

Evaluation of Salivary Alpha-Amylase Levels for Determining Stress Variations in Patients Undergoing Spinal Anesthesia for Infra-Umbilical Surgery

Abstract

Aims and Objectives: To evaluate salivary alpha-amylase (sAA) levels for determining stress variations in patients undergoing spinal anesthesia for infra-umbilical surgery. **Materials and Methods:** One hundred and twenty subjects (age 18–65 years) planned for infra-umbilical surgery under spinal anesthesia were included and allocated to Groups A and B ensuring age and sex matching. In both groups, sAA levels (S1 to S4) were assessed sequentially at different times (E1 to E4). S1 and S2 were collected on the evening before surgery (E1) and in the preoperative room on the day of surgery (E2), respectively. Thereafter, in Group A, S3 and S4 were collected before (E3) and 15 min after spinal anesthesia (E4), following which intravenous Midazolam was given. In Group B, intravenous Midazolam was administered first, S3 was collected 5 min later (E3), spinal anesthesia was administered and S4 was collected after 15 min (E4). **Results:** In both groups, sAA levels showed a mild increase from E1 to E2 (not significant). Thereafter from E2 to E3 and E3 to E4, a significant sharp rise in sAA levels in Group A and a significantly acute decline in Group B was noted. Mean sAA levels in Group A were higher as compared to group B ($P < 0.005$) in E3 and E4. **Conclusion:** Sequential documentation of increase in sAA levels in our study, starting with the baseline levels, presents a comprehensive report of the stress that the patients experience during preoperative period and reinforces the need of anxiolytic before spinal anesthesia.

Keywords: Biomarkers, midazolam, preoperative stress, salivary alpha-amylase, salivary cortisol

Introduction

Stress is a ubiquitous phenomenon in our everyday lives and can be defined as any extrinsic or intrinsic stimulus that evokes a biological response. Body's physiological and psychological responses are activated in different ways to help the person to adapt to stressful situation.^[1] This compensatory response, also known as stress response, helps the body to cope with stressful situation.^[2] However, if the intensity of stress passes beyond a threshold, it has the potential to cause health problems.^[3]

The stress response system comprises the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, both of which act in a coordinated manner in response to activation by a stressor. ANS quickly promotes physiological changes, leading to the release of catecholamines into blood circulation. Activation of the

HPA axis, a hormonal system, culminates with the release of cortisol, its downstream hormone, from the adrenal cortex, minutes after activation. Activation of HPA and ANS induce dramatic changes in the constituents of secreted saliva, as a result of which salivary proteins, such as Alpha-amylase and Cortisol undergo corresponding changes. Salivary cortisol is the standard indicator of HPA axis activity,^[4] while ANS activity can be measured in humans using sAA as a surrogate marker. Changes in these salivary parameters sensitively reflect variations in stress levels and since saliva can be sampled noninvasively, these can be utilized as sensitive and reliable stress indicators. Cortisol reaches its peak in a longer time, shows diurnal variation, carry-over effect and is difficult to measure.^[5] This study plans to use salivary alpha-amylase (sAA) as noninvasive objective biomarker to sequentially document preoperative stress level

Shagufta Bano,
Renu Garg,
Meenu Agrawal¹,
Ruchi Agarwal²,
Ashutosh Kumar,
Praveen Prashant

Departments of Biochemistry,
¹Anaesthesia and ²Pathology,
Bhagat Phool Singh Government
Medical College for Women,
Khanpur Kalan, Sonapat,
Haryana, India

Submitted: 15-Feb-2021

Revised: 01-Jul-2021

Accepted: 06-Sep-2021

Published: 17-Nov-2021

Address for correspondence:

Dr. Renu Garg,
Department of Biochemistry,
Bhagat Phool Singh
Government Medical College
for Women, Khanpur Kalan,
Sonapat, Haryana, India.
E-mail: renu4garg@gmail.com

Access this article online

Website:
www.ijabmr.org

DOI:
10.4103/ijabmr.ijabmr_126_21

Quick Response Code:



How to cite this article: Bano S, Garg R, Agrawal M, Agarwal R, Kumar A, Prashant P. Evaluation of salivary alpha-amylase levels for determining stress variations in patients undergoing spinal anesthesia for infra-umbilical surgery. Int J App Basic Med Res 2021;11:253-7.

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variations in patients planned for infra-umbilical surgery. We plan to further validate our results by giving intravenous Midazolam at different points of time preoperatively and compare the respective sAA levels to generate objective evidence for the most appropriate time in preoperative period for administration of anxiolytics. It is assumed that not just surgical procedures, but the whole atmosphere of the operation theater and anesthetic procedure will induce stress in patients. We hope that sequential documentation will help in having a holistic understanding of the preoperative stress and assist in developing individualized treatment plans for the patient.

