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Original Article

Anaesthesia Section

Substance P Level in Patients with Degenerative Lumbar Spine Disorder undergoing Decompressive Surgery: An Observational Study

AMRITA PANDA[1], RAJLAXMI SARANGI[2], DEBADYUTI SAHU[3], SAURABH SHARMA[4], NARENDRA KUMAR DAS[5], SIBANARAYAN RATH[6]

ABSTRACT

Introduction: Patients with degenerative lumbar spine disorders experience Chronic Low Back Pain (CLBP) for which they undergo decompressive surgery. Substance P (SP), a neurotransmitter which acts as a modulator of pain perception and transmits nociceptive signals via primary afferent fibers to the spinal cord and brainstem. In chronic painful conditions, SP level can be associated with the severity of pain and gets altered; however, this correlation is not present in acute pain.

Aim: To evaluate serum SP level in CLBP patients undergoing decompressive spine surgery.

Materials and Methods: The present study was a prospective observational study, in which 30 patients with CLBP undergoing decompressive spine surgery were enrolled. Along with them, one first-degree relative of each patient and an equal number of healthy volunteers were included in the study group. Patients were followed-up on the 5th postoperative day and at two months after surgery for the evaluation of SP levels and Visual Analogue Scale (VAS) scores. Statistical data analysis was carried out using IBM PASW Statistics (SPSS) 25.0 version software. SP levels followed a non normal distribution and were compared by Mann-Whitney U or Kruskal Wallis test (for 2 or more groups, respectively) and correlated using Spearman rank correlation.

Results: Patients undergoing decompressive spine surgery had a significantly higher SP level (97.5 picogram/mL (pg/mL)) than healthy volunteers (23.22 pg/mL), p-value <0.001. The serum SP levels in patients were found to be significantly reduced on the 5th postoperative day (31.7 pg/mL) and at two months after surgery (48.5 pg/mL), p-value=0.043. In contrast, there was no discernible change in the VAS score, which did not correlate with the fall in SP levels on the 5th postoperative day.

Conclusion: SP level was elevated in subjects with degenerative lumbar spine disorders undergoing decompressive surgery. Higher levels of SP can be attributed to CLBP in those patients. SP can be contemplated as a biomarker of pain due to degenerative lumbar spine pathology. However, further studies are warranted to substantiate this.

Keywords: Low back pain, Neurokinin-1 receptor, Visual analogue scale

INTRODUCTION

Degenerative pathology involving the lumbar spine results in CLBP, which in turn affects the quality of life. Surgical measures are recommended in those cases of CLBP not responding to conservative therapy [1]. Degeneration of the intervertebral disc results in an increased level of inflammatory markers, upregulation of proalgesic factors, and promotes nerve fiber growth [2]. Cellular degeneration and damage lead to the activation of Reactive Oxygen Species (ROS). The upregulation of ROS is contemplated in the synaptic transmission of pain [3]. The earliest and thoroughly understood function of SP is that of a neurotransmitter and modulator of pain perception. SP is an 11-amino acid neuropeptide that acts on the Neurokinin-1 (NK-1) receptor, which transmits nociceptive signals via primary afferent fibers to spinal cord and brainstem second-order neurons. SP aids in sensitising the neurotransmitter glutamate in the postsynaptic neurons and facilitates the transmission of pain signals to the somatosensory area of the brain [4,5]. It chiefly acts on the NK-1 receptors, which are primarily G-protein-coupled in nature [6]. Low back pain in patients with degenerative lumbar spine disorders is chronic in nature. Increased levels of SP are linked to such chronic painful conditions, but SP does not rise substantially in response to acute pain [7].

SP binds to the NK-1 receptor, causing an increase in levels of cyclic Adenosine Monophosphate (cAMP), which in turn influences the levels of release of inflammatory cytokines [8]. Unbound SP is hydrolysed by peptidases in the extracellular fluid and by Angiotensin-Converting Enzyme (ACE) in plasma [9]. The half-life of SP is longer in plasma (in hours) compared to that in tissues. In neurogenic inflammation, SP apparently has significant role because of its presence in the damaged tissue area in response to pain signals that travel along the axons of somatosensory areas of the brain, which in turn stimulates the expression of Interleukin-8 involved in neutrophil recruitment [10].

