The Biobehavioral Relationship between Pain and Stress in Postoperative Oropharyngeal Cancer Patients

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Introduction

Pain is a symptom that accompanies many illnesses and disease processes (Harkreader, & Hogan, 2004). It alerts the body that tissue damage has occurred. Pain can occur as either acute with a short term onset and duration lasting for less than six months, or chronic, lasting for longer periods of time of more than six months (Jarvis, 2004). Both acute and chronic pain are commonly present in cancer patients at all stages of the disease process. According to the American Cancer Society, about 30% of patients with a new diagnosis of cancer experience pain, however, reports of pain increase as the disease progresses with pain being experienced by 30%-50% of patients undergoing treatments including chemotherapy, radiation and surgery. If pain is not relieved adequately, it can then lead to further discomforts and medical complications including tachycardia, hypoxia, nausea, vomiting, decrease in immunity and wound healing ability, fatigue, depression, anxiety and stress (American Cancer Society, 2007; Jarvis, 2004). Thus, because of these complications, it is important that healthcare providers perform complete pain assessments, treat the symptom of pain and properly communicate pain management plans to patients (Portenoy, & Lesage, 1999).

Another physiological response that can exacerbate illness and can influence the cardiac, respiratory and gastrointestinal systems of the body is stress (Harkreader, et al., 2004). Research has demonstrated that there is a positive correlation between subjective measures of pain and stress, but less is known about the relationship between pain and physiological stress measures. In addition, little research has been conducted to determine the relationship between pain and stress levels in postoperative head and neck cancer patients. Thus, the following study will explore the relationship between the pain experienced by postoperative head and neck cancer patients who have undergone otolaryngological surgery and stress hormones cortisol and
catecholamines. This will help determine the biological stress levels that head and neck cancer patients may encounter while recovering from surgery and provide further insight into the pain and stress relationship.

Review of Literature

The Pain Process

The body perceives pain sensations using both the peripheral and central nervous system. The cells that perceive pain in the peripheral nervous system are called nociceptors. When a painful stimulus occurs, nociceptor cells receive the stimulus which is carried by A-delta and C-fibers to the central nervous system. A-delta fibers are myelinated and transmit signals rapidly, carrying sharp pain signals whereas C-fibers are unmyelinated and slower, carrying achy and dull pain signals. Once signals are carried to the central nervous system, they ascend the spinal column via the anterolateral spinothalamic tract, terminate in the thalamus, processed in the cortex and the painful sensation is perceived (Jarvis, 2004). However, the quantity and speed of competing sensory stimuli influences whether or not pain information continues to travel up the spinal column and if it is perceived by the cortex. The gate control theory, introduced by Melzack and Wall, states that the substantia gelatinosa in the dorsal horn of the spinal column acts as a gate, which allows faster stimuli to override the sensation of weaker stimuli (Melzack, & Wall, 1965).

Prevalence studies have shown that pain is a common factor of cancer (American Cancer Society, 2007) and research studies have been conducted to further understand how pain is perceived by cancer patients. A recent systematic review looked at 54 research studies that were interested in determining the presence of pain in cancer patients. Their findings conclude that more than half of all cancer patients experience pain, with 64 percent of those who had cancer in
the advanced stages reporting pain (Van Den Beuken-van Everdingen, De Rijke, Kessels, Schouten, Van Kleef, & Patijn, 2007). In 2,266 patients who were referred to a pain service with various cancer diagnoses, 98% reported having pain when first evaluated by the pain service, with 77% rating their pain as severe. In head and neck cancer patients specifically, the most frequent causes of pain were neuropathic and soft tissue pain occurring after surgery (Grond, Zech, Diefenbach, Radbruch, & Lehmann, 1996). Pain levels from head and neck cancer are also highly prevalent at the time of diagnosis and range in severity based on the specific type of cancer (Chaplin, & Morton, 1999). Finally, when analyzing the effectiveness of pain regimens after surgical resections for head and neck cancer patients, pain was experienced by a majority of patients immediately after surgery, with the average pain level reported being a six on a pain scale of zero to ten (Gil, Smith, Marouani, Khafif, & Fliss, 2006). Thus, these studies reinforce that pain is a very real symptom for cancer patients, including the pain experienced by head and neck cancer patients.