Materials and Methods

This comparative study was conducted in the Department of Biochemistry, (institute name removed for blinding) in collaboration with the Department of Anesthesia after approval from the Institutional Ethical Committee and in accordance with the Helsinki declaration. Patients in the age group of 18–65 years, admitted for infra-umbilical elective surgery under spinal anesthesia from July 2019 to February 2020, were enrolled for the study after taking written informed consent. Patients with expected unpredictable levels of preoperative stress such as those undergoing onco-surgery/emergency surgery and those on preoperative analgesic therapy or having any other associated systemic factor which may alter levels of stress biomarkers such as hormonal therapy, antidepressants, antipsychotics, anticholinergic drugs, and pregnancy were excluded from the study.

A total of 120 patients fulfilling the inclusion and exclusion criteria were included. Subjects were assigned to Group A and B by the anesthetist, ensuring matching of baseline parameters such as age and sex. In both groups, four saliva samples were collected at four different events (E1 to E4) for the estimation of sAA and samples were numbered (S1 to S4) corresponding to the events. For both groups, S1 and S2 were collected on the evening before surgery (E1) and in preoperative room on the day of surgery (E2), respectively. Thereafter in patients assigned to Group-A, S3 was collected on OT table before spinal tap (E3). Then, spinal anesthesia was administered and S4 was collected 15 min later (E4). This was followed by administration of I/V Midazolam (0.04 mg/kg diluted to a total volume of

5 ml) for intra operative anti-anxiety and sedative effect. In patients assigned to Group B, I/V Midazolam in the same dose was first administered on OT table before the spinal tap. After 5 min of the same, S3 was collected (E3). Spinal anesthesia was then administered and S4 was collected after 15 min (E4).

Specimen (saliva) collection and salivary alpha-amylase analysis

Strict methodological recommendations were used to collect saliva sample, so that factors that influence and add variance to saliva-based stress biomarker measurement outcomes may be avoided.^[6] After explaining the procedure to the patients, saliva was collected using a test strip placed carefully under the patient's tongue for exactly 30 s. It was then transferred to test tube containing pre-measured phosphate buffer and was analyzed for sAA on Erba-Transasia Semi Autoanalyzer.^[7] Appropriate internal and external quality controls were run before analyzing samples.

Statistical analysis

Statistical analysis was done using the Statistical Package for the Social Sciences software version 26 (SPSS Inc, Chicago, IL, U.S.A.). The collected data was analyzed using descriptive (mean \pm standard deviation for continuous and frequency for categorical variables) and inferential statistics (independent *t*-test and Chi-square test). Changes in sAA over the time were analyzed using Repeated Measures Analysis of Variance (ANOVA).

Results

Table 1 summarizes the variations in mean values of sAA at all four different time events in Groups A and B, respectively. The two groups were comparable with respect to age, sex, and all included patients underwent spinal anesthesia for infra-umbilical surgery.

A repeated-measures ANOVA was conducted to compare the sAA in both groups. Mauchly's test indicated that the assumption of sphericity had been violated, therefore the Huynh–Feldt corrected tests are reported. There was a significant interaction between time and group ($F [2.42, 285.65] = 145.1, P < 0.0001$). *Post hoc* comparisons indicated that there was no significant difference in sAA

Table 1: Variations in mean values (estimated marginal means reported) of salivary alpha-amylase from E1 to E4 in both groups

Characteristics	sAA (U/ml)		Mean difference (95% CI)	P
	Mean (SE)			
	Group A	Group B		
Event 1	158.36 (11.72)	159.37 (11.72)	-1.008 (-33.830-31.813)	0.95
Event 2	184.08 (15.56)	177.37 (15.56)	6.728 (36.858-50.315)	0.76
Event 3	258.37 (14.55)	113.88 (14.55)	144.487 (103.714-185.259)	0.001
Event 4	504.40 (16.51)	86.58 (16.51)	417.823 (371.572-464.074)	0.001

sAA: Salivary alpha amylase; SE: Standard error; CI: Confidence interval

level between the two groups at time E1 ($P = 0.95$) and time E2 ($P = 0.76$). There was a significant difference between the two groups at time E3 and E4, with Group A having higher sAA levels than Group B ($P = 0.001$ and $P = 0.001$, respectively) [Table 1 and Figure 1].

