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Research Article

The effect of general or spinal anaesthesia on pro- and anti-inflammatory intracellular cytokines in patients undergoing appendicectomy using flowcytometric method

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KEYWORDS

Stress;
Intracellular cytokines;
General;
Spinal anaesthesia

Abstract *Background:* Cytokines are glycoproteins which have important role in immunological changes that occur in perioperative period. The balance between pro- and anti-inflammatory cytokines controls the clinical course and outcome.
Objectives: Compare intracellular levels of pro- and anti-inflammatory cytokines with general or spinal anaesthesia.
Patients and methods: Forty patients ASA physical status I–II between 20 and 35 years old, 23 males and 17 females undergoing appendicectomy were included in this comparative prospective study. Patients were randomly allocated into two equal groups: Group G under general anaesthesia induced by thiopental 5 mg/kg, fentanyl 2 μg/kg. Tracheal intubation facilitated by 0.8 mg/kg rocuronium and the maintenance with isoflurane 1.5% plus rocuronium 0.15 mg/kg, Group S under spinal anaesthesia with hyperbaric bupivacaine 0.5% 3–4 ml. Arterial blood pressure, heart rate and oxygen saturation were measured pre, post-induction of anaesthesia, then every 15 min till the end of operation. Tumour Necrosis Factor-α, Interleukin-6 and Inter-

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leukin-10 levels were measured before induction of anaesthesia, at the end of surgery and 12 h postoperatively.

Results: The study recorded a significant increase in pro-inflammatory (TNFα and IL-6) and the anti-inflammatory (IL-10) cytokine levels at the end of surgery when compared with pre-anaesthetic level in both groups with more significant increase of IL-10 in spinal group than general group. At 12 h postoperatively, TNF-α returned to normal in both groups; IL-6 and IL-10 increased significantly in all patients of both groups with more significant increase in IL-10 level in spinal group than general group. These findings show that there is significant difference between spinal and general anaesthesia as regards to IL-10 which increased more in spinal group than general group at the end of surgery and 12 h postoperatively.

Conclusion: The inflammatory response to surgery was suppressed during spinal anaesthesia to a greater extent than during general anaesthesia.

1. Introduction

Surgical stress causes variable effects on haemodynamic and immunologic responses with the release of various stress hormones [1]. Also, anaesthesia affects the immune response with the release of different cytokines which affect the inflammatory response in the postoperative period.

Among the cytokines which affect these responses were the Tumour Necrosis Factor α (TNF-α), Interleukin-6 (IL-6) and Interleukin-10 (IL-10) [2–4].

Cytokines are intracellular regulatory proteins acting through specific receptors, and have a major role in the immune response and inflammatory changes caused by surgery or infection [5].

There are two main groups of cytokines; proinflammatory cytokines produced by macrophages mainly acting through upregulation of inflammatory reactions and anti-inflammatory cytokines acting through the downregulation of inflammatory reactions [4].

The balance between the proinflammatory cytokines (TNF-α and IL-6) and the anti-inflammatory cytokines (IL-10) limit the spread of infection, tissue injury and promote tissue healing and repair by their local and systemic effects [4–6].

As regards the cytokines which were examined in the current study, TNF-α is the early mediator in the inflammatory response and is responsible for production of other cytokines as proinflammatory IL-6 and anti-inflammatory IL-10.

TNF-α is produced by a variety of other cell types including lymphoid cells, mast cells, endothelial cells and others. The local increase of its concentration causes cardinal signs of inflammation as heat, swelling, redness and pain. Also, TNF-α is responsible for some haemodynamic changes such as tachycardia and hypotension with some physiological changes as fever, oliguria, and changes in the level of consciousness in septic patients [7–9].

Interleukin-6 is a soluble glycoprotein secreted by T-cells and macrophages. It detects the extent of tissue injury as it is an important mediator for development of acute phase response during inflammation and immune response, also has an important role in host defence and haematopoiesis [10].

Interleukin-10 also known as human Cytokine Synthesis Inhibitory Factor (CSIF) is a heterogeneous protein produced by monocytes mainly and to a lesser extent by lymphocytes, it has anti-inflammatory effect by inhibiting synthesis of proinflammatory cytokines, also has stimulatory effect towards certain T-cells, mast cells, stimulates B-cell maturation and antibody production [11].

Different anaesthetic methods may affect the cytokine response to surgery as previous study showed that total intravenous anaesthesia using propofol and remifentanyl suppresses inflammatory response caused by surgery to a greater extent than a balanced inhalational technique using isoflurane [12].

Previous study proved that the addition of the spinal block has an advantage over the use of general anaesthesia alone; reducing neuroendocrinal response to surgery [13].

