Evaluation of Stress related to Invasive Medical Procedures in Children With Cancer using Salivary α-Amylase Activity

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The purpose of this study is to investigate the usefulness of salivary α-amylase activity (sAA) as an index of stress related to invasive medical procedure in children with cancer. sAA and heart rate (HR) were measured before and after bone marrow aspirations or lumbar punctures in pediatric cancer inpatients. Child’s self-report, parents’ and physicians’ reports were also collected to evaluate children’s fear and distress during the procedure. As a result, sAA immediately before and immediately after procedure were significantly higher than 30 minutes before procedure and after recovery from sedation. HR showed no significant differences. Physician’s rating of child distress was significantly related with sAA. In this study, sAA was shown to sensitively reflect children’s physical and mental stress, and was measurable non-invasively and easily. It can be a useful index of stress related to invasive medical procedure in children with cancer.

Key Words: children with cancer, invasive medical procedures, stress, salivary biomarker, salivary α-amylase activity (sAA)

Introduction

In pediatric cancer treatment, invasive medical procedures such as bone marrow aspiration (BMA) and lumbar puncture (LP) are performed repeatedly and regularly. For children with cancer, these procedures which cause them great fear and distress may be a serious stressor. It has been reported that, if insufficient pharmacological or psychological intervention is conducted for reducing the fear and distress, several negative effects will develop on the child’s psychological well-being and subsequent medical procedures (Bruce, 2006; Chen, Zeltzer, Craske, & Katz, 2000; Cohen, Blount, Cohen, Ball, McClellan, & Bernard, 2001; Weisman, Bernstein, & Schechter, 1998). To provide an optimal intervention for each child, precise evaluation of child’s stress is essential. Thus, an accurate and practical method for measuring stress is needed (Blount, Simons, Devine, Jaaniste, Cohen, Chambers, & Hayutin, 2008).

It is said that the best method of evaluating stress related to medical procedure is patient’s self-report. However, in the case of children, such subjective evaluation is difficult due to limitations in understanding or communication. Therefore, objective methods such as assessment by parents or medical staffs, behavioral observations, and physiological measures (ex. heart rate, blood pressure, hormones in blood) are commonly used. However, these methods have problems respectively; assessment by parents or medical staffs do not always agree with the child’s own assessment; behavioral evaluation takes much time and effort; ways used for physiological evaluation are often invasive themselves and equipment for measurements can incur significant costs (Blount, Piira, Cohen, & Cheng, 2006; Franck, Greenberg, & Stevens, 2000). In other words, in addition to reliability and validity, simplicity, non-invasiveness of use, and availability in cost are required for measures which are used in clinical medical settings (Morison, Grunau, Oberlander, & Whitfield, 2001; Treadwell, Franck, & Vichinsky, 2002).

In recent years, several markers analyzable from saliva (salivary biomarkers) have gained attention
as a non-invasive index of stress. Salivary biomarkers have an advantage that, unlike blood, sampling does not cause stress. It allows repeated and long-term measurements in subjects (Granger, Kivlighan, Blair, El-Sheikh, Mize, Lisonbee, Buckhalt, Stroud, Handwerger, & Schwartz, 2006). In particular, Groza, Zamfir, and Lungu (1971) first reported transient elevation of salivary \(\alpha\)-amylase activity (sAA) levels as a marker for postoperative stress in pediatric patients. Other experimental researches have also demonstrated elevation of sAA during physical or mental stress, suggesting its possible role as a stress index that reflects the activation state of the sympathoadrenal medullary (SAM) system (Chatterton, Vogeslong, Lu, Ellman, & Hudgens, 1996; Granger, Kivlighan, el-Sheikh, Gordis, & Stroud, 2007; Nater & Rohleder, 2009; Nater, Rohleder, Gaab, Berger, Jud, Kirschbaum, & Ehlert, 2005). Further, a portable device for analyzing sAA, which enabled real-time measurements at patient’s bedside has been developed (Yamaguchi, Kanemori, Kenemaru, Takai, Mizuno, & Yoshida, 2004). Studies using this device in medical settings reported its utility for evaluating patient’s physical and mental stress (Takeda, Onishi, Yamaguchi, & Takeya, 2006; Takeda, Watanabe, Onishi, & Yamaguchi, 2008).

In the present study, to investigate the usefulness of sAA as an index of stress related invasive medical procedures in pediatric cancer patients, we examined relationships between sAA and traditional stress measures including heart rate (HR), child’s self-report, and parent’s and physician’s ratings.

### Method

#### Participants

The subjects were 10 children with cancer (3 males, 7 females), who had been admitted to the XXX Hospital. We excluded children diagnosed with the developmentally-disabled. Participants’ age at the start of the study ranged from 3 years 6 months to 15 years 4 months (mean = 9 years 2 months ± 4 years 2 months). Length of hospitalization ranged from 0.5 to 8 months (mean = 3.1 ± 2.9 months). Seven patients were diagnosed as acute lymphatic leukemia (ALL), 2 were lymphatic malignancy, and 1 was brain tumor (see Table 1).

