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Reaffirming the Peripheral Action of Local Anaesthesia (LA): A Prospective Clinical Study

Dr. B L Himaja Reddy MDS[1], Dr. Kavitha Prasad MDS[2], Dr. Vineeth Kumar K MDS[3], Dr. Sanah Tazeen MDS[4], Dr. Rajanikanth B.R MDS[5], Dr Parimala Sagar MDS[6], Dr. K Ranganath MDS[7]

[1]Oral & Maxillofacial Surgeon, Faculty of Dental Sciences- RUAS Bangalore. India
Mail ID: *lhimajareddy93[at]gmail.com*

[2]Oral & Maxillofacial Surgeon, Professor and Associate Dean, Faculty of Dental Sciences -RUAS Bangalore. India
Mail ID: *kavithaprasad.os.ds[at]msruas.ac.in*

[3]Oral & Maxillofacial Surgeon, Fellow in Oral Oncosurgery - RCS-UK, Health Care Global Enterprises Ltd. Hospital Bangalore
Mail ID: *Sanah_tazeen[at]yahoon.com*

[4]Oral & Maxillofacial Surgeon, Associate Professor, Faculty of Dental Sciences- RUAS Bangalore, India
Mail ID: *drvineeth.os.ds[at]msruas.ac.in*

[5]Oral & Maxillofacial Surgeon, Associate Professor, Faculty of Dental Sciences- RUAS Bangalore, India
Mail ID: *rajanikanth.os.ds[at]msruas.ac.in*

[6]Oral and Maxillofacial Surgery, Associate Professor, Faculty of Dental Sciences. Bangalore, India
Mail ID: *drparimala11[at]gmail.com*

[7]Oral Maxillofacial Surgeon, Professor and Head of the Department, Faculty of Dental Sciences. Bangalore, India
Mail ID: *ranganth.os.ds[at]msruas.ac.in*

Abstract: ***Background: As per existing literature Local Anaesthetics (LA) had only peripheral action .In study by All saffar et.al poskulated that the opiorphins in saliva were elevated after the administration of Local Anaesthetic(LA) .To investigate the proposed central mechanism of LA this study was conducted. Purpose: To investigate the proposed Central mechanism of action of Local anaesthetic and association with salivary opiorphin's. Study Design: This was a prospective study done in the Department of Oral and Maxillofacial Surgery, FDS, Ramaiah University of Applied Sciences, Bangalore in patient milieu of 44 patients (22 males, 22 females), aged between 18-69years, indicated for prophylactic extractions under Local Anaesthesia between November 2019 to February 2021. Predictor: salivary opiorphin's assesses to be increased after administration of Local Anaesthetics. Main outcome variable(s): The mean levels of salivary opiorphin's were decreased and increased but ranged in normal limit. Analyses: The P value was 0.96, with no statistical significance. Results: The mean level of salivary opiorphin's before was 28.38ng/ml and 26.24ng/ml, after administration of local anaesthesia. Therefore, there was a mean decrease of 2.14ng/ml in salivary opiorphin levels after administration of LA. The P value was 0.96, with no statistical significance. Conclusion: Our study results indicate that local anaesthetics have only peripheral action. There was a mean decrease in opiorphin levels after administration of local anaesthesia. The change in opiorphin levels was not statistically significant; negating any association between administration of local anaesthesia (2% lignocaine hydrochloride) by IANB technique and salivary opiorphin levels.***

Keywords: Salivary opiorphin's ,opiorphin's, Endorphin's Neuroectopeptidases ,Central Mechanism of action

1. Introduction

In Maxillofacial surgery and other fields of medicine the most commonly experienced symptoms ispain[1 4 5 6 12 14]. Currently, the analgesics, corticosteroids, anaesthetics are playing a major role in treating the pain [12 3 4 67 8 14 21]. Rapid advancements and discoveries in the field of pain research necessitate updating and redefining on a regular basis [12].

Identifying the linked markers of nociception and pain would help with pain diagnosis and management[2356]. Painful inflammatory, viral, autoimmune, premalignant, and malignant diseases can cause ulcers and erosions of the oral mucosa[1 2 3 4 5 9 10 11 14 15 16 17 18 19 20 21 22 23 24]. As the primary salivary glands are part of the neuroendocrine system, the content of saliva changes in response to a variety of local and systemic situations[10 11 12 14]. Some salivary components secreted have been used as biomarkers for physiological and pathological states, even for pain .[1 2 3 4 8 9 10 11 13 14 15 16 17 18 19 20 21 22 23 24].

The Maxillofacial Surgeon now has access to a wide selection of local anaesthetics, and it would be judicious to choose the most potent anaesthetic for efficient pain control. They are used to treat acute, inflammatory, cancer-related, and chronic pain [3 5 7 8 16]. They work by preventing the propagation of action potential in axons in a reversible manner.[1 2 3 4 5 6 7 8 10 11 13 14 15 16 17 18 19 20 21 22 23 24] Local anaesthetics can be used to target nociceptive and neuropathic pain in any area of the nervous system, from the periphery to the brain, to create the desired anaesthetic or analgesic effect [1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24].

