INTRODUCTION
Opioids, inhalational agents, intravenous, and local anesthetics have shown different effects on immune system and cytokine expression [1]. General anesthesia accompanied by surgical stress is considered to suppress immunity, presumably by directly affecting the immune system or activating the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system [2]. Surgical stress and general anesthesia may suppress natural killer and cytotoxic T cells and also activate sympathetic nervous system [3]. Cytokines play an important role in selectively recruiting monocytes, neutrophils, and lymphocytes, as well as in inducing chemotaxis through the activation of G-protein-coupled receptors [5]. MCP-1 almost made by all cells and tissues upon stimulation by different agents, but it mainly released by monocyte cells, that is why MCP-1 was first designated as monocyte chemotactic and activating factors that could lead to kill tumor targets and considered as the major chemoattractant agent in the human body [6]. The primary aim of this study was to evaluate the effects of different anesthesia techniques on some innate immunity components in orthopedic patients.

METHODS
This study was conducted on 30 patients with orthopedic surgeries and arthroscopy; 16 males (53.3%) and 14 females (46.7%) with the age range of 10–72 years old recruited from the Orthopedic and Rheumatology Department of Al-Diwaniyah Teaching Hospital during period from January 1, 2018, to April 30, 2018. Three types of anesthesia were used: 10 patients anesthetized with general anesthetics, 10 patients with local anesthesia, and 10 patients with regional anesthesia with duration of anesthesia ranging 75 min (15–90 min). Sample was collected at three timing intervals 24 h before, during, and 12 h after surgery. The study population was assessed by questionnaire regarding age, gender, type of surgery, duration of anesthesia, and clinical history of other disease. Kits of ELISA are used in this study depending on sandwich enzyme immunoassay method. Micro ELISA plate provided in this kit has been pre-coated with an antibody specific to TNFα and INFγ. OD for each well is calculated at once using a microplate reader spectrophotometer at the wavelength of 450 nm.

Flow cytometry
Flow cytometry assay kits that have been used in this study are flow cytometry kit for CD16 and hematological white blood cell (WBC) count.

Statistical analysis
Data were translated into a computerized database structure. An expert statistical advice was sought for. All data were analyzed using Statistical Package for the Social Sciences software version 20 in association with Microsoft Excel 2016. To measure the strength of association between categorical variables, such as the effect of anesthetic techniques on cellular response, the odds ratio was used. Log transformation was carried out to make the distribution of variables related to CD16 natural killer cells and the level of MCP-1, normal.

RESULTS
According to the type of anesthesia, this study enrolled 10 patients with general anesthesia, 10 patients with regional anesthesia, and 10 patients with local anesthesia. The mean duration of anesthesia was 44.33±19.85 min and it ranged from 15 to 90 min. Hypertension was seen in three patients (10%), diabetes was seen also in three patients (10%), ischemic heart disease was seen in a single patient (3.3%), asthma was seen in single patient (3.3%), and agranulocytosis was seen in single patient (3.3%), as explained in Table 1.
DISCUSSION

Variation in the type of surgical operation was proved by several authors to be associated with different types of responses to the same anesthesia techniques [7]. In the current study, there is a significant rise in neutrophil count after anesthesia in comparison with baseline level. This is in agreement with Deirmengian et al. [8]. The proposed mechanism of raising neutrophil count is mostly probably due to surgical trauma and associated stress with neurohumoral effect in addition to variations in cell cycle due to stress hormones (e.g., epinephrine and corticosteroids).

It was also observed that the level of the chemotactic chemokine MCP-1 remains insignificantly altered before, within, and after operation (p>0.05). Neither CD16 natural killer cells nor MCP-1 showed significant correlation with age of patients (p>0.05), as demonstrated in Table 9. CD16 NK cells did not vary significantly in relation to type of anesthesia, whether local, regional, or general, in all situations whether before, at time, or after operation (p>0.05), also the level of the chemotactic chemokine MCP-1 showed no significant difference with respect to the type of anesthesia, general versus regional versus local, whatever the time in relation to anesthesia was, pre-operatively, peri-operatively and post-operatively, (p>0.05), as demonstrated in Table 10. Regarding the correlation of immune marker with time duration of anesthesia, the results showed that immune cells, lymphocytes, showed no statistical significant correlation with duration of anesthesia (p>0.05) [Table 11]. There was a highly significant rise in CRP blood level post-operatively when compared with pre-operative blood level, 2.65 (4.99) and 299 (5.64), respectively, (p<0.001) as shown in Fig. 3.

