Journal of Cardiovascular Disease Research

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

Assessment of Salivary Alfa Amylase (SAA) and Salivary Cortisol level in in Patients Undergoing Spinal Anesthesia for Infra-Umbilical Surgery

Ashutosh Kumar,* Research Scholar Medical Biochemistry, Malwanchal University (Index Medical College Hospital & Research Centre, Indore)

Dr Shreya Nigoskar, Prof & Head Dept. of Biochemistry, Malwanchal University (Index Medical College Hospital & Research Centre, Indore)

Dr Ruhi Charak, Assistant Professor, Department of Biochemistry, National Institute of Medical Sciences, Jaipur Rajasthan.

Dr Rimpy Charak, Assistant Professor, Department of Biochemistry, Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur, Rajasthan.

Corresponding Author*
Address- Ashutosh Kumar, Research Scholar Medical Biochemistry, Index Medical College

Hospital & Research Centre, Malwanchal University, Indore. Phone: 9992735517

Email: ashu16989@gmail.com

Introduction

An outside or inside stimulus that causes a biological reaction can be called stress. The way our bodies react to these stresses is called the stress reaction.[1] As part of the body's response to injury, this includes a lot of different biochemical, endocrine, immunological, and hemodynamic affects.[2] Injured tissues can stay alive by breaking down their own stored body fuels as part of the stress reaction. After an operation, the body goes through a series of changes that are called the metabolic reaction to injury. These changes have a big effect on the body's fluids and electrolytes.[3] It is possible for stress to turn on or off many hormonal processes that are connected to the gonads, thyroid, adrenal glands, hypothalamus, and the adrenergic system. An incredibly little quantity of stress can turn on the hypothalamic-pituitary-adrenal axis, which in turns activates a variety of distinct hormone-secreting systems.[3] Several different ways in which stress can have an effect on the body include the following: increased levels of stress hormones can cause the pituitary gland to release more hormones and the sympathetic nervous system to become more active.[2] Alterations in pituitary secretion have the ability to exert downstream effects on the release of hormones from organs designated as targets. The levels of inorganic electrolytes, such as sodium, potassium, chloride, and calcium, see an increase when the body is under stress. While the concentration of transferrin, albumin, zinc, and iron in the serum decreases, the concentration of copper and transport proteins in the serum can be found to increase.[4] High blood glucose levels are a common consequence of decreased

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

insulin synthesis, which occurs frequently in the presence of stressful situations. Both hepatic glycogenolysis and gluconeogenesis are increased when stress hormones like cortisol and catecholamines are present.

Stress and salivary glands

The salivary glands are responsible for the production of a fluid that is rich in bicarbonate and features digesting and protective proteins, among other components. This fluid is then discharged into the gastrointestinal system. It is the autonomous nervous system that is accountable for the stringent management of its operating procedures. The secretion of fluids and electrolytes in saliva is mostly controlled by parasympathetic activity, whereas the release of proteins is primarily driven by sympathetic stimulation.[5] The hypothalamic-pituitary-adrenal axis is activated when we are under stress. The stress response system consists of the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis, which function in a coordinated way when activated by a stressor. The autonomic nervous system rapidly induces physiological changes, resulting in the secretion of catecholamines into the bloodstream. The activation of the HPA axis, a hormonal system, results in the release of cortisol, its downstream hormone, from the adrenal cortex within minutes of activation. The activation of the HPA axis and ANS precipitates significant alterations in the composition of produced saliva, leading to matching modifications in salivary proteins, including Alpha-amylase and Cortisol.[6] Salivary cortisol serves as the primary indication of HPA axis activity, but ANS activity in humans may be assessed using Salivary alfa amylase as a proxy marker. Alterations in these salivary characteristics accurately indicate fluctuations in stress levels, and due to the non-invasive nature of saliva sampling, they can serve as sensitive and dependable markers of stress.[7] So in this study we use salivary alpha amylase and salivary cortisol as a biomarker for Pre-operative and intraoperative stress in patients undergoing infra- umbilical elective surgery with spinal anaesthesia.

