Randomized Trial

Immunosuppressive Effect of Intrathecal Morphine, Dexmedetomidine, or Both in Combination with Bupivacaine on Patients Undergoing Major Abdominal Cancer Surgery

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Background: An impaired immune system in the perioperative period has important clinical implications in patients with cancer. Despite the immunosuppressive properties of opioid therapy, it is still commonly utilized in the intrathecal or epidural space for the treatment of postoperative pain. Also, intrathecal dexmedetomidine has extended analgesic efficacy in postoperative pain; it can significantly affect immune function in perioperative patients.

Objective: To investigate the effect of intrathecal morphine, dexmedetomidine, or both in combination with bupivacaine on cellular immunity and cytokine production in cancer surgical patients.

Study Design: A prospective randomized clinical study.

Setting: South Egypt Cancer Institute, Assiut University.

Methods: Ninety patients were randomly assigned to receive intrathecal morphine 0.5 mg (Group M, n = 30), dexmedetomidine 0.5 μg/kg (Group D, n = 30) or morphine 0.5 mg with dexmedetomidine 0.5 μg/kg (Group MD n = 30); 2 mL bupivacaine 0.5% was added to injected drugs in all groups. Blood samples were collected preoperative (T0), immediate postoperative (T1), 4 hours postoperative (T2), and 24 hours postoperative (T3) for measurement of CD3, CD4, CD4/CD8 and CD16+56(NK), interleukin(IL)-1beta (IL-1β), IL-6, IL-10 and tumor necrosis factor alpha (TNF-α).

Results: A significant reduction in cellular immunity (CD3, CD4, CD8, CD4/CD8, CD 16+56) was noticed in the 24-hour postoperative period in all 3 studied groups, with a marked reduction in Group M in comparison to Group MD and Group D. Regarding inflammatory mediators, IL-10 and IL-1β showed significant reduction in Group M in the first 24-hour postoperative period in comparison to Group MD and Group D, while IL-6 was significantly reduced in Group MD and Group D in comparison to Group M in the same period. TNF-α was significantly increased postoperative at T1 and T2 in the 3 studied groups, then at T3 it decreased without a statistically significant difference with the preoperative level.

Limitations: Our study has some limitations, such as the short period of follow-up and lack of postoperative clinical follow-up of patients to discover the association between immunity and patient outcomes.

Conclusion: Intrathecal dexmedetomidine has the least immunosuppressive effect than morphine and morphine-dexmedetomidine, in combination with bupivacaine.

Key words: Cancer surgery, postoperative, intrathecal, morphine, dexmedetomidine, immunity

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When considering pain and analgesia, it is important to recognize the complex relations between the immune and nervous systems. It has been supposed that a well-regulated neuroimmune response to infection, painful stimuli, and tissue damage represents a cohesive system for host protection and tissue healing (1). However, the effect of pain resulting from tissue injury and the direct effect of tissue damage on immune function cannot be separated (2).

Surgical procedures have well proved their marked effects on the function of the immune system in humans, including increased vulnerability to infection, delayed wound healing, and enhanced tumor growth and spread of metastatic cancer (3). The mechanisms underlying immunosuppression in surgery are complex. They include pain, hypothalamic-pituitary-axis activation, sympathetic nervous system activation, tissue damage, and the effects of anesthesia and analgesia (4).

Opioid administrations in the intrathecal or epidural space are commonly utilized for treatment of postoperative pain (5,6). Intrathecal injection of morphine to obtain adequate postoperative analgesia during the first postoperative 24 hours is an extensively used technique (7,8); however, opioid therapy has been proved to have immunosuppressive properties (9).

Dexmedetomidine is a selective $\alpha 2$ agonist with pharmacological properties such as analgesia, drowsiness, anxiolysis, and sympatholysis. However, it may cause hypotension, hypertension, bradycardia, atrial fibrillation, nausea and hypoxia (10). A few clinical investigations have indicated that intrathecal dexmedetomidine has extended analgesic efficacy in the postoperative phase (11,12). Dexmedetomidine also can block the overproduction of a range of inflammatory molecules such as tumor necrosis factor (TNF-α) interleukin (IL)-1, and IL-6 in numerous acute inflammatory animal models, according to recent findings (13,14).

We therefore investigated the effect of intrathecal administration of morphine, dexmedetomidine, or both in combination with bupivacaine on cellular immunity and cytokine production in patients undergoing major abdominal cancer surgeries at postoperative 24 hours follow-up.

Methods

Study Design and Ethics

This prospective randomized double-blind comparative study was approved by the Research Ethics Committee of the South Egypt Cancer Institute, Assiut University, Assiut, Egypt. Our protocol was prospectively registered with the Clinical Trials.gov trial registry (identifier: NCT03024957) and strictly followed the regulations and amendments of the Helsinki Declaration. All study patients provided written informed consent. The trial report complies with the Consolidated Standards of Reporting Trials (CONSORT) checklist.