Table 1: Association of cytokine level and gender

| Cytokine | Male (n=16) | Female (n=14) | p |
|----------|------------|--------------|---|
|          | Median     | IQR          | Median | IQR |
| TNFα pre | 58.08      | 46.84        | 61.02  | 28.41 | 0.835 |
| TNFα peri| 53.50      | 31.62        | 56.55  | 13.14 | 0.560 |
| TNFα post| 55.02      | 47.03        | 51.97  | 26.13 | 0.519 |
| IFNγ pre | 140.82     | 106.51       | 147.52 | 64.60 | 0.835 |
| IFNγ peri| 130.40     | 71.91        | 137.35 | 29.88 | 0.560 |
| IFNγ post| 133.88     | 32.35        | 126.93 | 59.39 | 0.519 |

Table 2: Association of cytokine level and gender

Table 3: Correlation between age and cytokine levels

| Cytokine | r | p |
|----------|---|---|
| TNFα pre | -0.109 | 0.65 |
| TNFα peri | 0.091 | 0.63 |
| TNFα post | -0.052 | 0.784 |
| IFNγ pre | -0.109 | 0.568 |
| IFNγ peri | 0.087 | 0.649 |
| IFNγ post | -0.047 | 0.803 |

Table 3: Correlation between age and cytokine levels

Table 4: Correlation between cytokine levels and type of anesthesia

| Cytokine | General anesthesia | Local anesthesia | Regional anesthesia | p |
|----------|--------------------|------------------|---------------------|---|
|          | Median IQR         | Median IQR       | Median IQR          |   |
| TNFα pre | 62.11 (3.12, 63.64) | 51.53 (19.63, 58.51) | 50.88 (22.31, 54.58) | 0.115 |
| TNFα peri | 55.02 (22.31, 54.58) | 47.20 (52.62, 12.54) | 55.02 (22.31, 54.58) | 0.558 |
| IFNγ pre | 150.00 (79.85, 153.46) | 93.99 (113.04, 131.07) | 31.12 (133.88, 65.59) | 0.686 |
| IFNγ peri | 125.94 (44.63, 141.81) | 97.07 (136.61, 28.52) | 125.94 (44.63, 141.81) | 0.558 |
| IFNγ post | 124.46 (50.71, 132.88) | 71.91 (137.35, 29.88) | 124.46 (50.71, 132.88) | 0.686 |

*Significant at p<0.05. Values were expressed as median (IQR); n: Number of the cases; †Kruskal–Wallis H-test; TNFs: Tumor necrosis factor alpha, IFNγ: Interferon gamma, IQR: Interquartile range.
to the possibility of post-operative infection [9]. In the current study, there is a significant decline in lymphocyte count after anesthesia in comparison with baseline level. This is in agreement with Dąbrowska and Slońowski 2014. The proposed mechanism for the reduction in lymphocyte count is the disturbance in apoptosis of lymphocyte through bcl2-dependent mechanism, by dysregulation of antiapoptosis and proapoptosis signal equilibrium [10].

In the current study, there is a significant decline in monocyte count after anesthesia in comparison with baseline level. The reason for that is most probably due to changes in immune mediators as a response to tissue injury and stress accompanying surgery, and also, there is a significant decline in eosinophil count after anesthesia in comparison with baseline level. The reason for that is most probably due to changes in immune mediators as a response to tissue injury and stress accompanying surgery. Moreover, in the current study, there is no significant change in basophil count after anesthesia in comparison with baseline level. The reason for that is most probably due to changes in immune mediators as a response to tissue injury and stress accompanying surgery This is in agreement with Valiathan et al. [13]. The present study showed that post-operative WBC count and differential counts were not significantly correlated to the gender of the patient. This finding disagrees with Chen et al. [11]. The concept of aging of the immune system is recent and controversial. Several suggestions have been proposed to explain the reduced number of some cell types that are involved in adaptive and innate immune response, and the most widely accepted explanation is the aging of bone marrow and the source of all cells involved in immune system [12]. The present study showed that post-operative WBC count and differential were not significantly correlated to the gender of the patient. This is in agreement with Valiathan et al. [13].

