The effect of preoperative information in relieving anxiety in oral surgery patients.

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Running Title: Alleviation of anxiety during dentoalveolar surgery

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Abstract – Appropriate stress management of patients is essential for smooth running of invasive or surgical dental procedures conducted under local anaesthesia. **Objective:** The current study analyzed the effectiveness of pre-operative information provision for anxiety reduction during dentoalveolar surgery in patients with high or low trait anxiety. **Methods:** Patients scheduled for oral surgical procedures performed by six private dental practitioners were invited to participate in the study. They were randomly assigned to four groups who received the following pre-operative information: i) basic information only, ii) basic information with details of the operative procedures, iii) basic information with details of the expected recovery, and iv) basic information with details of both the operative procedures and recovery. The participants’ trait anxiety level was measured with the Depression Anxiety Stress Scales (DASS), then they were divided into high or low trait anxiety groups with the method of median-split on the basis of the DASS score. **Self-rated anxiety was recorded** immediately before, during and 10 minutes after the surgical procedures. **Results:** High trait anxiety subjects gave higher self-reported anxiety levels (repeated measures ANOVA, \( p < 0.05 \)). Pre-operative provision of details about the expected recovery only or details concerning both the operative procedures and recovery led to significant reduction in
self-reported anxiety among the participants throughout the procedure ($p < 0.01$). However, information on operative procedures led to anxiety reduction in low ($p < 0.05$) but not high trait anxiety participants. **Conclusion:** Provision of pre-operative information of the recovery process leads to significant anxiety reduction in all patients who undergo surgical/invasive procedures with local anaesthesia.

Key words: trait anxiety; dental anxiety; pre-operative information; oral surgery

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Anxiety is a prevalent phenomenon in dental practice (1). Vassend reported that among a sample of individuals of age 15 or above, 4.2% to 7.1% had high dental anxiety, and 60% had experienced at least one very painful dental visit (2). Such dental anxiety may result in patient's avoidance behaviors in the form of delaying making an appointment, canceling and failing to show up for booked appointments (1). These behaviors pose a significant impact on the dental health of the population (3, 4, 5). Preventive care or early intervention is in turn compromised and, as a consequence, oral health deteriorates (6). For the community, such avoidance behaviors may also cause a great loss in productivity and cast a huge burden on the medical and health sector (7).

Anxiety has been shown to be related to a person's perception of and tolerance for pain. Pre-operative anxiety is correlated with post-operative pain and anxiety, and lowers the threshold in pain perception (8). Patients with high dental anxiety also expect higher levels of pain (9). Dental anxiety, however, has a multifactorial aetiology (10) and research studies often failed to establish a significant correlation between the level of anxiety and the type of dental treatment to be performed (11). A report summarized 19 American studies published during the period 1954-2000 had shown that despite an observed increase in general anxiety of the US population with time, dental anxiety seems to have remained stable throughout the period of review (12).

Patients' anxiety reactions have also been shown to be a potent source of stress for dental
practitioners. These may in turn impair practitioners' performance in delicate and complex treatment procedures. It has been found that dentists consider dental treatment as technically superior when dental patients experience less distress (13). Anxious dental patients also require up to 20% more chair time than those who are calm and relaxed (14).

Psychologists have looked into cognitive factors underlying anxiety disorders (15). It has been suggested that several cognitive biases contribute to high anxiety: selective attention to threatening information, enhanced memory of threatening information, negative interpretations of ambiguous information, and a tendency to perceive a higher likelihood of negative events happening to oneself (16). Such cognitive biases may be removed or minimized by reducing the ambiguity in the event (17, 18).

Over the last two decades, psychological techniques have been increasingly applied to patients who are about to undergo noxious medical treatments, such as some dental procedures (19). Common methods involve providing patients with information about the upcoming procedures, teaching relaxation and coping techniques; as well as methods following the cognitive, behavioral and modeling approaches (7). Unfortunately, there is little agreement on the optimum strategies, despite all the severe consequences of dental anxiety (20).

Rosengarten (21) posted preparatory information to parents who were due to bring their children to dentist for the first time, which included a short booklet for the children. A brief introductory visit without any examination or treatment procedures followed.
This preparation resulted in more cooperation, yet it was not clear whether the booklet, the visit, or both were important for these children. Other studies failed to replicate the results. Compliance with appointment keeping was found improved yet there was little effect on the actual behaviour of the children (22, 23).