Patients

Ninety patients were enrolled. They were classified as I or II according to the American Society of Anesthesiologists physical status classification system. They were aged 20-70 years old and were scheduled for a major abdominal cancer surgery. Excluded from the study were patients with a known allergy to the study drugs; significant cardiac, respiratory, renal, hepatic, or immune system disease; a body mass index $\geq$ 30 kg/m^2; a history of opioid use for pain management at the time of enrollment or drug addiction; a history of taking any drug affecting the immune system; previous stroke; a psychiatric disease that could affect pain perception.

Outcome

The primary outcome was the effect of intrathecal administration of morphine, dexmedetomidine, or both in combination with bupivacaine on cellular immunity (CD3+, CD4+, CD8+), and natural killer cells (NK) (CD16+56). The secondary outcome was cytokine production IL-1β, IL-6, IL-10, and TNF-α).

Randomization and Blinding

Ninety patients were randomly allocated into one of 3 groups of 30 patients each, based on a computer-generated randomization table.

- Morphine group (Group M) (30 patients).These patients received 10 mg of hyperbaric bupivacaine 0.5% in 2 mL volume and 0.5 mg morphine in 1 mL volume intrathecally.
- Dexmedetomidine group (Group D) (30 patients). These patients received 10 mg of hyperbaric bupivacaine 0.5% in 2 mL volume and 5 µg of dexmedetomidine in 1 mL volume intrathecally.
- Morphine + dexmedetomidine group (Group MD) (30 patients). These patients received 10 mg of hyperbaric bupivacaine 0.5% in 2 mL volume and 0.5 mg of morphine plus 5 µg of dexmedetomidine in 1 mL volume intrathecally.

Total volume in the 3 groups was 3 mL.

The investigated drugs were prepared in a ster-

ile syringe by the hospital pharmacy and given to an investigator who was blinded to the identity of the drugs. The attending anesthesiologist, surgeon, data collection personnel, and the patient were all blinded to the patient group assignment. We tried to replicate the same conditions for all patients in order to obtain accurate results.

Procedures

Patients took oral diazepam (5 mg) the night before surgery. Upon arrival at the operative theater, a 16G catheter was introduced intravenously at the dorsum of the hand; lactated Ringer's solution 10 mL/kg was infused intravenously over 10 minutes before initiation of spinal anesthesia. Basic monitoring probes (electrocardiography, noninvasive blood pressure, SpO_2, and temperature) were applied. Patients were placed in a sitting position. A 25G Quincke needle was placed in either the L2-L3 or L3-L4 interspaces and the injected drugs were given according to the group allocation.

Immediately after successful spinal anesthesia, patients were repositioned supine; general anesthesia was induced with fentanyl 1.5–2 µg/kg, propofol 2–3mg/kg, and lidocaine 1.5 mg/kg. Endotracheal intubation was facilitated by cisatracurium 0.15 mg/kg.

Anesthesia and muscle relaxation were maintained by isoflurane 1–1.5 minimum alveolar concentration (MAC) in a 50% oxygen/air mixture. A cisatracurium 0.03 mg/kg bolus was given every 30 minutes. At the end of surgery, muscle relaxation was reversed by neostigmine 50 µg/kg and atropine 10 µg/kg. Patients were extubated and transferred to the postanesthesia care unit.

Outcome Assessments and Data Collection

All patients were followed for the first postoperative 24 hours in the postanesthesia care unit. Five mL of venous blood at T0 (preoperative), T1 (immediate postoperative), T2 (4 hours postoperative), and T3 (24 hours postoperative) were collected respectively and blended in anticoagulant tubes. Then, after ambient-temperature centrifugation at 3,000 r/min for 5 minutes by low-speed centrifuge, plasma was taken and reserved in a refrigerator at -40°C for backup.

Flow cytometric analysis for the anticoagulated blood samples was tested by flow cytometer (FACS Calibur, BD company) to count T lymphocytes subsets (CD3+, CD4+, CD8+), and NK (CD56+).

Cytokine analysis was done by Human Premixed Multi-Analyte Kit, Luminex Assay (catalog no. LXSAH-10; R&D Systems). All of the samples were analyzed at the same time. A list of analytes available in the polystyrene or magnetic bead formats for the Luminex were used. Microparticles, standards, and samples were pipetted into wells and the immobilized antibodies bound with the biomarkers of interest. According to the manufacturer's protocol, bead-based multiplex assay for the Luminex platform were used. It was used to detect concentrations of IL-1β), IL-6, IL-10, and TNF-α.

Statistical Analysis

The sample size was calculated using G*Power version 3.1.9.2.Software (15). A calculated sample size of 28 would have 80% power and a type I error of 0.05 using a 95% CI to detect a difference at a level 0.05 of significance. Considering potential drop-outs, we decided to enroll 30 patients in each group for the study.

Data entry and data analysis were done using SPSS version 19 (Statistical Package for Social Science). Data were presented as number, percentage, mean, and standard deviation. The χ^2 test was used to compare between qualitative variables. The Mann-Whitney U test was used to compare quantitative variables between 2 groups. Wilcoxon's signed rank test was done to compare between pre- and postoperation quantitative variables in the case of nonparametric data. A P value of < 0.05 was considered statistically significant.