The majority of the available literature on the mechanism of action of local anaesthesia focuses on its peripheral effects at the injection site, with no evidence of a central effect [3714]. In the recent past, few authors have indicated opiorphin mediated central action of local anaesthetics which in turn prolongs the duration of endogenous opioids [7].

Annie Wiser et.al. identified opiorphin as an endogenous pentapeptide molecule from human saliva in 2016[1 2 3 4 5 7 8 10 11 12 13 14 15 16 20 21 23 24].

Opiorphin works by preventing the breakdown of enkephalin, a natural opioid analgesic. As a result, the presence of opiorphin is thought to improve pain blockade, and current research is focused on evaluating the potential of opiorphin as an exogenous analgesic.[12 3 4 5 7 8 10 11 12 13 14 15 16 20 21 23 24]

Local anaesthetics play a major role in reducing pain while performing maxillofacial surgical procedures.[3 6 7]Opiorphin's are the biomarkers present in the saliva and indicate the pain severity [37 10 14]. Al-Saffar et al. proposed a new central mechanism of action for local anaesthetics in 2013, based on increased salivary opiorphin levels following LA administration [13 10 1114].

The local anaesthetics are used in surgical practise for their peripheral action [1,3,8]. There is insufficient evidence in the literature regarding the existence if any; of its central action. Therefore, there is a need to explore the co-relation between salivary opiorphin levels and local anaesthetic administration.[12 10 141521]The present study was done to assess the levels of salivary opiorphin before and after administration of LA in patients undergoing prophylactic extractions.

2. Materials and Methods

This was a prospective study ,observational ,in vivo study with convinence sampling done in the Department of Oral and Maxillofacial Surgery, FDS, Ramaiah University of Applied Sciences, Bangalore in patient milieu of 44 patients (22 males, 22 females), aged between 18-69years, indicated for prophylactic extractions under Local Anaesthesia between November 2019 to February 2021 . The study was approved by University Ethics Committee for Human Trials, Ramaiah University of Applied Sciences followed by Helnski protocol . The patients were informed about the procedure and informed consent was taken. Patient with chronic inflammatory condition, organ transplants, antipsychotic and antidepressant drugs. Chronic alcoholics and smokers, immune compromised disorders, who have taken antibiotics & analgesics in the previous one week, Xerostomia patients were excluded.

Sample Collection

The 2ml unstimulated saliva was taken through micropipette /syringe before administration of LA and after administration of LA thorough IANB. Salivary samples were collected twice from each patient. The first saliva sample was collected before the administration of LA. Second saliva sample was collected into the vaccutainer 5 mins after the administration of LA. Both the saliva sample were collected were unstimulated salivary samples. The salivary samples were centrifuged at 3000 rpm, cooled and ELISA was performed to assess the opiorphin levels in saliva by using HUMAN OPI ELISA KIT and analysed by spectrophotometry technique. The collection of samples was thorough micropipette (fig 1) and stored at -80° centigrade till completion of study period. The method employed was sandwich ELISA technique with GENLISA™ kit, as it employs double antibodies and has higher specificity and sensitivity compared to conventional kits

1) Microtiter Plate Reader able to measure absorbance at 450 nm.
2) Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25µl to 1000µl
3) Deionized (DI) water
4) Wash bottle or automated microplate washer
5) Graph paper or software for data analysis
6) Timer
7) Absorbent Paper. All reagents were used with care after cooling at room temperature and stored in cool place as they were light sensitive.

Assay Procedure: It was strongly recommended that all Standards and Samples run in duplicates or triplicates. For each sample standard curve was required, therefore, 50 ul Standard antibody solution was added to standard well. Thereafter, 40 ul of test sample was added to respective sample wells. Next pipetting of 10 ul Biotinylated Opiorphin Antibody to respective sample wells was done, pipetting of 50 ul Streptavidin-HRP Conjugate to respective sample wells and standard wells was done and mixed well. The plate was covered with a sealer and incubated for 60 minutes at 37°C. Aspiration and washing the plate for 4 times with diluted Wash Buffer (1X) was done and residual buffer was blotted by firmly tapping plate upside down on absorbent paper. Any liquid from the bottom outside of the microtiter wells was wiped as any residue can interfere in the reading step. Pipetting of 50 ul Substrate A, followed by 50 ul Substrate B in all the wells was done and was incubated at 37°C for 10 minutes. Positive wells turned bluish in color, then 50 ul of Stop Solution was pipetted into all wells. The wells turned from blue to yellow in color. Absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution was attained.