Event-wise percentage changes in sAA level in Group A and B [Figure 2] in both groups showed a mild increase from E1 to E2 (P value not significant). Thereafter from E2 to E3 and E3 to E4, a significant sharp rise in sAA levels in Group A and a significantly acute decline in Group B were observed. Mean sAA levels in Group A were higher as compared to Group B ($P < 0.005$) in E3 and E4.

Discussion

Recently there has been an increased focus on evaluating the impact of stress on specific health outcomes which may assist in planning timely interventions to improve quality of life in patients. Subjective tools based on patients' self-reporting in the form of psychological questionnaires, for example, state-trait anxiety inventory score and visual analogue score (VAS) have been conventionally utilized for stress evaluation. These, however, tend to be unreliable and compromised. On the other hand, biomarkers are quantifiable and reliable indicators of a physiological process. Body fluids such as plasma, serum, urine, and saliva are increasingly being used for stress assessment.^[3] However, invasive procedures such as venepuncture may induce mental stress^[8] and thus act as confounding factor. Noninvasive and easy to measure stress-responsive biomarkers are now being preferred as objective tools to predict and monitor stress both in research and in clinical practice.^[3,9] For our study, in the process of looking for a biomarker which is valid, noninvasive, and easy to collect, we did an extensive literature search and shortlisted three most commonly used biomarkers i.e. sAA, salivary cortisol and plasma catecholamines. After a lot of deliberation, we decided to use sAA as an established sensitive, reliable and valid biomarker for stress. Researchers have reported

an association between plasma catecholamines and sAA in both physical and mental stress, indicating the potential of sAA as a marker of ANS activity.^[10] An extensive meta-analysis by Batista *et al.*^[3] conferred salivary cortisol and sAA as efficient biomarkers for evaluating stress, emphasized their use to monitor and prevent stress-related pathologies and reported sAA to elicit a more sensitive response than cortisol. Due to minimally invasive nature of collection and the ease of analysis, sAA samples could be collected repeatedly in subjects of all age groups with minimal training.

Many studies have reported an increase in stress in response to varied nonsurgical situations such as mild-to-moderate exercises,^[11-13] sky diving,^[14] mental arithmetic test,^[15] noise exposure,^[16] Trier Social Stress Test,^[4,17] academic assessment stress,^[18] stressful videos viewing,^[19] etc. Studies have also evaluated variations in stress during surgery^[5,6,20-25] and during anesthesia.^[26] Guglielminotti *et al.*^[27] evaluated stress in pregnant females scheduled for caesarean section on being shifted to the operating room. In our study, to generate clear evidence of variations in preoperative stress levels, patients were divided into two groups; one received anxiolytic before spinal anesthesia and the other received it after spinal anesthesia but before the surgical procedure. The anxiolytic was advised in low dose to achieve conscious sedation, resulting in stress relief while providing the easily controllable level of sedation, anterograde amnesia, rapid and clear-headed recovery and with no side effects.^[28,29]

A mild increase in sAA level was observed in both groups on being shifted to the preoperative room from the comfort of the ward. This shot up sharply in Group-A indicating an acute rise in stress as he/she faced the operation theatre environment and underwent spinal tap. Contrastingly, in Group B, there was a sharp fall in sAA levels after anxiolytic administration. It is an interesting conclusion that stress in the preoperative period, as evident by sAA levels, increased significantly on being shifted to the operative room and still more before receiving spinal

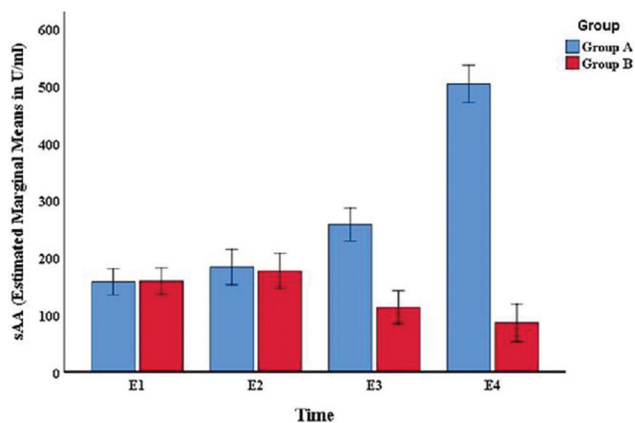


Figure 1: Comparison of salivary alpha amylase levels in the study participants of both groups at the time points E1, E2, E3, and E4