Results

Patients' Characteristics

Ninety-four patients were assessed for eligibility to participate in this study. Four patients were excluded from the study because they refused to participate. A final number of 90 patients completed this study and were subjected to statistical analysis. These patients were equally distributed into 3 groups (n = 30 per group) as shown in the flow diagram (Fig. 1). There were no significant differences among the 3 studied groups as regards demographic (age, gender) and clinical characteristics (diagnosis) ($P > 0.05$) (Table 1).

Study Endpoints

Primary Outcome

A significant reduction in the serum level of CD3, CD4, CD8, CD4/CD8, and CD 16+56 was noticed at T2 and T3 in the 3 studied groups, except CD8 when the significant reduction started early at T2 ($P < 0.05$). The reduction was statistically significant in Group

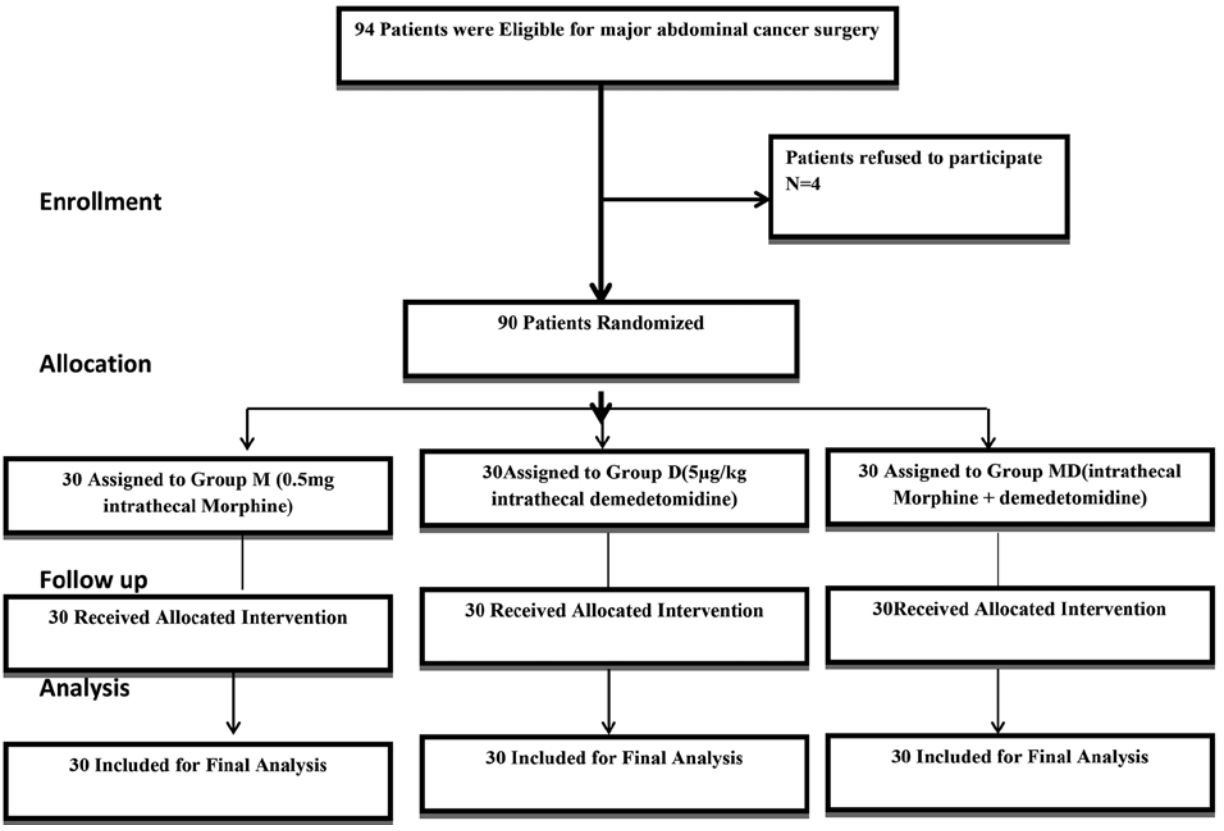

Fig. 1. *Flow diagram of the study patients.*

Table 1. *Personal data and type of operation of the studied group.*

	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Age: Mean ± SD Range	 49.33 ± 8.34 32.0 – 66.0	 48.93 ± 8.40 33.0 – 65.0	 49.33 ± 8.21 33.0 – 64.0	0.830	0.988	0.824
Gender: No. (%) Men Women	 15 (50.0%) 15 (50.0%)	 13 (43.3%) 17 (56.7%)	 14 (46.7%) 16 (53.3%)	0.605	0.796	0.795
Type of Operation: No. (%) Colectomy	 7 (23.3%)	 6 (20.0%)	 6 (20.0%)	0.754	0.754	1.000
Anterior Resection of sigmoid and rectosigmoid colon	6 (20.0%)	5 (16.7%)	6 (20.0%)	0.739	1.000	0.739
Ovariectomy	8 (26.7%)	9 (30.0%)	10 (33.3%)	0.774	0.573	0.781
Cystectomy	9 (30.0%)	10 (33.3%)	8 (26.7%)	0.781	0.774	0.573

Data expressed as mean ± SD (range) number and percentage. *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD. * = $P < 0.05$.