Figure 1: Micropipette Used For Saliva Collection and Human OPI Elisa Kit

Calculation of Results

By using spectrophotometry, the determination of the mean Absorbance for each set of duplicate or triplicate Standards and Samples was done. Samples were diluted, multiplied by the appropriate dilution factor Using Graph Paper, plotting of the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis was done. The best fit curve was drawn through the standard points and unknown Human Opiorphin concentration was determined and the unknown's mean Absorbance value on the Y-axis and a horizontal line

was drawn to the standard curve. At the point of intersection, a vertical line was drawn to the X-axis and the Human Opiorphin concentration was determined. Software which could generate a cubic spline curve fit was used for automated results

3. Results

This prospective study was conducted to assess the relation between administration of local anaesthesia and salivary opiorphin level on 44 patients who reported to the Department of Oral & Maxillofacial Surgery, Faculty of Dental Sciences, Ramaiah University of Applied Sciences.

Table 1

Comparison of mean Opiorphin (ng/ml) before and after LA administration using M C NEMARS Rank Test					
Time	N	Mean	SD	Mean Diff	P-Value
Before LA	44	28.38	27.23	2.14	0.96
After LA	44	26.24	19.63		

Table 2

Gender wise comparison of mean Opiorphin levels (ng/ml) before and after LA administration using McNemar's Test						
Gender	Time	N	Mean	SD	Mean Diff	P-Value
Males	Before LA	22	24.48	19.09	-3.13	0.57
	After LA	22	27.61	17.2		
Females	Before LA	22	32.29	33.49	7.41	0.78
	After LA	22	24.88	22.12		
Comparison of Salivary Opiorphin levels based on the gender of study patients using chi square Test						
Time	Sal. Opiorphin	Before LA		After LA		P-Value
		n	%	n	%	
Males	Normal	15	68.20%	11	50.00%	0.21
	Increased	7	31.80%	11	50.00%	
Females	Normal	16	72.70%	18	81.80%	0.48
	Increased	6	27.30%	4	18.20%	

Table 3

Age wise comparison of mean Opiorphin levels (ng/ml) before and after LA administration using McNemar Rank Test						
Age	Time	N	Mean	SD	Mean Diff	P-Value
≤ 27 yrs.	Before LA	23	25.3	23.42	0.2	0.69
	After LA	23	25.1	16.47		
> 27 yrs.	Before LA	21	31.76	31.1	4.27	0.85
	After LA	21	27.5	22.97		
Comparison of Salivary Opiorphin levels based on the age group of study patients using Chi Square Test						
Time	Sal. Opiorphin	Before LA		After LA		P-Value
		n	%	n	%	
≤ 27 yrs.	Normal	17	73.90%	15	65.20%	0.48
	Increased	6	26.10%	8	34.80%	
> 27 yrs.	Normal	14	66.70%	14	66.70%	1
	Increased	7	33.30%	7	33.30%	

4. Discussion

In clinical maxillofacial surgery practice, local anaesthetics play a major role in intraoperative and postoperative pain control till today [1 2 6 7 8 14 15]. A probable new concept in the mechanism of local anaesthesia was explored by Al-Saffar et al in 2013 wherein they proposed a link between opiorphin levels in the saliva and local anaesthetic agents, where they found that after administration of local anaesthetic there was significant rise in levels of salivary opiorphin [1 2 3 7 8 10 14 15]. There are many theories postulated for the mechanism of action of LA, however the specific receptor and membrane expansion theory are given importance today [1,3,815]. Pain is classified as acute and chronic pain, vary in their pathophysiology and etiology and experienced subjectively. [1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24]

Although pain is a subjective experience, there are two basic types of pain differing in their etiology and physiopathology: acute and chronic pain. Acute pain is self-limiting, usually concordant with the degree of on-going tissue damage, and remitting with resolution of the injury. It is nociceptive in nature. Chronic pain is not self-limiting, inadequately treated, and predominantly neuropathic in nature, leading to peripheral and/or central nociceptive sensitization[1 3 7].

The endogenous nociceptive-modulating system which mostly comprises of the physiological opioid pathways counteracts in the pain activity in transmission pathways [1 2 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24]. On the synaptic membranes of opioid and target neurons, both mu and delta (d)-opioid receptors are present, and enkephalins interact with them with considerable affinity. Because of its high intrinsic effectiveness, enkephalins require a smaller proportion of opioid receptors to elicit the same antinociceptive responses as morphine [1 2 8 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24].

All opioid receptors had binding capacity to metallo ectopeptidases binds to the membranes and gathered results in inactivation of enkephalins pathway aminopeptidase-N receptors and neutral endopeptidase (NEP EC3.4.21.11) [1-5,9-24]. By inhibiting the degradation of enkephalins in circulation, released in response to a noxious stimulus, the analgesic potency can be enhanced. Both human and animal aminopeptidase were able to bind to and inhibit opiorphin) [1 2 8 10 11 12 13 14 15 16 17 18 191 20 21 22 23 24]. Proteases inhibited by

opiorphin they are ecto-aminopeptidase N (ANPEP), neutral ecto-endopeptidase (MME), and maybe also a dipeptidyl peptidase (DPP3), resulting in extended enkephalin effects and the release of endogenous pain medications. The co-relation among local anaesthetic with analgesic consequences and the enkephalin breakdown pathway reasons as marginal elevation in mean opiorphin levels in saliva after administration of local anaesthesia [1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24].

