Self perceived work related stress and the relation with salivary IgA and lysozyme among emergency department nurses

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Aims: To assess and compare the self perceived work related stress among emergency department (ED) and general ward (GW) nurses, and to investigate its relation with salivary IgA and lysozyme. 

Methods: One hundred and thirty two of 208 (63.5%) registered female ED and GW nurses participated in this study. A modified mental health professional stress scale (PSS) was used to measure self perceived stress. EUSA methods were used to determine the salivary IgA and lysozyme levels. 

Results: On PSS, ED nurses had higher scores (mean 1.51) than GW nurses (1.30). The scores of PSS subscales such as organisational structure and processes (OS), lack of resources (RES), and conflict with other professionals (COF) were higher in ED than in GW nurses. ED nurses had lower secretion rates of IgA (geometric mean (GM) 49.1 µg/min) and lysozyme (GM 20.0 µg/min) than GW nurses [68.2 µg/min; 30.5 µg/min]. Significant correlations were observed between PSS and log IgA and lysozyme secretion rates. OS, RES, and COF were correlated with log IgA and lysozyme levels.

Conclusion: ED nurses, who reported a higher level of professional stress, showed significantly lower secretion rates of salivary IgA and lysozyme compared to GW nurses. Salivary IgA and lysozyme were inversely correlated with self perceived work related stress. As these salivary biomarkers are reflective of the mucosal immunity, results support the inverse relation between stress and mucosal immunity. 

Research has shown that nursing is a stressful occupation.1-3 Growing evidence suggests that it might be increasing in severity.4 Emergency department (ED) nursing is a special kind of nursing. In addition to the stressors that are encountered by nurses such as work overload, dealing with difficult patients, role conflict, lack of support, and home-work conflicts,4,5 ED nurses are in a position that is expected to deal with additional stressors. These include unexpected numbers of patients at any time, unexpected rapid changes in patients' situations, and response to distressing or traumatic incidents such as sudden death, patient violence, inappropriate attendees, and physical or verbal abuse on a daily basis.6,7 Emergency nursing is a highly stressful profession.

It is believed that nurse stress could lead to a decrease in quality of care, increased staff turnover, and impairment of nurses' health.8 Studies that relate to nurse working stress within the specialty of ED unit have mostly been conducted using subjective questionnaire or interview methods. Furthermore, different questionnaires have been used in different studies, making it difficult to compare the study results. Studies have mainly been carried out in the USA, UK, and Canada.8

Recently, various correlational and longitudinal studies have lent support to the hypothesis that chronic psychological stress could lead to impairment of immune function, leaving the individuals exposed to greater risk of infection and illness.9-12 Secretory immunoglobulin A (sIgA) in saliva, the main immunological defence of mucosal surfaces, had repeatedly been shown to be sensitive to psychological variables. It is believed to be representative of functional status of the entire mucosal immune system.13

Lysozyme, or muramidase, one of the major non-immunological antimicrobial proteins in saliva, is widely distributed in human tissues and secretions.14 It is considered to belong to a primitive defence system, known as the innate immune system. Lysozyme is a cationic protein with wide antimicrobial activities. The mechanism of the antimicrobial function of lysozyme is through its enzymatic activity, which cleaves (-1,4) glycosidic bonds between muramic acid and N-acetylglucosamine residues in the peptidoglycan of the bacterial cell wall.15-16 Other proposed antimicrobial activities of lysozyme include inhibition of bacterial growth, metabolism, and dechaining.17

The relation between stress and salivary lysozyme is not clear. Recently, Perera et al assessed the relation between stress and salivary lysozyme in two studies. One study showed a decreased salivary lysozyme after academic examinations in 15 students.18 The other study, on 15 students, showed increased salivary lysozyme level after exposure to various relaxation strategies.19 However, Ng et al found no significant correlation between the self perceived stress levels and salivary lysozyme in 124 female Singapore nurses.20 Their study utilised a general stress questionnaire that focused on the average Asian population, and was not specific for nurses. In all the above mentioned studies, lysozyme was measured using a lysoilate method, which is thought to be less sensitive than the enzyme linked immunosorbent assay (ELISA) method. However, significantly different levels of salivary lysozyme were found in different nursing units, similar to those obtained with IgA.20 A close linkage between salivary lysozyme and sIgA has also been suggested in students, nurses, and patients with psoriasis.21-23

The objectives of this study were: (1) to assess and compare the self perceived work related stress of female ED and general ward (GW) nurses; and (2) to investigate the relation between salivary IgA and lysozyme with self perceived work related stress.