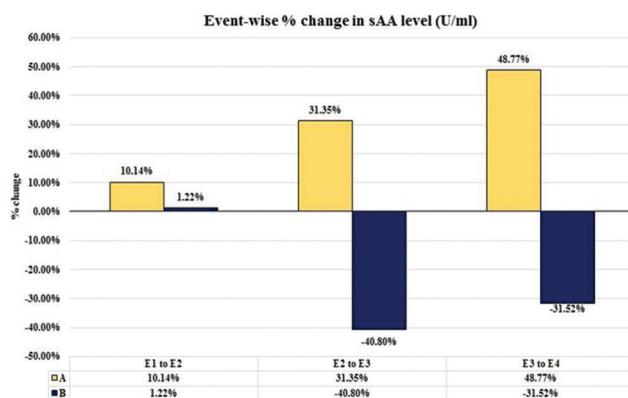


Figure 2: Comparison of event-wise percentage change in salivary alpha-amylase levels in study participants of both groups

anesthesia, thus reinforcing the fact that not just the surgical process, but acute stress of spinal tap, impending surgery or related environmental variables are equally stressful to patients. Our results also revalidate and reinforce the most appropriate time for giving an anxiolytic to be, not just before the surgical procedure but, before the spinal tap (anesthetic procedure) itself.

Evaluation of stress, that the patient is undergoing, is important to be assessed, as high anxiety in the preoperative phase may not only increase dissatisfaction but also prolong the duration of the procedure, increase the risk of complications and requirement of sedative/analgesic drugs.^[30] At this point, we argue that documentation of stress only in the operative room, as done in many studies, lacks evidence of baseline sAA activity which varies from patient to patient. Sequential documentation of stress levels starting from the day before surgery in the ward and ending with spinal anesthesia helped us to comprehensively evaluate the stress that the patient went through.

We agree that despite ensuring matching of baseline parameters such as age and sex, some other variables might still act as confounding factors, and this is a definite limitation of our study. To the best of our knowledge, this is the first study of its kind where the progressive stress experienced by the patient consecutively at four different times preoperatively, including the baseline levels, was measured using objective marker in a comparative manner. We emphasize that sequential documentation of stress levels, through a noninvasive technique like sAA levels, can help in individualizing treatment plans for the patients and to improve the overall treatment outcome.

Conclusion

In this study, sequential documentation of sAA, starting with the baseline levels, comprehensively represents stress that the patients experience during the preoperative period. This includes the stress of spinal tap, impending surgery, or related environmental variables. Our results reinforce the most appropriate time for giving an anxiolytic; not only before the surgical procedure but before the spinal tap. We hope that an all-inclusive evaluation of preoperative stress may help in optimizing patient care.