Opiorphin inhibits three matrix metallo peptidases followed by dipeptidyl peptidases and extends the action of encephalins which results in enhancement of pain control. Increase in opiorphin levels during local anaesthetic administration suggest the possible correlation b/w local anaesthetic effect and enkephalin degradation pathway.anaesthesia [1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24]. In view of the proposed new central mechanism of action of local anaesthetic agents, we designed a study, to assess the mean opiorphin levels of saliva before and after administration of local anaesthesia. This would determine whether the central mechanism theory of LA was true. There are in-vitro and in- vivo studies in literature on opiorphin as a biomarker of pain. Opiorphin was isolated from saliva in 2006 [12101113 14 15 16 17 18 19 20 2124]. Regulation of this chemical in painful conditions and its analgesic effect has to be studied further [12101113 14 15 16 17 181 19 20 2124].

The current study aimed at providing a co-relation between levels of opiorphin in saliva and the utilization of single local anaesthetic drug and Inferior Alveolar Nerve Block approach [1 14 21]

This was an observational study which included 44 patients (22 males and 22 females) who underwent prophylactic extraction/s with Inferior Alveolar Nerve Block (surgical extraction of third molar, orthodontic extraction, extraction of grossly decayed teeth) in the Department of Oral and Maxillofacial Surgery followed Helinski declaration and ethical board of Ramaiah clearance was obtained . Patients of ASA 1 were included and patients on oral antibiotics and analgesics for previous 1 week, patients with xerostomia, organ transplantation, patients with history of co-morbidities, alcoholism, smoking and pregnancy were excluded from the study [110 14 15 16]. Parida et.al conducted a study, which determined by providing a co-relation between opiorphin levels of saliva and the utilization of various local anaesthetic drugs (Lignocaine, Bupivacaine, Articaine) and approaches by using both mandibular and maxillary nerve blocks[1 14 15]. However, statistically significant results were not obtained [1 2 3 4 8 10 11 14 1520]. In their study, opiorphin level in saliva were elevated after local infiltration inferior alveolar nerve block [1 2 3 4 8 10 11 14 15 20]. This could be because of multiple needle pricks for inferior alveolar, lingual and buccal, resulted in increasing the patients pain perception.

Opiorphin levels decreased after posterior superior alveolar nerve blocks and infraorbital block [18111520]. The two reasons for the decrease of opiorphin level after administration of LA were, one, LA causes pain control Second, when numbness sets in, a psychological situation can provide alleviation, which could have contributed to opiorphin down-regulation [18111520]. They concluded that there was no significant co-relation between opiorphin levels and various local anaesthetic agents, techniques [18111520].

Nilofar.et.al study assessed opiorphin levels in saliva of patients aged 20 to 75 years old with painful soft tissue ulcers in the oral cavity caused by trauma, oral submucous fibrosis, oral candidiasis, potentially malignant disorders (PMDS) such as, oral submucous fibrosis, oral carcinoma, lichen planus recurrent aphthous ulcer and Burning Mouth Syndrome (BMS)was measured [18111520]. They discovered a positive correlation (r=0.28) between levels of opiorphin in saliva and the patient's age. Patients develop a natural compensating adaptive mechanism to pain threshold as they get older. However, there is a paucity of research on the elevation in salivary opiorphin levels as people get older. In individuals with OPMDs and oral cancer, increased opiorphin levels were induced by a natural protective mechanism. They discovered that salivary opiorphin levels and the patient's age have a favourable connection (r=0.28). As people get older, they have a natural compensating adaptive mechanism for pain threshold. There is, however, a scarcity of studies on the rise in salivary opiorphin levels as people age increases. [18111520].

Salari et al. measured opiorphin levels in saliva of people with and without BMS in their study [124111520]. They discovered that BMS participants had a considerably greater level of opiorphin in UWS (8.129ng/ml-6.445 ng/mL) than the control group (5.017 ng/mL -2.585 ng/ml). Although the BMS group had higher opiorphin levels in stimulated whole saliva (SWS) than the controls, the difference was not statistically significant. Similarly, despite the fact that UWS had greater opiorphin levels than SWS, the variability in opiorphin levels between the two groups was not statistically significant [12411 14 1520]. Due to the antibody adhesion potential of immature translational products formed from PROL 1 protein, ELISA-based analyses demonstrated a larger increase in opiorphin levels in saliva than liquid chromatography mass spectroscopy-based assessments [12411 14 1520].

In BMS patients the rise in opiorphin levels was explained as a robust response to the chronicity of pain. Individuals psychological condition might also contribute to the changed pain threshold in these persons, which results in the alteration of local and central mechanisms [1241114 15 20]. BMS is a psychosomatic disorder linked to the patient's emotional state, adds to the decreased threshold for pain and explains anti-depressant properties of opiorphin [12411 14 1520].