Baldwin and Barnes (24) compared the reactions of children who had extractions on the same day right after being informed about the treatment need versus those whose extractions were done with one week’s advance notice. Recovery was found to be quicker in those with prior notice. Janis (25) argued that patients who are informed about their operations are able to engage in what he called “the work of worrying”, a kind of inner preparation for the stress to come. Accordingly, patients could plan their coping methods and rehearse them mentally if they know what to expect.

Herbertt and Innes (26) varied the amount of information they gave to child patients about treatment procedures. Direct reduction of uncertainty with provision of more treatment information was shown effective in reducing dental anxiety. The relationship between anxiety and information was curvilinear: too much or too little treatment information resulted in higher anxiety. It has also been suggested that the recovery of some people is actually hindered by giving them more information than they wanted (27). It was suggested that unwanted information can override patients’ ways of coping, making coping more difficult for them.
Among the individual characteristics which warrant consideration, trait anxiety is a very important one. Trait anxiety refers to a relatively permanent and stable individual predisposition or proneness to anxiety, or a tendency to respond to stressful situations. State anxiety, in contrast, is the transient subjective feelings of tension, apprehension and increased autonomic activities. Trait anxiety is reactive and remains latent until activated by some form of stressor associated with a threat or danger (28). As a personality variable, it may be inferred from the frequency and intensity at which an individual experiences state anxiety. People with high trait anxiety may remain chronically in a generalized state of anxiety. They are more likely to develop clinical anxiety (29, 30), more vulnerable to stress and respond to a wider range of situations as dangerous or threatening (28). Trait anxiety has been shown to also affect the effectiveness of intervention procedures in other areas of anxiety research (31).

Lovibond and his colleagues found that ambiguity about situational factors biases individuals with high trait anxiety towards a more threatening appraisal of the environment; whereas those of low trait anxiety tend to appraise the same environment as less threatening. Nevertheless, individuals with different levels of trait anxiety all exhibit an appreciable reduction in state anxiety in response to unambiguous situational information (31, 32). To reduce situational ambiguity and therefore anxiety, provision of relevant information and clarification of the situation may help.

The present study looks into how provision of different categories of pre-operative
information may reduce dental anxiety among high and low trait anxiety adult patients in a real life dental setting. It is hypothesized that pre-operative information about the procedures and expected recovery leads to a better understanding of the treatment as well as a less ambiguous appraisal of the situation, and therefore reduces anxiety. Besides, the reduction in anxiety should be more distinct for high trait anxiety patients as their original anxiety level is higher. However, information about the operative procedures should have different effects on high and low trait anxiety patients. As the details of the treatment procedures are associated with frightening images like bloody scenes, surgical sites, and incisions of body tissues, it is possible that treatment details would provoke, rather than reduce anxiety in some patients, in particular, those with high trait anxiety.

Material and methods

Participants, grouping and dentists calibration

Six general dental practitioners took part in the study. The dentists were all educated and trained at the Faculty of Dentistry, the University of Hong Kong, with 7-9 years of general practice experience when recruited. The study was conducted within a four-week period during which recruitment notices were posted in the participating dentists’ waiting rooms. A verbal explanation was given to patients who were interested in participating in the study and who were to receive dental alveolar surgery involving alveolar bone removal. The target group
size was 200. Patients who agreed to participate were asked to read and sign a consent form which summarized the experimental procedures and highlighted their right to terminate the experiment at any time without detriment to their treatment. All participants should have no systemic diseases and were screened by SKSN before the surgery to ensure that they had no positive psychiatric history. Dental attendance pattern and previous dental surgery experience of the participants were also recorded.

The participants were assigned to one of the four groups using a random table. The groups were: i) N - receiving “basic information only”, ii) P - receiving “basic information with details of operative procedures”, iii) R - receiving “basic information with details of expected recovery” and iv) PR - receiving “basic information with details of both operative procedures and recovery”.

