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Assessing the Early Post-Operative Analgesic Effects of Intra-Operative Lidocaine-Bupivacaine Use at the Incision Line and/or Around the Ovary in Ovariohysterectomy Operations of Dogs on Pain Mediators

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GU, ZP, ZKS, İE, FZ, FEÖ and SS presented the concept and planned the methodology. FEÖ, GU, ZP, ZKS, İE, and SS performed investigation and data curation. GU, ZP, ZKS and FEÖ did formal analysis. ZKS, GU and FEÖ wrote the manuscript. ZP, ZKS and FEÖ validated and supervised the study. FEÖ administered the project.

Key words
Glasgow composite pain scale short form, Pain mediators, Ovariohysterectomy, Lidocaine, Bupivacaine

ABSTRACT

The present study investigated the effectiveness of intra-operative bupivacaine–lidocaine (BLK) combination administration concurrently with meloxicam, a non-steroidal anti-inflammatory drug (NSAID), on the Glasgow Composite Pain Scale Short Form (GCPS-SF) scores and pain mediators in the early post-operative ovariohysterectomy (OHE) period in 30 female dogs of different breeds and ages divided into three equal groups. OHE is reportedly associated with moderate pain. BLK was administered inside the ovarian bursa 10 min before ovary removal in Groups (G) 1 and G2 and linear to the incision line 10 min before entering the abdomen in G1. G3 was the control group. The intergroup comparison of pain mediators and GCPS-SF scores showed no significant difference between the GCPS-SF scores at postop0, postop2, postop4, postop8 and postop24 h and the cortisol, TNF-α, IL1-β and NO levels determined at the same timepoints. TNF-α at postop24 h showed a significant positive correlation with the postop0 h GCPS-SF score. NO at postop8 h showed a significant negative correlation with the postop4 h GCPS-SF score. However, since these results were not simultaneous, they were disregarded. Although there was no statistically significant difference in post-operative pain and stress among the three groups, surgical stress was higher in G3, as indicated by high post-operative cortisol levels, which suggested the other two protocols involving BLK to be remarkable. Hence, using G1 and G2 protocols appeared feasible considering the post-operative cortisol stress hormone values. Nevertheless, further studies with larger samples are warranted to confirm these inferences.

INTRODUCTION

Ovariohysterectomy (OHE) is the most prevalent elective surgery performed under general anaesthesia in canines. Moderate pain during the operation occurs due to surgical incision, manipulation of the abdominal viscera and stretching of the ovarian ligaments (Deschamps, 2001; Otto, 2001; Carpenter *et al*., 2004; Hardie *et al*., 1997; Gaynor and Muir, 2014).

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Although pain is an individual experience with a subjective effect, it also occurs independently of the fact that different pathologies feature unique triggering mechanisms to elicit the pain response. In particular, the tumour necrosis factor alpha (TNF-α), interleukin (IL)-1 and IL-6 have been associated with neuropathic pain in various domains. TNF-α is a cytokine involved in systemic inflammation. The animal models of neuropathic pain based on various nerve injuries have suggested that TNF-α plays a crucial role in the extent of peripheral and central sensitization. Pro-inflammatory cytokine, IL-1β and TNF-α levels have also been reported to be considerably higher in incidences of non-healing wounds. Nitric oxide (NO) is a diatomic free radical that readily interacts with molecular oxygen and reactive oxygen species, thereby playing a role in producing inflammatory and immune responses (Kingo *et al*., 2018; Dray, 1995; Widgerow and Kalaria, 2012).

The present study investigated the effects of intra-operative local anaesthetic combination (bupivacaine–lidocaine, BLK) administration along with meloxicam, a non-steroidal anti-inflammatory drug (NSAID) on intra- and post-operative pain, surgical stress and acute phase inflammatory mediators in canines (n= 30) who underwent OHE.