M in comparison with the other 2 groups at T3 ($P < 0.05$).

There was a significant reduction in the serum level of CD3 and CD4 at T3 in Group M in comparison to both Group D and Group MD. Also, there was a significant reduction in CD3 in Group M and Group D at T2 and T3

in comparison to T0, and a significant reduction in CD3 in Group MD at T2 compared to the T0 level ($P < 0.05$) (Table 2).

The serum level of CD4 showed a significant reduction in Group M and Group MD at T2 and T3 in comparison to T0; there was a significant reduction in CD4 in Group D at T2 in comparison to the T0 level ($P < 0.05$) (Table 3).

The serum level of CD8 was significantly reduced in Group M at T2 and T3 in comparison to Group D and at T3 in comparison to Group MD. There was a

Table 2. *Changes in the level of CD3 in the 3 studied groups during the study period.*

CD3	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	50.83 ± 15.35	51.07 ± 15.15	50.93 ± 14.98	0.935	0.959	0.959
Median (Range)	52.5 (17.0-72.0)	53.5 (17.0-72.0)	53.0 (18.0-73.0)			
Immediate PO:						
Mean ± SD	51.78 ± 14.88	51.63 ± 15.05	51.74 ± 14.70	0.935	0.935	0.935
Median (Range)	53.5 (18.0-73.0)	53.5 (20.0-73.0)	55.0 (16.0-72.0)			
P Value[4]	0.200	0.872	0.234			
4 hours PO:						
Mean ± SD	39.87 ± 14.34	43.57 ± 14.14	41.47 ± 15.13	0.307	0.652	0.584
Median (Range)	43.0 (14.0-60.0)	47.5 (12.0-65.0)	43.0 (11.0-65.0)			
P Value[4]	0.000*	0.000*	0.000*			
24 hours PO:						
Mean ± SD	36.20 ± 13.82	48.30 ± 14.70	49.33 ± 14.90	0.003*	0.002*	0.819
Median (Range)	40.0 (12.0-57.0)	47.5 (11.0-72.0)	52.0 (16.0-70.0)			
P Value[4]	0.000*	0.033*	0.330			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$. PO, postoperative.

Table 3. *Changes in the level of CD4 in the 3 studied groups during the study period.*

CD4	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	32.37 ± 9.25	32.57 ± 9.09	32.73 ± 8.69	0.865	0.959	0.778
Median (Range)	30.5 (16.0-46.0)	31.0 (11.2-46.0)	31.0 (15.0-45.0)			
Immediate PO:						
Mean ± SD	32.43 ± 9.47	33.60 ± 8.91	33.43 ± 8.56	0.630	0.761	0.761
Median (Range)	31.5 (13.0-47.0)	32.0 (14.0-45.0)	31.0 (16.0-47.0)			
P Value[4]	0.761	0.050*	0.418			
4 hours PO:						
Mean ± SD	24.60 ± 8.64	26.33 ± 8.03	23.38 ± 7.91	0.367	0.529	0.079
Median (Range)	23.3 (10.5-38.0)	25.0 (9.5-41.0)	21.0 (11.5-38.0)			
P Value[4]	0.000*	0.001*	0.000*			
24 hours PO:						
Mean ± SD	22.90 ± 8.34	31.88 ± 8.31	30.00 ± 8.51	0.000*	0.005*	0.293
Median (Range)	21.0 (8.0-37.0)	30.5 (10.0-45.0)	30.0 (16.0-44.0)			
P Value[4]	0.000*	0.502	0.000*			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$. PO, postoperative.

significant reduction in postoperative CD8 in comparison to the T0 level in the 3 studied groups (Table 4).

The serum levels of CD4/CD8 and CD16+56 were significantly reduced at T2 and T3 in comparison to the T0 level in the 3 studied groups (Tables 5, 6).

Table 4. *Changes in the level of CD8 in the 3 studied groups during the study period.*

CD8	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	15.52 ± 7.64	16.03 ± 7.83	14.73 ± 4.40	0.750	0.911	0.841
Median (Range)	14.0 (7.0-41.0)	14.0 (7.0-42.0)	13.0 (8.0-27.0)			
Immediate PO:						
Mean ± SD	15.87 ± 7.51	16.53 ± 6.98	15.33 ± 4.46	0.468	0.683	0.699
Median (Range)	14.0 (7.5-40.5)	15.0 (9.0-42.8)	14.0 (9.0-30.0)			
P Value[4]	0.294	0.007*	0.005*			
4 hours PO:						
Mean ± SD	12.20 ± 3.69	14.92 ± 6.90	12.57 ± 3.59	0.047*	0.504	0.201
Median (Range)	11.0 (6.0-21.0)	13.1 (7.7-37.0)	11.6 (6.0-22.0)			
P Value[4]	0.000*	0.042*	0.011*			
24 hours PO:						
Mean ± SD	11.26 ± 3.29	14.64 ± 5.33	13.99 ± 4.25	0.001*	0.004*	0.733
Median (Range)	10.4 (5.5-20.0)	13.2 (7.8-34.0)	13.0 (7.0-28.0)			
P Value[4]	0.001*	0.101	0.038*			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$. PO, postoperative.