Clear briefing sessions with written materials were provided for the participating dentists and their clinical staff. Training was given to the clinicians on the exact content and proper delivery of the pre-operative information (N, P, R or PR) to the participants. The content of pre-operative information was calibrated against SKSN to minimize possible confounding effects such as difference in background training, practice experience and individual preference. All participating dentists reached consent on the surgical protocols so as to reduce inconsistencies as much as possible.
Assignment of low and high trait subgroups

The Depression Anxiety Stress Scales (DASS) were used to assess participants' level of trait anxiety. It is composed of three scales: anxiety, depression, and stress, each consisting of 14 items (33). These scales were designed to provide relatively pure measures of the related negative affective states of depression, anxiety and stress (34). The DASS has shown good convergent validity with other scales designed to measure selectively anxiety and depression. The correlation between the DASS Anxiety scale and the Beck Anxiety Inventory (35) is 0.81; and, the correlation between the DASS Depression scale and the Beck Depression Inventory (36) is 0.74 (34). The DASS has also shown good reliability with alpha values for the three scales between 0.84 and 0.91 (34). This instrument was translated, validated and successfully used in the Hong Kong Chinese population (37) and it was shown that, out of the 692 individuals surveyed, the mean (±SD) anxiety scale was 6.57 ± 5.39.

In this study, only the data of the anxiety (DASS anxiety) scale were analyzed. The scale focused on the physical or psychological indicators of anxiety (e.g., "I was aware of dryness of my mouth." and "I felt terrified."). The respondent indicated how often the item applied to him/her in the last one or two years (0=Not applicable at all, 3 = Always applies). The participants completed the DASS anxiety scale before receiving the pre-operative information.
The scaled score was the sum of the ratings of all 14 items measuring anxiety. A median-split of the DASS anxiety scaled scores (38) was used to assign participants to the high (H) or low (L) trait anxiety groups.

**Provision of pre-operative information**

According to the standard professional ethical requirements for dental practice, basic information including indications for and outcomes of the operation, scale of procedures, when and how to use analgesics for pain control and estimated duration of recovery were explained to all participants (N and other groups). Details of operative procedure (P group) included, for example, where to incise, how long the incision is, the bone to be removed or the tooth sectioned, and how the wound would be cleaned and closed. The information was presented in both verbal and standard pictorial formats in the form of simple illustrations so as to provide a clear explanation to the participants. Details of expected recovery and prognosis (R group) included, for example, the recovery process, when the swelling peaks and subsides, possible bruising on the face and when it resolves, chances of complications and what they are. All the pre-operative information was given by the dentist performing the surgical procedures immediately prior to the commencement of the surgical treatment.

*Self-reported anxiety score*
The immediately pre-, the intra- and the immediately post-operative anxiety levels were taken. **On each measurement**, participants were asked by an uninvolved dental surgery assistant to rate, on a 0-100 scale, their subjective anxiety at that moment, with 0 as none and 100 as most intense (39). Measurements were taken at seven time points: i) prior to the injection of local anesthetics (Pre-LA), ii) prior to the commencement of the surgical procedures, iii) 10, 20 and 30 minutes after the commencement of the surgical procedures, iv) at the completion of the surgery, i.e. right after placement of the last suture, and v) ten minutes after the completion of the surgery. The total time taken for the operation, starting from the surgical incision, was recorded using a stopwatch. After the surgery, participants were asked to describe their feelings and comment on what they had experienced to the dental surgery assistant who recorded the anxiety levels.

**Data Analysis**

One-way ANOVA were performed for continuous variables and Chi-Square test was performed for categorical variables to compare the group differences, including demographic data and trait anxiety levels, across subjects treated by the six different dentists and across the four groups provided with the four different formats of pre-operative information. Repeated measures ANOVA (40) was performed on the self-reported anxiety ratings. The between-subjects factors were the content of pre-operative information (4 levels) and trait
anxiety level (2 levels). The only within-subjects factor was time of measurement (7 levels). All post hoc comparisons were performed using the Tukey’s HSD test with a significance level of 0.05.

Ethics

The study was designed according to the Helsinki Declaration of 1964 as revised in 2002 (41). The project received approval from the Ethics Committee, Department of Psychology, the University of Hong Kong.

Results

A convenient sample of one hundred and ninety six subjects, who fulfilled the inclusion criteria, consented to participate. Four cases were dropped because no alveolar bone removal was needed in their surgery. A total of 192 individuals (90 or 47% female; 18-59 years, mean 35.2 ± 11.4 years) participated in the study. They were evenly distributed over the six general dental practitioners, i.e. 32 individuals for each practitioner. The high trait anxiety group consisted of 48 males and 48 females, while the low trait anxiety group had 54 males and 42 females. Statistical analyses of demographic data and trait anxiety scores (DASS anxiety) revealed no statistical significant difference amongst the patients recruited by the six dental practitioners.