MATERIALS AND METHODS

Animals

The present study was approved by the Experimental Animals Ethics Committee of the Near East University (Approval No.: SBE/2019-148-21). Elective OHE was planned for 30 female dogs of different breeds with a mean age of 3 years (6 months−7 years) and a mean weight of 14.65 kg (5−31 kg). The relevant consent documents were obtained and included in the study. The inclusion criterion included patients without clinical pain of any origin and normal complete blood count (CBC) parameters, including alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), albumin (Alb.), urea and creatinine levels, which provided information regarding the general health condition and fitness for anaesthesia of the animals.

Study design

The dogs were randomly divided into three groups. A combination of BLK was prepared comprising equal amounts of bupivacaine (Buvicaine, 0.5 mg/ml POLİFARMA, Tekirdağ, Turkey) and lidocaine (Jetokaine, 20 mg/ml, ADEKA, Samsun, Turkey) in each millilitre. Accordingly, 1 unit of lidocaine and 4 units of bupivacaine were mixed and 0.5 ml/kg of this mixture was administered to the relevant site. In group (G) 1 and G2, BLK was administered inside the ovarian bursa, which is an extension of the mesovarium, at a distance from the vessels and ligaments (Bubalo *et al*., 2008) 10 min before the removal of the ovaries. It was also administered in G1 linear to the incision line 10 min before entering the abdomen. G3 was the control group, and hence, BLK was not applied.

Anaesthesia and surgery

A catheter was placed inside the vena cephalica antebrachia in the dogs before the operation and 20 mg/kg cephalosporin (Sefazol, Mustafa Nevzat; Istanbul, Turkey) IV was administered via it for prophylactic purposes. An anaesthesia device (Hasvet AM852 with Neptune automatic ventilator, Hamburg, Germany) with a double vaporizer that operated with a semi-open/closed circuit system and had carbon dioxide trapping canisters in double chambers was used during the operation for all the groups. Anaesthesia was induced by 4–6 mg/kg IV injection of propofol (Propofol, Fresenius, Istanbul, Turkey), following IV injection of midazolam (0.1 mg/kg) and butorphanol (0.2 mg/kg) as pre-anaesthetic agents. Anaesthesia was maintained by the inhalation of 3%–5% sevoflurane (Sevorane Liquid, Abbott, Turkey). In all the cases, 0.2 mg/kg meloxicam was subcutaneously administered 5 min before the operation. The dogs were then intubated via the orotracheal route using endotracheal tubes (ETTs) having a 4.5–9 mm internal diameter width and a Murphy eye at the end. Ringer's lactate infusion was administered at a dose of 5 ml/kg/h throughout the operation. All surgical operations and BLK administrations were performed by the same physician. All the dogs underwent standard OHE (Tobias, 2010). In G1, BLK was administered linear to the incision line 10 min before entering the abdominal cavity and to the ovarian bursa 10 min before the removal of the ovary, whereas in G2, BLK was administered only to the ovarian bursa. Rescue analgesia (meloxicam) was administered in cases with an expected Glasgow composite pain scale short form (GCPS-SF) score of 11 or higher during the post-operative period.

Evaluation of the GCPS-SF scores and blood parameters

The GCPS-SF include certain clinical symptoms, such as behaviour, body postures, vocalisation, attention toward environment and caregiver, defecation, salivation, vomiting and appetite, as criteria for evaluating the pain felt by dogs on a scale of 0–24 points. Following the completion of all the surgical procedures, the sevoflurane vaporizer was switched off and the dogs were allowed to breathe oxygen until the swallowing reflex was triggered. Thereafter, the GCPS-SF was used to evaluate pain at postop 0, postop 0.5, postop 1, postop 1.5, postop 2, postop 2.5, postop 3, postop