Abbreviations: ED, emergency department; GM, geometric mean; GW, general ward; PBS, phosphate buffered saline; PSS, professional stress scale; sIgA, secretory immunoglobulin A; URI, upper respiratory tract infection

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METHODS

Subjects
This cross sectional study was conducted in the ED departments of three big public hospitals in Singapore. There were 142 eligible female nurses employed in these ED departments. In one of the hospitals, 66 eligible female nurses in three general wards were chosen as a control group. Participation was voluntary and anonymous. The objectives of the study were explained to the nursing officers and the nurses of the departments involved. The nursing officers helped to deliver the questionnaires and reminded nurses about filling in the questionnaire. In order to maintain confidentiality, the completed questionnaires were sealed before being handed to the nursing officers.

Questionnaire
The self administered questionnaire included items of information on sociodemographic characteristics such as age, marital status, educational level, nursing appointment, and years of working experience in their respective departments.

Mental health professionals stress scale (PSS)
A questionnaire developed by Cushway et al was used to assess the self perceived work related stress for mental health professionals. It included almost all the stressors encountered by the nursing profession, even in ED nursing. With some modifications to those questions that were unique to mental health professionals, it was used to assess the work related stress of ED nurses in this study. The questionnaire included seven parts with 42 items. Nurses were asked to indicate on a four point scale how frequently they experienced such stressful situations in the present department. The score was from 0 (does not apply to me) to 3 (does apply to me) (table 1). As suggested by the author, the total score was obtained by summing all the scores and dividing by the total number of questions to derive the average. Internal consistency coefficients for these scales range from 0.87 to 0.94. The concurrent valid- ity of the questionnaire is good; there is a good relation with suggestions by the author, the total score was obtained by summing all the scores and dividing by the total number of questions to derive the average. Internal consistency coefficients for these scales range from 0.87 to 0.94. The concurrent validity of the questionnaire is good; there is a good relation with the self perceived work related stress for mental health professionals.

Saliva collection
A written protocol on how to collect the saliva was given to the nurses. In addition, they were also briefed on the collection method and supervised on the day of collection. A single timed five minute unstimulated total saliva was obtained from nurses who participated in the study when they were on their morning shift. The time of saliva collection was between 1 pm and 3 pm, after they finished their work. This timing was chosen to match the circadian rhythm of saliva production (0.5 ml/min). The samples were immediately taken to the laboratory and stored frozen at −70°C until required for assay.

Valid subjects
To ensure validity of the results, exclusion criteria for participation were: pregnancy; chronic diseases affecting the immune system; regular medication with known effects on the immune system; and present or past (one week) history of URTI, as URTI will affect the level of IgA and lysozyme in the saliva.

Furthermore, the flow rate of saliva of valid subjects had to be at least 0.1 ml/min (under basal conditions, the rate of saliva production is 0.5 ml/min). Subjects with a flow rate of less than 0.1 ml/min would probably not have collected the saliva properly for the five minute period.