This study aims to correlate salivary alpha-amylase (SAA) and salivary cortisol level in in Patients Undergoing Spinal Anaesthesia for Infra-Umbilical Surgery. We intend to further validate our findings by administering intravenous Midazolam at various preoperative intervals and comparing the corresponding SAA levels and salivary cortisol levels to provide objective evidence for the optimal timing of anxiolytic administration. It is posited that not only surgical procedures but also the overall environment of the operating theatre and aesthetic processes will elicit stress in patients. We anticipate that sequential documentation will facilitate a comprehensive understanding of preoperative stress and aid in formulating individualized treatment plans for patients.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

Materials and Methods

The study was conducted at the Department of Biochemistry and Anaesthesia Index Medical College and Research Centre, Malwanchal University, Indore, for three years after ethical approval. The study involved 240 patients aged 18 to 65 who underwent elective infra-umbilical surgery under spinal anaesthesia. The sample size was 120 patients each in two groups (a total of 240 patients). Patients were divided into two groups: Group A, who underwent spinal tap and then received I/V Midazolam for intra-operative sedation, and Group B, who received I/V Midazolam and then spinal tap. The study excluded patients undergoing onco-surgery, emergency surgery, pre-operative analgesic therapy, pregnant patients, patients with endocrine disorders, severe systemic disorders, renal pathology, antidepressants, antipsychotic drugs, and those who refused spinal anaesthesia or failed it within 15 minutes. Patients were informed about the process of taking saliva samples for measuring salivary alpha amylase and cortisol activity and given written consent.

Saliva sample collection is a non-invasive technique used to assess patients for disorders or drug history. The collection of samples using a Whatman No.1 filter paper strip placed under the patient's tongue for 30 seconds. Then the strip was transferred to a test tube containing 3ml phosphate buffer, the supernatant is used for the SAA.

Sample Collection: 1st Sample are collected on the evening before surgery, with vital parameters recorded. For evaluation of anxiety, the State Trait Anxiety Inventory scale (STAI-s) was utilized for the purpose of assessing stress and anxiety. This scale was utilized by asking respondents how they were feeling at that particular time. This was done in order to examine components of anxiety proneness that are typically consistent, such as overall states of tranquillity, confidence, and security. In terms of the score, the lowest and maximum values are respectively 20 and 80.

2nd Samples are taken in the morning, after the patient is shifted to the pre-operative room. All patients were counselled about O.T atmosphere, procedure being done and due assurance was given by the anaesthetist.

3rd Sample: Patients divided into two group. Group A received normal saline, while Group B received Midazolam. After 5 minutes, vital parameters were recorded, and salivary sample was taken.

4th Sample: After spinal tap, vital parameters and saliva samples collected. Patients in Group A received Midazolam for intra-operative sedation, while Group B received Normal saline.

Salivary Alpha Amylase (sAA) was analysed on EM- 360 fully autoanalyzer. Appropriate internal and external quality controls were run before analysing samples.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

Statical Analysis

Version 24 of the Statistical Package for the Social Sciences (SPSS) software was used for statistical analysis. Descriptive statistics (mean ± standard deviation for continuous variables and frequency for categorical variables) and inferential statistics (independent t-test and Chi-square test) were used to examine the gathered data.

Results

Sample	**Group A** (Mean ± S.D (U/ml)	**Group B** (Mean ± S.D (U/ml)	**p- value**
Sample 1	158.90 ± 77.50	160.45 ± 100.20	0.952
Sample 2	182.50 ± 136.45	178.60 ± 101.80	0.761
Sample 3	260.35 ± 152.10	114.85 ± 42.00	0.001
Sample 4	505.80 ± 178.80	88.70 ± 21.50	0.001

Table 1: Comparison of study subjects of both groups according to SAA level.