Ethical clearance

BPSGMCW/RC389/IEC/18 dated 22.11.18.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Ali N, Nater UM. Salivary alpha-amylase as a biomarker of stress in behavioral medicine. *Int J Behav Med* 2020;27:337-42.
2. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: A review. *EXCLI J* 2017;16:1057-72.
3. Batista P, Pereira A, Vaz AB. Salivary biomarkers in psychological stress diagnosis. *ARC J Pharm Sci* 2017;3:9-18.
4. Petrakova L, Doering BK, Vits S, Engler H, Rief W, Schedlowski M, *et al.* Psychosocial stress increases salivary alpha-amylase activity independently from plasma noradrenaline levels. *PLoS One* 2015;10:e0134561.
5. Uesato M, Nabeya Y, Akai T, Inoue M, Watanabe Y, Kawahira H, *et al.* Salivary amylase activity is useful for assessing perioperative stress in response to pain in patients undergoing endoscopic submucosal dissection of gastric tumors under deep sedation. *Gastric Cancer* 2010;13:84-9.
6. Minowa C, Koitabashi K. Salivary alpha-amylase activity – An indicator of relaxation response in perioperative patients. *Open J Nurs* 2012;2:208-14.
7. Lorentz K. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes part 9. IFCC method for α -amylase (1,4- α -D-Glucan 4-Glucanohydrolase, EC 3.2.1.1). *Clin Chem Lab Med* 1998;36:185-203.
8. Koh D, Ng V, Naing L. Alpha amylase as a salivary biomarker of acute stress of venepuncture from periodic medical examinations. *Front Public Health* 2014;2:121.
9. Cozma S, Cozma LC, Ghiciuc CM, Pasquali V, Saponaro A, Patacchioli FR. Salivary cortisol and α -amylase: Subclinical indicators of stress as cardiometabolic risk. *Braz J Med Biol Res* 2017;50:e5577.
10. Hensten A, Jacobsen N. Salivary alpha amylase as a stress biomarker. *OSP J Dent Sci* 2019;1:JDS-1-103.
11. Chatterton RT Jr., Vogelsong KM, Lu YC, Ellman AB, Hudgens GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 1996;16:433-48.
12. Allen, Shaelee, "Salivary Alpha-Amylase as an Indicator of Body Stress Following an Acute Session of Repetitive Jumping" (2014). UNLV Theses, Dissertations, Professional Papers, and Capstones. 2055. Doi: <http://dx.doi.org/10.34917/5836074>.
13. Gallina S, Di Mauro M, D'Amico MA, D'Angelo E, Sablone A, Di Fonso A, *et al.* Salivary chromogranin A, but not alpha-amylase, correlates with cardiovascular parameters during high-intensity exercise. *Clin Endocrinol (Oxf)* 2011;75:747-52.
14. Chatterton RT Jr., Vogelsong KM, Lu YC, Hudgens GA. Hormonal responses to psychological stress in men preparing for skydiving. *J Clin Endocrinol Metab* 1997;82:2503-9.
15. Noto Y, Sato T, Kudo M, Kurata K, Hirota K. The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: A pilot study. *Anesth Analg* 2005;101:1873-6.
16. Walker ED, Brammer A, Cherniack MG, Laden F, Cavallari JM. Cardiovascular and stress responses to short-term noise exposures – A panel study in healthy males. *Environ Res* 2016;150:391-7.
17. Nater UM, Marca RL, Florin L, Moses A, Langhans W, Koller MM, *et al.* Stress-induced changes in human salivary alpha-amylase activity – Associations with adrenergic activity. *Psychoneuroendocrinology* 2006;31:49-58.
18. Ouda S, Alaki S, Safi MA, Nadhreen A, Johani KA. Salivary stress biomarkers – Are they predictors of academic assessment exams stress? *J Clin Exp Pathol* 2016;15:276-9.
19. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K, Nishikawa Y. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Arch Oral Biol* 2004;49:963-8.

20. Noorani H, Joshi HV, Shivaprakash P. Salivary alpha amylase as a noninvasive biomarker for dental fear and its correlation with behavior of children during dental treatment. *Int J Clin Pediatr Dent* 2014;7:19-23.
21. Robles TF, Sharma R, Park KS, Harrell L, Yamaguchi M, Shetty V. Utility of a salivary biosensor for objective assessment of surgery-related stress. *J Oral Maxillofac Surg* 2012;70:2256-63.
22. Jakushenko N, Nagobade D, Mihelons M, Kopeika U, Putnina A. Comparison of stress assist to saliva amylase in intubations with the GlideScope videolaryngoscope, Macintosh laryngoscope and fiberoptic bronchoscope. *Eur J Anaesth* 2008;25:247.
23. Ertürk EB, Ünlü H. Effects of pre-operative individualized education on anxiety and pain severity in patients following open-heart surgery. *Int J Health Sci (Qassim)* 2018;12:26-34.
24. Chowdhry V, Padhi M, Mohanty BB, Biswal S. Perioperative challenges in management of a deaf and dumb patient posted for high-risk cardiac surgery. *Ann Card Anaesth* 2016;19:564-7.
25. Ozalp G, Sarioglu R, Tuncel G, Aslan K, Kadiogullari N. Preoperative emotional states in patients with breast cancer and postoperative pain. *Acta Anaesthesiol Scand* 2003;47:26-9.
26. Mingir T, Ervatan Z, Turgut N. Spinal anaesthesia and perioperative anxiety. *Turk J Anaesthesiol Reanim* 2014;42:190-5.
27. Guglielminotti J, Dehoux M, Mentré F, Bedairia E, Montravers P, Desmots JM, *et al.* Assessment of salivary amylase as a stress biomarker in pregnant patients. *Int J Obstet Anesth* 2012;21:35-9.
28. Kamata K, Hagihira S, Komatsu R, Ozaki M. Predominant effects of midazolam for conscious sedation: Benefits beyond the early postoperative period. *J Anesth* 2010;24:869-76.
29. Patki A, Shelgaonkar VC. A comparison of equisedative infusions of propofol and midazolam for conscious sedation during spinal anesthesia – A prospective randomized study. *J Anaesthesiol Clin Pharmacol* 2011;27:47-53.
30. Gürbulak B, Üçüncü MZ, Yardımcı E, Kırılı E, Tüzüner F. Impact of anxiety on sedative medication dosage in patients undergoing esophagogastroduodenoscopy. *Wideochir Inne Tech Maloinwazyjne* 2018;13:192-8.