So, different types of anaesthesia affect the function of the immune system either by disturbing the function of the immune competent cells or by its effect on the stress response [14].

The present study, continuing the previous research on the effect of anaesthetic technique on pro- and anti-inflammatory cytokines, compares the effect of spinal and general anaesthesia on TNF-α, IL-6 and IL-10 levels in patients undergoing appendicectomy.

2. Patients and methods

Forty patients between 20 and 35 years old, 23 males and 17 females (ASA physical status I–II) undergoing appendicectomy in Zagazig University Hospitals were enrolled in the study after written informed consent from each patient and approval by local ethics committee. Sample sizes were estimated by using Epi-Info version, software computer package.

Exclusion criteria include systemic illness known to affect the inflammatory changes such as rheumatoid arthritis, patients under steroid treatment, also patients with Congestive heart failure, diabetes mellitus, hypertension, malignancy and any contraindications for spinal anaesthesia.

Patients were randomly allocated to receive either General anaesthesia (group G, $n = 20$) or Spinal anaesthesia (group S, $n = 20$) by using random table, even number for general anaesthesia and odd number for spinal anaesthesia.

Continuous monitoring of noninvasive arterial blood pressure, heart rate and oxygen saturation was recorded at pre-induction of anaesthesia, postinduction then every 15 min till the end of operation and peripheral nerve stimulator was used for monitoring of the degree of neuromuscular blockade.

Adverse effects as change in arterial blood pressure or heart rate more than 20% of basal level were recorded and hypotension with MAP less than 60 mmHg was corrected by fluid administration (Ringer lactate) with ephedrine incremental IV injection.

In General anaesthesia group (group G), we started by insertion of a peripheral Intravenous (IV) cannula after

sterilization of the skin, then preoxygenation with 100% O_2 and started induction by IV thiopental 5 mg/kg and fentanyl 2 μg/kg. Patients received IV rocuronium 0.8 mg/kg to facilitate endotracheal intubation. The lungs were ventilated using volume-controlled ventilation mode, with tidal volume 10 ml/kg and maximum airway pressure 30 mmHg.

After intubation, oxygen with 1.5% isoflurane was used for maintenance and rocuronium 0.15 mg/kg to maintain muscle relaxation (train of four stimulation of ulnar nerve was done every half an hour).

In Spinal anaesthesia group (group S), peripheral IV cannula was inserted and 500 cc Ringer lactate was given before spinal anaesthesia, patients were placed in sitting position and a 25 gauge spinal needle was inserted through the L_{3-4} intervertebral space after complete aseptic preparation of the skin. Hyperbaric bupivacaine 0.5% 3–4 ml was injected into the subarachnoid space.

Samples: 2 ml of venous blood in sterile EDTA vacutanier were taken just before induction of anaesthesia (B_1), at end of surgery (B_2) and 12 h postoperatively (B_3).

Flowcytometry was used for detection of IL-6, IL-10 and TNF-α [15].

Intracellular staining of cytokines was performed using intracellular staining reagent (R&D system Inc.). For IL-6, anti-human IL-6-fluorescein monoclonal antibody (cat number: IC206F) was used, anti-human IL-10-fluorescein monoclonal antibody (cat number: IC2171F) was used for IL-10 and anti-human TNFα-phycoerythrin monoclonal antibody was used for TNF-α. Each monoclonal antibody was prepared in a separate tube.

2.1. Procedure

Whole blood samples were collected in evacuated tubes containing EDTA as the anticoagulant. Contaminating serum components were removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 2000 per rpm for 5 min. 200 μl of packed cells is then transferred to a 5 ml tube for staining with the monoclonal. Whole blood cells will require lysis of RBC by adding 1 ml of lysing solution (1 × 10) and incubation for 10 min at room temperature, then the cells were washed twice and the supernatant was discarded to get the cell pellet.

Cells were fixed in 0.5 ml of cold 4% paraformaldehyde fixative and incubated at room temperature for 10 min. The cells were vortex intermittently in order to maintain a single cell suspension. Following fixation, the cells were washed twice in PBS by centrifuging at 1200 per rpm for 7 min. Supernatant was discarded and the cell pellet resuspended in 2 ml of SAP buffer (0.1% saponin in a balanced salt solution provided by the kit). Then, the cells were centrifuged at 1000 per rpm for 7 min. The supernatant decanted, ensuring that approximately 200 μl of SAP buffer remains in the tube. The cells were gently resuspended in the remaining SAP buffer and added 10 μl (or a previously titrated amount) of the monoclonal antibody. The tube was briefly vortexed and incubated for 30-45 min at room temperature *in the dark*. The cells were washed twice using 2 ml of SAP buffer each time. The cells were again centrifuged at 1200 per rpm for 7 min. The cells were resuspended in each tube with 200–400 μl of PBS for final flow cytometric analysis.