Table 5. *Changes in the level of CD4/CD8 ratio in the 3 studied groups during the study period.*

CD4/CD8	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	2.34 ± 0.88	2.22 ± 0.66	2.32 ± 0.67	0.600	1.000	0.900
Median (Range)	2.3 (1.1-3.9)	2.3 (0.7-3.3)	2.2 (1.4-3.9)			
Immediate PO:						
Mean ± SD	2.24 ± 0.77	2.15 ± 0.59	2.26 ± 0.63	0.647	0.959	0.819
Median (Range)	2.3 (1.1-3.5)	2.3 (0.7-3.0)	2.2 (1.3-3.9)			
P Value[4]	0.431	0.079	0.119			
4 hours PO:						
Mean ± SD	2.04 ± 0.64	1.91 ± 0.64	2.00 ± 0.70	0.559	0.751	0.717
Median (Range)	2.0 (1.1-3.6)	2.0 (0.6-3.0)	1.8 (0.8-3.5)			
P Value[4]	0.017*	0.003*	0.001*			
24 hours PO:						
Mean ± SD	1.97 ± 0.56	2.36 ± 0.85	2.23 ± 0.68	0.075	0.158	0.679
Median (Range)	1.9 (1.1-2.9)	2.3 (0.9-3.8)	2.2 (1.2-3.7)			
P Value[4]	0.015*	0.604	0.035*			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$. PO, postoperative.

Secondary Outcome

All the measured inflammatory mediators were markedly increased at T1 in the 3 studied groups, which may be due to surgical stress.

The serum level of IL-1β was significantly reduced in Group M in comparison to Group D at T2 (Table 7).

Regarding the serum level of IL-10, there was a significant reduction at T1 in Group M and Group MD

Table 6. *Changes in the level of CD16+56 in the 3 studied groups during the study period.*

CD16+56	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	21.83 ± 7.03	22.03 ± 7.78	21.93 ± 7.25	1.000	0.976	0.882
Median (Range)	20.5 (12.0-37.0)	19.0 (12.0-37.0)	20.5 (12.0-35.0)			
Immediate PO:						
Mean ± SD	22.70 ± 6.53	22.14 ± 7.57	22.63 ± 6.96	0.574	0.795	0.733
Median (Range)	22.0 (12.0-36.0)	20.4 (12.0-38.0)	20.4 (14.0-35.0)			
P Value[4]	0.142	0.672	0.004*			
4 hours PO:						
Mean ± SD	19.77 ± 7.70	20.57 ± 7.89	18.50 ± 7.31	0.641	0.558	0.173
Median (Range)	18.0 (10.0-35.0)	18.0 (8.1-36.0)	17.5 (8.0-31.0)			
P Value[4]	0.000*	0.000*	0.000*			
24 hours PO:						
Mean ± SD	19.53 ± 7.98	21.14 ± 7.88	20.59 ± 7.38	0.407	0.463	0.717
Median (Range)	17.5 (10.0-36.0)	18.0 (10.7-35.0)	19.5 (9.2-33.0)			
P Value[4]	0.000*	0.003*	0.000*			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$. PO, postoperative.

Table 7. *Changes in the level of IL-1β in the 3 studied groups during the study period.*

IL-1β	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	1.70 ± 0.88	1.59 ± 0.86	1.58 ± 0.90	0.679	0.605	0.824
Median (Range)	1.4 (0.7-4.0)	1.4 (0.6-4.6)	1.4 (0.6-4.6)			
Immediate PO:						
Mean ± SD	1.74 ± 0.75	1.79 ± 0.75	1.69 ± 0.75	0.848	0.756	0.631
Median (Range)	1.6 (0.8-3.2)	1.6 (0.8-3.2)	1.5 (0.8-3.2)			
P Value[4]	0.629	0.178	0.309			
4 hours PO:						
Mean ± SD	1.31 ± 0.24	1.76 ± 0.74	1.71 ± 0.77	0.026*	0.143	0.906
Median (Range)	1.4 (0.8-1.7)	1.6 (0.8-3.1)	1.5 (0.8-3.1)			
P Value[4]	0.339	0.781	0.469			
24 hours PO:						
Mean ± SD	1.68 ± 0.85	1.73 ± 0.85	1.70 ± 0.84	0.706	0.796	0.871
Median (Range)	1.4 (0.7-4.0)	1.5 (0.7-4.0)	1.5 (0.5-4.0)			
P Value[4]	0.658	0.629	0.629			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$. PO, postoperative.

in comparison to Group D. At T2 there was a significant reduction in Group M in comparison to Group D. Also, at T3 there was a significant reduction in Group M in comparison to both Group D and Group MD (Table 8).

The serum level of IL-6 was significantly reduced in both Group D and Group MD in comparison to group M at T2 and T3 ($P < 0.05$) (Table 9).

TNF-α serum level was significantly increased at T1 and T2 in comparison to the T0 level in the 3 studied groups (Table 10).