**Stress Mechanisms**

Stress is a physiologic phenomenon that occurs when the homeostatic balance of the body is threatened (Goldstein, 2003). Many hormones such as cortisol, epinephrine and norepinephrine take part in the stress response. Cortisol regulates the amount of plasma glucose in the body and has metabolic, anti-inflammatory, immune and central nervous system effects. When the body becomes stressed, the cerebral cortex tells the hypothalamus to release Cortocotropin-releasing hormone (CRH) which leads to the pituitary gland releasing Adrenocorticotropic hormone (ACTH) that stimulates the adrenal cortex to release cortisol. In addition, the catecholamines epinephrine and norepinephrine stimulate the alpha and beta adrenergic receptors. When stimulated, the alpha-1 receptors cause the contraction of smooth
muscle including blood vessels and beta receptors cause an increase in heart rate, contractility and bronchodilation. During a stressful experience, the catecholamines cause a blood pressure to rise, pupils to dilate, increase heart rate and contractility and inhibit the gastrointestinal system. This reaction to stress is known as the fight or flight response (Huether, & McCance, 2004). Recently, levels of plasma epinephrine and norepinephrine have been shown to correlate with a salivary enzyme called alpha-amylase (Nater, La Marca, Florin, Moses, Langhans, Koller, & Ehlert, 2006) and recent studies have demonstrated that it is present during times of psychological stress (Van-Stegern, Rohleder, Everaerd, & Wolf, 2006; Nater, et al., 2006; Gordis, Granger, Susman, & Trickett, 2006). Since saliva secretion is controlled by the autonomic nervous system, saliva secretions of alpha amylase and cortisol can represent a sympathetic nervous system and neuro-endocrine system response during times of stress (Chiappelli, Iribarren, & Prolo, 2006).

Recent studies have tested salivary cortisol and alpha-amylase to study their presence during stressful events. One study tested alpha-amylase response by showing to groups of participants a series of stressful images. One group of participants received a betablocker while the other received a placebo. Results indicated that the betablocker reduced amylase levels whereas the group receiving the placebo had high levels of amylase after viewing the stressful images (Van-Stegern, et al., 2006). A study by Gordis, et al., 2006, demonstrated a pattern between alpha-amylase and salivary cortisol. The study showed that 64.2 % of the sample had greater than a 10% increase of alpha amylase from the relaxation period immediately before the stressful event to the period immediately after the stress. From the sample, 68.2 % had cortisol levels increase from immediately before the stress and peaking 25 minutes after the stress (Gordis, et al., 2006). Similar patterns of alpha amylase increasing and peaking earlier than
cortisol have been found in other studies as well. A study by Nater, et al., 2006, had participants undergo the Trier Social Stress Test by having the subjects participate in a job interview and perform arithmetic problems in front of a crowd. This study also demonstrated that levels of alpha amylase significantly increased during the stressful interview and math equation. Salivary cortisol levels were measured as well and were shown to increase and peak after the stressful event (Nater, et al., 2006). These studies demonstrate that salivary cortisol and alpha amylase are consistently present during times of stress and are good, non-invasive measures to determining psychological stress (Chiappelli, et al., 2006; Gordis, et al., 2006; Nater, et al., 2006; Van-Stegern, et al., 2006).

The Relationship between Pain and Stress

Research studies have been conducted to determine the relationship between pain and stress and, more specifically, the relationship between pain and stress in cancer patients. In a study conducted by Syrjala and Chapko, 1995, multiple scales and questionnaires measuring pain, degree of oral mucositis severity, a complication of chemotherapy, and many psychological variables of distress were used in order to meet the objective of predicting pain related to treatment using physical, psychological and biomedical measures. The researchers used bone marrow transplant patients as subjects because they begin free of pain and develop pain once the treatment starts. The findings demonstrated that many factors affected pain, one of the strongest being distress prior to the initiation of the bone marrow transplant. The researchers do state that this study only investigates the effects that biomedical, psychological and physical factors have on pain and not the effects of pain on these variables (Syrjala, & Chapko, 1995). A similar study done by Schulz-Kindermann, Hennings, Ramm, Zander and Hasenbring, 2002 used the study by Syrjala and Chapko, 1995, as a model in an attempt to confirm their results and used a similar
hypothesis. This study did test many of the same variables as Syrjala and Chapko, 1995 and confirmed their results of distress being related to pain. The level of distress pre-transplant was reported to have the closest correlation with, and predicted mouth pain levels occurring later in the patients post-transplant (Schulz-Kindermann, Hennings, Ramm, Zander, & Hasenbring, 2002). Overall, these two research studies conclude that many factors and variables can affect pain, one of which is psychological variables such as distress levels (Schulz-Kindermann, et al., 2002; Syrjala, et al., 1995).