One hundred and forty five (75.5%) participants underwent surgical procedure for
removal of tooth/root (including impacted third molars, impacted canines), 35 (18.2%) had periodontal surgery for root resection or crown lengthening procedures, and 12 (6.3%) had other minor surgical treatments such as surgical endodontic therapy. Of all participants, 118 (61.5%) had regular dental check-ups during the previous five years and 98 (51%) of them had previous experience of dental surgery or extraction. The surgical procedures in this study lasted from 30 to 49 minutes with a mean of $39.1 \pm 6.1$ minutes.

*No statistical significant difference was found between the demographic data and the trait anxiety levels across the four groups of subjects provided with different formats of pre-operative information (Table 1).* The mean trait anxiety score was $6.5 \pm 4.9$ with range from 3 to 30, while the mean scores for high (H, $7.9 \pm 2.4$) was significantly higher than that of the low (L, $5.2 \pm 2.0$) trait anxiety groups ($t(190) = 20.8, P < 0.0001$). With reference to the median value of the Hong Kong population, 6.0 (37), the groups in our study indeed had high and low levels of anxiety. In addition, the two groups were more than one standard deviation apart, suggesting that this difference should be of psychological significance.

Mean self-reported anxiety in various treatment groups at different time points are shown in Figure 1. The main effect of trait anxiety level was significant, $F(1,179) = 224.4, MSE = 442.3, P < 0.05$, so was the main effect of the content of pre-operative information, $F(3,179) = 56.2, MSE = 442.3, P < 0.05$. The interaction between trait anxiety level and the content of
pre-operative information was also significant, $F(3,179) = 3.91, MSE = 442.3, P < 0.05$.

High trait anxiety patients reported significantly higher level of overall self-reported anxiety (69.5 ± 13.5) than low anxiety trait patients (32.6 ± 12.1). For the main effect of the content of pre-operative information, post hoc test showed that the N group reported the highest level of overall self-reported anxiety (61.8 ± 21.0), followed by P group (53.4 ± 23.9), who in turn reported higher anxiety than the R and PR groups (45.5 ± 21.5, and 43.5 ± 18.5, respectively).

Post hoc analyses were conducted to further examine the interaction. Among the high trait anxiety participants, the mean anxiety scores of the R or PR groups were significantly lower than those receiving N or P information. No difference was observed between those receiving N and P information, or between those receiving R and PR information. Among the low trait anxiety participants, provision of P, R or PR information led to lower anxiety than participants receiving N information; and no difference was obtained between those receiving P, R or PR information. The results indicated that provision of details of operative procedures (P) worked for the low but not for the high trait anxiety participants (Figure 2).

The main effect of time of measurement indicated a significant decreasing trend in self-reported anxiety across time, $F(6, 1104) = 240.8, MSE = 20.3, P < 0.05$. No interaction was found between time of measurement and the other two independent variables. Self-reported anxiety was not different among the first three time points, i.e. pre-LA, commencement and 10
minutes into the operation, whereas all other pairwise comparisons were significant ($P < 0.05$, Figure 1).

**Discussion**

The present research was designed to examine the role of pre-operative information in reduction of adult dental anxiety and for the first time investigated the possibility of difference or bias of such reduction in patients with high or low trait anxiety. Clinically, the format of pre-operative information was designed as basic information (N), while the other three conditions, basic information plus operative procedures (P), basic information with details of expected recovery (R), and basic information with details of operative procedures and expected recovery (PR) provided extra information. In this study, anxiety was measured by self-reported anxiety level which is a common practice in human studies (32, 42, 43).

The subjects, despite convenient sampling, showed mean (and SD) DASS-anxiety scores comparable with the general population of the Hong Kong Chinese (37). A possible source of bias in the present study would be the patient pools of the different dentists/clinics, which would be unaffected by calibration of the dentists on their surgical procedures. Analysis of the characteristics of patients receiving treatments from the six dentists yet did not reveal any statistical significant difference. Therefore, the sampling and process of data collection as a whole should be regarded as adequate.
The results showed that for all participants, pre-operative provision of information regarding the post-operative expected recovery details (R) or operative procedural details with post-operative expected recovery details (PR) led to significant reduction in self-reported anxiety. Provision of procedural details (P) led to anxiety reduction in the low but not in the high trait anxiety participants.