4, postop 5, postop 6, postop 8, postop 12 and postop 24 h. Blood samples were collected from the animals via the vena cephalica antebrachii in K2EDTA tubes and CBC tests were performed using the Haematology Analyzer (Mindray, Shenzhen, China). The serum ALT, ALP, TP, Alb, urea and creatinine levels were measured using BS-120 automatic analyser (Mindray, Shenzhen, China) with ready-to-use commercial test kits. Based on the result of the abovementioned parameters, the animals were evaluated for cortisol, TNF-α, IL-1β and NO for anaesthesia. Cortisol, considered the primary stress hormone (Kingo *et al*., 2018), was examined by enzyme-linked immunosorbent assay (ELISA) using a commercial test kit (DEH3388, Demeditec Diagnostics, Kiel, Germany). The concentrations of the pro-inflammatory cytokines TNF-α and IL-1β, which are known to cause strong hyperalgesia (Dray, 1995), were measured using canine-specific ready to use commercial ELISA test kits (SEA133CA, SEA563CA, USCN, Wuhan, China, respectively). NO concentration, involved in the up-regulation of the cytokine cascade (Widgerow and Kalaria, 2012), was measured using a ready-to-use test kit based on the colorimetric principle (E-BC-K036, Elabscience, Houston, USA). The MW-12A Microplate Washer and MR-96 Microplate Reader (Mindray, Shenzhen, China) devices were used for analysing the cortisol, TNF-α, IL-1β and NO levels. The cortisol, TNF-α, IL-1β and NO level analyses were repeated at postop 0, postop 2, postop 4, postop 8 and postop 24 h for all the cases, and 0 h was set as the time when the dogs first lifted their heads following the removal of the ETT.

Statistical analysis

Friedman test was used for intragroup periodic comparisons and the different periods were determined using the post-hoc Conover test. Kruskal–Wallis test was used to compare the parameters of the three groups for each measurement period. Conover post-hoc test was employed to determine the significant difference between the groups. A p value of ≤ 0.05 was considered statistically significant. Statistical Package for the Social Sciences (SPSS) version 23 (BM® SPSS® Statistics) software was used for the statistical analyses. Descriptive statistics pertaining to the study data were calculated as median, 25th and 75th percentiles, minimum and maximum values and mean rank numbers and are summarized in the tables.

RESULTS

The intergroup comparison of the pain mediators TNF-α, IL1β, NO and cortisol

TNF-α, IL1β and NO showed no significant difference among the groups (Table I, P > 0.05).

Table I. Serum TNF-α, IL-Iβ, NO and cortisol levels (Mean±SD) in groups 1, 2 and 3.

Time	G1 (n=10)	G2 (n=10)	G3 (n=10)
TNF-α			
Preop	214.74±204.87 (25.61-659.57)	217.03±223.26 (36.37-696.92)	168.59±249.71 (35.31-872.56)
Postop 0	198.09±178.36 (18.98-566.80)	203.53±209.29 (33.21-673.16)	155.59±226.19 (34.79-787.51)
2	211.37±197.69 (27.10-641.49)	209.22±213.00 (51.24-661.70)	169.63±244.25 (48.98-858.67)
4	215.75±199.80 (18.08-612.41)	194.52±195.20 (43.96-603.43)	154.45±242.30 (47.86-838.15)
8	223.71±194.44 (33.21-597.12)	241.17±246.10 (52.95-646.26)	167.77±251.77 (48.42-873.25)
24	192.57±193.57 (18.98-589.39)	222.64±249.44 (46.18-732.93)	151.21±227.16 (41.22-786.76)
IL1-β			
Preop	8.08±2.87 (5.16-13.49)	7.29±3.88 (4.55-15.59)	6.95±2.8 (4.38-12.36)
Postop 0	5.59±096 (4.64-7.33)	5.26±1.26 (3.90-8.10)	7.35±4.01 (4.47-16.53)
2	7.80±2.56 (4.72-12.09)	6.28±2.38 (4.38-12.50)	8.03±3.76 (4.38-16.68)
4	7.05±4.03 (4.47-16.53)	6.99±4.28 (4.14-16.68)	7.78±4.72 (4.22-19.50)
8	30.63±73.47 (4.72-239.63)	6.19±1.99 (4.14-10.12)	8.78±4.72 (4.47-17.98)
24	8.50±4.62 (4.81-17.49)	6.88±3.39 (4.30-14.67)	7.60±4.12 (4.47-17.49)
NO			
Preop	13.25±3.95 (8.32-20.11)	17.17±5.60 (7.61-26.54)	21.03±6.91 (14.39-35.46)
Postop 0	18.96±7.44 (8.64-30.11)	21.60±12.17 (10.82-45.11)	19.92±6.28 (13.32-34.39)
2	42.85±65.66 (9.39-227.60)	17.17±3.71 (11.89-23.32)	16.39±2.97 (10.82-20.82)
4	18.85±9.21 (8.32-33.68)	17.17±5.53 (8.32-26.89)	28.78±22.06 (11.89-80.82)
8	20.48±6.46 (10.82-30.71)	20.31±8.39 (12.96-35.11)	16.92±6.40 (8.32-28.68)
24	34.89±60.36 (8.32-206.17)	18.07±4.17 (12.25-27.25)	21.00±10.84 (11.89-45.11)
Cortisol			
Preop	3.24±2.24^{e} (0.40-6.30)	4.39±3.83^{de} (1.40-12.90)	4.49±2.62^{de} (2.20-9.30)