3. Statistical analysis

Data were checked, entered and analysed by using (SPSS version 19) software computer package. Data were expressed as mean ± standard deviation for quantitative variable, number and percentage in qualitative one. T-test, paired T-test and chi-squared test were used as appropriate. $p < 0.05$ was considered significant.

4. Results

As regards the demographic data (the age, sex) and the duration of surgery, there was no significant differences between the two groups (Table 1).

The level of proinflammatory cytokines (TNFα and IL-6) and anti-inflammatory cytokine (IL-10) before the induction of anaesthesia did not differ significantly between the general and spinal groups (Table 2).

At the end of surgery, there was significant increase in the level of both proinflammatory cytokines (TNFα and IL-6) and the anti-inflammatory cytokines (IL-10) when compared to the pre-induction level in both groups ($p < 0.05$) (Table 2) with significant increase in the level of anti-inflammatory cytokine (IL-10) in spinal group than general group ($p < 0.05$).

Table 1 Demographic data and duration of surgery between the two groups.

Data	General group ($n = 20$)	Spinal group ($n = 20$)
Age (years)		
Mean ± SD	29.2 ± 6.4	30.8 ± 6.1
Gender		
Male *N* (%)	12 (60.0)	11 (55.0)
Female *N* (%)	8 (40.0)	9 (45.0)
Duration (min)		
Mean ± SD	75 ± 14.6	73.6 ± 15.4

Data expressed by Mean ± SD and percentage.

Table 2 The level of TNF, IL-6 and IL-10 in both groups at pre-induction, end of surgery and 12 h postoperatively (mean ± SD), TNF, IL-6 and IL-10 presented as percentage in the flowcytometry.

Cytokines	Pre-induction	At end of surgery	12 h postoperatively
TNFα			
General anaesthesia	21 ± 12.2%	42* ± 14.8%	20 ± 13%
Spinal anaesthesia	22.1 ± 13.4%	41* ± 13.2%	21 ± 11.2%
IL-6			
General anaesthesia	19 ± 11.5%	35* ± 12.9%	30* ± 13.3%
Spinal anaesthesia	20.1 ± 12.1%	64* ± 13.3%	51* ± 14.7%
IL-10			
General anaesthesia	14 ± 12.3%	30* ± 8.2%	27* ± 10.2%
Spinal anaesthesia	15 ± 12.1%	48* ± 9.1%+	44* ± 11.1%+

* $p < 0.05$ when compared with preoperative level.
\+ $p < 0.05$ when compared between both groups.

Table 3 Haemodynamic table of general anaesthesia group. Data expressed by Mean ± SD.

	Pre-induction	Post-induction	15 min	30 min	45 min	60 min	75 min	90 min
Systolic blood pressure (mmHg)	124 ± 6.1	126.2 ± 5.8	125.2 ± 5.3	123.2 ± 6.1	124.1 ± 6.7	126.1 ± 5.6	126 ± 5.6	124.6 ± 6
Diastolic blood pressure (mmHg)	74.4 ± 5.6	76.5 ± 4.2	74.3 ± 6.2	72.1 ± 6.1	73.1 ± 6.1	73.6 ± 5.8	74.1 ± 6	72.1 ± 6
Heart rate (beat/minute)	94 ± 5.1	101 ± 4.9	100 ± 8.2	96 ± 6.7	95.1 ± 5.3	97.1 ± 6.2	96 ± 5.9	95.5 ± 6.2
O_2 saturation (%)	96.4 ± 0.8	97 ± 0.8	98.1 ± 0.8	96.5 ± 0.7	96.4 ± 0.89	96.5 ± 0.8	97.3 ± 0.6	96.6 ± 0.8

Table 4 Haemodynamic table of spinal anaesthesia group. Data expressed by Mean ± SD.