In view of the proposed new central mechanism of action of local anaesthetic agents, we designed a study, to assess the mean opiorphin levels of saliva before and after administration of local anaesthesia. This would determine whether the central mechanism theory was true.

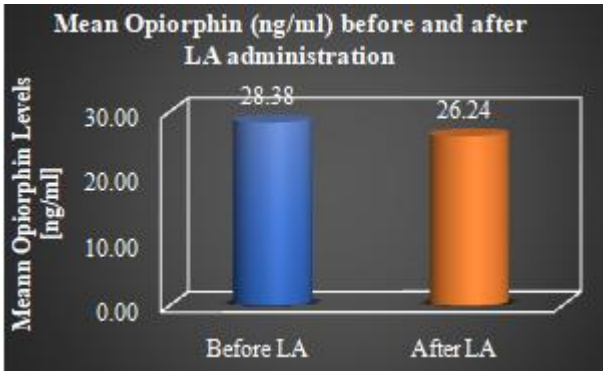
The current study aimed at providing a co relation between levels of opiorphin in saliva and the utilization of single local anaesthetic drug and Inferior Alveolar Nerve Block approach [115]. This was an observational study which included 44 patients (22 males and 22 females) who underwent prophylactic extraction/s with Inferior Alveolar

Nerve Block (surgical extraction of third molar, orthodontic extraction, extraction of grossly decayed teeth) in the Department of Oral and Maxillofacial Surgery [115]. Patients of ASA 1 were included and patients on oral antibiotics and analgesics for previous 1 week, patients with xerostomia, organ transplantation, patients with history of co-morbidities, alcoholism, smoking and pregnancy were excluded from the study [115].

In our study the mean age group selected was between 18-69 years, with equal female and male distribution [1 2 10 15]. In this study we aimed to assess the range of salivary opiorphins levels before and after the administration of Local Anaesthetic who underwent asymptomatic prophylactic extractions. The normal range of opiorphin is between 2.5 – 25.9 ng/ml. In the current study before administration of local anaesthetic the mean levels of opiorphin in saliva was 28.38ng/ml and 26.24ng/ml, five minutes after administration of LA [1 2 10 15].

Table 1

Comparison of mean Opiorphin (ng/ml) before and after LA administration using MC NEMAR Test					
Time	N	Mean	SD	Mean Diff	P-Value
Before LA	44	28.38	27.23	2.14	0.96
After LA	44	26.24	19.63		



Graph 1

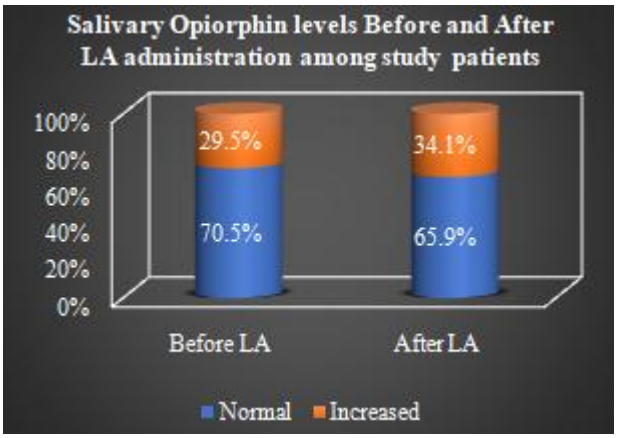
Mean Opiorphin levels before administration was 28.38 ng/ml and after administration was 26.24ng/ml. There was no statistical significance in opiorphin levels before and after administration.

In patients below 27 years of age Mean Opiorphin levels before administration of LA was 25.30ng/ml and after administration of LA was 25.10 ng/ml. In the patients above 27 years of age was 31.76 ng/ml before administration and after administration was 27.50ng/ml.

The opiorphin levels were reduced after administration of LA, but was within the normal range. The decrease in opiorphin levels was not significant statistically (p=0.96). This finding was similar to other studies conducted by Parida.et.al,Florence et.al, Boucher.et.al [12415]. Contradictory to our results, in the study conducted by Al-Saffar et al., the mean opiorphin levels in patients increased from 5.96 ng/ml to 14.5 ng/ml and their results suggested that local anaesthetics have an effect on the enkephalin pathway, causing increased opiorphin secretion and thereby enhancing pain control.Even though there is increase in the salivary opiorphin levels in their study ,the opiorphin levels before and after administration of LA range within the normal limits of opiorphin secretion in the saliva, that is, between 2.5ng/ml-25.9ng/ml. So, the possibility of LA causing the increase of opiorphin levels is questionable.