Table continued on next page............

Time	G1 (n=10)	G2 (n=10)	G3 (n=10)
Postop 0	9.30±2.81^{ab} (5.20-14.20)	9.33±2.25 ^{ab} (6.20-14.30)	10.20±2.85^{a} (4.20-13.40)
2	8.76±4.56^{abc} (0.90-13.80)	5.75±3.60^{bcde} (1.70-12.90)	8.17±3.25^{abcd} (3.70-13.90)
4	3.90±2.28^{e} (1.10-7.80)	3.63±1.79^{e} (0.60-6.20)	5.16±2.61^{cde} (2.00-11.30)
8	2.14±2.07^{e} (0.10-6.90)	268±1.60^{e} (0.50-4.80)	3.92±1.37^{e} (1.80-6.10)
24	2.13±1.63^{e} (0.40-6.30)	2.13±1.067^{e} (0.80-4.10)	2.80±1.19^{e} (1.00-4.20)

TNF-α, tumour necrosis factor alpha; IL1-β, interleukin-1 beta; preop, pre-operative; postop, post-operative; SD, standard deviation, NO, nitric oxide.
For TNF-α, IL1-β, NO p>0.05, for control p< 0.001.

The serum cortisol levels showed a significant difference in the post-operative values of G1 and G2 (3.24 ± 2.24, 4.39 ± 3.83, respectively; P < 0.001). The cortisol levels, measured at postop0 for G1, G2 and G3 was 9.30 ± 2.81, 9.33 ± 2.25 and 10.20 ± 2.85, respectively, were not significantly different between G1 and G2; however, the cortisol levels in G3 was statistically significantly different (P < 0.001). At postop 2 h, the cortisol levels were 8.76 ± 4.56, 5.75 ± 3.60 and 8.17 ± 3.25 in G1, G2 and G3, respectively, and were statistically significantly lower in G2 (P < 0.001). At postop 4 h, the cortisol levels were 3.90±2.28, 3.63±1.79 and 5.16±2.61 in G1, G2 and G3, respectively, showing a statistically significant difference in G3 (P < 0.001; Table I).

Intragroup comparison of each group for GCPS-SF scores

The mean GCPS-SF score at postop0 h was significantly higher compared with the scores at other the measurement timepoints in G1 (p < 0.001). This was followed by the score at postop 0.5 h, which was similar to postop 1 h, but significantly higher than the score in the subsequent measurement timepoints (p < 0.001). No significant differences were observed in the scores among the remaining periods.

Similarly, in G2, the mean GCPS-SF score at postop0 h was significantly higher than in the other measurement periods (p < 0.001), followed by that in the posto 0.5 and postop 1 h periods. There was no significant difference in the scores between those two (postop 0.5 and postop 1 h) periods. There was no significant change in the scores in the postop 1.5 h and the subsequent timepoints, and the highest value was seen at the first awakening.