No previous literature was available on the estimation of the serum level of SP in patients with degenerative lumbar spine disorders undergoing decompressive surgery. Again, studies have been conducted on Caucasian controls and African American controls, but to date, no other literature is available with respect to the Indian population [11]. This study presents a sincere attempt to provide insight into the Indian scenario. Considering all these factors, the primary objective of this study was to evaluate the serum SP level among CLBP patients undergoing decompressive spine surgery, both preoperatively as well as on the 5th day and two months after-surgery. The secondary objective was to compare the presurgery serum SP levels of patients with those of their first-degree relatives and available pain-free healthy volunteers (acting as controls). The secondary outcome objective of the study was to correlate the changes in SP levels in patients postsurgery (over time) with VAS used for pain perception by individuals.

MATERIALS AND METHODS

This prospective observational study was undertaken during the period January 2022 to August 2022 in association with the Departments of Anaesthesiology, Biochemistry, and Neurosciences at Kalinga Institute of Medical Sciences (KIMS), KIIT DU, Bhubaneswar, Odisha, India. The study was in line with the principles of the Declaration of Helsinki, after approval from the Institutional Ethics Committee (IEC) (KIIT/KIMS/IEC/782/2021) and registration with the Clinical Trial Registry India (CTRI/2022/01/039780), written informed consent was taken from all study participants (i.e., patients with CLBP scheduled for decompressive surgery, their corresponding first-degree relatives, and healthy volunteers). Study participants were recruited as per inclusion and exclusion criteria in the Department of Anaesthesiology.

Inclusion criteria: Individuals with American Society of Anaesthesiologists (ASA) physical status I and II in the age group of 30 to 70 years with a history of low back pain lasting ≥3 months duration posted for decompressive spine surgery were included in the study.

Exclusion criteria: Patients with other co-existing chronic painful conditions and those under ACE inhibitor drugs were excluded from the study.

Sample size: Based on a study by Brandow AM et al., the average serum SP level were 22.99±7.6 and 32.5±11.6 in the control group (N=35) and the case (SCD) group (N=25), respectively. The effect size was calculated to be 0.97. Considering a 95% level of significance (alpha=0.05) with a study power of 95% and effect size of 0.97 [11], the sample size was calculated to be 29. Hence, 30 patients were recruited as cases (15 males and 15 females). The participants in the healthy volunteer group were attempted to be matched with the patient group in terms of age and gender. Healthy and pain-free volunteers who were neither related to the patients nor accompanying them were recruited (30 in total: 15 male and 15 female) as the healthy control group.

Simultaneously, 30 of their corresponding first-degree relatives were also recruited as controls. Matching both age and gender was not possible for the patient relative group, as the available first-degree relative of the patient was chosen. They can act as one of the control groups with similar pain perception to the patients studied. Thus, the levels of study parameters in patient relatives can be considered as the baseline for the respective patients.

Study Procedure

Peripheral venous blood samples were collected from all study participants in the Department of Anaesthesiology and sent to the Department of Biochemistry for storage and further analysis. Demographic data from all study participants were analysed. Under strict aseptic measures, 1.5 mL of venous blood samples were collected from the patients thrice: 24 hours prior to surgery, on the 5th postoperative day, and then around two months after surgery. Simultaneously, 1.5 mL of blood was collected from the first-degree relatives of patients at the time of admission. Concurrently, 1.5 mL blood samples were also collected from healthy volunteers. All venous blood samples were collected at the Anaesthesiology department in serum separator tubes and sent to the biochemistry laboratory. Blood samples were allowed to clot, then centrifuged at a speed of 2000 to 3000 rpm, aliquoted, and stored at -80°C. SP levels were estimated using the Human SP ELISA Kit (ELK Biotechnology, Catalog number: ELK1039) by sandwich enzyme-linked immunoassay. The analytical measurement range of SP in the ELISA kit was between 15.63 to 1000 pg/mL.