Table 2

Comparison of Salivary Opiorphin levels Before and After LA administration among study patients using McNemar's Test					
Sal. Opiorphin	Before LA		After LA		P-Value
	n	%	n	%	
Normal	31	70.50%	29	65.90%	0.82
Increased	13	29.50%	15	34.10%	



Graph 2

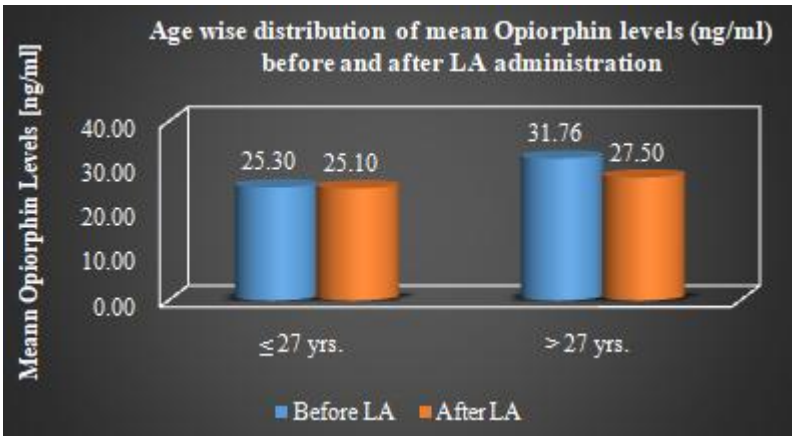
In this study, majority of the patient showed no changes in opiorphin levels, few patients showed marginal increase in the levels of opiorphin, before and after administration of local anaesthetic. However, the difference in the opiorphin levels before and after administration showed no statistical significance.

In our study, with respect to age wise distribution, in patients below the age group of 27 years, before administration of LA, salivary opiorphin levels was 73.9% in 17 patients, and after administration of local anaesthesia the opiorphin levels in saliva was reduced to 65.2% in 15 patients. Among them, in 6 patients showed marginal rise in salivary opiorphin levels from 26.1% to 34.8% after administration of LA. However, this rise in salivary opiorphin levels ranged in normal limits. The (p=0.48) Value was not statistically significant [2 411 14 21].

In our study, with respect to age wise distribution, in patients above 27 years of age, in 14 patients, the salivary opiorphin levels before administration of LA was 66.7%. 7 patients showed increased levels of salivary opiorphin (33.3%) before and after the administration of LA. However, this rise in salivary opiorphin levels ranged in normal limits and (p=1.00) was not statistically significant [2 411 14 20].[table 3-graph -3]

Table 3

Age wise comparison of mean Opiorphin levels (ng/ml) before and after LA administration using McNemar Rank Test						
Age	Time	N	Mean	SD	Mean Diff	P-Value
≤ 27 yrs.	Before LA	23	25.3	23.42	0.2	0.69
	After LA	23	25.1	16.47		
> 27 yrs.	Before LA	21	31.76	31.1	4.27	0.85
	After LA	21	27.5	22.97		
Comparison of Salivary Opiorphin levels based on the age group of study patients using Chi Square Test						
Time	Sal. Opiorphin	Before LA		After LA		P-Value
		n	%	n	%	
≤ 27 yrs.	Normal	17	73.90%	15	65.20%	0.48
	Increased	6	26.10%	8	34.80%	
> 27 yrs.	Normal	14	66.70%	14	66.70%	1
	Increased	7	33.30%	7	33.30%	



Graph 3 (a)

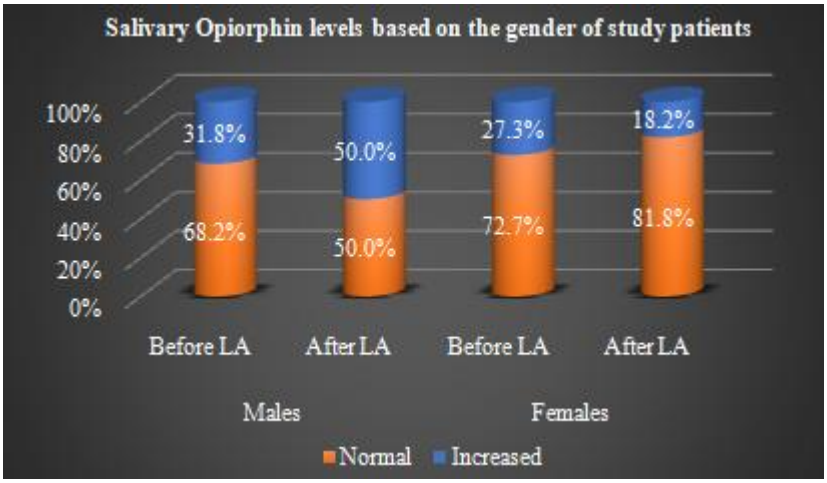
In patients below 27 years of age Mean Opiorphin levels before administration of LA was 25.30ng/ml and after administration of LA was 25.10 ng/ml. In the patients above 27 years of age was 31.76 ng/ml before administration and after administration was 27.50ng/ml.

In our study with respect to gender wise distribution in male patients the salivary opiorphin levels before administration of LA was 68.2% in 15 patients, after administration of LA the salivary opiorphin levels were decreased to 50.0%. In 7 patients the opiorphin levels before administration was 31.8% after administration of LA was 50.0% and in 11 patients after and before administration of LA was 50.0%.