The mean GCPS-SF score at postop0 was significantly higher than at the other measurement timepoints in G3. This was followed by postop 0.5 and postop 1 h, although there was no significant difference in the scores between these two periods. The scores at postop 1.5 h did not significantly differ from that at postop1 and postop 2 h and the subsequent measurement timepoints. No significant change was observed among the scores at postop 2 h and the later measurement timepoints (Table II).

Table II. Periodic changes in the Glasgow composite pain scale short form in each group separately.

Variable	G1 (n=10)						G2 (n=10)						G3 (n=10)					
	Me-dian	25 %	75 %	Min.	Max.	Average rank number**	Medi-an	25 %	75 %	Min.	Max.	Average rank numbe**	Me-dian	25 %	75 %	Min.	Max.	Average rank number**
Glasgow postop 0	6.00	3.75	7.25	2.00	8.00	12.70 ^{a}	5.00	2.75	7.50	2.00	12.00	12.95 ^{a}	5.50	3.75	8.50	2.00	10.00	12.90 ^{a}
Glasgow postop 0.5	2.00	0	4.25	0	10.00	10.35^{b}	1.50	1.00	4.50	0	10.00	11.40^{b}	3.00	2.00	4.00	0	5.00	11.35 ^{b}
1	.50	0	3.00	0	7.00	8.60^{bc}	1.00	0	1.25	0	4.00	9.15^{b}	2.00	1.50	3.25	0	4.00	10.25 ^{bc}
1.5	0	0	1.00	0	13.00	7.55^{c}	0	0	0	0	1.00	6.20^{c}	0	0	1.00	0	3.00	7.30 ^{cds}
2	0	0	.25	0	2.00	6.45 ^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	3.00	5.80 ^{d}
2.5	0	0	0	0	2.00	5.95 ^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	3.00	5.80 ^{d}
3	0	0	0	0	2.00	5.95 ^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	3.00	5.80 ^{d}
4	0	0	0	0	2.00	5.95 ^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	2.00	5.50 ^{d}
5	0	0	0	0	0	5.45 ^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	2.00	5.50 ^{d}
6	0	0	0	0	1.00	5.70^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	0	5.20 ^{d}
8	0	0	0	0	0	5.45^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	0	5.20 ^{d}
12	0	0	0	0	0	5.45^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	0	5.20 ^{d}
24	0	0	0	0	0	5.45^{c}	0	0	0	0	0	5.70^{c}	0	0	0	0	0	5.20 ^{d}

*, Friedman's test; Conover post-hoc test; **, Significantly different periods have completely different letters. Preop, pre-operative; postop, post-operative. p*<0.001.

Intergroup comparison of the GCPS-SF scores

The separate comparison of the three groups at each measurement timepoint did not reveal any significant intergroup difference at any timepoint in GCPS-SF scores (Table III). Nevertheless, although the highest GCPS-SF score was 12 at postop 0 h in G2 N3, rescue analgesia was not administered because the postop 0.5 h GCPS-SF score was 10.

Intergroup comparison of pain mediators and the GCPS-SF scores

No significant difference was observed between the GCPS-SF scores at postop 0, postop 2, postop 4, postop 8 and postop 24 h and cortisol, TNF- α, IL1-β and NO levels at the same measurement timepoints (Table IV). Although a significant positive correlation was found between the TNF- α level at postop 24 h and the GCPS-SF score at postop 0 h, and a significant negative correlation was found between the NO value at postop 8 h and the GCPS-SF score at postop 4 h, this was not taken into consideration since the measurements were not taken simultaneously.

Table III. Intergroup comparison of the Glasgow composite pain scale short form scores.

Pain score	P
Glasgow postop 0	0.86
Glasgow postop 0.5	0.53
1	0.17
1.5	0.30
2	0.36
2.5	0.59
3	0.59
4	0.59
5	0.36
6	0.36
8	1.00
12	1.00
24	1.00

Preop, pre-operative; postop, post-operative.

Table IV. Intergroup comparison of pain mediators and the Glasgow composite pain scale short form scores.