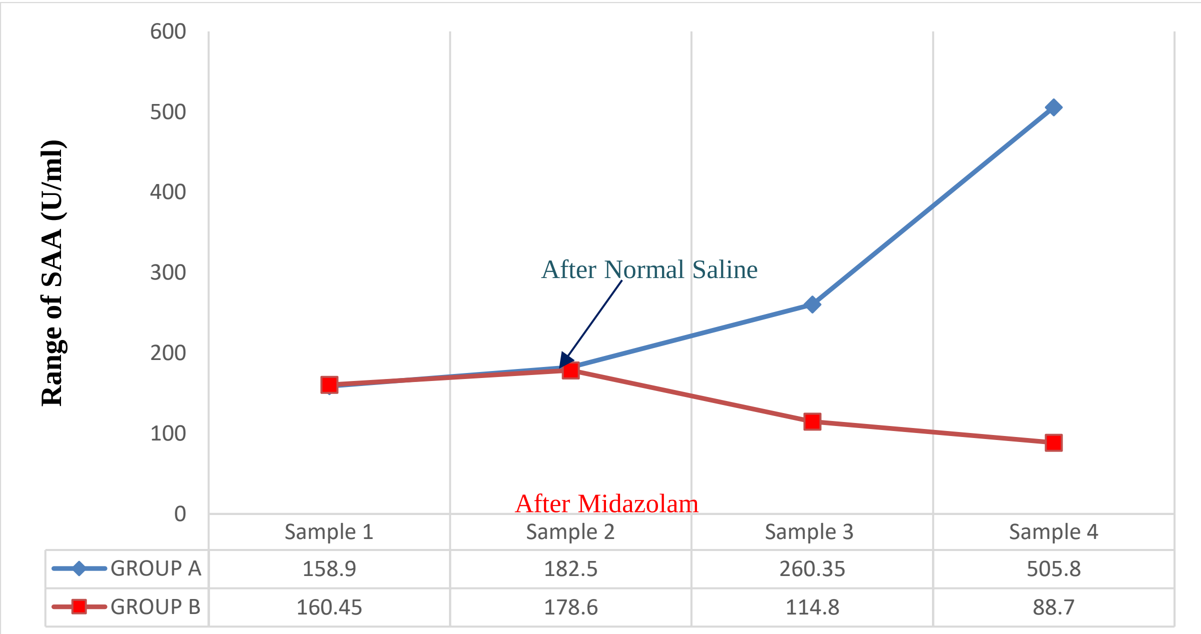

Figure 1: Comparison of study subjects of both groups according to SAA level

Mean difference of SAA in Sample 1 and Sample 2 in Group A and B was found to be statistically not significant (p>0.05). However, in Sample 3, the difference in mean SAA level in Group A (**260.35 ± 152.10 U/ml**) and in Group B (**114.85 ± 42.00 U/ml**) was statistically highly significant (p<0.005). Similarly, in Sample 4, the mean SAA levels in Group A and

Group B were **505.80 ± 178.80 U/ml** and **88.70 ± 21.50 U/ml**, respectively, showing the difference to be statistically highly significant ($p<0.005$) (**Table 1** and **Figure 1**).

Sample	**Group A** Mean ± S.D	**Group B** Mean ± S.D	**p- value**
Sample 1	18.50 ± 2.30	17.80 ± 2.10	0.120
Sample 2	21.85 ± 3.50	19.75 ± 3.10	0.032
Sample 3	27.56 ± 2.80	15.57 ± 2.20	0.001
Sample 4	36.63 ± 4.50	13.21 ± 2.05	0.001

Table 2: Comparison of study subjects of both groups according to Salivary Cortisol level.