	Pre-spinal	Post-spinal puncture	15 min	30 min	45 min	60 min	75 min	90 min
Systolic blood pressure (mmHg)	120.1 ± 4.3	119 ± 4.3	118 ± 5.2	115 ± 6.2	116.1 ± 6.3	119.3 ± 5.8	120.7 ± 5.1	121.1 ± 6.5
Diastolic blood pressure (mmHg)	73 ± 5.3	72.8 ± 4.1	70.1 ± 4.3	69.1 ± 4	70 ± 5.1	72.2 ± 6.1	73.2 ± 4.5	73.1 ± 5.2
Heart rate (beat/minute)	93.6 ± 4.3	94.1 ± 5.1	100.1 ± 5.3	103.4 ± 8.5	96.1 ± 4.9	95.1 ± 6.1	94.1 ± 5.6	93.6 ± 3.2
O_2 saturation (%)	96.3 ± 0.67	97.6 ± 0.9	98.2 ± 0.8	97.9 ± 0.8	96.3 ± 0.8	97.3 ± 0.8	98.1 ± 0.5	97.2 ± 0.3

At 12 h postoperatively, TNF returns to the pre-induction level in both groups and IL-6 and IL-10 still with significant higher concentration than the pre-induction level ($p < 0.05$). Also, there is significant increase in the level of IL-10 in spinal group compared to general group ($p < 0.05$) (Table 2).

As regards the haemodynamic data, there was no significant differences between the two groups (Tables 3 and 4).

As regards the complications (hypotension, bradycardia and decrease in O_2 saturation), there was only hypotension in two patients of spinal group and was corrected by fluid and ephedrine.

5. Discussion

Surgery and anaesthesia lead to stress response which causes inflammatory changes and immune response during and after surgery that is responsible for postoperative complications such as infection and hypercatabolism [14].

This stress response is initiated by two mechanisms, the stimulation of the afferent nerve and the release of humoral substances such as cytokines (IL-1 and TNFα), prostaglandins, kinins and others.

The most important is the neural pathway which is responsible for the release of catabolic hormones while the humoral response is responsible for the hyperthermic reactions, change in capillary permeability and coagulation changes.

The type of anaesthesia has a role in this response as spinal anaesthesia will block the neural response so that it reduces the endocrine-metabolic response [16].

Cytokine mediators which are released as a result of surgical trauma or anaesthesia have a role in infection and tissue repair.

The increase in the proinflammatory cytokines leads to increase in postoperative complications. The increase in the anti-inflammatory cytokines has protective effect against infection and tissue trauma. So, the balance between the two mediators is important to decrease the postoperative complications and help tissue repair [4].

In the present study, there was an increase in both pro- and anti-inflammatory cytokines at the end of surgery with more increase in the anti-inflammatory interleukin-10 in the spinal group (Table 2).

So, the anaesthetic technique affects the release of cytokines. This result is in accordance with the result of Ke et al. [12] which compared the effect of total intravenous anaesthesia and inhalational anaesthesia on the release of pro- and anti-inflammatory cytokines during open cholecystectomy. They found that the proinflammatory cytokine levels (TNFα and IL-6) and the anti-inflammatory cytokine (IL-10) showed a significant increase in their concentrations at the end of anaesthesia compared with pre-induction levels [12].

At 12 h postoperatively, IL-6 and IL-10 levels are still higher than pre-induction level as in the present study.

In a previous study comparing the effect of general and spinal anaesthesia on stress response, it was found that there was no significant difference as regards the anaesthetic techniques on proinflammatory cytokines [17] and these results were not in accordance with the result of the present study, as the previous study measured the TNFα and IL-6 before induction and 24hours after surgery so they found that TNFα not elevated and in the present study TNFα was elevated at the end of surgery and returned to normal at 12 h postoperative beside that in the present study we measured IL-10 and it was significantly different in both groups but the previous study of Unase et al. [17] not measure the IL-10 so they not found significant difference between the both groups.

Indeed, the study of Marijana et al. [18] showed that the proinflammatory cytokine IL-6 was increased in patients after spinal anaesthesia. This was in accordance with the result of the present study, also showed that serum concentration of anti-inflammatory cytokines had no statistical difference in values before and after surgery and this was not in accordance with the results of the present study, as the study of Marijana et al. measured the cytokines level 2 h before surgery and in the first, third, fifth days postoperatively and this time interval was longer than the sampling time of the present study beside that the previous study was on small number of patients and not comparing general anaesthesia with spinal anaesthesia but they concluded that spinal anaesthesia results in less immunosuppression, thus stimulating the cell immunity [18].

Dimopoulou et al. [2] found that the concentration of TNFα, IL-6 and IL-10 were elevated postoperatively compared to preoperative level and this was in accordance with the result of present study.

In summary, the present study found that the type of anaesthesia affects the pro- and anti-inflammatory cytokine levels as both general and spinal anaesthesia cause an increase in the pro- and anti-inflammatory cytokines with significant increase in anti-inflammatory cytokine IL-10 in spinal anaesthesia than in general anaesthesia.

So, it is preferred to use spinal anaesthesia when it is possible.

We recommend study on different types of operation and measure other types of cytokines or measure cytokine after longer period (days). Also, search about the postoperative complications as infection or disorder in wound healing.

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