### Table 8: Immune markers in relation to gender

| Marker          | Total n=30 mean±SD | Male n=16 mean±SD | Female n=14 mean±SD | p* |
|-----------------|---------------------|-------------------|---------------------|----|
| CD16Pr          | 12.45±8.14          | 11.57±8.74        | 12.84±8.76          | 0.934 |
| CD16Pe          | 21.05±13.36         | 21.16±14.18       | 20.89±15.78         | 0.662 |
| CD16Po          | 13.25±5.79          | 11.10±7.22        | 14.66±5.92          | 0.934 |
| MCP-1Pr         | 11.14±12.80         | 11.25±13.27       | 9.94±12.84          | 1.000 |
| MCP-1Pe         | 13.24±7.13          | 13.24±11.13       | 13.65±6.56          | 0.771 |
| MCP-1Po         | 10.79±11.64         | 11.02±10.10       | 10.65±11.23         | 0.394 |

*p: Correlation coefficient, CD: Cluster of designation

### Table 9: Correlation of immune markers with age

| Marker          | R       | p      |
|-----------------|---------|--------|
| Log CD16Pr      | 0.209   | 0.268  |
| Log CD16Pe      | -0.011  | 0.953  |
| Log CD16Po      | 0.127   | 0.503  |
| Log MCP-1Pr     | 0.020   | 0.915  |
| Log MCP-1Pe     | -0.170  | 0.370  |
| Log MCP-1Po     | -0.007  | 0.696  |

*p: Correlation coefficient, CD: Cluster of designation

### Table 10: Immune markers in relation to type of anesthesia

| Marker          | General | Local | Regional | P   |
|-----------------|---------|-------|----------|-----|
| CD16Pr          | 13.24   | 15.09 | 6.47     | 14.33 | 0.777 | 0.145 |
| CD16Pe          | 20.09   | 22.98 | 15.83    | 21.16 | 11.99 | 0.557 |
| CD16Po          | 12.85   | 13.35 | 7.70     | 13.28 | 5.88  | 0.866 |
| MCP-1Pr         | 2.52    | 11.72 | 6.23     | 11.70 | 13.62 | 0.004 |
| MCP-1Pe         | 13.98   | 15.19 | 5.00     | 12.46 | 6.50  | 0.673 |
| MCP-1Po         | 5.01    | 10.41 | 8.77     | 10.97 | 9.82  | 0.093 |

*p: Correlation coefficient, CD: Cluster of designation

### Table 11: Correlation of immune markers with duration of anesthesia

| Marker          | r       | p      |
|-----------------|---------|--------|
| Log CD16Per     | -0.508  | 0.174  |
| Log CD16Post    | 0.049   | 0.799  |
| Log MCP-1Per    | 0.069   | 0.719  |
| Log MCP-1Post   | 0.095   | 0.618  |

*p: Correlation coefficient, CD: Cluster of designation

### Table 7: Median level of immune markers in relation to operation timeline

| Marker          | Pre-operatively | Perioperatively | Post-operatively | p* |
|-----------------|-----------------|-----------------|------------------|----|
| CD16, median (IQR) | 12.45 (8.14)    | 21.05 (13.36)   | 13.35 (5.79)     | <0.001 |
| MCP-1, median (IQR) | 11.14 (12.80)   | 13.24 (7.13)    | 10.79 (11.64)    | <0.001 |