A standard general anaesthesia protocol was followed for all patients. All patients were premedicated with intravenous (i.v.) glycopyrrolate 0.2 mg and fentanyl 2 µg/kg. Induction of anaesthesia was done with i.v. propofol 1.5-2.5 mg/kg. Endotracheal intubation was done with i.v. vecuronium 0.1 mg/kg. Anaesthesia was maintained with nitrous oxide, oxygen, and Isoflurane 0.8-1 MAC. The effect of the muscle relaxant was reversed using i.v. neostigmine 0.05 mg/kg along with glycopyrrolate 0.01 mg/kg. One gram of i.v. paracetamol was administered 10 minutes prior to reversal. Pain was estimated using the VAS. VAS static (without any movement in the supine posture) and VAS dynamic (with movement in the supine posture) were used for the assessment of postoperative pain. The patient was asked to rate his/her pain on a scale of 1 to 10. A score of 0/10 indicates no pain, while a score of 10/10 indicates the worst pain. VAS score was assessed at different time intervals: presurgery, at "0" minutes, and 5 days postsurgery, with both VAS static and VAS dynamic scores [12].

STATISTICAL ANALYSIS

All continuous parameters were analysed for normality by Shapiro-Wilk test. Normally distributed parameters were expressed as mean ± standard deviation (SD) and compared using Student's t-test or one-way Analysis of Variance (ANOVA) for two or more than two groups, respectively. Paired parameters were compared by paired t-test, and correlations were tested using Pearson's correlation analysis. Parameters with non normal distribution were expressed as median (interquartile range), compared using the Mann-Whitney U test for unpaired parameters, the Wilcoxon Signed Rank test for paired parameters, and correlations were tested using Spearman's rank correlation. Non continuous parameters were analysed for differences using the Chi-square test. Data analysis was carried out by using IBM PASW Statistics (SPSS) version 25.0 software. A p-value <0.05 was considered statistically significant.

RESULTS

After collecting the data, a comparative analysis was done for the demographic variables of patients undergoing surgery. The Shapiro-Wilk test was used to assess the normality of continuous variables. Demographic variables like age, height, weight, and Body Mass Index (BMI) followed a Gaussian distribution, while serum SP levels did not. A total of 30 patients with lumbar degenerative disease were enrolled in the study, with 15 males and 15 females. Relatives were selected as per availability. Healthy controls were only gender-matched but not age-matched, as being free of any kind of pain was an essential inclusion criterion for the healthy control group. Age, Body Mass Index (BMI), and serum SP levels were significantly higher in patients at the presurgery state than in the other two groups (p-value<0.05) [Table/Fig-1].

Variables	Patients (30)	Relatives (30)	Healthy (30)	F	p-value
Age (in years)	43.30±12.15	37±11.94	33.80±8.95	5.676	0.005$^{\#\#}$
Height (in cm)	165.23±6.51	168.87±7.85	168.03±5.40	2.450	0.092$^{\#\#}$
Weight (in kg)	64.70±7.12	65.03±9.93	61.13±6.22	2.237	0.113$^{\#\#}$
BMI (kg/m^2)	23.78±3.00	22.72±2.44	21.64±1.85	5.636	0.005$^{\#\#}$
Gender distribution (M:F)	15:15	17:13	15:15	0.356	0.837$^{\$}$
SP (pg/mL)	97.5 (36.25-170.09)	59.5 (16.25-95.2)	23.22 (16.14-32.04)	35.094	<0.001$^{\#}$
Pain duration (in months)	11 (6-36)	NA	NA	NA	NA

[Table/Fig-1]: Demographic variables details in three study groups and comparison among them.
SP: Substance P; BMI: Body mass index; $^{\#\#}$: One-way ANOVA; $^{\$}$: Chi-square test; $^{\#}$: Kruskal Wallis test

Gender had no effect on serum SP levels in all three study groups (p-value>0.05) [Table/Fig-2]. Also, serum SP levels among patients before surgery, with a median value of 97.5 pg/mL, were higher than among their relatives (median: 59.5 pg/mL), although not statistically significant (p-value=0.084), and significantly higher (p-value<0.001) than among healthy subjects [Table/Fig-3].