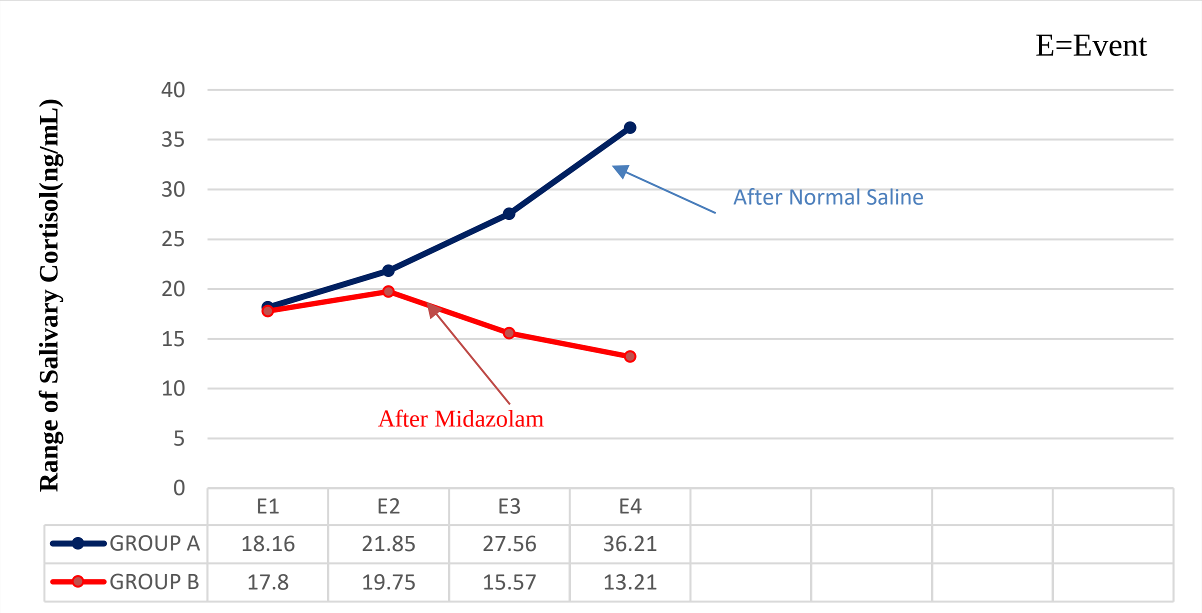

Figure 2: Comparison of study subjects of both groups according to STAI score.

Mean difference of Salivary cortisol in Sample 1 and Sample 2 in Group A and B was found to be statistically not significant ($p>0.05$). However, in Sample 3, the difference in mean Salivary Cortisol level in Group A (**27.56 ± 2.80 ng/mL**) and in Group B (**15.57 ± 2.20 ng/mL**) was statistically highly significant ($p<0.005$). Similarly, in Sample 4, the mean Salivary cortisol levels in Group A and Group B were **36.21 ± 4.50 ng/mL** and **13.21 ± 2.05 ng/mL**, respectively, showing the difference to be statistically highly significant ($p<0.005$) (**Table 2** and **Figure 2**).

Discussion

In recent times, there has been a heightened emphasis placed on the evaluation of the influence that stress has on certain health outcomes. This evaluation has the potential to aid

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

in the development of timely treatments that aim to enhance the quality of life of patients. Subjective techniques, which are based on patients' self-reporting and are presented in the form of psychological questionnaires, have traditionally been applied for the purpose of stress evaluation. Some examples of these instruments are the State-Trait Anxiety (STA) inventory score and the Visual Analogue Scale (VAS). Nevertheless, these are typically faulty and corrupted in some way. On the other hand, biomarkers are signs of a physiological process that can be quantified and are dependable. Plasma, serum, urine, and saliva are some of the essential body fluids that are increasingly being utilized for the purpose of stress evaluation.[8] However, intrusive treatments like venipuncture have the potential to cause mental stress[9] and as a result, they can operate as a factor that causes stress.

In both research and clinical practice, stress-responsive biomarkers that are non-invasive and simple to assess are increasingly being favored as objective instruments for the purpose of predicting and monitoring stress.[8,10]

The study examined the variation in salivary alpha amylase (SAA) activity in patients undergoing infraumbilical elective surgery under spinal anesthesia. The mean SAA levels in both groups were similar during the first and second events, but not statistically significant. The percentage increase in SAA from sample 1 to sample 2 was 10.50% in group A and 1.30% in group B. Stress during surgery and waiting in the pre-operative room led to increased SAA levels in both events. In group A, SAA levels increased by 32.40% compared to event 2, while in group B, they dropped by 41.00%. Anxiolytic drugs before spinal tap reduced stress, resulting in lower SAA levels. In group A, SAA levels increased by 49.85% from event 3, attributed to increased stress from fear of the procedure, needle pain, and operative fear. However, SAA levels in group B patients decreased by 32.00% after spinal anesthesia, indicating the sedative and anxiolytic effect of midazolam.