However, the increase/decreases/no change in salivary opiorphin levels after administration of LA ranged in normal limit there was no statistically significant (p=0.21)[2 4 10 11 14 20]. In female patients (n=16) the salivary opiorphin levels before administration of LA was 72.7% and after administration of LA the salivary opiorphin levels was 81.8%. In 6 patients the salivary opiorphin levels were 27.3% before and decreased to 18.2% after administration of LA. However, the increase/decrease in the mean opiorphin levels showed no statistical significance (p=0.48) [4 10 11 14 20].

Table 4

Gender wise comparison of mean Opiorphin levels (ng/ml) before and after LA administration using McNemar's Test						
Gender	Time	N	Mean	SD	Mean Diff	P-Value
Males	Before LA	22	24.48	19.09	-3.13	0.57
	After LA	22	27.61	17.2		
Females	Before LA	22	32.29	33.49	7.41	0.78
	After LA	22	24.88	22.12		
Comparison of Salivary Opiorphin levels based on the gender of study patients using chi square Test						
Time	Sal. Opiorphin	Before LA		After LA		P-Value
		n	%	n	%	
Males	Normal	15	68.20%	11	50.00%	0.21
	Increased	7	31.80%	11	50.00%	
Females	Normal	16	72.70%	18	81.80%	0.48
	Increased	6	27.30%	4	18.20%	



Graph 4

In males, patients (n=15) the salivary opiorphin levels before administration of LA was 68.2% and after administration of local anaesthetic was 50.0% normal range. The p value was not statistically significant. In females, the levels of salivary opiorphins before administration was 72.7% and there marginal rise to 81.8% after administration of LA, but was within the normal range. The P value was not statistically significant. (Table4).

With respect to Gender comparison between opiorphin levels before and after administration of local anaesthetic, females showed higher opiorphin levels when compared to males. The findings of our study were in concurrence to the study done by Doufer.et.al [124 10 13 14 1520]{table -4 graph-4}. The salivary opiorphin levels before and after administration of LA were within normal range. However, opiorphin levels were marginally higher in patients above 27yrs of age and in female patients both before and after administration of LA.

5. Conclusion

The findings of our study show that local anaesthetics have only peripheral action and there was no co-relation between salivary opiorphin levels and LA action. The Current study does not show an association between administration of local anaesthesia (2% lignocaine hydrochloride) by IANB technique and salivary opiorphin levels. There was a mean decrease in salivary opiorphin levels after administration of LA. The mean salivary opiorphin levels are 2.5ng/ml-25.9ng/ml in normal individuals. The mean salivary opiorphin levels were more with advancing age in our study; probably due to adaptive mechanism to withstand pain. Also, the opiorphin levels were more in females both before and after administration of LA in our study and could be attributed to psychosocial and emotional factors.

6. Future Directions

Synthetic formulation of salivary opiorphin which has analgesic and antianxiety action has to be explored

References

[1] Al-Saffar MT, Al-Sandook TA, Y-Taha M. A possible new concept in the mechanism of action of local anesthesia. Am J Med Biol Res. 2013;1(4):134-7.
[2] Boucher Y, Braud A, Dufour E, Agbo-Godeau S, Baaroun V, Descroix V, Guinnepain MT, Ungeheuer MN, Ottone C, Rougeot C. Opiorphin levels in fluids of burning mouth syndrome patients: a case-control study. Clinical Oral Investigations. 2017 Sep;21(7):2157-64
[3] Bennett CR, MONHEIM S. Local anesthesia and pain control in dental practice. St. Louis: CV Mosby Company. 1984.
[4] Dufour E, Villard-Saussine S, Mellon V, Leandri R, Jouannet P, Ungeheuer MN, Rougeot C. Opiorphin secretion pattern in healthy volunteers: gender difference and organ specificity. Biochem Anal Biochem. 2013;2(3):2-11.
[5] Gnaneswaran MR, Varadarajan U, Srinivasan R, Kamatchi S. Accessory oral cavity. National Journal of Maxillofacial Surgery. 2012 Jul;3(2):232.
[6] Kumari K, Kaur G, Arora SS, Pandher PK, Singh R, Bhat RA. To compare the effect of ice and the lignocaine gel in reducing pain of needle prick before palatal nerve block. Oral Surgery. 2021 Feb;14(1):23-8.
[7] Malamed SF. Handbook of local anesthesia-e-book. Elsevier health sciences; 2019 Mar 28.
[8] Marini M, Roda LG. Neuropeptide enzyme hydrolysis in human saliva. Archives of Oral Biology. 2000 Sep 1;45(9):775-86.
[9] Mennini N, Mura P, Nativi C, Richichi B, Di Cesare Mannelli L, Ghelardini C. Injectable liposomal formulations of opiorphin as a new therapeutic strategy in pain management. Future Science OA. 2015 Nov;1(3).
[10] Nejad NK, Ramakrishna P, Kar A, Sujatha S. Quantitative analysis and expression of salivary opiorphin in painful oral soft-tissue conditions: A descriptive study. Journal of Global Oral Health• Volume. 2020 Jul;3(2):123.
[11] Noble F, Roques BP. Protection of endogenous enkephalin catabolism as natural approach to novel