Table 8. *Changes in the level of IL-10 in the three studied groups during the study period.*

IL-10	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	1.61 ± 0.96	1.56 ± 0.93	1.52 ± 0.90	0.882	0.668	0.830
Median (Range)	1.4 (0.6-4.6)	1.4 (0.5-4.3)	1.4 (0.5-4.2)			
Immediate PO:						
Mean ± SD	43.89 ± 28.58	69.17 ± 25.89	48.55 ± 19.91	0.000*	0.060	0.001*
Median (Range)	35.2 (18.2-129.8)	62.2 (20.3-120.7)	42.3 (16.0-91.0)			
P Value[4]	0.000*	0.000*	0.000*			
4 hours PO:						
Mean ± SD	32.07 ± 17.02	44.83 ± 20.25	36.70 ± 17.40	0.009*	0.225	0.132
Median (Range)	30.0 (9.7-81.2)	38.8 (17.5-80.5)	33.1 (12.1-83.4)			
P Value[4]	0.000*	0.000*	0.000*			
24 hours PO:						
Mean ± SD	17.84 ± 9.97	25.19 ± 12.42	25.41 ± 14.88	0.006*	0.003*	0.626
Median (Range)	16.0 (7.2-50.0)	24.3 (8.8-66.6)	21.6 (11.0-80.8)			
P Value[4]	0.000*	0.000*	0.000*			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$.

Table 9. *Changes in the level of IL-6 in the 3 studied groups during the study period.*

IL-6	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	1.33 ± 0.21	1.34 ± 0.21	1.34 ± 0.22	0.647	0.690	0.953
Median (Range)	1.4 (0.9-1.7)	1.4 (0.9-1.7)	1.4 (0.9-1.7)			
Immediate PO:						
Mean ± SD	98.30 ± 63.42	100.06 ± 67.10	99.49 ± 73.78	0.929	0.918	0.912
Median (Range)	84.3 (22.3-302.4)	88.9 (22.6-346.5)	87.4 (22.8-333.1)			
P Value[4]	0.000*	0.000*	0.000*			
4 hours PO:						
Mean ± SD	79.54 ± 48.55	37.72 ± 24.97	57.64 ± 34.08	0.000*	0.030*	0.006*
Median (Range)	70.4 (22.0-205.2)	29.7 (11.3-100.2)	48.9 (22.3-160.2)			
P Value[4]	0.000*	0.000*	0.000*			
24 hours PO:						
Mean ± SD	61.21 ± 20.86	34.69 ± 13.68	52.09 ± 19.11	0.000*	0.099	0.000*
Median (Range)	62.7 (20.3-100.0)	38.5 (10.9-60.2)	54.4 (15.5-85.0)			
P Value[4]	0.000*	0.000*	0.000*			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$.

Table 10. *Changes in the level of TNF-α in the three studied groups during the study period.*

TNF{should this be TNF-α?}	Group M (n = 30)	Group D (n = 30)	Group MD (n = 30)	*P* Value[1]	*P* Value[2]	*P* Value[3]
Pre-operative:						
Mean ± SD	37.33 ± 11.00	37.73 ± 11.75	37.16 ± 10.56	0.929	0.959	0.802
Median (Range)	36.2 (17.0-55.8)	37.1 (15.8-63.3)	36.4 (17.7-54.1)			
Immediate PO:						
Mean ± SD	65.47 ± 36.08	64.09 ± 38.35	64.08 ± 35.59	0.595	0.756	0.813
Median (Range)	61.6 (21.0-200.1)	59.7 (20.4-205.4)	61.9 (20.2-180.1)			
P Value[4]	0.000*	0.000*	0.000*			
4 hours PO:						
Mean ± SD	58.66 ± 20.11	51.53 ± 20.65	57.58 ± 20.16	0.110	0.923	0.154
Median (Range)	55.5 (32.0-133.0)	52.7 (20.2-120.0)	55.5 (27.1-130.4)			
P Value[4]	0.000*	0.000*	0.000*			
24 hours PO:						
Mean ± SD	39.40 ± 12.86	32.92 ± 13.11	36.02 ± 13.50	0.117	0.220	0.264
Median (Range)	37.0 (10.0-75.2)	30.5 (13.2-75.0)	33.7 (8.6-70.9)			
P Value[4]	0.614	0.057	0.530			

Data expressed as mean ± SD, median (range). *P* value[1]: Significance between Group M and Group D; *P* value[2]: Significance between Group M and Group MD; *P* value[3]: Significance between Group D and Group MD; *P* value[4]: Significance between baseline and each time point of assessment * = $P < 0.05$.

Discussion

An impaired immune system in the perioperative period has important clinical implications because it is associated with an increased risk of developing postoperative infections and sepsis, and it has been suggested that it increases the risk of disease progression in patients with cancer (16). Regional analgesia has potential beneficial effects on the immune system because it reduces anesthetic consumption, ameliorates surgical stress, facilitates pain control, and reduces opioid consumption (17).