Variables	Male (15)	Female (15)	MWU	p-value
SP (Patient Pre-Sx)	100.41 (25.85-210.75) pg/mL	92.97 (33.56-195.14) pg/mL	1.080	0.293
SP (Relative)	60 (32.9-96.4) pg/mL	37 (9-93.7) pg/mL	22.0	0.314
SP (Healthy control)	25 (16.2-43) pg/mL	26.6 (16.6-58.96) pg/mL	1041	0.620

[Table/Fig-2]: Gender wise comparison of study variable.
Pre-Sx: Presurgery; SP: Substance P; MWU: Mann-Whitney U test; Mann-Whitney U test was used for serum SP (as non-normally distributed)

Groups compared	Mean difference	MWU	p-value
Patient pre-sx vs patient relative	174.249	111.0	0.084
Patient pre-sx vs healthy control	206.212	394.5	<0.001
Patient relative vs healthy control	31.962	408.0	0.006

[Table/Fig-3]: Comparison of Substance-P (pg/mL) levels between study groups.
Pre-Sx: Presurgery; MWU: Mann-Whitney U test; Patient Pre-Sx SP (pg/mL): 97.5 (36.25-170.095); Patient Relative SP (pg/mL): 59.5 (16.25-95.2); Healthy Control SP (pg/mL): 23.225 (16.136-32.044)

Serum SP levels among patients two months postsurgery were significantly lower than presurgery levels (p-value=0.043). The five-day postsurgery levels of serum SP were still lower, which could be due to the effect of analgesia. Then, at two months postsurgery (i.e., after the weaning of the analgesia effect), serum SP levels, though raised than during immediate postsurgery state, were still lower than the patients' relative levels (which can be considered as the baseline for respective patients) [Table/Fig-4].

Patient pre-Sx	Patient five day post-Sx	Patient two month post-Sx	Z	p-value
97.5 (36.25-170.095) pg/mL	31.7 (17.24-56.37) pg/mL	48.5 (26.75-71.06) pg/mL	-2.023	0.043

[Table/Fig-4]: Comparison of substance P level pre and postsurgery (Wilcoxon Signed Ranks Test).
Pre-Sx: Presurgery; Post-Sx: Postsurgery

Both static and dynamic VAS scores exhibited a significant alteration following surgery (p-value<0.001) [Table/Fig-5]. In the presurgery stage, SP levels did not exhibit a significant correlation with age, BMI, or pain duration suffered by the patient (p-value>0.05) [Table/Fig-6]. The association of presurgery serum SP levels with gender, static, and dynamic VAS scores

VAS static				
Presurgery	Postsurgery (0 minute)	Postsurgery (5 day)	KWH	p-value
3 (1-4)	2 (1-3)	4 (1-6)	40.178	<0.001
VAS dynamic				
Presurgery	Postsurgery (0 minute)	Postsurgery (5 day)	KWH	p-value
4 (3-6)	5 (3-6)	6 (4-9)	19.163	<0.001

[Table/Fig-5]: VAS static and dynamic data and their comparison over time.
KW: Kruskal Wallis test; VAS score represented as median (range)

Correlation	N	ρ	p-value
Substance-P and age	30	-0.060	0.785
Substance-P and BMI	30	0.181	0.409
Substance-P and pain duration	30	0.051	0.818

[Table/Fig-6]: Correlation of Substance-P (SP) with demographic variables among patients (presurgery) (Spearman Rank Correlation).