analgesic and antidepressant drugs. Expert opinion on therapeutic targets. 2007 Feb 1;11(2):145-59.
[12] Ozdogan MS, Gungormus M, Yusufoglu SI, Ertem SY, Sonmez C, Orhan M. Salivary opiorphin in dental pain: A potential biomarker for dental disease. Archives of Oral Biology. 2019 Mar 1; 99:15-21.
[13] Ozdogan S, Sonmez C, Yolcu D, Gungormus M. Tear Opiorphin Levels in Ocular Pain Caused by Corneal Foreign Body. Cornea. 2020 Nov 1;39(11):1377-80.
[14] Parida SK, Guruprasad T, Krishnakumar VB, Ravi RP. A study of salivary opiorphin levels using different anaesthetic drugs and techniques–A randomized controlled clinical study. Journal of Stomatology, Oral and Maxillofacial Surgery. 2018 Jun 1;119(3):169-71.
[15] Pinto M, Rougeot C, Gracia L, Rosa M, García A , Arsequell G, Valencia G, Centeno NB. Proposed bioactive conformations of opiorphin, an endogenous dual APN/NEP inhibitor. ACS medicinal chemistry letters. 2012 Jan 12;3(1):20-4.
[16] Paszynska E, Roszak M, Slopien A, Boucher Y, Dutkiewicz A, Tyszkiewicz-Nwafor M, Gawriolek M, Otulakowska-Skrzynska J, Rzatowski S, Dmitrzak-Weglarz M. Is there a link between stress and immune biomarkers and salivary opiorphin in patients with a restrictivetype of anorexia nervosa?. The World Journal of Biological Psychiatry. 2020 Mar 15;21(3):220- 9.
[17] Rougeot C, Robert F, Menz L, Bisson JF, Messaoudi M. Systemically active human opiorphin is a potent yet non-addictive analgesic without drug tolerance effects. Journal of physiology and pharmacology. 2010 Aug 1;61(4):483.
[18] Roques BP, Noble FL, Fournie-Zaluski MC. Endogenous opioid peptides and analgesia. Opioids in Pain Control: Mechanisms and Clinical Implications. 1999:21-46.
[19] Sarfaraz S, Ramzan S, Bano T, Fatima W, Sabir A. Evaluation of Analgesic Effect of Human Saliva. J. Anal. Pharm. Res. 2017;6(3):00175.
[20] Salarić I, Sabalić M, Alajbeg I. Opiorphin in burning mouth syndrome patients: a case-control study. Clinical Oral Investigations. 2017 Sep;21(7):2363-70.
[21] Sestile CC, Maraschin JC, Rangel MP, Santana RG, Zangrossi Jr H, Graeff FG, Audi EA. B2- kinin receptors in the dorsal periaqueductal gray are implicated in the panicolytic-like effect of opiorphin. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2017 Oct 3;79:493-8.
[22] Sitbon P, Van Elstraete A, Hamdi L, Juarez-Perez V, Mazoit JX, Benhamou D, Rougeot C. STR-324, a stable analog of opiorphin, causes analgesia in postoperative pain by activating endogenous opioid receptor–dependent pathways. Anesthesiology. 2016 Nov;125(5):1017-29.
[23] Yang QZ, Lu SS, Tian XZ, Yang AM, Ge WW, Chen Q. The antidepressant-like effect of human opiorphin via opioid-dependent pathways in mice. Neuroscience letters. 2011 Feb 4;489(2):131-5.
[24] Wisner A, Dufour E, Messaoudi M, Nejdi A, Marcel A, Ungeheuer MN, Rougeot C. Human Opiorphin, a natural antinociceptive modulator of opioid-dependent pathways. Proceedings of the National Academy of Sciences. 2006 Nov 21;103(47):17979-84

Legends

Figure 1: Micropippetteand HumanOpi Elisa Kit

Table 1: Comparison of mean Opiorphin (ng/ml) before and after LA administration using M C NEMAR'S Test-(graph-1).

Table 2: Comparison of Salivary Opiorphin levels Before and After LA administration among study patients using M C NEMAR'S Test (graph-2).

Table 3: Age wise comparison of mean Opiorphin levels (ng/ml) before and after LA administration using MC NEMAR'S Test and Comparison of Salivary Opiorphin levels based on the age group of study patients using M C NEMAR'S Test (graph-3).

Table 4: Gender wise comparison of mean Opiorphin levels (ng/ml) before and after LA administration using M C NEMAR'S Test and Comparison of Salivary Opiorphin levels based on the gender of study patients using M C NEMAR'STest(graph-4).