The goals of other research studies in this area have been to determine the relationship between pain and psychological variables such as anxiety and distress in hospitalized cancer patients. An older study examined the multiple aspects and components of pain in cancer patients, which included psychological aspects such as stress and anxiety. The study compared inpatient and outpatient pain-free cancer patients to inpatient and outpatients who had cancer related pain. The data for this study was collected throughout the treatment period. The results concluded that the inpatients with pain reported a higher stress and anxiety level than the inpatients without pain or the two outpatient groups with and without pain (Ahles, Blanchard, & Ruckdeschel, 1983). Another study completed by Zimmerman, Turner-Story, Gaston-Johnson and Rowles, 1996, also sought to determine if cancer patients with pain experienced more psychological symptoms such as anxiety, depression and hostility than those without pain. Unlike the other two pieces of research, this study design only contained one data collection period. Nevertheless, results from this study indicated that cancer patients with pain demonstrated a higher correlation between pain and the psychological factors described earlier, which included anxiety, than those patients without pain (Zimmerman, Turner-Story, Gaston-Johnson, & Rowles, 1996). Thus, these studies have all displayed similar conclusions about pain
and psychological variables, demonstrating that there is a relationship and correlation between pain and psychological variables such as anxiety and distress in cancer patients (Ahles, et al., 1983; Zimmerman, et al., 1996).

In a recent study, researchers were interested in using salivary alpha amylase levels to determine if this objective measure could be used to predict pain when using the visual analogue scale (VAS) for pain. The sample of the study included people with chronic lower back or leg pain and people with no chronic pain. All participants received an epidural block. The VAS, saliva samples, heart rates and blood pressures were collected before the epidural and again at 30 and 45 minutes afterward. Results indicated that the correlation between pain and stress among all of the participants was $r = 0.561$ with $P < 0.01$. Thus, the researchers conclude that salivary alpha amylase and the VAS pain scale correlate, but more research is needed to confirm the relationship. Also, the researchers reported high false-positive rates in the alpha amylase (Shirasaki, Fujii, Takahashi, Sato, Ebina, Noto, et al., 2007).

Overall, studies have demonstrated that pain is a serious side affect of cancer and, more specifically, head and neck cancer (Van Den Beuken-van Everdingen, et al., 2007; Grond, et al., 1996; Gil, et al., 2006; Chaplin, et al., 1999). Studies have been conducted to determine the stress levels of cancer patients and the relationship between these two factors. These correlations between pain and stress levels were found subjectively, and there has been no research articles found that use the newer method of measuring objective stress levels in surgical cancer patients using the non-invasive method of collecting saliva cortisol and alpha amylase levels. Thus, the goal of this study is to explore the relationship of pain and stress postoperatively in head and neck cancer patients who have undergone otolaryngological surgery by utilizing self-report questionnaires as well as saliva samples for alpha amylase and cortisol levels. This study will
expand on previous research studies and hopefully bring more understanding to this relationship of pain, stress, and the effects they have on one another.

Methods

This pilot study is a component of a larger, two year research study examining that compares the effects of two different dressing types on inflammation, epithelialization and pain for anterior thigh split-thickness skin graft (STSG) donor sites in head and neck cancer patients who have undergone otolaryngological surgery. This specific study utilizes the psychoneuroimmunological (PNI) framework and will measure the relationship between pain and stress using behavioral, psychological and biological measures. The sample consisted of 13 hospitalized head and neck cancer patients who had undergone otolaryngological surgery with a split-thickness skin graft at the Arthur G. James and Richard J. Solove Cancer Hospital at The Ohio State University Medical Center. Inclusion criteria included having otolaryngological surgery with an anterior thigh STSG, having a Glasgow Coma Score of 15, or 10 if the patient has a tracheostomy, no documented circulatory deficits and at least 18 years of age with the ability to read and write English. Exclusion criteria included patients unable to give independent consent, inability to complete a self reported pain scale, patients who were prisoners, active alcoholics, and taking medications such as steroids that affect the healing process. Consent to participate in the study was obtained from the patient preoperatively.