		Glasgow postop 0			Glasgow postop 2			Glasgow postop 4		
		r	P	n	r	P	n	r	P	n
Spearman's rho	Cortisol post 0	-.027	.888	30	.264	.159	30	.124	.515	30
	Cortisol postop 2	-.135	.477	30	.012	.948	30	-.046	.808	30
	4	-.147	.439	30	-.074	.698	30	-.108	.569	30
	8	-.001	.994	29	-.076	.693	29	.065	.737	29
	24	-.310	.095	30	-.195	.303	30	-.108	.569	30
	TNF-α postop 0	.274	.143	30	-.116	.542	30	.046	.808	30
	TNF-α postop 2	.228	.226	30	-.021	.913	30	.154	.415	30
	4	.230	.221	30	-.023	.905	30	.154	.415	30
	8	.251	.189	29	-.105	.588	29	.081	.675	29
	24	.360	.050	30	-.036	.849	30	.124	.515	30
	IL1-β postop 0	.256	.173	30	.164	.386	30	.077	.685	30
	IL1-β postop 2	.033	.861	30	.344	.063	30	.263	.161	30
	4	-.098	.605	30	.308	.098	30	.247	.188	30
	8	.171	.375	29	.163	.399	29	.212	.271	29
	24	.056	.767	30	.321	.084	30	.278	.137	30
	NO postop 0	-.305	.101	30	-.009	.961	30	.162	.392	30
	NO postop 2	-.052	.785	30	.103	.588	30	.325	.080	30
	4	-.106	.577	30	.089	.639	30	.201	.287	30
	8	.062	.750	29	-.274	.151	29	-407	.029	29
	24	-.179	.343	30	-.203	.282	30	-.046	.808	30

Preop, pre-operative; postop, post-operative.

DISCUSSION

The present study aimed to investigate the effects of different local anaesthetic applications on intra- and post-operative pain, surgical stress and acute phase inflammation mediators with regard to the same general anaesthesia protocol in the three groups (n= 10) of dogs that underwent OHE. It is well-established that inflammation and pain may occur during the first 24 h after OHE and that post-operative analgesia is required not only for welfare practices but also because uncontrolled pain may cause complications, including cardiovascular stress, immunosuppression, delayed wound healing and anorexia, as well as behavioural changes, which might lead to self-mutilation following the operation and increased duration of hospital stay, thereby increasing expenses. Pain is also considered a vital sign of life forms in addition to body temperature, pulse, respiration and blood pressure (Hancock *et al*., 2005; Bonnet and Marret, 2005; Wagner *et al*., 2008). Therefore, the use of drug combinations with different mechanisms of action for a multimodal analgesic protocol is prevalent in veterinary medicine (Lamont, 2008).

The GCPS-SF scores were used to evaluate the pain intensities experienced by the dogs in the present study and no statistical difference was observed between the groups as per the measurements, even at postop 24 h ($p > 0.05$). The fact that the three groups did not differ in terms of pain scores and that pain was at the lowest level based on the GCPS-SF scores suggested both the convenience of the anaesthesia protocol and that local anaesthesia reduced post-operative pain.

The techniques involved in the OHE operation involve the ligation and removal of the ovarian pedicle. The traction and ligation of the ovarian ligament is considered a nociceptive (painful) stimulus, and in cases where anaesthetic depth and/or analgesic therapy is insufficient during this stage, nociceptive stimulation may manifest in different ways and degrees, producing symptoms ranging from increased heart and respiratory rates to considerable abdominal tension and movement (Deschamps, 2001). In this study, no symptoms of severe pain were observed from the recovery period in the post-operative period until postop 24 h, which indicated that all the three protocols were successful in post-operative pain control. Additionally, the statistically significant decrease ($p < 0.001$) in the GCPS-SF scores at the post-operative measurement timepoints compared with that at postop 0 h indicated that the three protocols used in this study successfully controlled post-operative pain.