Laboratory methods and analyses
An ELISA method was used to determine the salivary IgA concentration (µg/ml). The salivary lysozyme concentration was measured using an ELISA method that was developed in our laboratory. In brief, a 96 well microtitre ELISA plate was coated overnight at 4°C with 200 µl of rabbit anti-human lysozyme at a concentration of 7.0 mg/l in sodium carbonate-bicarbonate buffer (pH 9.6). The well was washed with phosphate buffered saline (PBS) containing 0.05% (w/v) Tween 20 (PBS/Tween), and blot dried. The plate was used
immediately, or stored at −20°C for up to two months. A 200 µl aliquot of 2% (w/v) bovine serum albumin (radioimmunoassay grade, Sigma, USA) in PBS/Tween was added and left at room temperature for 120 minutes. The well was washed with PBS/Tween 20; 100 µl of specimen diluted in PBS (1/50) and 100 µl biotinylated lysozyme (1/1000) (radioimmunoassay grade, Sigma, USA) was added to each well. The plate was incubated at 37°C for 60 minutes (standard at 50, 25, 12.5, 6.25, 3.125 µg/ml; biotinylated lysozyme 1/10000). The well was washed with PBS/Tween 20; 200 µl avidin alkaline phosphatase diluted in 1/3000 in PBS was added and incubated at 37°C for 60 minutes. After washing, 200 µl of enzyme substrate p-nitrophenyl phosphate sodium (0.1% w/v) in diethanolamine buffer (DEA buffer, pH 9.8) was added and incubated for 30 minutes at room temperature (kept in the dark). The reaction was stopped with 50 µl 1.0 M NaOH. The absorbance was read on a Bio-Rad microplate reader at 405 nm. Each run included five twofold dilutions of purified human urine lysozyme (stock concentration 50 mg/l; Sigma Chemical Co., St Louis, MO) from which a standard curve was generated. The salivary IgA and lysozyme secretion rate (µg/min) were computed by multiplying the absolute IgA and lysozyme concentration with the absolute saliva flow rate.

Statistical analysis
Data are presented as mean (SD); all p values and confidence intervals are two sided, and the level of statistical significance was considered to be p < 0.05. Two sample independent t tests were used in the univariate analysis. General linear models were used in the multivariate analysis for controlling the possible effect of marital status and work experience, as marital status and work experience might affect the level of work related stress. Partial correlations between stress scores and biomarkers were calculated after controlling for marital status and work experience. A Bonferroni correction was made to control for type I error inflation. As the data of salivary IgA and lysozyme showed positively skewed distributions, logarithmic transformations were performed for all data for further statistical analyses. The data analyses were performed with SPSS software.

RESULTS
Response rate
One hundred and sixty two nurses of 208 eligible nurses took part in this study, of which 106 of 142 (74.6%) ED nurses and 56 of 66 (84.8%) GW nurses completed a questionnaire and provided a salivary sample. The overall response rate was 77.9%.

Eventually 132 nurses who did not have URTI and who had properly collected saliva samples were selected for further analyses, 82 in the ED and 50 in the GW nurse group. This represents an overall response rate of 63.5%. Of the excluded nurses, 16 had a salivary flow rate of less than 0.1 ml/min, and 13 had suffered from an URTI either currently or during the past week. One nurse was excluded because of pregnancy. Comparisons between the 132 participants and the 30 subjects who dropped out of the study revealed no significant differences with respect to demographic or psychological variables.

Sociodemographic characteristics
Among the 132 selected nurses, most (85.6%) were 20–40 years old. The ED nurses were slightly older (mean age 32.1 years) compared to the GW nurses (27.6 years) (p = 0.002). Eighty per cent of the nurses had obtained a nursing diploma or higher; the others had at least completed secondary school. Forty three per cent of nurses in ED were married, as were 22% of nurses in GW (p = 0.016). ED nurses had slightly more working experience (5.3 years) than GW nurses (2.9 years) (p = 0.000). Marital status and working experience were used as covariates to adjust for their possible effects. There were no obvious differences in educational level and other factors between the two groups. Table 2 shows the sociodemographic characteristics.

