Assessment of Salivary Alfa Amylase (SAA) and The State-Trait Anxiety Inventory (STAI) level in in Patients Undergoing Spinal Anesthesia for Infra-Umbilical Surgery

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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

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.

STAI: There are 2 subscales within this measure. First, the State Anxiety Scale (S Anxiety) evaluates the current state of anxiety, asking how respondents feel "right now," using items that measure subjective feelings of apprehension, tension, nervousness, worry, and activation/arousal of the autonomic nervous system. The Trait Anxiety Scale (T Anxiety) evaluates relatively stable aspects of "anxiety proneness," including general states of calmness, confidence, and security.[8] (Annexure-1)

This study aims to correlate salivary alpha-amylase (SAA) and the State-Trait Anxiety Inventory (STAI) 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 STAI 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.

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 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.
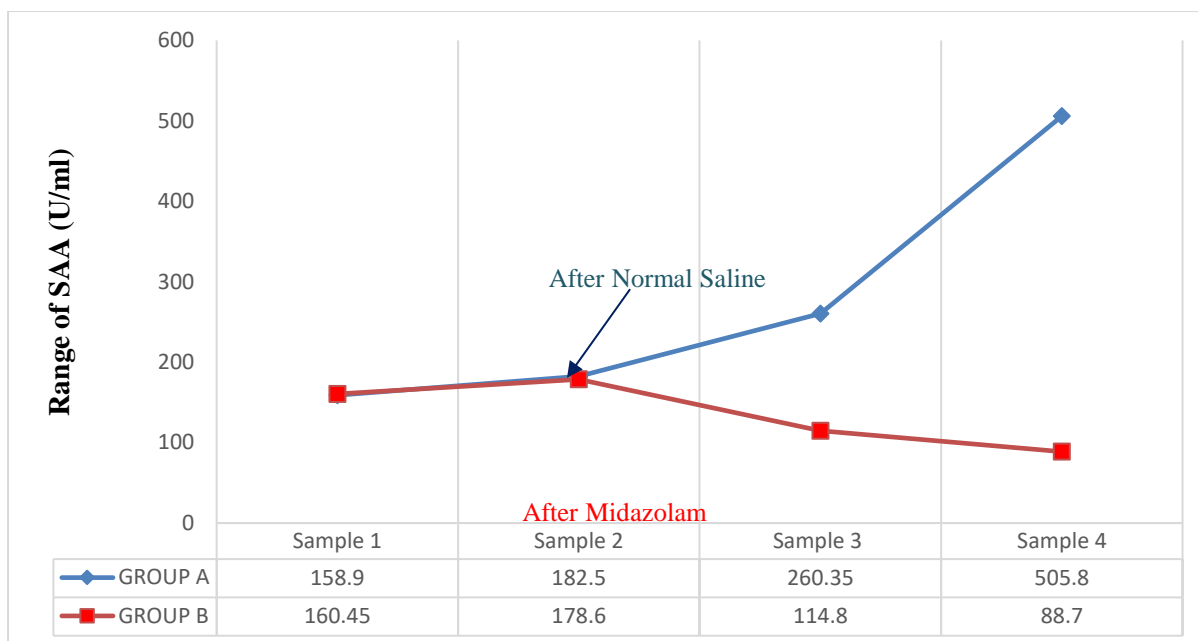
Statical Analysis

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.