#### Measurement and Protocol

When a participant received invasive medical procedure (BMA or/and LP), the following measurements and ratings were conducted (see Fig. 1). All procedures took place in a treatment room. Midazolam, ketamine (when required) and lidocaine in a dose appropriate to child’s age and weight were used to provide sedation and analgesia. Sedation was performed in the patient’s room with monitoring of oxygen saturation and HR.

Salivary \(\alpha\)-amylase activity. sAA was measured with a portable sAA monitor (\(\alpha\)-amy; Yamaha Motor Co. Ltd., Japan) consisting of a disposable test strip and an optical analyzer. This measurement was conducted at following four phases during medical procedure; (i) more than 30 minutes before (phase-i), (ii) immediately before (during skin antisepsis)(phase-ii), (iii) immediately after the procedure (within 10 min) (phase-iii), and (iv) after recovery from sedation.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age (year, month)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Hospitalization (month)</th>
<th>Procedures (times)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3y6m</td>
<td>F</td>
<td>Acute lymphatic leukemia (ALL)</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>4y1m</td>
<td>F</td>
<td>ALL</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>5y7m</td>
<td>F</td>
<td>ALL</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>6y5m</td>
<td>F</td>
<td>ALL</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>E</td>
<td>8y6m</td>
<td>M</td>
<td>Lymphatic Malignancy</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>F</td>
<td>11y1m</td>
<td>F</td>
<td>ALL</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>11y5m</td>
<td>F</td>
<td>Brain Tumor</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>11y7m</td>
<td>M</td>
<td>ALL</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>13y10m</td>
<td>F</td>
<td>ALL</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>J</td>
<td>15y4m</td>
<td>M</td>
<td>Lymphatic Malignancy</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>
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Measurement at phase-i was conducted before administration of sedative agents: phase-iv was conducted when patient was at recovery level “5=fully awake and responsive” as in Tobias, Phipps, Smith, and Mulhern (1992).

Heart rate. HR was measured using pulse oximeter (PULSOX 300; Konica Minolta Sensing, Inc., Japan). HR during saliva sampling (approximately one minute) was recorded and then averaged.

Child self report. The Wong & Baker’s faces scale (Wong & Baker, 1988) was used to evaluate child’s feelings of fear. Prior to the procedure, children were instructed to “select one from six faces that best shows how you feel now” in terms of fear. They were explained that smiling face (0) expresses “No fear at all” and frowning face (5) expresses “Very fearful.” This survey was administered once at phase-i, more than 30 minutes before the procedure.

Parent report. Parent ratings of child’s fear were obtained using a visual analog scale (VAS) (Blount, Bunke, Cohen, & Forbes, 2001). The VAS consisted of a 10-cm line anchored on the left with “No fear at all” and on the right with “Most fear possible.” At phase-iii (Immediately after the procedure), parents were asked to indicate their rating by marking a vertical mark along the line. The distance from the left anchor to the vertical mark served as the parent’s VAS score.

Physician report. Physician ratings of child’s distress during the procedure were obtained using a VAS (Blount et al., 2001), a linear scale consisted of a 10-cm line anchored on the left with “No distress at all” and on the right with “Most distress possible.” At phase-iii(immediately after the procedure), physicians were asked to indicate their rating by marking a vertical mark along the line. The distance from the left anchor to the vertical mark served as the physician’s VAS score.

Statistical Analysis

In this study, measurements were conducted during the medical procedures of the participants, and consequently it was difficult to unify the number of measurement times of participants. Therefore, with reference to Sievers, Yee, Foley, Blanding, and Berde (1991), the data obtained from a total of 29 procedures in the 10 participants (mean number of procedures 2.9±1.9) were individually processed as a single datum and used in the analysis in this study. Eleven procedures were LP only, 9 dates were BMA only, 7 procedures were BMA and LP , and 2 procedures were BMA and central venous catheter extraction. The average time required for a medical procedure was 25.4±10.3 minutes.

sAA and HR were log-transformed to approximate a normal distribution. These measures were analyzed using one-way ANOVA for repeated measures followed by a Tukey-Kramer HSD test. All reported results were corrected by the Greenhouse-Geisser procedure. Correlation between sAA and HR was assessed by Pearson’s correlation coefficient. In addition, we assessed whether children’s age affects their sAA and HR. For this, we divided participants into two groups; younger children (younger than 6 years of age) and older children (6 years or older). Comparison of mean value between groups was analyzed using Student t test.