(presurgery and 0 minutes postsurgery) were analysed. In all cases, the associations were found to be statistically not significant (p-value>0.05). Similarly, the association between five days postsurgery serum SP levels and corresponding static and dynamic VAS scores were also statistically not significant [Table/Fig-7].

Association	N	χ^2	p-value
Substance-P (Pre-Sx) and gender	30	χ^2 (21) = 29.069	0.112
Substance-P and VAS static (both Pre-Sx)	30	χ^2 (126) = 40.324	0.998
Substance-P (Pre-Sx) and VAS static ("0" minute Post-Sx)	30	χ^2 (42) = 31.492	0.066
Substance-P and VAS static (both 5-Day Post-Sx)	30	χ^2 (90) = 32.189	0.924
Substance-P and VAS dynamic (both Pre-Sx)	30	χ^2 (126) = 40.324	0.988
Substance-P (Pre-Sx) and VAS dynamic ("0" minute Post-Sx)	30	χ^2 (126) = 45.779	0.950
Substance-P and VAS dynamic (both 5-Day Post-Sx)	30	χ^2 (36) = 29.416	0.773

[Table/Fig-7]: Association of serum SP levels with discrete variables among patients (Chi-square test).
Pre-Sx: Presurgery; Sub-P: Substance P; VAS: Visual analogue scale

DISCUSSION

The findings of this study reveal that serum SP levels among patients (presurgery) and their relatives were significantly higher than in healthy controls. Presurgery SP levels in patients were higher than in their respective first-degree relatives, but not statistically significant. Postsurgery (5-day) SP levels in patients were significantly lower than presurgery levels. Two months postsurgery, SP levels were higher than 5-day postsurgery levels but significantly lower than presurgery SP levels.

SP belongs to the Tachykinin (TAC) family of neuropeptides and is encoded by the TAC1 gene. In animals, SP, Neurokinin A (NKA), and Neurokinin B (NKB) are the three primary TAC members. All mammalian tissues and body fluids are distributed with TAC family members, but the central nervous system and peripheral nervous system have the highest densities of SP [13,14]. SP is synthesised in ribosomes as a large non functional protein and undergoes post-translational modification to produce active SP [15]. A cell surface metalloendopeptidase breaks down unbound SP, indicating a shortened half-life of SP in tissues [16,17]. SP plays a major role in immune cell migration and expression of chemokines and adhesion molecules [8]. It has been established that mediators of chronic pain are emitted from both neuronal and non neuronal components.

When considering the demographic details in the present study, the age and BMI of patients undergoing surgery were higher and significantly different from healthy individuals and their relatives. However, in this study, gender had no effect on serum SP levels in all three groups. A study done by Racine M et al., showed that there was no difference in human pain sensitivity between genders, even with the use of deep, tonic, long-lasting stimuli that mimic clinical pain [18].

Studies have demonstrated that SP is known to be associated with chronic painful conditions, inducing the release of proinflammatory mediators like prostaglandins [5,9]. This neuropeptide is released from pain-sensing fibers to increase pain sensitivity through its actions on the dorsal horn of the spinal cord and is a key element in neurogenic inflammation [19,20]. Liswowska B et al., in their study, found a positive correlation between serum SP concentration and chronic pain intensity. SP and its receptor are involved in chronic pain and are associated with joint and tissue inflammation, triggering an inflammatory cascade that causes

the release of a variety of inflammatory mediators, one of them being SP [21]. Therefore, in degenerative spine disorders, SP can be contemplated as a neurotransmitter attributed to CLBP and underlying inflammatory pathology. Patients with chronic degenerative lumbar spine disorders undergoing decompressive surgery often experience chronic pain. In the present study, SP levels were significantly higher among CLBP patients during the presurgery state {97.5 (36.25-170.09) pg/mL} compared to healthy controls {23.22 (16.14-32.04) pg/mL} (who were free from any painful conditions). Similarly, the presurgery SP levels of CLBP patients were higher compared to their relatives {59.5 (16.25-95.2) pg/mL, although the value was not statistically significant.

