors (Cummings and Cooper 1979). This study has a female subjugate occupation with results consistent with other studies on nurses (female), examining work pressure and salivary IgA. Home–work conflict is still significant in the age of work-related pressure. Also, it seems noteworthy that teaching higher secondary students adds to work pressure and participating in the relaxation response programme will assist in mitigating stress, thus leading to lower salivary IgA levels. A comparable outcome was found by (Schulz et al. 1998), where significant correlations were found between cortisol and perceived stress levels.

Stress has been linked to less healthy dietary habits, a higher body mass, and a less healthy diet, all of which are recognised risk factors for higher cholesterol. There is early proof of the benefits of workplace wellness programmes among schoolteachers (Stiefel et al. 2017). The current study displayed a reduction in the cholesterol level in post-treatment groups of teachers. When the body faces stress, definite physiological reactions take place, which lead to changes in levels of hormones and blood components. Both of these events may result in higher cholesterol (Kulig et al. 2015). The blood components comprising cholesterol become more concentrated. This might be one way in which stress leads to higher cholesterol levels in the short term (Austin et al. 2015).

Stress can also cause an important and extended increase in the level of blood sugar. Stress blocks body from insulin release, thus letting glucose accumulate in the blood. If stressed for a long time, sugar levels increase (Wenjuan et al. 2019). The present study also observed such an elevation in the fasting blood sugar levels among stressed teachers. Stress management techniques help people regulate their blood sugar levels (Singh 2016). A decrease in FBS has been observed after participation in the relaxation response programme in the current study among urban and rural schoolteachers.

Stress can result either from low levels of antioxidants and/or from an increased reactive species production. Various models of oxidation point out that albumin plays key role in antioxidant functions (Munne-Bosch and Pinto-Marijuan 2017). The present study specified an increase in the levels of albumin among the stressed groups and found that the levels were reduced after relaxation response.

The majority of research on teacher stress and burnout focuses on urban schoolteachers. Little research has examined differences in stress and burnout between rural and urban schoolteachers despite the fact that rural and urban school systems are different (Chaudhary and Srivastava 2017). The studies that do exist find greater stress among urban teachers; rural schools offer a less stressful learning environment. Furthermore, the primary sources of stress appear to be different for rural versus urban schoolteachers (Ouellette et al. 2018), observed that stress from insufficient discipline policies, noisy pupils, inadequate
salaries, and too much work were the primary sources of stress for urban teachers, whereas stress from only inadequate discipline policies and too much work were the major sources of stress for rural teachers.

Some problems and demands confronting teachers in rural and urban school systems are similar; yet, many are different (Dawn et al. 2017) examined urban schoolteachers and found that they experienced significantly more stress from poor working conditions and poor staff relations than rural schoolteachers did. Time pressure was expressively greater than stress from a poor working situation and poor staff relations for both urban and rural schoolteachers. While a great deal of research exists in regard to urban teacher stress, very few solutions and their effectiveness have been reported.

Teacher stress interventions have varied widely over the past few decades. Throughout the relaxation response, the body moves near a state of physiological relaxation, where heart rate, digestive functioning, blood pressure, and hormonal levels yield normal levels (von der Embse et al. 2019). During stress, the fight-or-flight response happens naturally. In modern times, the fight-or-flight response is triggered several times throughout the day due to a wide range of stressors, many of which are perhaps more situational than truly based on survival.

Encompassing more schools would have permitted a larger sample size and better speculation of results. Looking at age and length of teaching experience could contribute significantly to evaluating the stress levels among teachers.

Since the study has focused on higher secondary schools under private leadership, there is a need to take up equivalent studies in government schools in Tamil Nadu.

5. Conclusions

The relaxation response programme resulted in quantifiable improvements in physiological markers of stress. Thus, the relaxation response is considered a helpful way to turn off the fight-or-flight response and bring the body back to pre-stress levels among rural and urban schoolteachers.

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