Faces scale score, and parent’s VAS score was matched to physiological measures (sAA and HR) at phase-ii (immediately before the procedure). Physician’s VAS score was matched to these at phase-iii (immediately after the procedure) to determine whether relationships exist between children’s sAA
and HR, Faces Scale, and VAS score. Correlation analyses were conducted using Pearson’s correlation coefficient for VAS score, Spearman’s rank-correlation coefficient for Faces scale score.

All statistical analyses were performed using JMP 8.0 (SAS Institute, CA, USA) with \( p < .05 \) taken as statistically significant.

**Ethical Considerations**

This study was approved by the Ethics Committee at XXX Hospital. The purpose and protocol of the study was fully explained to participants and their parents in both spoken and written forms, and written informed consent was obtained from each participant and the parent.

**Results**

**Changes in sAA and HR during Invasive Medical Procedures**

Mean sAA of each phases was 12.0±5.7 (KU/L) at phase-i (more than 30 minutes before procedure), 30.8±25.8 (KU/L) at phase-ii (immediately before procedure), 53.1±45.8 (KU/L) at phase-iii (immediately after procedure), 15.6±8.8 (KU/L) at phase-iv (after recovery from sedation).

Mean HR of each phases was 100.7±19.0 (bpm) at phase-i (more than 30 minutes before procedure), 101.2±25.2 (bpm) at phase-ii (immediately before procedure), 106.8±28.2 (bpm) at phase-iii (immediately after procedure), 103.6±22.0 (bpm) at phase-iv (after recovery from sedation).

After sAA and HR were log-transformed to approximate a normal distribution, changes in sAA and HR were analyzed. As a result, sAA immediately before the procedure (phase-ii) was significantly higher \((p<.01)\) compared to more than 30 minutes before the procedure (phase-i). And sAA immediately after the procedure (phase-iii) was significantly higher \((p<.01)\) compared to more than 30 minutes before the procedure (phase-i) and after recovery from sedation (phase-iv). HR showed no significant changes between any measurement phase (see Fig. 2).

**Relationship between sAA and HR**

There was no significant correlation between sAA and HR except for immediately after the procedure (phase-iii; \( r = .479, p < .01 \)). However, after excluding the sample of younger children (3 participants, \( n = 10 \)), significant positive correlations were shown at the following three phases; immediately before the procedure (phase-ii; \( r = .625, p < .01 \)), immediately after the procedure (phase-iii; \( r = .667, p < .01 \)), and after recovery from sedation (phase-iv; \( r = .513, p < .05 \); see Fig. 3).

**Comparison of sAA and HR between Younger and Older Children**

We found no significant difference in sAA between younger and older children, whereas HR of younger children were significantly higher than the older children at all measurement phases (see Table 2).

**Correlation among Physiological Measures and Self, Parent, Physicians Rating**

We found significant positive correlations between physician’s rating of child distress (physician’s VAS score) and sAA \( (r = .415, p < .05) \) and HR \( (r = .549, p < .01) \) immediately after the procedure (phase-iii; see Fig. 2 Changes in Salivary \( \alpha \)-amylase Activity (sAA) (A) and Heart Rate (HR) (B) During Invasive Medical Procedure in Children With Cancer (mean±SD, \( n = 29 \))

Note. Data are presented as log-transformed value. **\( p < .01 \).
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Fig. 3 Correlation Between Salivary α-amylase Activity (sAA) and Heart Rate (HR) at; (a) Phase-i (more than 30 minutes before), (b) Phase-ii (immediately before procedure), (c) Phase-iii (immediately after procedure), and (d) Phase-iv (after recovery from sedation)

Note. The figure consists of data from older (≥6 years) children (n=19) and data are presented as log-transformed value.

Table 2 Comparison of Salivary α-amylase Activity (sAA) and Heart Rate (HR) Between Younger (n=10) and Older (n=19) Children

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Phase-i 30 min before</th>
<th>Phase-ii Immediately before</th>
<th>Phase-iii Immediately after</th>
<th>Phase-iv After recovery from sedation</th>
</tr>
</thead>
<tbody>
<tr>
<td>sAA</td>
<td>Younger</td>
<td>10.3 (7.1–14.9)</td>
<td>22.9 (9.7–49.7)</td>
<td>38.4 (15.5–95.3)</td>
<td>11.1 (7.1–17.4)</td>
</tr>
<tr>
<td></td>
<td>Older</td>
<td>11.4 (7.2–17.9)</td>
<td>22.8 (9.6–54.1)</td>
<td>30.1 (9.3–97.3)</td>
<td>14.9 (8.0–27.8)</td>
</tr>
<tr>
<td>HR</td>
<td>Younger</td>
<td>112.8* (97.8–130.1)</td>
<td>122.8* (104.3–144.6)</td>
<td>127.3** (104.8–154.7)</td>
<td>115.2** (95.7–138.6)</td>
</tr>
<tr>
<td></td>
<td>Older</td>
<td>90.7 (76.7–107.4)</td>
<td>85.6 (70.8–103.6)</td>
<td>91 (73.6–112.5)</td>
<td>93.7 (77.0–113.9)</td>
</tr>
</tbody>
</table>

Note. Data are an inverse transformation of mean and mean±standard deviation value after logarithmic conversion. Approximately 70% of data is within the standard deviation.