Cellular immunity, commonly known as T lymphocyte immunity, is characterized by CD3+, an antigen-expressing signal found on all mature T cells (18). T helper cells, also known as CD4+ T cells, aid in the activation of cellular and humoral immunity (19). CD8+ T cells are mostly immunosuppressive lymphocytes that prevent other immune cells from performing their tasks. A significant drop in the CD4+/CD8+ ratio usually implies disease severity and a bad prognosis (20). NK cells, a subset of lymphocytes, play a critical role in the immune system's defense against viral and bacterial infection, as well as mediating spontaneous cytotoxicity against tumor cells (21).

Th1 type and Th2 type CD4+ positive cells can be divided into 2 subgroups with distinct functions. Unless a tumor develops, Th1/Th2 is reasonably balanced in normal conditions. When a tumor is present, Th2 cells predominate, but the cellular immune response mediated by Th1 is blocked, resulting in immunological suppression, weakening of antitumor activity, and immune escape of tumor cells, leading to tumor recurrence (22). Th1 secretes IL-2, TNF-β, and interferon-gamma, stimulates T cells and macrophages, mediates the cellular immune response, and decreases infection after surgery. Th2 primarily produces IL-4, IL-6, and IL-10, as well as inducing B-lymphocytes to produce immunoglobulin and mediate humoral immunity (23). IL-6 is the "gold index" for postoperative stress; it plays both an anti-inflammatory and pro-inflammatory role in the stress response and immune response by activating the hypothalamic-pituitary-adrenocortical axis system (24). According to studies, IL-10 can both cause inflammation and trigger anti-inflammation, allowing the immune function to remain relatively stable (25).

This study was designed to evaluate the effect of intrathecal administration of morphine, dexmedetomidine, or both in combination with bupivacaine on cellular immunity and cytokine production in patients undergoing major abdominal cancer surgeries in the postoperative 24 hour follow-up period. Our results postulate that intrathecal dexmedetomidine provided a lower immune-suppressant effect than the combina-

tion (dexmedetomidine and morphine) and morphine groups.

In Group M, there were significant reductions in CD3, CD4, CD8, CD4/CD8, and CD16+56 at T2 and T3. Regarding inflammatory mediators (IL-10, IL-6, and TNF-α), they were significantly reduced at T2 and T3 except at T3 TNF-α the reduction was not significant. A slight reduction in IL-1β was noticed in T2 and T3.

In Group D, there was a significant reduction at T1 in CD4 and CD8, while in CD3 and CD16+56 the significance started at T2. The CD4/CD8 ratio also was reduced in the postoperative period; the reduction was only significant at T2. All the inflammatory mediators (IL-6, IL-10, TNF-α) were significantly reduced at T2 and T3. IL1β did not show any significant changes.

In Group MD, there were minimal changes in the level of CD3, CD4, and CD4/CD8 in the postoperative period. Regarding CD3, a significant reduction was detected at T2. CD4 and CD4/CD8 were significantly reduced at T2 and T3. However, CD8 and CD 16+56 showed a significant reduction at T2 and T3. The inflammatory mediators (IL-6, IL-10, TNF-α) were significantly increased at T1, T2, and T3. IL-1β did not show any significant changes. So its effect on the pro- and anti-inflammatory mediators is close to the effect of Group D, with a slight decrease in cellular immunity.

In clinical trials, dexmedetomidine reduces plasma IL-6 concentration during the postoperative period (26). Studies have revealed that patients who received dexmedetomidine, postoperative levels of IL-1, IL-6 and TNF-α were decreased, suggesting that dexmedetomidine could effectively inhibit inflammatory responses, thereby ameliorating the cellular immune functions of patients to a certain degree (27,28).

In concordance with our study, Kawasaki et al (29) studied the effect of dexmedetomidine on the production of pro-inflammatory mediators (TNF-α, IL-6) in human blood induced by lipopolysaccharide and showed the inhibitory effect of dexmedetomidine on it. The mechanism by which dexmedetomidine inhibits the production of pro-inflammatory mediators can occur through α2-adrenergic receptors and inhibition of necrosis factor B.

Also, animal studies conducted by Xiang et al (30) led to a preventive administration of dexmedetomidine with survival in lipopolysaccharide-induced endotoxemia greatly improved. This was accompanied by a reduction in the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. However, the anti-inflammatory and protective effects of dexmedetomidine are not eliminated during vagotomy.

The effect of dexmedetomidine on the perioperative cellular immunity (CD3, CD4, CD8, CD4/CD8, NK) of children with brain tumors was studied by Wu et al (31). They found that intravenous infusion of dexmedetomidine during general anesthesia inhibits the perioperative stress response and cellular immune suppression.

Dong et al (32) found that dexmedetomidine effectively reduced the release of inflammatory factors of patients undergoing a resection due to gastric cancer, including IL-1β, TNF-α, and IL-6 and that it exerts its anti-inflammatory effect through down-regulating the expression of anti-inflammation pathways. They also found that dexmedetomidine can reduce the decline of CD3+ and CD4+ subgroup levels, improving impaired immune function.