Shivley *et al*. (2019) investigated the nociceptive effect of the excessive retraction of the ovarian ligament and sharp transection and monitored the increase of heart rate during the manipulation of the ligament in OHE operations in dogs. The pain was rated using pre- and post-operative pain scores and the GCPS-SF, which indicated that the sharp transection was faster and had a comparatively less impact on the heart rate (Shivley *et al*., 2019). The present study, consistent with the results of the relevant literature, showed no statistically significant change in the blood pressure and pulse in all the three groups during the dissection under the general anaesthesia protocol (G1, G2 and G3) as well as under local anaesthesia (G1 and G2).

Overcoming this nociceptive stimulation by only increasing the concentration of the anaesthetic agent would require very high concentrations of inhalation agents that might cause hypotension, hypothermia and concurrent prolonged restlessness. Therefore, as a general rule, a balanced anaesthesia protocol involving the use of different drugs with different properties is preferred. A combination of drugs allows the use of lower concentrations of each substance, thereby reducing side effects (Wenger *et al*., 2005). Another study used transdermal fentanyl patches on dogs 24 h before the operation and reported that epidural morphine provided better analgesia compared with morphine after the completion of OHE and that dog showed a higher incidence of adverse effects when fentanyl patches are applied following OHE (Pekcan and Koc, 2010). Similar to existing literature, the present study showed no statistically significant change in the blood pressure and pulse among all the three groups during dissection under the general (G1, G2 and G3) and local (G1 and G2) anaesthesia protocols.

NSAIDs and opioids are the most commonly used analgesics in canines (Cardozo *et al*., 2014). Despite the resistance of most clinicians, there has been an increase in the use of potent analgesics for controlling post-operative pain in OHE (Hewson *et al*., 2001). Most NSAIDs that are currently used for small animals produce analgesic effects by selectively inhibiting the cyclooxygenase-2 (COX-2) isoform. This selectivity is especially crucial for producing analgesia. It has minimal side effects on the stomach, kidney and platelet functions. Meloxicam is an NSAID belonging to the oxicam group with a COX-1:COX-2 selectivity of 1:3–77. It is the most widely used analgesics for dogs, exhibiting a prolonged action (Hawkey, 1999; Mathews *et al*., 2001). Meloxicam has been reported to be more effective compared with robenacoxib for pain control in the canine population (Bendinelli *et al*., 2019). Carprofen and meloxicam have provided satisfactory analgesia for 72 h in dogs after OHE (Leece *et al*., 2005). In the present study, meloxicam administration during the pre-operative period ensured pain control at the desired level during the intra- and post-operative periods, and its pre-emptive application was successful for peri-operative pain control.

Local anaesthetics and techniques form a part of the multimodal approach for post-operative pain management (Gurney, 2012). The local and infiltration anaesthesia of the wound area is an attractive method for relieving post-operative pain due to its simplicity and low cost (Moiniche *et al*., 1998). Accordingly, lidocaine, a local anaesthetic, is the most commonly used local (Jones, 2001; Almeida *et al*., 2010) and IV (Valverde *et al*., 2004; Columbano, 2012; Tsai *et al*., 2013) anaesthetic in veterinary practice. Local anaesthetics inhibit the conduction potential of the nerves by reversible blockade of the Na^+ channel (Ramsey, 2008). Local anaesthetics, including lidocaine, are potential components of balanced anaesthesia and their action of blocking Na^+ channels of the nerves inhibits the processing of increased noxious stimuli following topical or infiltrative administration. It has been reported in human medicine literature that intraperitoneal (IP) local anaesthetics, including bupivacaine, decreases early post-operative pain scores (Perniola *et al*., 2014; Roy *et al*., 2014; Arden *et al*., 2013) as well as post-operative pain scores in canine OHE (Carpenter *et al*., 2004; Campagnol *et al*., 2012; Kim *et al*., 2012) and a panel in dogs and cats (Mathews *et al*., 2014). For OHE, IP bupivacaine provided more effective analgesia compared with placebo (Campagnol *et al*., 2012; Carpenter *et al*., 2004). Another study compared the analgesic effect of post-operative continuous lidocaine administration in dogs that underwent OHE and that of intramuscular methadone and measured the dynamic interactive visual analogue scale, CMPS-SF, mechanical wound thresholds, heart rate, respiratory rate and blood pressure pre- and post-operatively at 2, 4, 6, 18 and 24 h and reported that continuous lidocaine administration via a wound catheter between the peritoneum and abdominal muscles provided effective analgesia in dogs and was considered a promising analgesic option in veterinary surgery (Morgaz *et al*., 2014). Tissue injury causes the activation of nociceptive and inflammatory responses that are frequently associated with pain, hyperalgesia and behavioural changes (Hansen *et al*., 1997; Beerda *et al*., 1998; Siracusa *et al*., 2008; Väisänen *et al*., 2002). In the present study, no significant intergroup differences were observed in the TNF-α and IL-1β levels measured post-operatively until postop 24 h ($p > 0.05$). This indicated that surgical trauma occurred at the same level in all the three groups based on the measurements obtained until postop 24 h and the pain sensation was similarly reflected in all the three groups.