Besides, self-reported anxiety throughout the surgical procedure was lower for the low than the high trait anxiety participants. Throughout the course of the surgical procedure, self-reported anxiety dropped towards the completion of the operation; and the rate of reduction was the same for the high and low trait anxiety participants and for various types of prior information.

The current findings are basically consistent with previous studies which have shown that reduction of ambiguity about the situation reduces the anxiety an individual would experience (32). This investigation, however, is the first to show that this is also the case under real-life dental practice situations. The provision of information about the operative procedure and the expected post-operative recovery helped improve the participants’ understanding of the operation and probably also reduced the ambiguity about the procedure which the participants were about to undergo. This was postulated to be the main reason why self reported anxiety was alleviated as a result of appropriate provision of pre-operative information.

The finding is different, however, to those of previous studies in that pre-operative
information of the details of the operational procedures per se failed to lower the anxiety of high trait anxiety patients. Supposedly, provision of relevant situational information, clarification of the situation and hence reduction of ambiguity about a potentially threatening event or situation should have a positive effect on the reduction of perceived anxiety as well as on the autonomic anxiety response in people of high or low trait anxiety (32). The lack of anxiety reduction among the high anxiety patients is attributable specifically to the vulnerability towards noxious and threatening information in such patients. The information included in the procedural details of the operation, like incisions of body tissues and bone removal, was rather fear-provoking. The intrinsic fear-provoking nature of these stimuli seemed to offset the effect of anxiety reduction produced by improvement of knowledge and understanding of the situation. For those high trait anxiety patients receiving information about the operative procedures and post-operative recovery, it was postulated that the effect in anxiety reduction due to information about the post-operative recovery probably out-weighed the fear provoked by the procedural details.

The differential impact of pre-operative information of the details of the operational procedures on high and low trait anxiety subjects is consistent with psychological theories of stress which maintain that that people are not very objective in their appraisals of potentially stressful events. Some people are more prone than others to feeling threatened by life's difficulties. One study of hospitalized patients awaiting surgery showed only a slight
correlation between the objective seriousness of a patient's upcoming surgery and the level of fear experienced by the patient (44). Studies also found that anxious, neurotic people report more stress than others (45), as do people who are relatively unhappy (46). Thus, stress lies in the eye (actually, the mind) of the beholder. People's appraisals of stressful events are highly subjective.

A logical conclusion of this theory is that if an individual's appraisal of a threatening situation can be altered, the stressful impact of the event can be reduced. That is, an event that is normally threatening can be rendered less so if the person's cognition of the event can be changed. The present study showed that it is possible to alter the appraisal with the provision of pre-operative information and therefore to reduce the anxiety arising from the stressful procedures of oral surgery.

The present study avoided the problem of poor ecological validity which has been raised against laboratory studies. It was suggested that laboratory models of anxiety may lack ecological validity as experimentally-induced "anxiety" does not have the emotional and cognitive connotations connected with "real-life" stressful situations. This might imply that a laboratory based study has little relevance for the understanding of naturally occurring anxiety (47, 48).

The real-life settings of dental surgery performed in dental clinics provided a rather ideal context for studying operation-related anxiety and evaluating various anxiety-reduction
techniques. Dento-alveolar surgery allows the investigation of naturally-occurring anxiety across all age groups, and nevertheless, within a semi-laboratory setting where many environmental variables can be rigorously controlled.

In this study, we only obtained evidence for biases of anxiety perception in patients of different levels of trait anxiety with self-report measures. No corresponding measures were taken on the autonomic responses. This limitation should not be ignored, as it could cast doubts on the functional significance of the cognitive biases observed. Therefore, it seems reasonable to suggest that further research is required to examine these issues before any conclusive statement can be made. In this regard, the psychophysiological approach has the virtue of providing direct assessment of the functional significance of cognitive biases with concurrent measurements of cognitive processes and physiological indices of anxiety.

The present study showed that the provision of pre-operative information can lead to significant anxiety reduction in high and low trait anxiety patients, except that the provision of pre-operative procedural details does not elicit any anxiety reduction among high trait subjects.