*p: Friedman test, CD: Cluster of designation, IQR: Interquartile range
The present study showed that the level of immune markers CD16 and MCP-1 became significantly higher during operation, whatever the type and duration of anesthesia, in comparison with their levels before operation, and that their level decreased significantly after operation; however, it did not return back to the same level before operation (with the exception of MCP-1 which returned back almost to the same level before operation). Natural killer cell is an important player of the innate immunity, and its count is expected to rise during physiologic stress [18]. In the present study, there was no significant correlation between gender and age with MCP-1 and CD16 immune markers, and these results are in agreement with Karadeniz et al. and De Toda et al. [22,25]. In the present study, there was no significant correlation between the duration of anesthesia and immune markers (MCP-1 and CD16), and these results are in agreement with Song et al. [26]. Therefore, the most likely explanation is that the trigger for the rise in cellular counts and immune marker is the tissue injury produced by the surgical operation and so, once tissue injury supervenes, the level of these cells and markers get a rise with disregard to the duration of anesthesia [27-29]. In addition, in the present study, there was no significant correlation between type anesthesia and immune markers (MCP-1 and CD16), and these results are in agreement with Berger et al. [20].

CONCLUSIONS

Primarily, there is no significant effect for anesthesia on immune response in patients undergoing orthopedic operations. Moreover, changes in cells, immune markers, and cytokines were mainly attributable to tissue trauma during operation that is mediated by neurohumoral response.

Ethical clearance

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.

AUTHORS’ CONTRIBUTIONS

Adnan Hamad Aubaid: Contributing to the study design, data interpretation, and writing of manuscript. Khalid Lahmood Yaseen: Contributing to sample collection, writing the manuscript, statistical analysis, and publication.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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The study showed that post-operative WBC count and differential were not significantly correlated to duration of anesthesia. This finding is in agreement with Costa et al. [14]. The present study showed that post-operative WBC count and differential were not significantly correlated to the type of anesthesia. This is in agreement with Cho et al. [15]. It appears that the changes in WBC counts happened as a response to the stress accompanying surgical operation that is the mirror of hemural and neural stimulation and that trauma and tissue damage associated with surgical incision is the main stimulant factor behind these stress responses [16]. Accordingly, there will be no significant correlation with the count of WBC and the type and duration of anesthesia. In addition, the present study showed that post-operative WBC count and differential were not significantly correlated to CRP. This finding is in accordance with Boersema et al. [17]. The CRP has been shown to rise significantly in the current study, a finding that is similar to Godoy et al. [18]. The explanation for the rise of CRP is most likely to inflammation that accompanies tissue injury at the time of surgery with an increase in hepatic production of this acute phase reactant [17].

The present study showed that the level of cytokines (IFNγ and TNFα) became significantly lower during operation, whatever the type and duration of anesthesia, in comparison with their levels before operation and that their level continued to fall insignificantly after operation; however, it did not return back to the same level before operation. These results are similar to the findings of Cheng et al. [19]. The explanation for the fall in the level of these cytokines is most probably due to the anti-inflammatory effect subjected by IL-10. IL-10 is an anti-inflammatory cytokine that acts by autocrine and paracrine mechanisms that cause the suppression of secretion of pro-inflammatory cytokines such as IFN γ and TNFα by the same cell secreting IL-10 and other nearby cells, an effect that is named as shifting from t-helper 1 into t-helper 2 predominance [2]. The current study showed no significant correlation between any of the cytokines and gender of the patients. This finding is in agreement with Berger et al. [20]. The explanation for that is that the main difference between male and female patients is represented by certain hormonal levels, namely estrogen, progesterone, and testosterone, and these hormones have no effect on the level of inflammatory mediators [5]. The current study showed no significant correlation between any of the cytokines and age of the patients which are in agreement with Godbek-Drugiewska et al. [21]. The explanation for the lack of significant correlation between these cytokines and the

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**Table 12: WBC count before and after anesthesia**

| WBC         | Before | After | P      |
|-------------|--------|-------|--------|
| Neutrophil  | 5.62±2.75 | 6.74±2.89 | <0.001 |
| Lymphocyte  | 3.03±1.15 | 2.85±1.13 | <0.001 |
| Monocyte    | 0.65±0.25 | 0.63±0.24 | <0.001 |
| Eosinophil  | 0.31±0.40 | 0.30±0.40 | <0.001 |
| Basophil    | 0.03±0.03 | 0.03±0.04 | 0.687  |

WBC: White blood cell; SD: Standard deviation

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**Fig. 3: C-reactive protein level before and after surgery**

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**Table 12: WBC count before and after anesthesia**

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