Findings of our results were comparable with study conducted by **Useato et al** [7] where he use of Salivary Alpha Amylase (SAA) as a stress level assessment tool during therapeutic

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

endoscopy. The study involved 40 gastric neoplasia patients undergoing endoscopic superior dissection under deep sedation and general anaesthesia. The DS group showed increased SAA levels immediately after the procedure, while the GA group experienced a drop in SAA levels. The researchers suggested that pain could contribute to increased stress, resulting in increased SAA levels.

The study examined the variation in salivary cortisol activity in patients undergoing infraumbilical elective surgery under spinal anaesthesia. The mean salivary cortisol levels in both groups during the first event were almost the same, but not statistically significant. The percentage rise from sample 1 to sample 2 was 18.16% in group A and 10.97% in group B. The stress of patients admitted to the ward for surgery and waiting in the pre-operative room reflected an increase in salivary cortisol levels in event 1 and event 2. The difference in salivary cortisol levels at event 3 was significant, with group A experiencing a 26.17% increase in salivary cortisol levels compared to event 2. In group B, it dropped by 21.14%. The increased salivary cortisol levels in group A were 32.94% from event 3, attributed to increased stress due to cumulative fear of the spinal procedure, needle pain, and operative fear. However, group B patients' salivary cortisol levels decreased 15.10% after spinal anaesthesia, reflecting the sedative and anxiolytic effect of midazolam.

Findings of our results were comparable with study conducted by **Sanja Vickovi et al.**[12] investigated the use of salivary cortisol as a biomarker for stress in surgery patients. The research encompassed 60 individuals slated for elective abdominal aortic aneurysm surgery. Patients were categorized into two groups: MI (intermittently administered morphine) and MPCA (morphine patient-controlled analgesia). The study found that pain intensity and hemodynamic instability increased post-surgery, with elevated salivary cortisol levels in MPCA group.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

Conclusion

This study sequentially documents sAA and salivary cortisol beginning with baseline levels, to thoroughly illustrate the stress experienced by patients during the preoperative period, encompassing the stress from the spinal tap, impending surgery, and associated environmental factors. SAA and salivary cortisol level assessment can be used as a good tool for estimation of psychological stress. It is an objective, quantitative test that can be used for assessment of stress in various situations where subjective tests may become compromised due to various human factors involved.

REFERENCES

1. Yaribeygi H, Panahi Y, Sahraei H, Johnston T, Sahebkar A. The impact of stress on body function:A review. Excli J 2017;16:1057.
2. Desborough PJ, Hall GM. Endocrine response to injury. In;kaufmanL.anaesthesia review, edinburgh:churchchill livingstone, 1993:10;131-48.
3. Singh RR, Shekhar Shudhanshu, Akhtar JM, Shankar V. Serum electrolyte changes in major surgical trauma. Int j Res Med Sci 2016; 7:2893.
4. Sheeran P, Hall Gm. Cytokines in anaesthesia. Br j Anaesth 1997; 78:201-19.
5. Keremi B, Beck A, Fabian TK, et al. Stress and Salivary Glands. Curr Pharm Des. 2017;23(27):4057-65.
6. 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.
7. 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.
8. Batista P, Pereira A, Vaz AB. Salivary biomarkers in psychological stress diagnosis. ARC J Pharm Sci 2017;3:9-18.
9. 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.
10. 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.
11. Gracial Navarrno, Blanca Marı ´n-Fernandez,b Vanessa de Carlos-Alegre,c Amparo, Martı ´nez-Oroz,a Ainhara Martorell-Gurucharri,a Esther Ordon ˜ez-Ortigosa,a. Preoperative Mood Disorders In Patients Undergoing Cardiac Surgery:Risk Factors and Postoperative Morbidity in The Intensive Care Unit. Rev Esp.2011;64(11):1005-10.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 10, 2024

12. Vicković S, Zdravković R, Maričić-Prijić S, Nikolić D, Pap D, Čolak E, Jovičić S. Salivary cortisol as a biomarker of stress in surgical patients. J Med Biochem. 2023 Aug 25;42(3):469-475. doi: 10.5937/jomb0-42011. PMID: 37790204; PMCID: PMC10543123.