Patient pain levels at the site of the STSG were reported to the nurse every four hours and documented daily using the Numeric Rating Scale of 0-10 or the Wong-Baker FACES Scale for pain. Stress was measured on postoperative day five using the Distress Checklist when the dressing was removed form the graft site. Overall pain levels and anxiety levels, along with the type and dose of any pain or anti-anxiety medication given to the patient were collected at the
four data collection time periods by chart review. These doses of medications were calculated to a Morphine Equivalent Dose or Benzodiazepine Equivalent Dose to better describe and compare the amount of medication each patient had received. The Wong-Baker FACES Pain Scale, typically used for pediatric patients, consists of a series of faces progressing from a smiley face or a neutral face expression indicating no pain to a crying face indicating the most pain. The reliability of the FACES Pain Scale \( (r = 0.704, p < 0.001) \) and validity \( (r = 0.81 \text{ to } 0.95, p < 0.001) \) is strong and statistically significant when correlated with other measurements of pain in mature, hospitalized adult patients (Stuppy, 1998). The Distress Checklist includes a list of seven behaviors of stress observed by a trained healthcare provider or research personnel during a medical procedure. If the stressful behaviors are present, it is marked on the Checklist. A score of 0 indicates no distress and a score of 7 indicates extreme distress. Reliability of the Checklist is \( r = 0.90 \) between observers/raters. Inter-item reliability ranges from \( r = 0.26 \) to 0.60. The validity of behavioral distress is also significant when correlated among observers/raters \( (r = 0.74, p = 0.01) \) and self-reported distress \( (r = 0.35, p = 0.05) \) (Wells, 2003).

Saliva samples approximately one milliliter in volume are currently being collected to measure salivary cortisol and alpha amylase as a biomarker for stress. Collection times occur between 1900-2300 on postoperative days two and four and between 0700-1100 on postoperative days three and five using the non-invasive passive drool technique into a contaminant free container. The salivary cortisol and alpha-amylase will be analyzed using an enzyme immunoassay (EIA) with user instructions and reagents from the Salimetrics commercial kit.

A statistical analysis of all data included using descriptive measurements of frequency, standard deviation, median and mean for the demographic, pain, stress and anxiety data. Repeated measures analysis of variance (ANOVA) was conducted along with Pearson
Correlation for the demographic data, overall pain, site pain, anxiety and distress measurements. The specific correlations between the overall and STSG site pain ratings with anxiety levels and the Distress Checklist were of particular interest for the pilot study.

Results

The sample consisted of 13 patients, nine male and four female. The average age of the subjects was 66 with a range of 38-84 years of age. Twelve of the subjects identified as White, non-Hispanic when asked their ethnicity. Ethnic data was missing on one of the subjects. When asked about tobacco use, 71.4% of the patients reporting use, 50% reporting alcohol use, with data missing on one of the subjects. Additional demographic data collected included information regarding the body mass index, STSG donor site length, width and the type of dressing used.

Overall pain levels and pain at the STSG site were compared over the four data collection times. The mean overall pain level on day two was 2.436 (N=11, range of 0-9 out of 10); day three, mean 2.864 (N=11, range 0-7.3 out of 10); day four, mean 1.9 (N=8, range 0-3.3 out of 10); day five, mean 2.225 (N=8, range 0-7.5 out of 10). The mean STSG site pain level on day two was 0.896 (N=8, range 0-3.3 out of 10); day three mean 0.524 (N=8, range 0-2.7 out of 10); day four mean 2.042 (N=6, range 0-5 out of 10); day five mean 1 (N=7, range 0-4.5 out of 10).

The mean opioid medication administered for each four hour observation period, which was measured in Morphine Equivalent Dose in milligrams for day two PM was 10.250 (N=12, range 0-30); day three AM mean 8.864 (N=11, range 0-30); day four PM mean 15.375 (N=8, range 0-33); day five AM mean 13.75 (N=8, range 0-35). The overall pain levels reported were higher than the STSG site pain levels on days two, three and five. The STSG site pain reported was higher than overall pain on day four. However, pain rating differences were not statistically significant. The Morphine Equivalent Dose was also not significantly different over the four
time periods. Repeated measures Analysis of Variance (ANOVA) showed no significant difference in pain ratings over time among the patients. Figure 1 shows a comparison of the overall pain levels with the STSG site pain levels over the four data collection time points.