It can be concluded from the present study that the provision of pre-operative information should be decided in accordance with the trait anxiety of the subjects. For low trait anxiety patients, prior information should be provided in any one of the formats P, R or PR. In contrast, for high trait anxiety patients, only the formats R or PR should be provided in prior information to help reduce the anxiety.
In dental practice, it is difficult to determine trait anxiety level of a patient. Certainly, a
trait anxiety scale could be administered on patients who are due to receive dento-alveolar
surgery to assess their trait anxiety level. However, scoring and interpretation of the scale
would be an extra workload and requirement for the staff involved. Given this constraint, the
results of the present study suggest that provision of pre-operative information should better
involve either the details of the expected recovery or to combine those with the procedural
details. Provision of procedural details per se without providing information about the
expected recovery should be avoided.

Although anxiety reduction was observed in both high and low trait subjects, the lowest
mean self reported anxiety level in high trait anxiety patients, 59.5, was still very high
compared with the corresponding mean self reported anxiety level in low trait subjects, 27.6
(Figure 2). Further research should be carried out to examine if any other pre-operative
information or any other ways of presentation could help reduce the anxiety of high trait
anxiety patients.

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Table 1. Demographic data and trait anxiety levels of the subjects.

<table>
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<tr>
<th>Group*</th>
<th>N</th>
<th>P</th>
<th>R</th>
<th>PR</th>
<th>Statistics</th>
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<td><strong>Overall</strong></td>
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<td>( n )</td>
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<tr>
<td>Sex (male:female)</td>
<td>25:23</td>
<td>24:24</td>
<td>31:17</td>
<td>22:26</td>
<td>( \chi^2 _3=3.8 )</td>
<td>0.288</td>
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<tr>
<td>Age</td>
<td>33.3</td>
<td>37.6</td>
<td>34.7</td>
<td>35.2</td>
<td>( F(3,188)=1.2 )</td>
<td>0.308</td>
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<tr>
<td>Education (years)</td>
<td>10.8</td>
<td>10.4</td>
<td>10.2</td>
<td>10.3</td>
<td>( F(3,188)=0.9 )</td>
<td>0.458</td>
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<tr>
<td>Trait Anxiety score</td>
<td>6.2</td>
<td>5.9</td>
<td>6.9</td>
<td>6.8</td>
<td>( F(3,188)=0.2 )</td>
<td>0.882</td>
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<td>(DASS – anxiety)</td>
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<td><strong>Subgroups</strong></td>
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<td>High Trait Anxiety Group (H)</td>
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<td>( n )</td>
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<tr>
<td>DASS – anxiety</td>
<td>8.0</td>
<td>8.1</td>
<td>7.9</td>
<td>7.5</td>
<td>( F(3,92)=0.5 )</td>
<td>0.653</td>
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<td>Low Trait Anxiety Group (L)</td>
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<td>( n )</td>
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<td>25</td>
<td>23</td>
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<tr>
<td>DASS – anxiety</td>
<td>5.3</td>
<td>5.0</td>
<td>4.9</td>
<td>5.6</td>
<td>( F(3,92)=0.4 )</td>
<td>0.753</td>
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*N: basic information, P: basic information with details of operative procedure, R: basic information with details of expected recovery, PR: basic information with details of operative procedures and expected recovery.
Legend

Fig. 1. Self-report anxiety scores (0-100 Likert scale) throughout the course of surgical procedure in various pre-operative information groups. The first letter(s) of the label represent(s) pre-operative information given (N: basic information, P: basic information with details of operative procedure, R: basic information with details of expected recovery, PR: basic information with details of operative procedures and expected recovery), the second letter stands for trait anxiety (H: high trait anxiety, solid lines, and L: low trait anxiety, dotted lines). The main effects of trait anxiety level, content of pre-operative information, and their interaction were significant (repeated measures ANOVA, $P < 0.05$). Self-reported anxiety was not different among the first three time points.

Fig. 2. Overall mean self-reported anxiety of various treatment groups. Treatment groups - N: basic information, P: basic information with details of operative procedure, R: basic information with details of expected recovery, PR: basic information with details of operative procedures and expected recovery. Clear bars: high trait anxiety group, grey bars: low trait anxiety group. *Significant difference from N and P groups (Tukey's HSD test, $P < 0.05$). †Significant difference from N group ($P < 0.05$).