*p<.05; **p<.01.

Discussion

In the present study, we found significant change in sAA of pediatric cancer patients who took invasive...
There are some studies on relationships between sAA and state of the SNS including HR in adult subjects, but conclusions have not yet to reach a consensus (Granger et al., 2007). Though HR is often used as an index of stress, its basal level varies with age in children. Thus we need to take the effect of the child's age into consideration when examining a relationship between HR and other measures. In fact, we found a significant correlation between sAA and HR after excluding samples of younger children (younger than 6 years of age), whose HR is higher. We also found that HR level of younger children were significantly higher than the older children in every four phase, as in Jay, Elliott, Katz, and Siegel (1987). For sAA, by contrast, there was no significant difference between the groups. These results imply that by using sAA we can detect children's physiological reaction to stress which is difficult to catch by monitoring of HR. And, these facts indicate that sAA can be a more useful index of stress in children.

The Faces Scale is widely used for assessment of fear and distress in children because of its usability for clinical settings, and understandability for young children (Goodenough, Addicott, Champion, McInerney, Young, Juniper, & Ziegler, 1997). However, we found no relationships between the Faces Scale score and either sAA or HR. It has been reported that infants would have difficulty in ratings on five or six level scales (Carr, Lemanek, & Armstrong, 1998). In addition, another study presented that children's fear for medical procedures is at its peak in anticipatory phase for older children, while immediately before the procedure for younger children (Kazak, Penati, Boyer, Himelstein, Brophy, Waibel, Blackall, Daller, & Johnson, 1996). In this study, we conducted the examination of child self-report just once, at least 30 minutes before the procedure in consideration for burden on children. Our result could be explained by children's difficulty in rating scales or difference in rating protocols between this study and previous studies. Similarly, parent's rating of child fear was not related to sAA and HR. It has been documented that the ratings made by parents are different with those by children, because parents have tendency to judge child's condition subjectively (Chambers, Giesbrecht, Craig, Bennett, & Huntsman, 1999; Chambers, Reid, Craig, McGrath, & Finley, 1998; Manne, Jacobsen, & Redd, 1992). We inferred that our result may have

<table>
<thead>
<tr>
<th>Measure</th>
<th>sAA</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child self report (fear)</td>
<td>.262</td>
<td>-.272</td>
</tr>
<tr>
<td>Parent rating (fear)</td>
<td>-.327</td>
<td>-.947</td>
</tr>
<tr>
<td>Physician rating (distress)</td>
<td>.415*</td>
<td>.549**</td>
</tr>
</tbody>
</table>

Note. * Spearman's rank correlation coefficient.  
** Pearson's correlation coefficient.
been caused by this characteristic of parent's assessment. On the other hand, physician's rating of child distress was related to sAA and HR. Several studies reported that medical staffs tend to estimate child's distress based on obvious distress behaviors of the child, so they can make relatively objective assessments (Holdsworth, Raisch, Winter, Chavez, Leasure, & Duncan, 1997; Manne et al., 1992). It indicates that sAA could be an objective index of stress related to medical procedures in children.

It is said that the degree of stress response is affected by various factors such as severity of the stressor, subject's age, sex, temperament and cognitive appraisal of the stressor (Chen, Joseph, & Zeltzer, 2000; Morse, 1995). In case of stress related to medical procedures in children, the relationship between coping behavior and stress response is often emphasized (Manne, Bakeman, Jacobsen, & Redd, 1993; Schechter, Bernstein, Beck, Hart, & Scherzer, 1991). In addition, when we investigate characteristics of stress of children with cancer, the effect of medicines related to cancer treatment is required to be taken into consideration as well. Future research on sAA including these factors are required. Another important subject is psychological or educational intervention which mitigate child's stress related to pediatric cancer. In this study, we suggest that there is possibility that sAA could be useful for practical use in psychological or educational intervention for children with cancer to evaluate mental stress. Therefore, investigations on the utility of sAA to evaluate effectiveness of such interventions are also required.

**Conclusions**

The following results were revealed by this study. (1) sAA elevated significantly during invasive medical procedure in children with cancer. No significant change was seen in HR. (2) sAA and HR was correlated, representing that sAA was closely related to sympathetic activity. (3) sAA was related to their distress level rated by physicians. In summary, sAA reflected children's physical and mental stress sensitively. And it was measurable non-invasively and easily by using a portable analysis device. Thus, sAA might be a useful index of acute stress related to invasive medical procedures in children with cancer.

**Acknowledgment**

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**References**