Figure 1. Mean pain ratings of overall pain vs. STSG site pain at the four data collection points from postoperative day 2 through postoperative day 5 (Error bars show standard error of the mean [SEM])

Anxiety levels and Benzodiazepine Equivalent Dose levels were collected on all four of the data collection days. The mean anxiety level on postoperative day two was 0.23 (N=13, range 0-3); day 3 mean 0 (N=13); day four mean 0.87 (N=8, range 0-7); day five mean 0.63 (N=8, range 0-5). The mean anxiety rating over all four time periods was 1.5 (range 0 to 7) on a scale of 0 to 10. The amount of anti-anxiety medications given to the patients postoperatively was measured by the Benzodiazepine Equivalent Dose. The mean Benzodiazepine Equivalent Dose on postoperative day two was 0 (N=13), day three mean 0 (N=11), day four mean 1.25 (N=8, range 0-10), day five mean 0.625 (N=8, range 0-5). The Distress Checklist used on
postoperative day five demonstrated an average rating of 0.1 with a range of 0-1 out of seven.
Repeated measures ANOVA demonstrated no significance in anxiety ratings between patients over time.

Pearson Correlations between the demographic data, overall pain ratings, STSG site pain ratings, Morphine Equivalent Dose, Benzodiazepine Equivalent Dose, anxiety, and the Distress Checklist showed no consistent, significant data between the pain and stress measures. The only significant correlation between overall pain and anxiety occurred on postoperative day two with $r = .665, p < 0.05$. A correlation between the mean postoperative day four anxiety and the mean day five STSG site pain occurred with $r = .853, p < 0.05$. Significant correlations between the mean overall pain ratings occurred between postoperative days two and three ($r = .935, p < 0.01$), postoperative days three and four ($r = .824, p < 0.05$) and postoperative days two and five ($r = .996, p < 0.01$). The mean Morphine Equivalent Dose for oral opioid medications was positively correlated with the mean overall pain ratings on postoperative day three ($r = .638, p < 0.05$) and postoperative day four ($r = .805, p < 0.05$). Postoperative days two and three showed a significant correlation among the mean STSG site pain ratings ($r = .987, p < 0.01$) as well as between postoperative days two and four ($r = .908, p < 0.05$).

Significant correlations between the mean overall pain ratings and mean STSG site pain ratings occurred on postoperative day four ($r = .819, p < 0.05$). Other significant correlations occurred between the Distress Checklist on postoperative day five when the dressing is removed and the STSG donor site area with $r = .874, p < 0.01$. In addition, the body mass index was negatively correlated with the age of the patients ($r = - .722, p < 0.05$) and the mean Morphine Equivalent Dose on postoperative day two was correlated with the weekly amount of alcohol consumption
(r = .704, p < 0.05). Table 1 demonstrates the significant correlations between the pain, stress and anxiety variables as well as the donor site area and the Distress Checklist on postoperative day five.

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Table 1. Correlations between Pain and Stress Measures. Table shows Pearson’s r values. D = Day, Pain and anxiety ratings are average ratings within a specific four hour time period. Bolded values: p < 0.05, bolded and underlined values: p < 0.01. a: Cannot be computed because one of the variables is constant.

Discussion

The results demonstrate that the STSG site pain ratings were lower than the overall pain ratings. This may be partly explained by Melzack and Wall’s gate control theory for pain. This theory explains why a smaller pain stimuli is barely felt, or blocked by a larger stimuli. In the case of the head and neck postoperative cancer patients, the overall pain experienced postoperatively from their surgical site becomes the main focus of their pain. Thus, the pain from the STSG donor site is hardly noticed. This is evident from the comparison of the patient’s overall pain ratings and the STSG site pain ratings. The STSG pain ratings on the Numeric
Rating Scale of zero to ten were low, compared to the higher overall pain ratings mostly from the surgical sites, which supports the gate control theory. Only postoperative day four showed a higher STSG site pain rating than the overall pain rating. Outliers in the data account for this finding. Due to the lack of clarity in the pain rating data collection sheets, overall pain levels may have been charted by the healthcare workers instead of the STSG site pain, since the outlying data was more consistent with the overall pain ratings on the other postoperative days. Finally, ANOVA showed no significant difference in pain ratings among the four time periods. A larger sample size may lead to significance between the subjects when analyzing pain ratings.