Self perceived work related stress
The score of PSS of ED nurses (mean 1.51) was higher than that of GW nurses (1.30) (p = 0.012). The difference was still observed after adjustment for marital status and working experience (p = 0.029). After finding a difference between ED and GW nurses on the PSS, we compared the subscales of PSS. Scores of organisational structure and processes, lack of resources, and conflict with other professions were significantly higher in ED compared to GW nurses (all p = 0.002). After adjustment for marital status and working experience, all three subscales and patient related difficulties showed significant differences (all p < 0.01). The other subscales showed no significant differences between ED and GW nurses. However, after Bonferroni adjustment, only organisational structure and processes showed significant differences (table 3).

Statistical analyses of salivary biomarkers
Salivary IgA
SIA concentration and secretion rate were lower in ED nurses (geometric mean (GM) 148.5, min-max 52.9–408.0 µg/ml; and 49.1, 5.4–47.7 µg/min) than that of GW nurses (GM 200.9, min-max 54.3–95.9 µg/ml; and 68.2, 29.8–220.1 µg/min)
Salivary lysozyme concentration and secretion rate were lower (p = 0.002, 0.001). The differences still existed even after adjustment for marital status and working experience (p = 0.002, 0.038) (table 4). After Bonferroni correction, slgA concentration still showed the difference between the two groups. Log salivary IgA secretion rate was negatively correlated with PSS (r = −0.28, p = 0.001).

With regard to the subscales of PSS, organisational structure and processes, lack of resources, conflict with other professionals, and home-work conflict had significant negative correlations with log slgA secretion rate (r = −0.24 to −0.33, all p < 0.01). Other subscales had no significant correlations with log slgA level. After Bonferroni correction, organisational structure and processes, lack of resources, and conflict with other professionals still showed significant correlations with log slgA secretion rate (table 5).

Salivary lysozyme

Salivary lysozyme concentration and secretion rate were lower in ED nurses (GM 62.4, min-max 12.9–307.5 µg/ml; and 20.00, 3.4–116.1 µg/min) than that of GW nurses (101.9, 16.2–258.6 µg/ml; and 30.5, 4.6–117.0 µg/min) (p = 0.000, 0.003). The differences could be observed even after adjustment for marital status and working experience (p = 0.000, 0.012) (table 4). After Bonferroni correction, salivary lysozyme concentration and secretion rate still showed difference between the two groups. Log salivary lysozyme secretion rate was negatively correlated with PSS (r = −0.28, p = 0.001).

With regard to the subscales of PSS, organisational structure and processes, lack of resources, and conflict with other professionals, personal self doubt, and home-work conflict had significantly negative correlations with log salivary lysozyme secretion rate (r = −0.18 to −0.39, all p < 0.05). The other two subscales had no significant correlations (table 5). After Bonferroni correction, only conflict with other professionals still showed significant correlation with log salivary lysozyme level. Furthermore, log salivary lysozyme secretion rate was moderately correlated with that of slgA (r = 0.35, p = 0.000).
DISCUSSION

Response rate

The response rate of 63.5% in this study was satisfactory, compared to other studies that had response rates of 27% and 33%. Only 16 of 162 nurses (9.9%) were excluded from the study because of improper collection of salivary samples. No obvious difference in response rate was noted between ED (74.6%) and GW (84.8%) nurses.

Sociodemographic characteristics

One of the differences between ED and GW nurses in the sociodemographic characteristics was age. The mean difference was less than five years. Previous reports have indicated no significant age effect on salivary IgA and immunity in adults aged 15–70 years. Thus, the age difference was unlikely to be a confounder. The second difference between ED and GW nurses was marital status. There were more married nurses in the ED (44%) than in the GW (22%) group. Fulhur et al. found that married women could get more social support from their family network, which could buffer the stress in their work. However, in a recent nursing stress study of 1043 Singapore nurses, Boey et al. found that married nurses would generally have more housework to do, a common situation in Asia. Inability to meet the family demands because of work was highly detrimental to the nurses’ emotional stability. Another difference was the working experience. ED nurses had slightly more working experience (5.3 years) than GW nurses (2.9 years). Again, in the study of Boey et al., nurses who had more than 10 years working experience reported significantly lower stress than the less experienced nurses. Thus, marital status and working experience were used as covariates to adjust for their possible effects.

