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ORIGINAL ARTICLE

Assessment of salivary amylase as a stress biomarker in pregnant patients

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ABSTRACT

Background: Chronic stress during pregnancy has been associated with worsened maternal and fetal outcomes. Acute stress immediately before spinal anaesthesia for caesarean section may contribute to hypotension. Therefore objective measures of acute stress may help identify women at risk of adverse outcomes. Salivary alpha-amylase is a stress biomarker that has so far been poorly investigated during pregnancy. The reference change value is the difference between two sequential results that must be exceeded for a change to be considered clinically relevant. Our first aim was to determine if salivary alpha-amylase increased in pregnant patients when subjected to the stress of transfer to the operating room. Our second aim was to determine if changes in salivary alpha-amylase were likely to be clinically significant by measuring reference change value in healthy volunteers.

Methods: In 15 pregnant patients undergoing planned caesarean section under spinal anaesthesia, salivary alpha-amylase, systolic blood pressure, heart rate, and immediate anxiety were measured on the morning of surgery on the ward and again in the operating room. The reference change value was calculated from 18 healthy volunteers.

Results: A median 220% increase in salivary alpha-amylase activity ($P = 0.0015$) and a 17% increase in systolic blood pressure ($P = 0.0006$) were observed between the ward and operating room. No changes of immediate anxiety or heart rate were observed. Reference change value was ±76% in volunteers and 13 of the 15 pregnant patients had a salivary alpha-amylase increase greater than the reference change value.

Conclusion: When pregnant women are taken to the operating room, a clinically and statistically significant increase in salivary alpha-amylase was observed. Further studies are required to define its clinical usefulness.

Keywords: Anxiety; Stress; Psychological; Saliva; Salivary alpha-amylases; Caesarean section

Introduction

Chronic stress during pregnancy is associated with worsened maternal and fetal outcome and poor pain relief during labour.[1–3] Moreover, acute stress immediately before spinal anaesthesia for elective caesarean section may contribute to the occurrence of hypotension.[1,4–6] Increased sympathetic activity before spinal anaesthesia, as shown by an increased low-to-high frequency ratio with off-line heart-rate variability analysis, is associated with hypotension; prophylactic treatment of hypotension guided by this ratio is effective.[4–6] Real-time, objective and non-invasive assessment of the level of acute stress may therefore help identify pregnant patients at risk of hypotension after spinal anaesthesia for caesarean section. Unfortunately, non-invasive assessment of stress during pregnancy is challenging. Self-administered questionnaires such as the Spielberger's State-Trait Anxiety Inventory (STAI) are unhelpful in uncooperative patients or where there are language or comprehension barriers, who represent up to 20% of patients in our institution.[7] Haemodynamic changes induced by stress, such as tachycardia, may be unreliable in pregnant patients due to attenuation of the chronotropic response to endogenous catecholamines and beta-adrenergic agents observed during pregnancy, as well as effects of patient medication.[8,9]

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Salivary components such as alpha-amylase (SAA) have recently gained interest as non-invasive indicators of body changes associated with stress. SAA, one of the principal salivary proteins secreted by highly differentiated epithelial acinar cells of the exocrine salivary glands following activation of beta-adrenergic receptors, is viewed as a measure of endogenous sympathetic activity. Since an association between SAA and low-to-high frequency ratio has been demonstrated,[10,11] it has been proposed as a stress biomarker in non-perioperative situations such as extreme sport activities or induced psychological stress.[12–15] Blunting of stress-induced increases in SAA, however, has been reported in the second and third trimesters of pregnancy when compared with non-pregnant patients and may make SAA unsuitable to assess stress during late pregnancy.[16] The first goal of this study was therefore to determine if SAA increases in pregnant patients submitted to the stressful environment of the operating room (OR) for a planned caesarean section under spinal anaesthesia.

To confer clinical relevance on any such changes in SAA activity, the difference between two sequential results that must be exceeded above the biological and analytical 'noise' must be determined. The reference change value (RCV) has been proposed as a means of comparing two consecutive biological measurements, and takes into account the variability related to the measurement techniques of SAA activity (analytical variability measured by the inter-assay coefficient of variation) and the intra-individual variability of SAA activity in patients (biological variability measured by the coefficient of intra-individual variation).[17,18] Analytical and biological variability values used to calculate RCV are obtained from a control population using standardized techniques of saliva collection, SAA measurement and a delay between two saliva samples similar to those used in the population of interest, in this case pregnant patients. The second goal of this study was therefore to calculate the RCV of SAA in healthy volunteers in order to determine if the change in SAA activity observed in pregnant patients exposed to the stress of the OR is greater than the biological and analytical variability, and hence clinically significant.

Methods

The study was approved by the Ethics Committee of Cochin Hospital, Assistance Publique-Hôpitaux de Paris, France. Informed consent was obtained from all patients. Fifteen American Society of Anesthesiologists status 1 or 2 women undergoing planned caesarean section under spinal anaesthesia were studied. Patients were fasted from midnight. Blood pressure, heart rate, anxiety and SAA activity were measured in the following sequence on the ward and in the OR. Anxiety was measured with the Spielberger's STAI form. This validated tool for self-reporting immediate anxiety comprises 20 statements related to the immediate anxiety state,[7] and includes statements such as "I feel calm" or "I am worried", to which the patient must select one of four responses: not at all, somewhat, moderately so or very much so. The score for the STAI-state changes as the context changes, and its completion takes about five min. The STAI score ranges between 20 and 80. Non-invasive blood pressure and heart rate were measured after at least 10 min bed rest with the patient lying supine with a 20° left tilt. The mean of three consecutive measurements was calculated.

A control group was composed of volunteers recruited from members of the medical staff of the Anesthesia and Biochemistry Departments of Bichat Hospital. To avoid stress related to working conditions, volunteers fasted from midnight, came to hospital on a day off, and samples were obtained in a quiet area after a 10 min rest.

Subjects were required to abstain from smoking, eating or drinking water for one h before salivary sampling; brushing teeth was not permitted for three h before sampling. No subject was on medication containing adrenergic agonists or antagonists. Saliva was collected into a Salivette® (Sarstedt, France) over a two-min period,[19] which was then centrifuged and the supernatant stored at −20°C until SAA assay. In pregnant patients, the first saliva sample was obtained on the day of surgery while the patient was on the surgical ward between 08:00 and 13:00 h, following which an 18-gauge intravenous catheter was inserted in the left hand. The second measurement was performed three h later in the OR immediately before spinal anaesthesia and after monitoring with non-invasive blood pressure, five-lead electrocardiogram and pulse oximetry was applied. In controls, two samples of saliva were obtained at three h intervals between 08:00 and 13:00 h. Salivary sampling was performed on all patients in the sitting position. Saliva was diluted 1:200 (vol:vol) in saline, and SAA activity measured with a colorimetric assay using 4,6-ethylidene-(G7)p-nitrophenyl-(G1)-D-maltoheptaoside as substrate on the Modular P® analyzer (Roche Diagnostics GmbH, Mannheim, Germany).[20] Imprecision was determined using two samples of healthy controls (mean 54 IU/mL and 139 IU/mL). For each sample, SAA was measured 10 times from 10 different salivary dilutions. The inter-assay coefficient of variation (CVa) for SAA in volunteers was 4% and 3% at 54 and 139 IU/mL, respectively.

Statistical analysis

Results are expressed as median [range] or number of patients (%). Comparisons of quantitative variables used the Mann–Whitney *U* test or the Wilcoxon test as appropriate. Associations between quantitative vari-