When analyzing the overall pain and STSG site pain ratings with the anxiety and distress ratings, no consistent, significant correlations were found. This is due to the small sample size as well as the lack of sensitive stress measures. The Distress Checklist was only used on postoperative day five and is an objective measure in which the healthcare provider observes for physical signs of stress including eye or mouth tension, making a fist or flinching. Distress ratings from the Checklist were low, with an average rating of 0.1 out of seven. Observed anxiety ratings were also low overall (mean 1.5 out of 10), but still higher than the Distress Checklist. During more recent data collection efforts, patients have been rating their anxiety levels from three to seven on a scale of ten when asked. This indicates that anxiety is present among this patient population, but the methods of obtaining stress and anxiety measures used in this study were not sensitive enough to detect actual patient anxiety levels. Utilizing a larger sample size, along with the use of a stress measure specifically for head and neck cancer patients would provide better assessment of stress levels in this patient population. Finally, implementing the collection of salivary cortisol and alpha amylase to the study would provide
biomarkers of stress levels patients may be experiencing, further increasing the accuracy and validity of the stress measurements.

Limitations to the study include the small sample size of thirteen patients, patients being discharged from the hospital early before the end of postoperative day five as well as variability of charting and documentation among healthcare workers. The inconsistency may be due to the generality of the data collection sheets, which led to inconsistencies among the data as well as missing data. Because of these limitations, this study contains many implications and suggestions to improve the pilot as well as the current parent study. Improvements to the pilot study include collecting the data earlier in the postoperative hospital stay. Beginning collection on postoperative day one instead of postoperative day two may help decrease the amount of missing data as a result of early patient discharge from the hospital. In addition, implementing the collection of saliva samples would provide a reliable biological measure of stress to the study that would strengthen the stress and anxiety results. It may also be beneficial to obtain a saliva sample from the patients prior to surgery to be used not only as a baseline, but to compare stress and anxiety levels both before and after surgery.

Improvements to the parent study include improving the consistency of charting and recording the data between healthcare providers to increase reliability and decrease the amount of missing data. This may be accomplished by adding specific guidelines as to the exact data that is needed and the times at which the documentation needs to be completed. In addition, improvements can be made to the data collection sheets as well. The demographic data questions should be more specific to obtain the most accurate description of the sample. For example, the questions regarding tobacco, drug and ETOH use should all be separate and have separate places to document the type and frequency used. Each patient diagnosis should also be
documented preoperatively, and the type of surgical procedure would also be beneficial to document in order to analyze any trends in pain and stress regarding the type of cancer and the extent of the surgical procedure. The data collection sheets for pain should include overall pain ratings as well as pain at the STSG site. Any pain medications and the specific dose given should also be placed on the form. Precise, consistent collection times for the pain levels should also be placed on the data collection forms. The data sheet used to describe the epithelialization of the STSG site should contain more specific characteristics of possible epithelialization that is coded with a wider range of numeric ratings. This would provide a more detailed description of the STSG site and as well as an easier statistical analysis. Finally, a new subjective measure of stress should be included in place of the Distress Checklist. Because the Distress Checklist is measured only by the observation of stress indicators, a patient may be stressed and anxious without demonstrating outward signs or characteristics. Simply asking the patients if they are experiencing stress or anxiety may be a more useful method, but the most reliable would be to develop or incorporate a tool that measures stress specifically for head and neck cancer patients.

Conclusion

Research has demonstrated that many cancer patients, including head and neck cancer patients, experience pain and stress throughout the cancer treatment process. Because limited research has been conducted among the head and neck cancer population, the continuation of research in this area is vital to continue to improve patient outcomes. Data from this pilot study indicate that acute postoperative head and neck patients do experience clinically significant levels of pain and stress, even though statistical significance was unable to be shown with the small sample size and limited methods of measurement. Lessons learned from the pilot study demonstrate the need for further research with this patient population regarding pain and stress.
levels, using more accurate instruments that are appropriate for this patient group and setting. In addition, research using the non-invasive method of saliva sampling to analyze salivary cortisol and alpha-amylase should be further evaluated and implemented in the parent study. Finally, it is recommended that improvements be made to the current parent study need to increase the validity and significance of its results.
References