Our findings are consistent with the meta-analysis of Wang et al (33). They concluded that dexmedetomidine can reduce perioperative stress and inflammation caused by surgical trauma, protect immune function, and has a variety of protective effects when administered as an anesthetic adjuvant. All these indicate that dexmedetomidine may provide benefits to patients undergoing surgery and improve the clinical outcomes of these patients during the perioperative period (33).

Yang et al (34) conducted a study on patients undergoing mastectomy to evaluate the effect of dexmedetomidine on perioperative immune function. The level of CD3+ and CD4+ in the dexmedetomidine group rose remarkably at 6, 48, and 72 hours postoperatively. The cellular level of CD8+ decreased significantly 24 hours postoperatively, while the level of NK cells increased markedly 6 hours and 24 hours after the operation; CD4+/CD8+ increased dramatically at all postoperative times. They concluded that intravenous dexmedetomidine can significantly reduce the inhibition of cellular immune function in the perioperative period of patients undergoing mastectomy and is important for the maintenance and improvement of the body's immune function, as well as for the prognosis of patients undergoing surgery (34).

Dexmedetomidine has a protective effect on immune stress which is manifested from the following aspects: 1) it reduces acute psychological stress reaction by sedation, thereby indirectly playing the role of immune protection. A study (35) showed that the effect of acute psychological stress on immune function is expressed as the increase of CD8+ and NK cells as well as proliferation decline of CD4+ and T-lymphocytes, which then lead to infectious diseases; 2) by lessening immune inhibition, which can be reached by dexmedetomidine

itself through inflammation reduction, it plays a role in postoperative immune protection (36).

Opioids, such as morphine, are used to relieve postoperative pain; however, they have side effects. One study demonstrated a relationship between morphine and immunosuppression (37). Moreover, the effect of acute opioid exposure on the immune system differs from that of chronic exposure (38). In humans, morphine has been shown to decrease NK cell activity throughout the postoperative period when given intravenously or intrathecally (39).

A study done by Zou et al (40), using an inflammatory pain model, showed that intrathecal administration of morphine can suppress immune function by changing the activities and the percentage of immune cells, and suggests that a spinal mechanism may be involved in morphine-induced immunosuppression.

Morphine has been extensively researched and is known as the classic opioid analgesic and the benchmark against which other opioids are measured (41). It has been widely studied, with reductions in functions of innate and adaptive immunity, as well as a significant reduction in cellular immunity, following acute and chronic morphine treatment (37).

In vivo morphine administration is linked to a decrease in innate immunity (42). In vivo treatment with morphine reduces the function of NK cells, T cells, B cells, and polymorphonuclear leukocytes (43). Yeager et al (44) undertook a clinical investigation to assess the effects of morphine on human immunity in vivo. Healthy volunteers were given either a low or high dose, continuous intravenous morphine exposure for 24 hours. To explore the effects of morphine on the immune system, peripheral blood was taken for investigation. At 2 and 24 hours after starting the intravenous morphine treatment, substantial inhibition of NK cytotoxicity was seen. These findings demonstrate that, at an analgesic dose range, morphine can produce a significant inhibition of the cellular immune system (44).

In patients undergoing hysterectomy, the effect of intrathecal morphine on NK cell activity was studied (45). Three groups were given 0.5 mg intrathecal morphine, 0.1 mg intrathecal morphine, or 10 mg intravenous morphine, respectively, whereas the control group was given an inhalational anesthetic. Blood samples were taken to measure the activity of NK cells. When compared to baseline, the group that received 0.5 mg intrathecal morphine exhibited lower NK cell activity on first postoperative day and demonstrated recovery on second postoperative day. The NK cell activity in the control group, the group given 0.1 mg intrathecal morphine, and the group given intravenous morphine revealed no significant differences (45). These findings are in line with those of Yokota et al (46), who found that NK cell activity decreased on first postoperative day in groups given 0.5 mg intrathecal morphine (46).

Cytokines are affected by signals released by cells after morphine administration (e.g., elevation in IL-6 concentrations) (47,48). This result indicates that immune responses are influenced by the interaction of morphine action in neural cells and peripheral tissue. Healthy postpartum women who had been given morphine through intravenous, epidural, or spinal route had their peripheral blood tested. The findings showed that morphine lowered IL-2 expression in CD4+ cells following activation, regardless of the route of administration, while this effect was not seen in CD8+ cells. IL-6 production was significantly boosted by spinal and intravenous morphine, whereas IL-10 production was inhibited by epidural morphine. Intravenous or epidural morphine may block the expression of different cytokines more effectively than spinal morphine, affecting immunological response. The generation of IL-2 was reduced by all 3 methods of morphine administration (38).

Limitation

Our study has some limitations, such as the short period of follow-up, only within 24 hours, and the lack of postoperative clinical follow-up to discover the association between immunity and patient outcomes.

Conclusion

The use of intrathecal dexmedetomidine either alone or in combination with morphine led to a lower immunosuppressive effect than intrathecal morphine alone. On comparing groups with each other we found that the lowest level of the proinflammatory mediator IL-6 was detected in Group D then Group MD and Group M respectively. Regarding the anti-inflammatory mediator IL-10, its level was higher in Group D and Group MD than Group M.

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