Relatives are genetically similar to the patient and may represent the patient in a pain-free (basal) condition. The SP levels in relatives were also significantly higher than in healthy controls (although both groups are free from pain). This can be explained by the hypothesis that the level of apprehension and/or stress among patients' relatives may be at similar levels to that of the patients, as they share either similar genetic makeup or environmental circumstances, or both. This hypothesis needs further validation. Thus, serum SP levels may be affected by psychological stress, apprehension, or emotions alongside the effect of pain. A study by Ebener K and Singewald N illustrated that stressful and aversive stimuli alter the content of SP in brain tissue and increase the efflux of SP in particular limbic regions like the amygdala and septum. The intensity of this effect depends on the severity of the stressor, which was similar to present study findings [22]. Many chronic pain syndromes like osteoarthritis and CLBP are associated with elevated SP levels. The present study yielded similar results, where serum SP levels were found to be elevated in subjects with degenerative spine pathology requiring decompressive surgery.

In this study, the serum SP level was significantly higher preoperatively {97.5 (36.25-170.09) pg/mL compared to its concentration at the 5th postoperative day {31.7 (17.24-56.37) pg/mL} as well as at two months {48.5 (26.75-71.06) pg/mL} following decompressive surgery. These findings were in agreement with a study by Smith HS and Clauw DJ who demonstrated that CLBP is associated with elevated SP levels [23]. A study conducted in patients with rheumatoid arthritis undergoing orthopaedic surgery concluded that there was a correlation between the intensity of acute pain and serum SP levels postoperatively, but there was no correlation between the intensity of acute pain and the concentration of SP in drainage fluid [24]. In the present study, serum SP levels exhibited a weak positive correlation with the duration of pain suffered by the patient, which was statistically not significant. This finding was supported by Izumi M et al., who reported that both the pain duration and shoulder pain VAS were not directly associated with serum SP levels in rotator cuff tear cases [25].

Regarding the association of VAS scores and SP levels in patients before surgery and on the 5th postoperative day, significant association was observed. The results of the present study do not correlate with the findings of the study by Brandow AM et al., where elevations in SP levels were linked with significantly higher preoperative VAS scores [11]. This is quite similar to the fact that chronic painful conditions such as CLBP are associated with elevated SP levels. Once elevated, SP levels do not fluctuate much, not even in response to acute painful stimuli. Thus, elevated levels of SP appear to be a biological marker for the presence of chronic pain.

Limitation(s)

In this study, only venous blood was utilised for the estimation of SP, although SP can be detected in other body fluids such as saliva. A more in-depth analysis of the role of SP as a biomarker for chronic pain is needed, involving a larger sample size. Also, the stress levels of study participants were not measured.

CONCLUSION(S)

Serum SP levels were substantially elevated in patients with CLBP requiring decompressive spine surgery compared to their first-degree relatives and healthy volunteers. There was a significant reduction in their SP levels following decompressive surgery. Hence, the raised level of SP can be attributed to chronic pain. However, SP levels are unaffected by the duration of pain suffered. Further studies are warranted to validate SP as a biomarker of pain, which can pave the way for the development of novel analgesics acting on NK-1 receptors.

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PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Anaesthesia, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India. **(ORCID ID: 0000-0002-4255-4672).**
2. Professor, Department of Biochemistry, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
3. Assistant Professor, Department of Biochemistry, Shri-Bagchi Shankara Cancer Hospital, Bhubaneswar, Odisha, India.
4. Assistant Professor, Department of Anaesthesia, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
5. Professor, Department of Neurosciences, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.
6. Assistant Professor, Department of Research and Developement, Kalinga Institute of Medical Sciences, Bhubaneswar, Odisha, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Rajlaxmi Sarangi,
Professor, Department of Biochemistry, Kalinga Institute of Medical Sciences,
Bhubaneswar-751024, Odisha, India.
E-mail: rajlaxmi.sarangi@kims.ac.in

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