Self perceived work related stress

ED nurses scored significantly higher on the PSS than GW nurses. This is in accordance with Revicki and Gershon’s study results of work related stress in emergency medicine workers. The results indicate that nurses working in ED departments perceived themselves to experience more stress than GW nurses. From the PSS subscales, patient related difficulty and workload were the two subscales that showed the highest score for both groups. The result was similar to other study results, even though no significant difference was shown between the two groups, ED nurses perceived higher levels of patient related difficulties, organisational structure, lack of resources, and conflict with other professionals than did GW nurses. This suggests that those subscales reflected important causes of work related stress.

The mean PSS score in this study was 1.51. The study of Cushway et al. studied where the mean PSS score of nurses was relatively low at 1.30, showed good internal consistency coefficients of 0.87 to 0.94. Thus, PSS score within this range should have similar internal consistency.

Salivary IgA

ED nurses, who perceived a higher level of work related stress, had a significantly lower sIgA concentration and secretion rate than that of GW nurses. sIgA secretion rate was significantly negatively correlated with PSS. These results confirmed the findings of other studies. sIgA was also significantly negatively correlated with the scores of organisational structure and process, lack of resources, conflict with other professionals, and home-work conflict. This is in line with the results that showed the differences between ED and GW nurses in answering the PSS. Furthermore, although home-work conflict showed no difference between ED and GW nurses, home-work conflict was still very important in the generation of work related stress. Asian women are the main force of domestic work. In the study of nurses by Boey et al., meeting family demands was significantly related to anxiety and depression ($r = 0.44$, $p < 0.001$) and sense of inadequacy ($r = 0.34$, $p < 0.01$). This could be the reason for the negative correlation between sIgA and the score of home-work conflict.

Salivary lysozyme

In this study, ED nurses who reported a higher level of work related stress had a significantly lower level of salivary lysozyme than GW nurses. The significant negative relationship between salivary lysozyme secretion rate and stress level on the PSS was also observed in the studies by Perera and colleagues. Furthermore, a positive correlation was found between sIgA and lysozyme secretion rate in all nurses ($r = 0.35$, $p = 0.000$). Lysozyme was also found to correlate with the subscales of PSS, as did sIgA. Except for workload and patient related difficulties, all other PSS subscales were negatively correlated with lysozyme secretion rate. As lysozyme is one of the components contributing to mucosal immunity, the results showed the negative relation between stress and immunity.

As this is a cross sectional study, it is not possible to infer a causal relation between stress and lowered lysozyme levels. However, a possible mechanism that explains the relation is proposed. Psychological stressors have been known to raise glucocorticoid levels through increased sympathetic activity. An increased level of glucocorticoid is responsible for the observed inhibition of macrophage and monocyte function. As the macrophage is one of the principal sources of salivary lysozyme, an increase in glucocorticoid level caused by stress can inhibit the production and secretion of lysozyme. A longitudinal study is needed to investigate the relation between lysozyme and stress.

Limitations

Some limitations of the study should be mentioned. Firstly, the cross sectional research design did not permit us to examine possible sequential relations between self perceived work related stress and the salivary biomarkers. Secondly, although we used a more detailed and specific questionnaire to assess nursing stress, we still could not separate the relative contributions of the individual and the non-working environment when examining the stress response. Longitudinal research that includes not only repeated measures of stress but also related physiological parameters (for example, autonomic nervous system and immunological measures), would provide additional insights into the potential implication of stress induced health impairment.

Conclusions

ED nurses reported higher levels of stress compared to GW nurses on the PSS. These findings support previous results that ED nursing is more stressful than GW nursing. ED nurses, who perceived a higher level of stress, showed a significantly lower level of salivary IgA and lysozyme than GW nurses. Salivary IgA and lysozyme were inversely correlated with self perceived work related stress. As these salivary biomarkers are reflective of the level of mucosal immunity, results support the inverse relation between work related stress and mucosal immunity found in other studies.
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