**Effect of deep neuromuscular block on stress response during laparoscopic gastrectomy**

**Trial registration:** NCT02100280

**Material and methods**

This prospective, randomized, and controlled study was done after approval of the institutional review board of Seoul National University Bundang Hospital (Seongnam, South Korea) and registration at clinicaltrials.gov (NCT02100280). Adult (≥18 yrs) patients, whose American Society of Anesthesiologists (ASA) physical status I or II, scheduled for elective laparoscopic gastrectomy who provided written informed consent were included. Patients with renal or hepatic dysfunction, history of neuromuscular disease, allergy for neuromuscular blocker, or family history or malignant hyperthermia were excluded. Patients were randomly assigned to groups moderate and deep using a computer-generated random numbers (Random Allocation Software, version 2.0). All of the primary and secondary outcome variables were checked by blinded investigator to patient group.

When patients arrived at the reception area, premedication was done with midazolam 0.03 mg kg⁻¹. Anaesthesia was induced and maintained with IV propofol, remifentanil, and rocuronium. The dose of propofol was adjusted to maintain bispectral index (BIS; A-2000 BIS™ monitor; Aspect Medical Systems, Inc., Natick, MA, USA) value of 40-60, remifentanil to maintain blood pressure and heart rate within 20% of preoperative value, and rocuronium to maintain train-of-four (TOF) 1-2 (moderate group) or post-tetanic count (PTC) 1-2 (deep group). The monitoring was composed of electrocardiography, noninvasive blood pressure, pulse oximetry, esophageal temperature, end tidal CO₂, BIS, and acceleromyography (TOF-Watch-SX; MSD BV,
Oss, The Netherlands).

The neuromuscular monitoring and management were done according to Good Clinical Research Practice guidelines. After induction of anaesthesia, continuous neuromuscular monitoring was started after calibration and stabilization of the signal as recommended in Good Clinical Research Practice guidelines; 50 Hz tetanic stimulation for 5 s, calibration, and stabilization for at least 2 min. After stabilization, rocuronium 0.6 mg / kg IV was administered for tracheal intubation. Maintenance dose of 0.1 - 0.2 mg / kg rocuronium was administered as needed for the maintenance of TOF1-2(moderateblock) or PTC1-2(deepblock). At the end of surgery, reversal of neuromuscular block was done according to current guidelines using IV neostigmine up to 50 μg / kg with glycopyrrolate in 5:1 ratio in moderate group and IV sugammadex 2-4mg / kg in deep group.

Blood samples were collected from the antecubital vein of the arm not used for IV infusion at preoperatively, at the end of peritoneal closure, and at 2 and 48hrs after the end of operation for analysis of TNF-α, IL-1β, IL-6, IL-8, and C-reactive protein (CRP) (Table 1). The blood samples were collected at the serum separating tubes and were left at room temperature for more than 30 minutes and centrifuged at 3,000 r.p.m for 10 minutes. Transfer the separated serum to Micro Tube 1.0ml each, and stored (less than -20 °C) in Deepfreezer. Cytokines were analysed with enzyme-linked immunosorbent assay kit(R&D Systems, Minneapolis, Minnesota, USA) and CRP was determined by an institutional chemistry analyser(Beckman Coulter, Brea, California, USA).

Intraoperative patient movement, the requirements for muscle relaxation by surgeon, the amount of rocuronium used, time from administration of reversal agent to TOF ratio of 0.9, operation time, and anesthesia time were recorded. A 5-point surgical rating scale (1 = excellent, 2 = good, 3 = acceptable, 4 =
poor, 5 = very poor) was rated by surgeon at the end of surgery. At post-anaesthesia care unit (PACU), symptoms or signs of residual paralysis were evaluated; hypoxemia (SpO2 < 95%), inability to maintain head elevation for 5 s, or diplopia.

Postoperative pain was evaluated using verbal numerical rating scale (VNRS, 0 = no pain, 10 = the severest pain imaginable) at postoperative 1, 2, 6, 24, 48 hr. Postoperative pain was controlled by IV patient controlled analgesia using fentanyl. If patient complained of severe pain (VNRS score of 7 or more), additional analgesics could be used by the attending physician. The amount of fentanyl used and additional analgesic drugs used were reported. Complications related to anticholinesterase, such as nausea or vomiting, dry mouth, and delayed gas out were also evaluate at the same time.

Statistica analysis

The primary outcome variable was the level of IL-6. To detect the difference in the IL-6 level of 10 pg/ml with α = 0.05 and β = 0.2, 43 patients per group were needed. This calculation was based on a between subject standard deviation of change of 16.3 pg/ml for IL-6 level from the previous study. Considering the 10% dropout rate, a total of 48 patients per group were decided to be recruited. Data were expressed as mean (SD) or median (range) and P < 0.05 were considered statistically significant. The interval data (cytokines levels) were analyzed by unpaired t-test, ordinal data (surgical rating scale, postoperative pain) by Mann-Whitney U test, and nominal data by test of Fisher’s exact test as appropriate. The comparison of data within the group was done by repeated measures ANOVA.