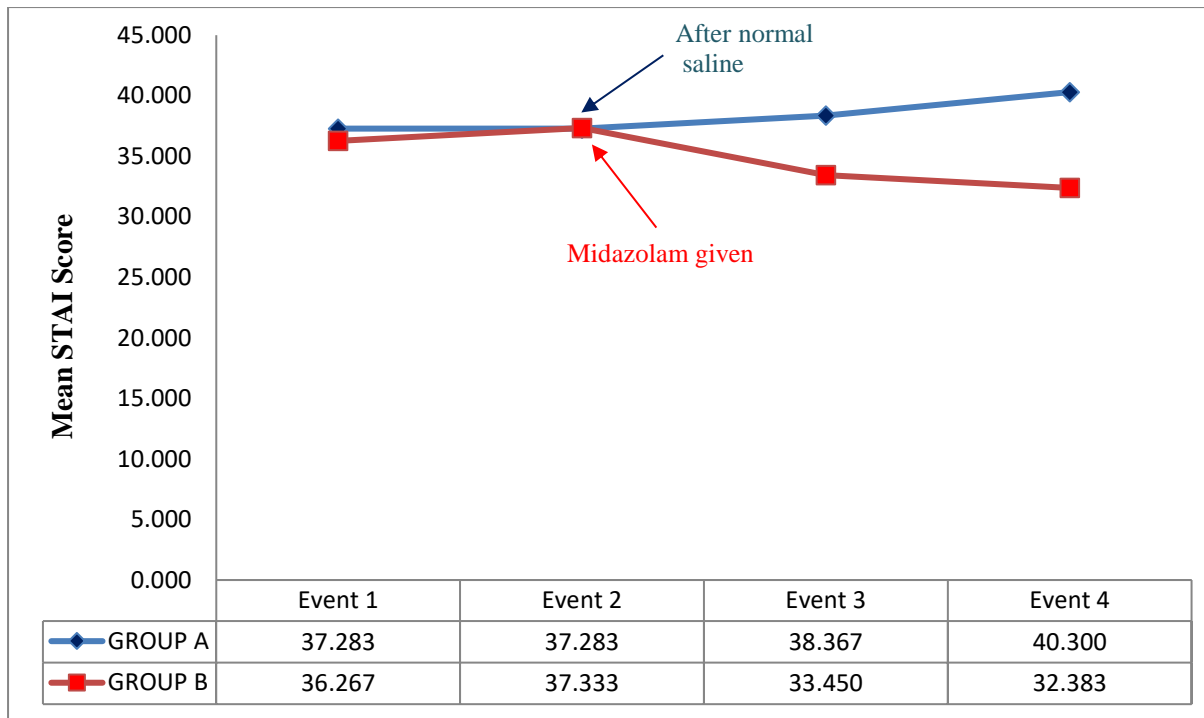|	Sample 1	Sample 2	Sample 3	Sample 4
GROUP A	158.9	182.5	260.35	505.8
GROUP B	160.45	178.6	114.8	88.7

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	37.50 ± 6.00	36.50 ± 5.00	0.293
Sample 2	37.50 ± 6.00	37.60 ± 5.90	0.963
Sample 3	38.50 ± 7.10	33.85 ± 2.00	0.001
Sample 4	40.50 ± 9.20	32.80 ± 1.90	0.001

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

	Event 1	Event 2	Event 3	Event 4
GROUP A	37.283	37.283	38.367	40.300
GROUP B	36.267	37.333	33.450	32.383

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

Table 2 and **Figure 2** show a comparison of the mean STAI scores among the study groups. The difference in the mean anxiety score was not significant (p>0.05) in Event 1 and Event 2. However, a significant difference (p<0.005) was observed in Event 3 (mean STAI: Group A **38.50 ± 7.10** and Group B **33.85 ± 2.00**) and in Event 4 (mean STAI: Group A **40.50 ± 9.20** and Group B **32.80 ± 1.90**).

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 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.[9]

However, intrusive treatments like venipuncture have the potential to cause mental stress[10] 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.[9,11]

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 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 State-Trait Anxiety Inventory (STAI) is a tool used to evaluate trait and state anxiety, aiding in the differential diagnosis of anxiety and depressive syndromes. In the present study, the STAI score in group A increased incrementally, while in group B, it declined consistently. The decrease in group B was attributed to the anxiolytic properties of Midazolam. A study by **Garcia et al.**[12] found a correlation between STAI scores and pre-operative anxiety in postoperative patients, leading to increased painkiller usage.

Conclusion

This study sequentially documents sAA, 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 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.

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