The stress reaction induced by the operation and the associated pain can be detrimental for patient recovery, and hence, steps should be taken to minimize the same. Similar to human beings, animals also respond to stress by activating the sympathetic–adrenal–medullary and hypothalamic–pituitary–adrenal axes (Moberg and Mench, 2000; Hekman *et al*., 2014). The activation of these systems has been associated with changes in physiological parameters, including heart and respiratory rates, cortisol and catecholamines levels and neuropeptide secretion. Although it is necessary to cope with the acute homeostatic changes of the body, stress, particularly long-term stress reactions, can be harmful. Surgery-induced stress reaction is usually proportional to the degree of tissue trauma (Marana *et al*., 2003; Chernow *et al*., 1987; Horta *et al*., 2015), and post-operative stress and pain severity may also be affected by other factors, such as surgical skills and techniques, analgesic protocol and complications (Michelsen *et al*., 2012; Mastrocinque *et al*., 2012; Mastrocinque and Fantoni, 2003). IP and incisional bupivacaine spraying in dogs has been reported to be very effective in preventing post-operative pain in OHE (Korkmaz *et al*., 2019).

In the present study, the intergroup comparison of serum cortisol levels revealed a statistically significant difference among the groups at postop 0, postop 2 and postop 4 h. No statistical significance was observed among the groups at postop 8 and postop 24 h ($P > 0.001$); however, a decrease was noted. The results showed that lower cortisol levels were recorded in G1 at postop 0, postop 4 and postop 24 h. G1 also exhibited lower levels of surgical stress, which is consistent with other data. This was considered important by the authors.

This study also determined the concentrations of NO, involved in the up-regulation of the cytokine cascade in the early wound healing phase (Widgerow and Kalaria, 2012), pro-inflammatory cytokines TNF-α and IL-1β, which cause strong hyperalgesia (Dray, 1995) and the stress marker cortisol (Kingo *et al*., 2018), revealing no statistically significant difference among the three groups based on a comparison of the levels of the abovementioned parameters at the different measurement time points ($p > 0.05$). Hence, it was considered that stress markers were present at equal levels after the application of the three protocols included in this study and that they contributed to significant post-operative pain control.

In conclusion, although it was observed that all the three protocols induced an equal effect on post-operative pain and stress, taking into consideration the post-operative cortisol levels, increased surgical stress in G3 suggested that the other two protocols (G1 and G2) were more prominent for pain control, and thus, we recommend their usage for anaesthetic pain control in veterinary surgery. Future studies involving g larger samples are warranted for further confirming and strengthening the results of the present study.

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IRB approval

The present study was approved by Near East University Graduate Education Institute Directorate.

Ethical statement

The present study was approved by the Experimental Animals Ethics Committee of the Near East University (Approval No.: SBE/2019-148-21).

Statement of conflict of interest

The authors have declared no conflict of interest.

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